The Role of the Circadian Clock in Hippocampal Aging and as a Therapeutic Target in Alzheimer's Disease

Paula Adler

A thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
in partial fulfillment of the requirements for the
Doctorate in Philosophy degree in Biochemistry

Ottawa Institute of Systems Biology
Department of Biochemistry, Microbiology and Immunology
Faculty of Medicine
University of Ottawa

© Paula Adler, Ottawa, Canada, 2019

Mieux vaut prévenir que guérir.

French proverb

Abstract

Aging-associated cognitive impairment and neurodegenerative brain diseases such as Alzheimer's disease (AD) have profound global consequences, including significant health, social, and economic impacts. Rational therapeutic interventions that can effectively address sleep disturbances and memory impairment in people with AD, as well as the complex etiopathogenesis of the disease, remain major unmet clinical needs.

Impaired function of the circadian system, which regulates memory and sleep, occurs during normal human aging and in several neurodegenerative brain diseases, including AD. There exists a complex interplay between the aging process, cognitive decline, and circadian rhythms. Yet, how aging influences rhythmic processes in the brain that underlie memory remains largely unknown. Moreover, given that the circadian clock regulates sleep, hippocampal function, and neurodegeneration, its dysfunction may contribute to the development and clinical manifestations of AD. Therefore, the circadian clock represents a new potential therapeutic target for AD. In particular, casein kinase 1δ and 1ε (CK1δ/ε) are key clock regulators and highly overexpressed in AD brains, making them feasible targets to improve sleep and cognition in people with AD.

In the first part of this thesis, young and middle-aged mice were compared using quantitative mass spectrometry in order to perform a proteome-wide screen for proteins with rhythmic abundances in the hippocampus and dissect changes in the temporal orchestration of biological pathways during aging. This proteomic analysis revealed that aging disrupted circadian regulation of the hippocampal proteome, leading to loss of circadian rhythms of proteins that participate in specific biological functions. Notably, proteins displaying loss of rhythmicity during aging were involved in processes critical for hippocampal function and memory, including energy metabolism, neurotransmission, and synaptic plasticity. Moreover, aging was found to alter the daily expression profiles of proteins implicated in

age-related neurodegenerative diseases and hallmarks of aging, including mitochondrial dysfunction and loss of protein homeostasis. These findings suggest that aging-induced changes in rhythmic physiology may play important roles in cognitive decline and aging-associated brain disorders such as AD.

In the second part of this thesis, the therapeutic potential of a small molecule CK1δ/ε inhibitor (PF-670462) was evaluated in a triple transgenic mouse model of AD (3xTg-AD). Mass spectrometry–based proteomic analyses revealed that PF-670462 administration in 3xTg-AD mice reversed hippocampal proteomic alterations in several AD-related and clock-regulated pathways, including synaptic plasticity and amyloid precursor protein processing. Furthermore, PF-670462 administration rescued working memory deficits and normalized behavioural circadian rhythm disturbances in 3xTg-AD mice. This study provides *in vivo* proof of concept for CK1δ/ε inhibition against AD-associated hippocampal proteomic changes, memory impairment, and circadian disturbances.

These studies provide a framework for understanding the links between age-related cognitive decline, neurodegenerative diseases, and the circadian clock. The aging-associated changes identified in the circadian regulation of biological processes underlying memory function in the hippocampus could represent new opportunities to correct dysregulated rhythms and thereby prevent or reverse age-related cognitive decline. Moreover, these findings suggest that CK1δ/ε inhibition or, more broadly, direct circadian clock modulation has neuroprotective disease-modifying potential and represents a viable therapeutic avenue to treat cognitive impairment and sleep disturbances in people with AD. Together, these studies highlight emerging roles for the circadian clock in hippocampal aging and as a therapeutic target for AD.

Acknowledgements

I would first like to thank my thesis supervisor, Daniel Figeys, for his continual support and guidance during my PhD studies, as well as for the opportunity to develop and work on these and other projects. I am especially grateful to him for having trusted me to work independently and creatively, while providing me with the right amount of guidance when I needed it. I could not have asked for a better mentor with whom to share this journey of discovery and learning.

I also wish to thank the members of my thesis advisory committee (Laura Trinkle-Mulcahy, Richard Bergeron, and John Woulfe), for contributing to my academic development with their constructive feedback and encouragement over the course of my PhD studies; and Michael Schlossmacher, for his support within the MD/PhD Program throughout my doctoral studies and for stimulating discussions about research and neurodegenerative diseases.

Moreover, I am grateful for the support and camaraderie of my current and former coworkers in the Figeys laboratory, several of whom have helped with experiments at all hours of the day and night for these and other circadian studies. In particular, thanks to Janice Mayne, for her guidance and meticulousness as well as for lively discussions about various topics; to Zhibin Ning, for his continual help with the mass spectrometers and his sense of humour; to Krystal Walker, for sacrificing many of her evenings and weekends to help with mouse experiments; and to Kerwin Chiang, for his help with proteomic analyses and his hard-working attitude. I am also thankful for the positive environment they have created in the laboratory, along with many other members of our group.

Thanks also to members of the University of Ottawa Behavioural Core, specifically Diane Lagace, Kerstin Ure, Mirela Barclay, and Chris Luckhart, for training as well as their support in the planning and execution of mouse behavioural and cognitive testing.

In addition, I am thankful for funding provided by the Alzheimer Society of Canada and the University of Ottawa throughout my PhD studies.

Lastly, I would like to thank my family, in particular for their continuous support in my personal and professional development, for helping define my values and principles, and for instilling in me a passion for learning.

Table of Contents

Abstract ... iii
Acknowledgements ... v
Table of Contents ... vii
List of Abbreviations ... x
List of Figures ... xiii
Chapter 1. Introduction ... 1
1.1. The circadian system ... 1
1.1.1. Terminology and history ... 1
1.1.2. Organization of the mammalian circadian system ... 3
1.1.3. Systems biology approaches to studying circadian rhythms ... 12
1.1.4. The circadian system's role in health, aging, and disease ... 14
1.2. The hippocampus and memory ... 17
1.2.1. Terminology and history ... 17
1.2.2. Anatomy, organization, and function of the hippocampus ... 19
1.2.3. Cellular and molecular mechanisms of hippocampal memory ... 21
1.2.4. Systems biology approaches to studying hippocampal memory ... 23
1.2.5. Circadian regulation of hippocampal function and memory ... 24
1.3. Aging ... 30
1.3.1. Terminology and history ... 30
1.3.2. Hallmarks of aging and brain aging ... 31
1.3.3. Aging and the circadian system ... 32
1.3.4. Aging, the hippocampus, and memory ... 38
1.3.5. Aging and neurodegenerative diseases ... 41
1.4. Alzheimer's disease ... 42
1.4.1. Terminology and history ... 42
1.4.2. Epidemiology and societal impact of AD ... 44
1.4.3. Classification and risk factors of AD ... 45
1.4.4. Diagnosis and clinical presentation of AD ... 46
1.4.5. Treatment of AD ... 49
1.4.6. Etiology and pathogenesis of AD ... 51
1.4.7. Alzheimer's disease and the circadian system ... 55

1.5. Rationale, hypotheses, and objectives 62
1.5.1. State of the art 62
1.5.2. Purpose 64
1.5.3. Hypothesis #1 and Objective #1 66
1.5.4. Hypothesis #2 and Objectives #2a, 2b, and 2c 66
Chapter 2. Aging-Induced Alterations in the Murine Circadian Hippocampal Proteome 67
2.1. Introduction 67
2.2. Materials and Methods 69
2.2.1. Animals and tissue collection 69
2.2.2. Proteomic analysis of hippocampal tissues 70
2.2.3. LC-MS/MS analysis 71
2.2.4. Mass spectrometry data processing 71
2.2.5. Bioinformatic and statistical analyses 72
2.2.6. Data availability 72
2.3. Results 73
2.3.1. Aging disrupts the hippocampal circadian proteome 73
2.3.2. Age-related changes in the circadian regulation of biological functions 79
2.3.3. Aging disrupts the circadian regulation of energy metabolism 88
2.3.4. Aging disrupts the circadian regulation of synaptic structure and function 91
2.3.5. Aging disrupts rhythmic proteins involved in hallmarks of aging and neurodegenerative diseases 94
2.4. Discussion 100
Chapter 3. Therapeutic Targeting of Casein Kinase 1δ/ε in an Alzheimer's Disease Mouse Model 105
3.1. Introduction 105
3.2. Materials and Methods 108
3.2.1. Animals 108
3.2.2. Stable isotope labelling by amino acids in cell culture 108
3.2.3. *In vivo* CK1δ/ε inhibition and tissue collection 109
3.2.4. Proteomic analysis of hippocampal tissues 109
3.2.5. *In vitro* CK1δ/ε inhibition 110
3.2.6. SILAC-based proteomic analysis of N2a cells 110
3.2.7. LC-MS/MS analysis 111

3.2.8. Mass spectrometry data processing 111
3.2.9. Cognitive testing 112
3.2.10. Behavioural circadian rhythm testing 113
3.2.11. Bioinformatic analysis 114
3.2.12. Statistical analysis 115
3.2.13. Data availability 115
3.3. Results 115
3.3.1. PF-670462 treatment alters expression of AD-related and clock-regulated proteins *in vitro* 115
3.3.2. PF-670462 administration *in vivo* normalizes hippocampal proteomic alterations associated with AD-like pathology 119
3.3.3. PF-670462 administration rescues hippocampal-dependent working memory deficits in 3xTg-AD mice 130
3.3.4. PF-670462 administration normalizes disrupted behavioural circadian rhythms in 3xTg-AD mice 134
3.4. Discussion 140
Chapter 4. Conclusion 143
4.1. Key findings 143
4.2. Significance and future directions 144
References 149
Contributions of Collaborators 183
Rights and Permissions 184
Appendices 185
Appendix A 185
Appendix B 194

List of Abbreviations

3xTg-AD	triple transgenic mouse model of Alzheimer's disease
Aβ	β-amyloid
ABC	ammonium bicarbonate
AChEI	acetylcholinesterase inhibitor
ACN	acetonitrile
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
APOE	apolipoprotein E
APP	amyloid precursor protein
Arg	arginine
ATP	adenosine triphosphate
AVP	arginine vasopressin
BACE1	β-site APP cleaving enzyme
BMAL1	brain and muscle ARNT-like 1
BPSD	behavioural and psychological symptoms of dementia
CA	*cornu ammonis*
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
cAMP	cyclic adenosine monophosphate
CK	casein kinase
CLOCK	circadian locomotor output cycles protein kaput
CNS	central nervous system
CRY	cryptochrome
CT	circadian time
DALY	disability-adjusted life year
DAVID	Database for Annotation, Visualization and Integrated Discovery
DBP	D-box binding protein
DD	constant darkness
DMSO	dimethyl sulfoxide
DMT	disease-modifying therapy
DNA	deoxyribonucleic acid

DSM	*Diagnostic and Statistical Manual of Mental Disorders*
DTT	dithiothreitol
ESI	electrospray ionization
EtOH	ethanol
FA	formic acid
FASP	filter-aided sample preparation
FBS	fetal bovine serum
FDR	false discovery rate
FTD	frontotemporal dementia
GABA	γ-aminobutyric acid
GHT	geniculohypothalamic tract
GO	Gene Ontology
GSK3	glycogen synthase kinase 3
HD	Huntington's disease
HDAC	histone deacetylase
HSP	heat shock protein
IAA	2-iodoacetamide
ICD	International Classification of Diseases
IDH	isocitrate dehydrogenase
IGL	intergeniculate leaflets
IWG	International Working Group
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC	liquid chromatography
LC-MS/MS	liquid chromatography tandem mass spectrometry
LD	light/dark
LFQ	label-free quantification
LTP	long-term potentiation
Lys	lysine
MAP	microtubule-associated protein
MAPK	mitogen-activated protein kinase
MCI	mild cognitive impairment
mRNA	messenger RNA

MS	mass spectrometry
m/z	mass-to-charge ratio
N2a	Neuro-2a
NAD	nicotinamide adenine dinucleotide
NFIL3	nuclear factor, interleukin-3 regulated
NMDAR	*N*-methyl-D-aspartate receptor
NPAS2	neuronal PAS domain protein 2
NTg	non-transgenic
PD	Parkinson's disease
PER	period
PKC	protein kinase C
PPI	protein–protein interaction
PTM	post-translational modification
RHT	retinohypothalamic tract
RNA	ribonucleic acid
ROR	retinoic acid-related orphan receptor
ROS	reactive oxygen species
s.c.	subcutaneous
SCN	suprachiasmatic nuclei
SCX	strong cation exchange
SDS	sodium dodecyl sulphate
SEM	standard error of the mean
SILAC	stable isotope labelling by amino acids in cell culture
siRNA	small interfering RNA
SV2A	synaptic vesicle glycoprotein 2A
TCA	tricarboxylic acid
TFA	trifluoroacetic acid
TTFL	transcription/translation feedback loop
VIP	vasoactive intestinal peptide
ZT	zeitgeber time

List of Figures

Figure 1.1.	Model of the mammalian cell-autonomous circadian clock.	7
Figure 1.2.	Circadian rhythms in processes that influence hippocampal memory.	26
Figure 1.3.	Influence of the circadian clock on hallmarks of aging and brain aging.	36
Figure 1.4.	Reciprocal interactions between the circadian system and Alzheimer's disease.	56
Figure 2.1.	Aging disrupts the hippocampal circadian proteome.	74
Figure 2.2.	Proteins gaining circadian rhythmicity in the hippocampus of middle-aged mice.	77
Figure 2.3.	Aging leads to loss of circadian oscillations in specific biological functions in the hippocampus.	80
Figure 2.4.	Protein interaction network analysis of proteins displaying loss of circadian rhythmicity in the hippocampus during aging.	82
Figure 2.5.	Aging disrupts the circadian oscillations of proteins involved in clock regulation.	86
Figure 2.6.	Aging disrupts the circadian regulation of proteins involved in energy metabolism in the hippocampus.	89
Figure 2.7.	Aging disrupts the circadian regulation of proteins involved in synaptic structure and function in the hippocampus.	92
Figure 2.8.	Aging alters hippocampal circadian oscillations of proteins implicated in Alzheimer's disease and hallmarks of aging.	96
Figure 3.1.	Chemical structure of PF-670462.	107
Figure 3.2.	PF-670462 treatment alters expression of AD-related and clock-regulated proteins *in vitro*.	117
Figure 3.3.	PF-670462 administration shifts the hippocampal proteomic profile of 3xTg-AD mice towards that of NTg mice.	120
Figure 3.4.	PF-670462 administration rescues AD-related protein abundance changes in the hippocampus of 3xTg-AD mice.	124
Figure 3.5.	PF-670462 administration alters expression of proteins involved in LTP and APP processing in the hippocampus of 3xTg-AD mice.	127
Figure 3.6.	PF-670462 administration rescues working memory deficits without altering anxiety-like behaviour in 3xTg-AD mice.	132
Figure 3.7.	Dose determination for PF-670462 running wheel studies in 3xTg-AD mice.	135
Figure 3.8.	PF-670462 administration normalizes disrupted behavioural circadian rhythms in 3xTg-AD mice.	138

Chapter 1. Introduction

1.1. The circadian system

1.1.1. Terminology and history

The **circadian system** (from the Latin *circa* meaning “around” and *diem* meaning “day”) is an endogenous timekeeping system that regulates several physiological and behavioural processes, allowing organisms to anticipate and adapt to recurring daily changes in the environment (Dibner et al., 2010; Bollinger and Schibler, 2014). While circadian timekeeping has been described in a variety of living organisms (Young and Kay, 2001), the focus here will be on the mammalian circadian system. This system may be conceptualized as a hierarchical multi-oscillator network in which signals from the hypothalamic **suprachiasmatic nuclei (SCN)**, which act as the master circadian pacemaker or **central clock**, entrain individual **peripheral clocks** outside the SCN to the environmental day/night cycle and synchronize them to one another (Liu et al., 2007; Dibner et al., 2010; Richards and Gumz, 2013).

Circadian clocks (or **circadian oscillators**) are biological timekeeping devices that produce **circadian rhythms** (or **circadian oscillations**) in physiology and behaviour that have a period of approximately one day when an organism is kept under free-running conditions (Hastings et al., 2003; Dibner et al., 2010; Welsh et al., 2010; Bollinger and Schibler, 2014). Organisms kept under constant environmental conditions lacking external timing cues (i.e., **free-running conditions**) are thus in temporal isolation (Hastings et al., 2003; Liu et al., 2007). In the context of *in vivo* circadian experiments, “free-running”

rhythms generally refer to the behavioural rhythms observed following release of animals from a **light/dark (LD)** cycle into **constant darkness (DD)** conditions (Liu et al., 2007). Under these "free-running" conditions, biological timing is expressed in **circadian time (CT)** with CT0 corresponding to subjective dawn and CT12 corresponding to subjective dusk (Hastings et al., 2003). In contrast, in the presence of environmental ***zeitgebers*** (from the German *Zeit* meaning "time" and *Geber* meaning "giver") or time cues such as the daily light/dark cycle that serve as inputs for the circadian system, these inputs can enable the entrainment of circadian oscillators, and biological timing is expressed in **zeitgeber time (ZT)** with ZT0 corresponding to "lights on" and ZT12 corresponding to "lights off" (Dibner et al., 2010; Eckel-Mahan and Sassone-Corsi, 2015).

These circadian rhythms are commonly described by three parameters: period, phase, and amplitude. The **period** refers to the time difference between two neighbouring peaks of an oscillation wave and the **phase** describes the initial angle of an oscillation wave relative to a reference point (Zhang and Kay, 2010). In genome-wide circadian studies, the **amplitude** of a circadian oscillation often represents the fold change (i.e., the peak abundance divided by the trough abundance) (Takahashi et al., 2008; Hughes et al., 2017), although some have alternatively defined it as the magnitude change measured from the midline to either the peak or trough (Bell-Pedersen et al., 2005).

Descriptions of circadian rhythms can be traced as far back as 1729, when the French astronomer Jean-Jacques d'Ortous de Mairan documented his observation that the daily patterns of movement of plant leaves can still occur when a plant is placed in constant darkness (de Mairan, 1729). More recently, circadian oscillations have been described in a number of unicellular and multicellular organisms (Bell-Pedersen et al., 2005; Richards and Gumz, 2013), although the focus here will be on mammalian circadian rhythms. In the early 1970s, lesion experiments in rats demonstrated the critical role of the SCN in regulating circadian rhythms in locomotor activity and hormone levels, and these and other

experiments have contributed to the current model of the circadian system that involves the SCN acting as the central pacemaker (Moore and Eichler, 1972; Stephan and Zucker, 1972; Dibner et al., 2010). The discovery of mammalian clock genes and the presence of self-sustained molecular oscillators in non-SCN cells in the late 1990s (Dunlap, 1999) paved the way for more recent systems biology–based approaches to systematically examine circadian rhythms at the cellular level (Mermet et al., 2017; Millius and Ueda, 2017).

Knowledge of the roles that circadian rhythms play in normal physiology and health, and of how circadian disruption contributes to increased disease risk and the progression of various diseases, could be leveraged to develop new disease prevention and treatment strategies (Bass and Lazar, 2016; Panda, 2019). The importance of studies on circadian biology and in the emerging field of circadian medicine (Panda, 2019) is underscored by the 2017 Nobel Prize in Physiology or Medicine, which was awarded for discoveries of molecular mechanisms controlling circadian rhythms.

1.1.2. Organization of the mammalian circadian system

The current model of the mammalian circadian system identifies three distinct components: (*1*) the inputs, (*2*) the multi-oscillator network, and (*3*) the outputs (Liu et al., 2007; Albrecht, 2012). In this model, external and environmental **inputs** (e.g., the photoperiod) serve mainly to entrain the SCN to geophysical time. In turn, the SCN coordinate local circadian physiology in other brain regions and organs by synchronizing peripheral clocks through a variety of resetting signals, including neuronal and humoral cues. Together, the central and peripheral clocks comprise the **multi-oscillator network** that regulates circadian overt rhythms in behaviour and physiology (the **outputs**), with implications for health and disease (Liu et al., 2007; Dibner et al., 2010). Although the mammalian circadian system is more complex than this simplified, linear input–oscillator–output pathway would suggest, this model may nevertheless be useful for representing the system's overall organization.

Working together, the different components of the circadian system allow biological clocks to have certain properties that are critical to their effectiveness, including periodicity, entrainment, robustness, accuracy, and temperature compensation (Hogenesch and Ueda, 2011; Mohawk et al., 2012; Millius and Ueda, 2017).

1.1.2.1. Inputs

The overall purpose of the inputs to the circadian system is to provide entraining cues that enable an organism to anticipate and adapt to changes in the environment, through regulation of circadian clocks located throughout the body (Albrecht, 2012). Inputs to the multi-oscillator network can occur at two levels: (*1*) to the SCN (central clock) and (*2*) to peripheral clocks (Liu et al., 2007; Dibner et al., 2010; Albrecht, 2012):

(1) Inputs to the SCN. Inputs to the SCN include photic signals and non-photic signals (Liu et al., 2007; Albrecht, 2012) that converge on three major input pathways: the retinohypothalamic tract (RHT), the geniculohypothalamic tract (GHT), and serotonergic inputs (Dibner et al., 2010). It has been established that photic signals, such as the daily light/dark cycle, act as the main *zeitgeber* and to be transmitted from the retina to the SCN in two ways: (*a*) directly, via glutamate and pituitary adenylate cyclase-activating polypeptide (PACAP) release from intrinsically photosensitive retinal ganglion cells (ipRGCs) that form the RHT at the optic chiasm, above which the SCN are located (Hastings et al., 2018); and (*b*) indirectly, via neuropeptide Y (NPY) and γ-aminobutyric acid (GABA) release from the intergeniculate leaflets (IGL), which receive photic signals from the RHT. Information from photic signals can lead to phase resetting of clock gene expression within SCN neurons, enabling synchronization of the central clock to the daily light/dark cycle (Liu et al., 2007). Non-photic signals, such as activity and temperature rhythms (Dardente and Cermakian, 2007), have been shown to be transmitted mainly from the IGL and serotonergic nuclei to the SCN along two pathways: (*a*) via the GHT, from the IGL; and (*b*) via serotonergic inputs, from serotonergic nuclei (Dibner et al., 2010). Thus, information from photic and non-photic

signals travelling along these pathways are integrated by the SCN, which in turn transmit this timing information to the rest of the body (see section 1.1.2.2 below) (Dibner et al., 2010).

(2) Inputs to peripheral clocks. Although less is known about external inputs to peripheral clocks, they include non-photic signals such as feeding/fasting cycles (which are indirectly regulated by the SCN through oscillating behaviour) that can act as *zeitgebers* for several peripheral organs, including the liver and pancreas (Damiola et al., 2000; Dibner et al., 2010; Albrecht, 2012). However, it seems that the major timekeeping signals to peripheral clocks emanate from or are coordinated by the SCN, which integrate both photic and non-photic information (Dibner et al., 2010). These signals are conveyed through a combination of behavioural, neuroendocrine, and neuronal pathways to coordinate the phases of peripheral clocks (Liu et al., 2007; Dibner et al., 2010).

1.1.2.2. The multi-oscillator network

The overall purpose of the multi-oscillator network is to enable the circadian system to integrate various time cues (inputs) and transmit this timing information to cells throughout the body in order to regulate circadian behaviour and physiology (outputs) (Liu et al., 2007; Hastings et al., 2018). Individual circadian clocks at the levels of (*1*) cells and (*2*) tissues and organs comprise the multi-oscillator network, which is synchronized into a coherent system by the central clock (Liu et al., 2007; Dibner et al., 2010):

(1) Cellular clocks. In mammals, virtually all cells are thought to harbour a cell-autonomous molecular clock, of which the current model involves a network of interlocking transcription/translation feedback loops (TTFLs) comprised of so-called clock genes that are necessary for sustaining circadian rhythms (Dardente and Cermakian, 2007; Takahashi et al., 2008; Mermet et al., 2017). In the current model, the circadian gene network is comprised of (*a*) three TTFLs, which represent the major regulators of clock-controlled genes and rhythmic biological processes; and (*b*) other clock modifiers (Takahashi, 2017):

(*a*) Three interlocked TTFLs in the cell-autonomous molecular clock have been described up to the present day (**Figure 1.1**) (Takahashi, 2017). In the first feedback loop, the basic helix–loop–helix (bHLH) Per-Arnt-Sim (PAS) domain–containing transcription factors **BMAL1** (brain and muscle ARNT-like 1) and **CLOCK** (circadian locomotor output cycles protein kaput) or its paralog **NPAS2** (neuronal PAS domain protein 2) activate the transcription of *Period* (*Per*) and *Cryptochrome* (*Cry*) genes, and the resulting **PER** and **CRY** proteins interact with one other and translocate to the nucleus, where they repress their own transcription. In the second feedback loop, **REV-ERBs** (which are under transcriptional control of the CLOCK–BMAL1 and NPAS2–BMAL1 complexes) rhythmically repress the gene transcription of *Bmal1* and *Nfil3* that is activated by **RORs** (retinoic acid-related orphan receptors). In the third feedback loop, **NFIL3** (nuclear factor, interleukin-3 regulated; also known as E4BP4) and **DBP** (D-box binding protein) together regulate the transcription D-box containing genes, including the *Rora* and *Rorb* genes that encode RORα and RORβ, respectively (Zhang and Kay, 2010).

(*b*) In parallel with these three interlocked feedback loops, core clock proteins interact with several other regulators that control their activity, subcellular localization, and degradation, among other things (Zhang and Kay, 2010; Takahashi, 2017). A genome-wide screen in human cells has identified hundreds of clock modifiers that can affect clock period or amplitude (Zhang et al., 2009), highlighting that the mammalian circadian clock is not based exclusively on the feedback loops described above. Notably, post-translational modifications have been shown to play major roles in mediating the effects of these regulators (Gallego and Virshup, 2007), which include the serine/threonine kinases casein kinases 1δ and 1ε (**CK1δ** and **CK1ε**) and glycogen synthase kinase 3 (**GSK3**) (Zhang and Kay, 2010). Ultimately, the interlocking TTFLs and clock modifiers maintain circadian rhythms of SCN electrical firing and circadian gene expression, thereby driving circadian oscillations at the cellular, tissue, and organ levels (Hastings et al., 2018).

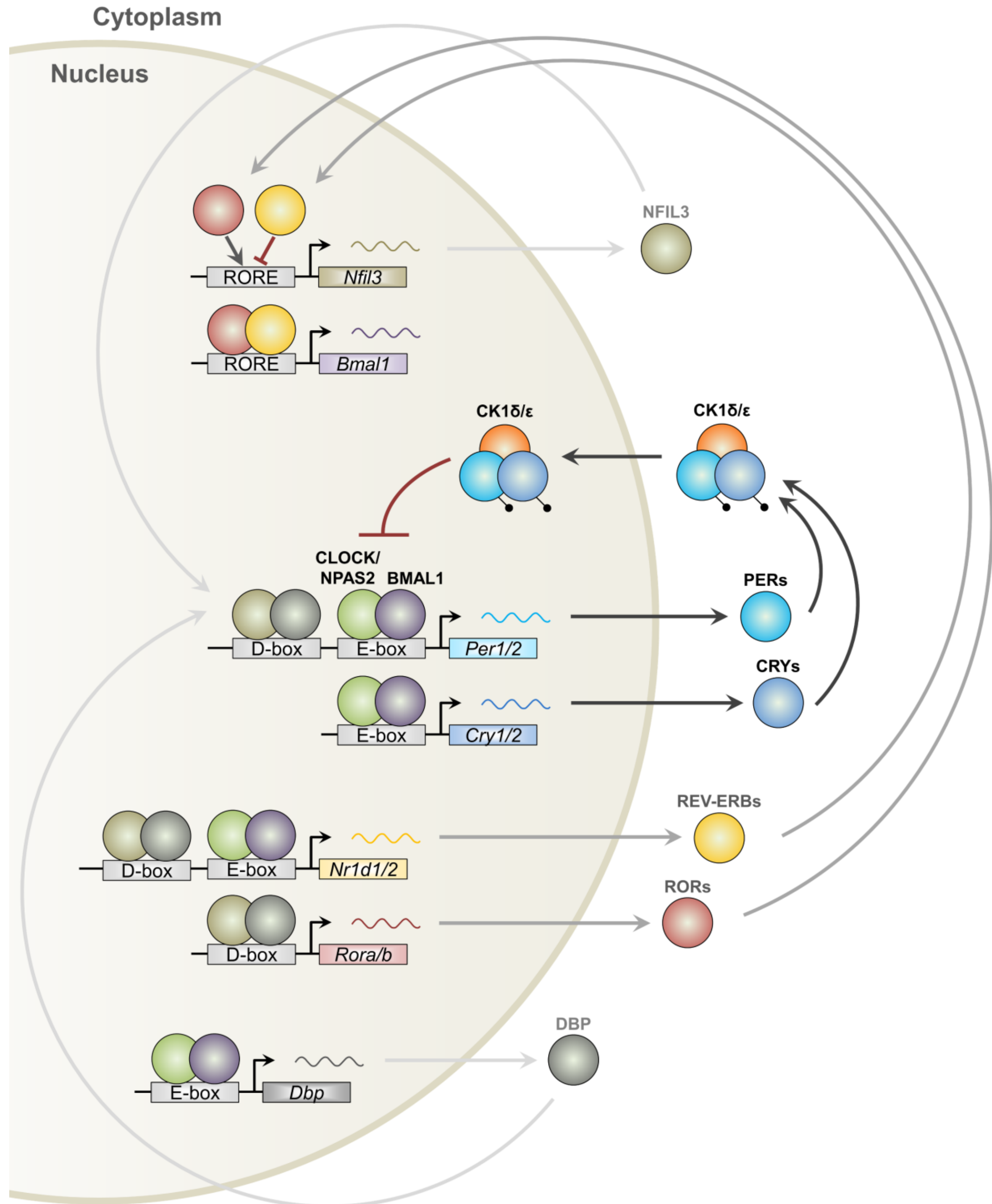

Cytoplasm
Nucleus
RORE
Nfil3
NFIL3
RORE
Bmal1
CK1δ/ε
CK1δ/ε
CLOCK/
NPAS2
BMAL1
D-box
E-box
Per1/2
PERs
CRYs
E-box
Cry1/2
REV-ERBs
D-box
E-box
Nr1d1/2
RORs
D-box
Rora/b
DBP
E-box
Dbp

Figure 1.1. Model of the mammalian cell-autonomous circadian clock.

The current model of the molecular circadian clock in mammals includes a network of three interlocking transcription/translation feedback loops. In the first feedback loop, BMAL1 and CLOCK (or NPAS2) heterodimerize and act at E-boxes to activate the transcription of *Per* and *Cry* genes, and the resulting PER and CRY proteins repress their own transcription. In the second feedback loop, REV-ERBs and RORs act at ROR-binding elements (ROREs) to regulate the transcription of *Bmal1* and *Nfil3*. In the third feedback loop, NFIL3 and DBP act at D-boxes to regulate the transcription of several clock genes. Also shown are CK1δ/ε, which phosphorylate several clock proteins (including PERs and CRYs) to regulate their subcellular localization and degradation. Hundreds of additional clock modifiers (not shown) control the activity, subcellular localization, and degradation of these core clock components. Ultimately, circadian clock proteins acting at E-box, D-box, and RORE sites drive rhythmic transcription of clock-controlled genes, which participate in various rhythmic biological processes.

(2) Tissue and organ clocks. Importantly, cellular oscillators within the SCN are coupled, allowing for their precise and robust pacemaker function at the tissue level as the central clock. On the other hand, peripheral cellular clocks are mainly coordinated by the SCN to produce coherent rhythms within (and between) peripheral oscillators at the tissue and organ levels (Liu et al., 2007; Dibner et al., 2010; Welsh et al., 2010; Hastings et al., 2018). Having provided an overview of the cell-autonomous molecular clock above, let us now focus on circadian oscillators at the levels of tissues and organs in (*a*) the central clock and (*b*) peripheral clocks:

(*a*) In the present model of the mammalian circadian system, the central clock consists of the hypothalamic SCN located just above the optic chiasm and on either side of the third ventricle (Hastings et al., 2018). Each of the SCN contain ~50,000 neurons in humans or ~10,000 neurons in mice that can be divided into two subregions based on their expression of various neuropeptides as well as their retinal and efferent connectivity: a ventral **"core"** and a dorsal **"shell"** (Hofman et al., 1988; Hastings et al., 2018). Neurons in the core region have been shown to receive retinal input, project to the shell, and mainly express vasoactive intestinal peptide (VIP) (Welsh et al., 2010). Neurons in the shell region have been shown to receive input from (and also project sparsely back to) the core and mainly contain arginine vasopressin (AVP) (Welsh et al., 2010). Although the vast majority of previous studies on SCN timekeeping have focused on the role of neurons, recent studies have been shifting the model of SCN function away from a neuron-centric view towards one in which both neurons and glia (astrocytes in particular) play important roles (Brancaccio et al., 2017; Hastings et al., 2018; Brancaccio et al., 2019). Intercellular coupling within the SCN produces a coherent oscillation at the tissue level, with individual rhythmic neurons adopting the same period and similar phases (Welsh et al., 2010). This circuit synchronization is thought to occur through a combination of chemical and electrical synapses (Liu et al.,

2007) and can be modulated by astrocytes, which confer an additional layer of regulation via signals such as glutamate, a major gliotransmitter (Hastings et al., 2018).

(*b*) Peripheral clocks consist of oscillators outside the SCN, including other brain regions (e.g., pineal gland, arcuate nucleus, hippocampus) as well as peripheral tissues and organs (e.g., liver, heart, kidneys, muscle) (Dardente and Cermakian, 2007; Liu et al., 2007). Signals from the SCN to peripheral clocks enable synchronization of peripheral oscillators to one another and to the environmental day/night cycle (Dibner et al., 2010), with the phases of peripheral clock oscillations delayed by 3–9 hours relative to the SCN (Morse and Sassone-Corsi, 2002). SCN output signals, together with direct inputs to peripheral tissues and organs, enable the coordination of cellular clocks within these peripheral oscillators (Dibner et al., 2010). It has been established that these SCN outputs are mediated through systemic signals as well as multiple interconnected neuronal, neuroendocrine, and behavioural pathways (Liu et al., 2007). These include systemic body temperature rhythms influenced by the SCN; efferent neuronal pathways terminating in other brain regions and peripheral organs, with the SCN firing rate rhythm as the major output; diffusible factors, including peptide hormones and cytokines, that are secreted from the SCN and its targets; and regulation of feeding behaviour (Liu et al., 2007; Dibner et al., 2010). Moreover, feedback signals from peripheral clocks to the SCN—including metabolic signals driven by 24-hour feeding rhythms—may in turn regulate circadian oscillations of the central clock (Greco and Sassone-Corsi, 2019). In summary, cell-autonomous molecular oscillators in central and peripheral clocks sustain coherent rhythms at the tissue and organ levels largely as a result of intercellular coupling (for the SCN) or signals from the SCN (for peripheral clocks).

1.1.2.3. Outputs

The overall purpose of the outputs of the circadian system is to provide organisms with an adaptive advantage through physiological and behavioural rhythms (Liu et al., 2007). A

variety of outputs have been previously described and may be broadly characterized as (*1*) behavioural, (*2*) physiological, or (*3*) cellular circadian rhythms:

(1) Behavioural circadian rhythms. In this context, we take a **behaviour** to mean an internally coordinated response of a whole living organism to internal and/or external stimuli (Levitis et al., 2009). Rhythmic behavioural processes that display circadian regulation include the sleep/wake cycle, locomotor activity, learning, and feeding (Mohawk et al., 2012; Bass and Lazar, 2016). Rhythmic behaviours can in turn influence physiology—such as when feeding affects endocrine function—as well as circadian clocks themselves, for instance by feedback through metabolic pathways (Mohawk et al., 2012).

(2) Physiological circadian rhythms. Most physiological processes in mammals are influenced by the circadian system, including physiology in the brain (see section 1.2.5 below) and periphery (Levi and Schibler, 2007; Bass and Lazar, 2016). One physiological circadian rhythm of the brain worth noting here is that of melatonin secretion by the pineal gland, which plays important roles in regulating circadian rhythms and sleep (Cajochen et al., 2003). In the periphery, rhythmic physiological functions that are subject to circadian oscillations contribute to the cardiovascular, immune, renal, endocrine, and reproductive systems (Richards and Gumz, 2013). These include metabolism, blood pressure, hepatic xenobiotic detoxification, intestinal lipid absorption, and cytokine expression (Levi and Schibler, 2007; Bass and Lazar, 2016).

(3) Cellular circadian rhythms. While the basic transcriptional clock mechanism that is thought to exist within virtually every cell in a mammalian organism seems to be the same across cells in a given organism, different tissues can exhibit tissue-specific clock-controlled outputs at the cellular level (Bass and Lazar, 2016; Mermet et al., 2017). As these include widespread circadian oscillations in the expression of clock-controlled genes, protein expression and modifications, and metabolite levels, systems biology approaches are

particularly well-suited for their study and to provide an integrative understanding of circadian rhythms at the cellular level (Millius and Ueda, 2017).

1.1.3. Systems biology approaches to studying circadian rhythms

In contrast to a reductionist approach, **systems biology** is a holistic approach that involves the application of tools from several disciplines with the goal of developing a coordinated, integrative view of the biological properties and functions of cells, tissues, and organisms, for instance in terms of their molecular constituents (Cowley, 2004; Kirschner, 2005; Millius and Ueda, 2017). The value of holistic approaches can be traced at least as far back as the fourth century B.C., when the Greek philosopher Aristotle wrote that "the totality is not, as it were, a mere heap, but the whole is something besides the parts" (Aristotle, *Metaphysics*, Book VIII).

More recently, systems biology studies of circadian rhythms using "-omics" technologies including transcriptomics, proteomics, and metabolomics have provided insights into the outputs of the mammalian circadian system at the cellular level, revealing widespread circadian regulation of cellular functions (Mermet et al., 2017; Millius and Ueda, 2017; Dyar et al., 2018). These large-scale studies have mainly focused on exploring tissue-specific circadian rhythms in young, healthy rodents, especially in the liver and SCN (Akhtar et al., 2002; Panda et al., 2002; Miller et al., 2007; Hughes et al., 2009; Masri et al., 2013; Chiang et al., 2014; Mauvoisin et al., 2014; Robles et al., 2014; Neufeld-Cohen et al., 2016; Robles et al., 2017; Wang et al., 2017). Some studies have also examined other tissues, such as the heart (Storch et al., 2002), skeletal muscle (McCarthy et al., 2007; Miller et al., 2007), hippocampus (Chiang et al., 2017), and a variety of other peripheral tissues (Zhang et al., 2014; Dyar et al., 2018), reinforcing the notion of tissue-specific circadian regulation of cellular functions. As revealed by two circadian transcriptomic atlases of mice and baboons, nearly half of all protein-coding genes in mice display circadian oscillations in expression in

at least one tissue (Zhang et al., 2014) while in baboons this figure is even higher, with over 80% of protein coding genes showing circadian rhythmicity of gene expression in at least one tissue (Mure et al., 2018). In mammals, clock-controlled genes are involved in several biological processes that are critical for normal cellular function, including transcription and DNA repair in the nucleus, energy metabolism and response to oxidative stress in the mitochondria, protein folding and secretion by the endoplasmic reticulum, and lysosomal function (Chaix et al., 2016; Mure et al., 2018).

These and other studies have also demonstrated an important role for post-transcriptional regulation of circadian rhythms (Chaix et al., 2016), underscoring the value of using proteomic technologies to investigate molecular circadian oscillations. This is especially important given that up to half of cycling proteins do not display concomitant oscillations at the mRNA level, while the remainder often display different phases of expression than their corresponding rhythmic transcripts (Lim and Allada, 2013; Mauvoisin et al., 2014; Robles et al., 2014; Mermet et al., 2017). The standard approach for large-scale studies of circadian rhythms at the protein level is currently shotgun bottom-up proteomics, which involves direct digestion of a protein mixture and separation of peptides by multidimensional chromatography followed by analysis by tandem mass spectrometry (MS) (Han et al., 2008). These proteomic studies have revealed that the abundances of proteins involved in tissue- and cell type specific physiological functions in mammals, such as xenobiotic detoxification in the liver (Robles et al., 2014) and cell migration and adhesion for wound healing in fibroblasts (Hoyle et al., 2017), are under circadian regulation.

Recent studies have expanded our understanding of the roles the circadian system under normal conditions and of ways to modulate the circadian clock. These include studies exploring the effects of genetic and environmental circadian disruption (Archer et al., 2014; Martino and Young, 2015; Depner et al., 2018; Kervezee et al., 2019; Resuehr et al., 2019), pharmacological circadian clock modulation (He et al., 2016), fasting and various diets (Sato

et al., 2017; Solanas et al., 2017; Tognini et al., 2017; Dyar et al., 2018; Kinouchi et al., 2018), and aging (Chen et al., 2016; Sato et al., 2017; Solanas et al., 2017) on circadian oscillations of cellular functions in humans and mice. Together, these studies have highlighted the widespread regulation of biological functions by the circadian system and begun to unravel how this regulation is altered under different conditions.

1.1.4. The circadian system's role in health, aging, and disease

Impairment of circadian function can arise from various sources, including circadian desynchronization, the aging process, diseases, and clock gene mutations (Takahashi et al., 2008; Froy, 2011; Golombek et al., 2013). Several lines of evidence from correlational and causation studies consistently support links between circadian disruption and various diseases, underscoring the importance of normal circadian function in health, and recent studies have begun to explore how to harness this knowledge for therapeutic purposes.

(1) Genetic clock disruption and disease. A number of studies have shown that dysfunction of clock genes is associated with a variety of deleterious health outcomes, including aging phenotypes (Kondratov, 2007; Hood and Amir, 2017a) (see section 1.3.3 below), cancer (Yu and Weaver, 2011; Ye et al., 2018), cardiometabolic diseases such as hypertension and type 2 diabetes (Takahashi et al., 2008; Crnko et al., 2019), and mood disorders (McClung, 2007). However, given that clock genes may be influencing functions outside of their roles in the molecular circadian oscillator, the specific contribution of circadian clock disruption to these health conditions is difficult to determine (Kondratov, 2007; Yu and Weaver, 2011). In addition, humans studies have provided evidence that clock gene mutations can cause heritable circadian rhythm sleep disorders such as familial advanced sleep phase syndrome (FASPS) (Toh et al., 2001; Xu et al., 2005).

(2) Environmental circadian disruption and disease. In humans, circadian rhythm disruption leads to cognitive impairment and increased likelihood of developing various

diseases, including aging-related neurodegenerative diseases, suggesting that clock-regulated processes play important roles in human physiology and disease (Reppert and Weaver, 2002; Kondratova and Kondratov, 2012; Golombek et al., 2013). In turn, circadian clock dysfunction is a feature of several neurodegenerative diseases, and accumulating evidence suggests that it might contribute directly to these pathologies (Kondratova and Kondratov, 2012; Musiek and Holtzman, 2016). However, sleep abnormalities may also contribute to cognitive impairment and neurodegenerative diseases, and the extent to which circadian-dependent mechanisms of sleep regulation contribute to these remains to be determined (Kondratova and Kondratov, 2012). Thus, additional studies are needed to determine how impaired circadian clock function may contribute to the development and progression of these conditions. Impaired circadian function is also associated with other health problems, including mild cognitive impairment (MCI) and dementia (Tranah et al., 2011; Diem et al., 2016), cardiovascular and gastrointestinal diseases (Hoogerwerf, 2009; Takeda and Maemura, 2011; Thosar et al., 2018), metabolic syndrome (Maury et al., 2010), intestinal dysbiosis (Voigt et al., 2014), obesity, diabetes (Kalsbeek et al., 2014), and cancer (Sahar and Sassone-Corsi, 2009; Golombek et al., 2013).

(3) Circadian rhythms in disease morbidity and mortality. Circadian variation in disease includes cardiovascular events (e.g., stroke, myocardial infarction, and sudden cardiac death) that peak in frequency in the morning (Cohen et al., 1997; Elliott, 1998); painful joint stiffness associated with rheumatoid arthritis that peaks in severity in the morning (Gibbs and Ray, 2013); exacerbations of chronic obstructive pulmonary disease and asthma that peak in severity at night and in the early morning hours (Sundar et al., 2015); and several other medical conditions (Smolensky and Peppas, 2007).

(4) Circadian rhythms and therapeutic interventions. Knowledge of how circadian regulation sustains health can be leveraged in three main ways to enable new preventative and therapeutic strategies for a variety of chronic diseases (Sulli et al., 2018a; Panda,

2019): (*a*) environmental and lifestyle changes to improve circadian rhythms, (*b*) optimization of the timing of administration of a therapeutic agent (**chronotherapy**), and (*c*) pharmacological targeting of the circadian system:

(*a*) Environmental and behavioural interventions to restore circadian rhythms, such as light therapy and time-restricted feeding, have been shown to be promising approaches for treating circadian rhythm sleep disorders (Gooley, 2008), sleep disturbances and cognitive impairment in dementia (Wu and Swaab, 2007; Hanford and Figueiro, 2013), motor symptoms associated with Huntington's disease (HD) (Wang et al., 2018), seasonal affective disorder (Magnusson and Boivin, 2003), and depression (Wirz-Justice et al., 2004), although other studies have shown no beneficial effects or only small changes with light therapy (Dowling et al., 2008; Friedman et al., 2012).

(*b*) It has been established that several drugs exhibit circadian variation in efficacy or toxicity, including various medications administered for hypertension, dyslipidemia, gastroesophageal reflux disease (GERD), and rheumatoid arthritis (Griffett and Burris, 2013; Kaur et al., 2013). As a result, it has been suggested that timing of drug administration be taken into account, with bedtime administration suggested for levothyroxine (Bolk et al., 2010) and telmisartan (Hermida et al., 2007) and evening administration suggested for simvastatin (Saito et al., 1991; Lund et al., 2002; Tharavanij et al., 2010), for instance.

(*c*) Lastly, pharmacological targeting of the circadian timing system with melatonin or small molecule circadian clock modifiers has been investigated as a potential therapeutic approach for various health conditions, including circadian rhythm sleep disorders (Dodson and Zee, 2010), insomnia (Buscemi et al., 2005), and sundowning in dementia (de Jonghe et al., 2010). Moreover, several high-throughput chemical biology screens have identified compounds affecting circadian rhythmicity (Wallach and Kramer, 2015), and other recent studies have been exploring pharmacological modulation of the circadian clock as a potential therapeutic approach in the context of cancer (Sulli et al., 2018b), metabolic

syndrome (He et al., 2016), and obesity (Solt et al., 2012). These studies not only support a link between circadian dysfunction and various diseases, but also highlight the potential of circadian clock modulation as a therapeutic approach for these diseases. Additional studies are needed to investigate the effects of pharmacological modulation of the circadian clock in the context of aging and neurodegenerative diseases associated with circadian dysfunction.

1.2. The hippocampus and memory

1.2.1. Terminology and history

The view adopted in this thesis is that the **hippocampal formation** (herein referred to as the **hippocampus**) of the medial temporal lobe of the mammalian brain comprises the dentate gyrus, CA fields (of the hippocampus proper), subiculum, presubiculum, parasubiculum, and entorhinal cortex (Andersen et al., 2007a). While homologous brain structures have been identified in non-mammalian vertebrates (Rodriguez et al., 2002), the focus here will be on the mammalian hippocampus. The hippocampus plays important roles in multiple cognitive functions, notably certain types of memory (Squire, 1992; Leuner and Gould, 2010). While **learning** may be described as the process of acquiring new knowledge and skills, **memory** refers to the mechanism of retaining and recognizing or recalling that information over time (Lynch, 2004; Kandel et al., 2014), or a mental impression of the thing remembered. Memory involves several processes, including acquisition, consolidation, and retrieval (Abel and Lattal, 2001), and may be categorized into short-term memory and long-term memory based on duration of persistence (Lynch, 2004).

Currently, the prevailing hypothesis of how information acquired during learning is encoded and stored in the brain is the synaptic plasticity and memory hypothesis, which asserts that activity-dependent **synaptic plasticity**—experience-dependent changes in synaptic connectivity (Ho et al., 2011)—is both necessary and sufficient for information storage underlying memory (Martin et al., 2000; Neves et al., 2008). **Long-term**

potentiation (LTP), a sustained increase in the efficiency of synaptic transmission that results from stimulation of afferent pathways, is one form of synaptic plasticity and is widely believed to be the synaptic basis of learning and memory in the hippocampus (Bliss and Collingridge, 1993; Lynch, 2004; Neves et al., 2008). LTP involves an alteration of **synaptic strength**, a measure of the amplitude of the postsynaptic potential produced by an action potential in the presynaptic neuron (Murthy, 1998; Zhang and Linden, 2003). Memory **engrams** are the proposed enduring physical and/or chemical changes that underlie newly formed memory associations in the brain (Tonegawa et al., 2015).

Studies on the hippocampus are thought to have begun at least as far back as the Alexandrian school of medicine (Andersen et al., 2007b), approximately two thousand years ago. The Italian anatomist and surgeon Giulio Cesare Aranzio (Arantius) is credited with the first written description of the hippocampus *circa* 1564 as well as with coining the name "hippocampus" (from the Ancient Greek *híppos* meaning "horse" and *kámpos* meaning "sea monster"), due to the resemblance of its three-dimensional form to a seahorse (Andresen et al., 2007b). In 1742, the French surgeon René-Jacques Croissant de Garengeot named the hippocampus "*cornu ammonis*" (horn of the ram), of which the abbreviation is still used in the present day to refer to the subdivisions (CA fields) of the hippocampus proper (Amaral and Lavenex, 2007).

The modern era of research on hippocampal-dependent memory may be considered to have begun in the 1950s with the publication of reports on patients who developed memory impairment following epilepsy surgery. Henry Molaison (patient H.M.), a patient of the American-Canadian neurosurgeon Wilder Penfield, underwent bilateral medial temporal lobe resection in an attempt to control his seizures, which had been refractory to medical treatment, and was left with a severe amnesic syndrome as a result of this procedure (Scoville and Milner, 1957). While researchers at the time attributed his amnesia solely to a loss of hippocampal function, more recent studies have revealed that the surgical procedure

performed on H.M. also damaged brain structures outside of the hippocampus (Corkin et al., 1997; Annese et al., 2014). Nevertheless, it has been shown that selective hippocampal lesions also result in memory impairment, albeit less pronounced than in H.M.'s case, indicating that memory function depends on the hippocampus working together with other brain structures (Spiers et al., 2001).

While the molecular, cellular, and circuit mechanisms underlying learning and memory are remarkably complex and not fully understood, modern investigations into these mechanisms within the hippocampus have been largely centred around hypotheses derived from Spanish neuroanatomist Santiago Ramón y Cajal's idea of neuronal plasticity (Cajal,1894) and, more recently, Canadian psychologist Donald Hebb's postulate of learning (Hebb, 1949). Knowledge of the mechanisms underlying hippocampal-dependent memory and how these pathways are altered in disease states could be harnessed in the development of new therapeutic strategies for diseases that involve impaired hippocampal function, including neurodegenerative diseases such as Alzheimer's disease (AD) (see section 1.4.6 below).

1.2.2. Anatomy, organization, and function of the hippocampus

The paired mammalian hippocampi are part of a system of medial temporal lobe structures that are important for memory (Squire and Zola-Morgan, 1991). The hippocampus is a curved cortical structure consisting of the dentate gyrus, CA fields of the hippocampus proper (CA1, CA2, and CA3), subiculum, presubiculum, parasubiculum, and entorhinal cortex (Andersen et al., 2007a). The major types of cells in the hippocampus include neurons, astrocytes, oligodendrocytes, microglia, vascular endothelial cells, mural cells, and ependymal cells (Zeisel et al., 2015). In contrast to most other cortical areas, the neuronal connections that link the various structures within the hippocampus seem to be for the most part unidirectional (Amaral and Lavenex, 2007), although several backprojections have also

been described (van Strien et al., 2009). Moreover, the hippocampus generally has a three-layered appearance (van Strien et al., 2009), although up to six layers have been described in some of its subregions (Insausti et al., 2017).

The hippocampus receives afferent inputs from virtually all cortical association areas that converge mainly on the entorhinal cortex (van Strien et al., 2009). In addition, direct inputs to the hippocampus from several other cortical and subcortical areas, as well as the brain stem, have been described (Vinogradova, 2001; Knierim, 2015). In the classic hippocampal trisynaptic circuit, the entorhinal cortex projects to granule cells of the dentate gyrus via the perforant pathway (Neves et al., 2008). Granule cells of the dentate gyrus then project via mossy fibres onto CA3 pyramidal neurons, which in turn project to CA1 pyramidal neurons through Schaffer collaterals (Neves et al., 2008). This standard depiction of hippocampal anatomical connectivity does not reflect its complexity as there are several other connections among these subregions and other subregions, consisting of both ipsilateral and contralateral projections, in addition to the trisynaptic circuit just described (Amaral, 1993; van Strien et al., 2009). Outputs from the hippocampus to other cortical regions have been shown to be conveyed by the entorhinal cortex, which receives hippocampally processed information via projections from CA1 and the subiculum (van Strien et al., 2009). Additional outputs from the hippocampus are carried via the fornix to several other targets, including the lateral septum and mammillary bodies, which in turn relay this information to other brain regions (Vinogradova, 2001).

The anatomical and organizational features of the hippocampus described above enable it to perform several functions. Evidence from electrophysiological, functional neuroimaging, cognitive, and neuropsychological studies have demonstrated roles for the hippocampus in episodic memory (Tulving and Markowitsch, 1998; Burgess et al., 2002; Rugg and Vilberg, 2013), spatial processing and memory (Burgess et al., 2002; Martin and Clark, 2007; Bird and Burgess, 2008), emotion (Phelps, 2004; Jin and Maren, 2015), motivation and goal-

directed behaviour (Tracy et al., 2001; Pennartz et al., 2011), and transitive inference (Bunsey and Eichenbaum, 1996; Heckers et al., 2004).

1.2.3. Cellular and molecular mechanisms of hippocampal memory

The role of the hippocampus in declarative (explicit) memory has been the focus of intensive study, as have the cellular and molecular substrates of hippocampal-dependent memory encoding, storage, and retrieval (Tulving and Markowitsch, 1998; Eichenbaum, 1999; Eichenbaum, 2004; Mayford et al., 2012). The cellular basis of learning and memory in the hippocampus seems to involve both synaptic and non-synaptic forms of neural plasticity, each of which may occur via a wide range of mechanisms (Abel and Lattal, 2001; Zhang and Linden, 2003; Malenka and Bear, 2004; Mozzachiodi and Byrne, 2010; Ho et al., 2011).

Additional mechanisms that might underlie hippocampal-dependent memory include synaptogenesis, synapse remodelling, and neurogenesis (Bruel-Jungerman et al., 2007; Caroni et al., 2012). In parallel with these structural and functional changes in hippocampal neurons, it has been shown that epigenetic modifications contribute to hippocampal memory function, including through modulation of synaptic plasticity (Levenson and Sweatt, 2005; Cortes-Mendoza et al., 2013; Zovkic et al., 2013). Moreover, given that studies have shown that astrocytes regulate hippocampal-dependent memory (Ota et al., 2013) and display plasticity (Pirttimaki and Parri, 2013), it is conceivable that astrocyte plasticity might also partially underlie hippocampal memory.

(1) Synaptic plasticity. It has been established that several different forms of hippocampal LTP contribute to learning and memory, and these may be categorized based on a number of variables, including: (*a*) decay time constants and dependence on protein synthesis and/or transcription, with LTP1, LTP2, and LTP3 (Raymond, 2007); (*b*) dependence on activation of *N*-methyl-D-aspartate receptors (**NMDARs**), with NMDAR-dependent LTP (Bliss and Collingridge, 1993; Luscher and Malenka, 2012) and NMDAR-

independent LTP (Nicoll and Schmitz, 2005; Sabeti and Gruol, 2008); (*c*) dependence on protein synthesis alone, with early-LTP and late-LTP phases (Frey et al., 1993); and (*d*) dependence on coincident activation of presynaptic and postsynaptic neurons for its induction (Hebb, 1949), with Hebbian LTP and non-Hebbian LTP (Urban and Barrionuevo, 1996). In general, major determinants of the type of LTP include the brain region, the type of neuron, and the type of input (Blundon and Zakharenko, 2008).

Regardless of the specific type, hippocampal LTP can involve both presynaptic and postsynaptic neuronal changes leading to an enhanced synaptic response (Lisman and Raghavachari, 2006; Blundon and Zakharenko, 2008; Bliss and Collingridge, 2013), with different mechanisms underlying LTP induction, maintenance, and expression (Miyamoto, 2006; Lisman and Raghavachari, 2015). LTP induction in the hippocampus has been shown to be mediated by the interplay of proteins from multiple signal transduction pathways, including kinases such as Ca^{2+}/calmodulin-dependent protein kinase II (**CaMKII**), protein kinase A (**PKA**), protein kinase C (**PKC**), and mitogen-activated protein kinase (**MAPK**) (Soderling and Derkach, 2000). Three main mechanisms have been proposed to underlie LTP expression in the hippocampus: changes in neurotransmitter release from the presynaptic neuron, changes in the properties of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (**AMPARs**) in the postsynaptic neuron, and changes in the number of AMPARs in the postsynaptic neuron (Bliss and Collingridge, 2013). Although research on synaptic plasticity in the hippocampus has thus far largely focused on neuronal mechanisms, studies have also demonstrated a role for glial cells, in particular astrocytes, in regulating synaptic plasticity (Ota et al., 2013; Haydon and Nedergaard, 2014). While much progress has been made in understanding the mechanisms of synaptic plasticity in the hippocampus that may mediate memory acquisition, the mechanisms distinguishing acquisition from consolidation and retrieval are less well understood (Abel and Lattal, 2001).

(2) Non-synaptic plasticity. The major focus of research on the cellular and molecular mechanisms of neural plasticity has been on synaptic plasticity, although there have also been some studies demonstrating a role for hippocampal non-synaptic plasticity in learning and memory (Moyer et al., 1996; Daoudal and Debanne, 2003; Mozzachiodi and Byrne, 2010). Non-synaptic plasticity can affect signal propagation in dendrites, axons, and somata of neurons (Mozzachiodi and Byrne, 2010) and includes modifications in neuronal intrinsic excitability, which may be mediated by changes in the properties, distribution, and number of ion channels (Daoudal and Debanne, 2003). How non-synaptic changes contribute to learning and memory remains unclear, although studies have supported roles for non-synaptic plasticity in both synapse-dependent and synapse-independent memory function (Mozzachiodi and Byrne, 2010).

1.2.4. Systems biology approaches to studying hippocampal memory

Along with the emerging trend in neuroscience of shifting away from a focus on individual neurons towards systems-based studies of neural circuits and networks to gain insight into how the brain works as a whole, recent studies using global “-omics” approaches have proved valuable in providing an integrative understanding of biological processes in the brain (Geschwind and Konopka, 2009).

Given the complex cellular and molecular mechanisms that have been shown to underlie hippocampal-dependent memory, systems biology approaches have been employed to better understand these events as well as how these processes are altered under different conditions, such as during aging, in disease states, or in response to drugs. Several transcriptomic and proteomic studies of the hippocampus have been conducted to explore the molecular correlates of memory (Vanguilder and Freeman, 2011; Valor and Barco, 2012; Pontes and de Sousa, 2016). For instance, a study using label-free quantitative proteomic analysis of the hippocampus to identify dynamic changes in protein expression

during long-term spatial memory formation in mice revealed that this process was associated with changes in the expression of proteins involved in synaptic transmission, cytoskeleton organization, neurotrophic signalling pathways, and metabolic activity (Borovok et al., 2016). In addition, these approaches have been used to elucidate alterations in the hippocampal transcriptome or proteome that are linked with aging and age-related cognitive impairment in rats (Freeman et al., 2009), memory impairment associated with AD-like pathology in 5XFAD mice (Neuner et al., 2017), and administration of the glutamate modulator riluzone in rats (Pereira et al., 2017) or acetylcholinesterase inhibitor (AChEI) donepezil in a triple transgenic mouse model of AD (3xTg-AD mice) (Zhou et al., 2018). Studies of the hippocampal proteome in humans and rodents have demonstrated that hippocampal aging involves dysregulation of glucose metabolism, impaired synaptic function, altered protein processing, and increased oxidative stress (Vanguilder and Freeman, 2011), all of which have previously been identified as hallmarks of aging or brain aging (Lopez-Otin et al., 2013; Mattson and Arumugam, 2018) (see section 1.3.2 below).

These discovery-based studies complement more focused, hypothesis-driven research by supporting findings regarding the involvement of proteins in hippocampal-dependent memory, identifying new potential therapeutic targets, and providing insight into changes in biological processes and pathways that occur under different conditions.

1.2.5. Circadian regulation of hippocampal function and memory

Interactions between biological rhythms and cognition have been studied in humans and animal models for decades (Davies et al., 1973; Holloway and Wansley, 1973; Tapp and Holloway, 1981; Monk et al., 1983; Hoffmann and Balschun, 1992). Several lines of evidence suggest that the circadian system modulates hippocampal function and certain types of memory, with mammalian studies demonstrating circadian rhythms in memory processing, circadian oscillations in cellular and molecular processes that can impact

memory, and memory impairment associated with circadian disruption (Eckel-Mahan and Storm, 2009; Gerstner et al., 2009; Gerstner and Yin, 2010; Smarr et al., 2014; Krishnan and Lyons, 2015; Rawashdeh et al., 2018; Snider et al., 2018):

(1) Circadian rhythms in memory processing. Due to the difficulty in separating the effects of the circadian system from those of time-of-day, activity cycles, body temperature, and sleep and wakefulness (Blatter and Cajochen, 2007; Kyriacou and Hastings, 2010; Krishnan and Lyons, 2015), providing direct evidence that conclusively demonstrates regulation of memory by the circadian system would be challenging. However, rodent studies have suggested that there are circadian oscillations in learning and memory (Chaudhury and Colwell, 2002; Eckel-Mahan et al., 2008), including hippocampal-dependent memory acquisition and retrieval, supporting the view that the circadian system directly and/or indirectly regulates memory processing.

(2) Clock genes in the hippocampus. In rodents, the expression of clock genes at the mRNA and protein levels in the hippocampus has been shown to vary as a function of time of day (Wakamatsu et al., 2001; Wang et al., 2009; Jilg et al., 2010; Gilhooley et al., 2011; Valnegri et al., 2011; Duncan et al., 2013; Chun et al., 2015). Moreover, hippocampal rhythms of *Per2* expression can persist in the absence of the SCN, as demonstrated by rhythms of PER2::LUC bioluminescence in mouse hippocampal slices maintained in culture (Wang et al., 2009) or PER2 oscillations following SCN lesions in rats (Lamont et al., 2005).

(3) Circadian rhythms in processes that can influence memory. Several cellular and molecular processes that have been shown to underlie or influence hippocampal function and memory (see section 1.2.3 above) display rhythmicity in mammals (**Figure 1.2**), including LTP and synaptic plasticity in general (Gerstner and Yin, 2010; Smarr et al., 2014; Frank, 2016; Besing et al., 2017; Snider et al., 2018), kinase signalling (Eckel-Mahan and Storm, 2009; Snider et al., 2018), hippocampal neuron excitability (Naseri Kouzehgarani et al., 2019), epigenetic modifications (Smarr et al., 2014), synapse remodelling

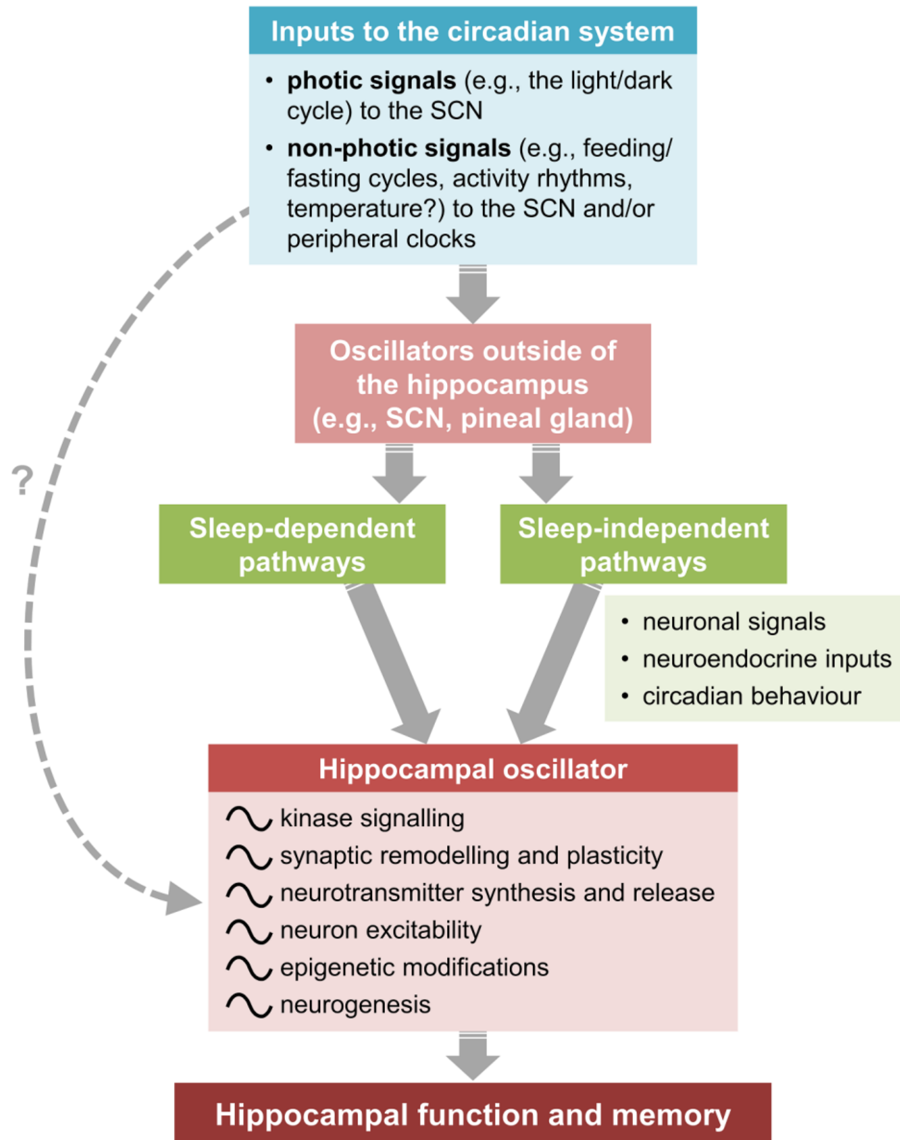

Inputs to the circadian system
• photic signals (e.g., the light/dark cycle) to the SCN
• non-photic signals (e.g., feeding/ fasting cycles, activity rhythms, temperature?) to the SCN and/or peripheral clocks
Oscillators outside of the hippocampus (e.g., SCN, pineal gland)
?
Sleep-dependent pathways
Sleep-independent pathways
• neuronal signals
• neuroendocrine inputs
• circadian behaviour
Hippocampal oscillator
kinase signalling
synaptic remodelling and plasticity
neurotransmitter synthesis and release
neuron excitability
epigenetic modifications
neurogenesis
Hippocampal function and memory

Figure 1.2. Circadian rhythms in processes that influence hippocampal memory.

Circadian rhythms in cellular and molecular processes that may influence hippocampal function and memory include rhythms in kinase signalling, synaptic plasticity and remodelling, neurotransmitter synthesis and release, neuron excitability, epigenetic modifications, and neurogenesis. These rhythms can be regulated through a combination of sleep-dependent and sleep-independent pathways, including rhythmic neuronal, neuroendocrine, and behavioural signals from the SCN, pineal gland, and other oscillators outside of the hippocampus.

(Perez-Cruz et al., 2009; Ikeno et al., 2013), and neurotransmitter synthesis and release (Panda et al., 2002). Moreover, secretion of hormones such as melatonin (Rawashdeh and Maronde, 2012; Smarr et al., 2014; Jilg et al., 2019), as well as hippocampal neurogenesis (Bouchard-Cannon et al., 2013; Smarr et al., 2014), exhibit daily rhythms and might influence memory.

At the molecular level, daily rhythms in the expression of diverse memory-related genes and proteins (or protein activity) have been observed in the mouse hippocampus. These include rhythms in synaptic components such as neurotransmitter regulators, synaptic vesicle proteins, receptors, and scaffolding molecules (Hannou et al., 2018). In addition, several signalling proteins and second messengers exhibit rhythmicity in the mouse hippocampus, including cyclic adenosine monophosphate (cAMP), protein kinase B (PKB, also known as Akt), PKC, CaMKII, cAMP response element-binding protein (CREB), and GSK3β (Rawashdeh et al., 2018; Snider et al., 2018). Notably, circadian oscillations in the cAMP/MAPK pathway have been described in the hippocampus of mice and play a role in normal hippocampal-dependent memory maintenance, as demonstrated by experiments in which ablation of MAPK rhythms interfered with memory persistence (Eckel-Mahan et al., 2008). Circadian rhythms in the phosphorylation of other proteins have also been shown in the hippocampus of mice, including proteins involved in cytoskeletal organization and PKA signalling (Chiang et al., 2017).

(4) Circadian disruption and memory. In mammals, cognitive impairment has been linked with disruption of the circadian system resulting from clock gene alterations, SCN lesions, environmental causes, certain diseases, and aging. Studies in mice have shown that genetic circadian disruption, through clock gene alterations or disruption of other genes critical to SCN function, is correlated with deficits in hippocampal-dependent memory. This includes impaired memory formation associated with siRNA knockdown of hippocampal *Per1* (Kwapis et al., 2018), impaired cued and contextual memory associated with *Npas2*

disruption (Garcia et al., 2000), impaired recall of contextual memory associated with disruption of the VIP gene (Chaudhury et al., 2008), and various memory impairments associated with global *Bmal1* deletion (Kondratova et al., 2010; Snider and Obrietan, 2018) or targeted deletion of *Bmal1* in astrocytes (Barca-Mayo et al., 2017) or excitatory forebrain neurons (Snider et al., 2016). However, given that these genes are also involved in functions outside of the circadian system, the specific contribution of circadian disruption to these cognitive deficits remains unclear. SCN lesions in rodents and environmental circadian disruption in rodents and humans (e.g., through phase shifting) (Smarr et al., 2014; Krishnan and Lyons, 2015) have also been shown to be associated with impairments in hippocampal-dependent memory (Tapp and Holloway, 1981; Fekete et al., 1985; Cho et al., 2000; Cho, 2001; Devan et al., 2001; Craig and McDonald, 2008; Ruby et al., 2008; Gibson et al., 2010; Loh et al., 2010; Phan et al., 2011; Ruby et al., 2013). Moreover, cognitive impairment associated with aging (Antoniadis et al., 2000; Haley et al., 2009; Oosterman et al., 2009; Kwapis et al., 2018) and AD (Bubu et al., 2017) has also been linked with the circadian system. Together, these studies have highlighted the complex interactions that exist between circadian rhythms, hippocampal function, and memory. Moreover, they suggest that disruption of the circadian system, as can occur during aging and in various neurodegenerative diseases, may contribute to impaired hippocampal function and memory.

1.3. Aging

1.3.1. Terminology and history

In this thesis, **aging** refers to the biological process of growing old and is characterized by **senescence**, an age-dependent functional decline that affects most living organisms over time (Flatt, 2012; Lopez-Otin et al., 2013; da Costa et al., 2016). Although the biological causes of aging remain unclear and several theories have been proposed over the years to explain mechanisms underlying the aging process (da Costa et al., 2016; McHugh and Gil, 2018), López-Otín and colleagues have proposed nine candidate **hallmarks of aging** that represent common cellular and molecular denominators of aging (Lopez-Otin et al., 2013). Similarly, Mattson and Arumugam highlighted ten interdependent **hallmarks of brain aging** that describe mechanisms of brain aging at the molecular, cellular, and systems levels (Mattson and Arumugam, 2018). In contrast to aging, **cellular senescence** refers to a state of permanent cell-cycle arrest that can occur in cells of both young and old organisms (Campisi and d'Adda di Fagagna, 2007).

The biological study of aging can be traced back to the writings of the Greek philosopher Aristotle in the fourth century B.C., including his work *On Longevity and Shortness of Life*. Hundreds of theories of aging have been proposed since then (Medvedev, 1990; Jin, 2010) and it has become apparent that there might not be a single or main cause of aging and, moreover, that the causes of aging might not be universal for all living organisms (Jin, 2010; da Costa et al., 2016). While several categorization systems have been described, a widely used one classifies modern biological theories of aging into two main categories: (*1*) programmed theories, which contend that a genetically-determined deterioration of bodily functions over time underlies aging and limits an organism's lifespan; and (*2*) damage theories, which are based on the idea that time-dependent accumulation of cellular damage underlies the aging process (Jin, 2010). Aging has been the focus of intense research

efforts over the past few decades, largely because age is a major risk factor for multiple prevalent diseases (Niccoli and Partridge, 2012).

The increase in life expectancy around the world since the beginning of the twentieth century, especially in high-income countries, has been accompanied by concomitant increases in the incidence and prevalence of chronic aging-associated diseases such as cancer, cardiovascular diseases, and neurodegenerative diseases (Crimmins, 2015; Franceschi et al., 2018). Given that age-related diseases have been estimated to account for over half of all global burden of disease among adults (Chang et al., 2019), there is a pressing need to address this challenge and foster healthy aging through a combination of various approaches, including developing new therapeutic interventions as well as making changes to health systems, social services, and public policy (Beard et al., 2016). In particular, increased emphasis should be placed on health promotion and chronic disease prevention (Lang et al., 2006; Kennedy et al., 2014).

1.3.2. Hallmarks of aging and brain aging

The hallmarks of aging (Lopez-Otin et al., 2013) and hallmarks of brain aging (Mattson and Arumugam, 2018) provide frameworks for studies investigating the cellular and molecular mechanisms of aging. The nine candidate hallmarks of aging are interdependent and can be categorized as primary, antagonistic, or integrative hallmarks. Genomic instability, telomere attrition, epigenetic alterations, and loss of protein homeostasis (or “proteostasis”) are tentative primary hallmarks (causes of damage). Dysregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence have been proposed as antagonistic hallmarks (responses to damage). Lastly, stem cell exhaustion and altered intercellular communication have been categorized as integrative hallmarks (culprits of the phenotype). The ten hallmarks of brain aging include several cellular and molecular mechanisms described by López-Otín and colleagues as hallmarks of aging in general, such as

mitochondrial dysfunction, dysregulated energy metabolism, oxidative damage, impaired proteostasis, inflammation, deficiencies in DNA repair, and stem cell exhaustion. Additional proposed hallmarks of brain aging include impaired cellular stress response, aberrant neuronal network activity, and dysregulated neuronal calcium homeostasis. These aging and brain aging hallmarks provide us with a framework for understanding the links between the aging process, neurodegenerative diseases, and the circadian system.

1.3.3. Aging and the circadian system

There exists a complex interplay between the aging process and the circadian system. On one hand, aging in mammals is associated with an overall functional weakening of the circadian system (Hofman and Swaab, 2006; Froy, 2011; Hood and Amir, 2017a; Popa-Wagner et al., 2017). On the other hand, accumulating evidence suggests that circadian clocks regulate several processes involved in the hallmarks of aging and brain aging (Kondratova and Kondratov, 2012; Fonseca Costa and Ripperger, 2015; Musiek and Holtzman, 2016), indicating that alterations in the circadian system could contribute to the aging process and the development of aging-associated diseases.

1.3.3.1. Changes in the circadian system in aging

In mammals, aging is associated with a decline in circadian function that is characterized by age-dependent changes in the circadian system's (*1*) response to inputs, (*2*) multi-oscillator network, and (*3*) output rhythms:

(1) Age-related changes in response to inputs. Aging is associated with decreased responsiveness to phase shifting and phase resetting signals (Zhang et al., 1996; Monk et al., 2000; Duffy et al., 2007), and decreased induction of *Per1* in particular seems to contribute to a reduction in SCN response to light in hamsters and rats (Asai et al., 2001; Kolker et al., 2003), although one study found that this was not the case in aged rats (Davidson et al., 2008). In addition, aging may have a modest effect on the sensitivity of

peripheral clocks to metabolic cues acting as *zeitgebers*, as demonstrated by studies on restricted food delivery in aged rats (Mistlberger et al., 1990; Walcott and Tate, 1996). Given the importance of time cues to the overall functioning of the circadian system, decreased responsiveness to these inputs could contribute to aging-associated circadian decline.

(2) Age-related changes in the multi-oscillator network. Changes to the multi-oscillator network in later life include changes in the SCN, changes in peripheral clocks, and changes in the synchronization of central and peripheral clocks. There is mixed evidence for changes in SCN structure during aging, with a cross-sectional study of the SCN in humans indicating that both SCN volume and total cell number are lower in aged subjects (Swaab et al., 1985), while studies in rats have been unable to replicate these findings (Madeira et al., 1995; Tsukahara et al., 2005). Age-related changes in neurochemical and electrophysiological outputs have also been characterized, with rodent and human studies indicating that aged subjects demonstrate reductions in AVP- and VIP-expressing cells in the SCN (Swaab et al., 1985; Roozendaal et al., 1987; Chee et al., 1988; Hofman and Swaab, 1994; Zhou et al., 1995), the amplitude of clock gene expression in the SCN (Kolker et al., 2003; Bonaconsa et al., 2014), the density of GABAergic axon terminals in the SCN (Palomba et al., 2008), the number and density of SCN cells expressing the MT1 melatonin receptor (Wu et al., 2007), and the amplitude and precision of the firing rhythms of SCN neurons (Satinoff et al., 1993; Watanabe et al., 1995; Aujard et al., 2001; Nakamura et al., 2011). Moreover, aging-associated SCN alterations in astrocyte and microglia activation (Deng et al., 2010) and cytokine and receptor levels (Beynon et al., 2009; Beynon and Coogan, 2010) may also play roles in impaired SCN function in later life. In addition to changes in the SCN, changes to the multi-oscillator network include dampening of peripheral oscillators and a slower rate of re-entrainment for peripheral tissues (Yamazaki et al., 2002; Davidson et al., 2008; Sellix et al., 2012). Differences between central and peripheral clocks in the rate of phase resetting following phase advances can result in

internal desynchronization (Davidson et al., 2008), which may have negative consequences for overall health (Golombek et al., 2013).

(3) Age-related changes in output rhythms. Changes to output rhythms that have been shown to occur with aging include changes to (*a*) behavioural, (*b*) physiological, and (*c*) cellular circadian rhythms. Overall, aging is associated with phase advances, fragmentation of rhythms, increased inter-day variability, and reduced internal synchronization of rhythms (Hofman and Swaab, 2006; Arellanes-Licea et al., 2014; Duncan, 2019). There is also evidence that aging can lead to reduced circadian rhythm amplitudes and shortened free-running periods, although some studies have shown that these changes are not consistently seen, especially in older women (Monk, 2005).

(*a*) Studies have shown that changes in behavioural circadian rhythms that occur during aging include alterations in sleep architecture (Mander et al., 2013), such as advanced sleep timing and increased sleep fragmentation (Roenneberg et al., 2007; Espiritu, 2008; Broms et al., 2014), although there is significant individual variability in the degree of sleep disruption. Aging has also been associated with a decrease in the amplitude of feeding and drinking rhythms in rats (Peng et al., 1990) and altered daily rhythms in cognitive performance in older adults (Schmidt et al., 2012).

(*b*) Circadian rhythms in various physiological parameters have also been shown to be altered with aging. These include plasma hormone levels (notably melatonin and cortisol) (Touitou and Haus, 2000), body temperature (Weinert, 2010), and blood pressure (O'Sullivan et al., 2003), which have been shown to display reductions in amplitudes and phase advances in older adults, although the evidence is not entirely consistent (Monk, 2005).

(*c*) At the cellular level, several studies have shown that aging is associated with tissue-specific changes in the daily patterns of expression of clock genes and clock modulators, including alterations in their amplitude and phase of expression in rodents (Kolker et al.,

2003; Wyse and Coogan, 2010; Chang and Guarente, 2013; Bonaconsa et al., 2014; Nakamura et al., 2015) and humans (Pagani et al., 2011; Chen et al., 2016). In addition, there have been both large-scale studies (Chen et al., 2016; Kuintzle et al., 2017; Sato et al., 2017; Solanas et al., 2017) and more focused studies (Gong et al., 2015; Lacoste et al., 2017) exploring the effects of aging on the circadian regulation of various biological processes in humans and model organisms.

1.3.3.2. Effects of the circadian system on the aging process

There is indirect evidence that age-related changes in the circadian system could contribute to the aging process, including links between circadian disruption and aging as well as links between the circadian clock and hallmarks of aging. Genetic and environmental disruption of circadian rhythms in fruit flies and mice, through mutations in some core clock genes or manipulation of the light/dark cycle, is associated with reduced lifespan and development of aging phenotypes (Kondratov, 2007; Yu and Weaver, 2011; Liu and Chang, 2017). However, it is important to note that the aging phenotypes in clock mutant mice are not consistent and also that there does not seem to be a good correlation between the extent of circadian disruption and aging phenotype (Yu and Weaver, 2011), suggesting that these effects are at least partially mediated by other, non-circadian mechanisms. Circadian regulation of processes and pathways involved in hallmarks of aging provide additional potential links between age-related circadian changes and the decline of various biological functions with advancing age (**Figure 1.3**) (Fonseca Costa and Ripperger, 2015). These include reciprocal interactions between the circadian clock and enzymes that regulate epigenetic modifications (Chang and Guarente, 2013; Fonseca Costa and Ripperger, 2015), as well as circadian regulation of proteostasis, oxidative phosphorylation, and redox homeostasis (Kondratov, 2007; Kondratova and Kondratov, 2012; Fonseca Costa and Ripperger, 2015).

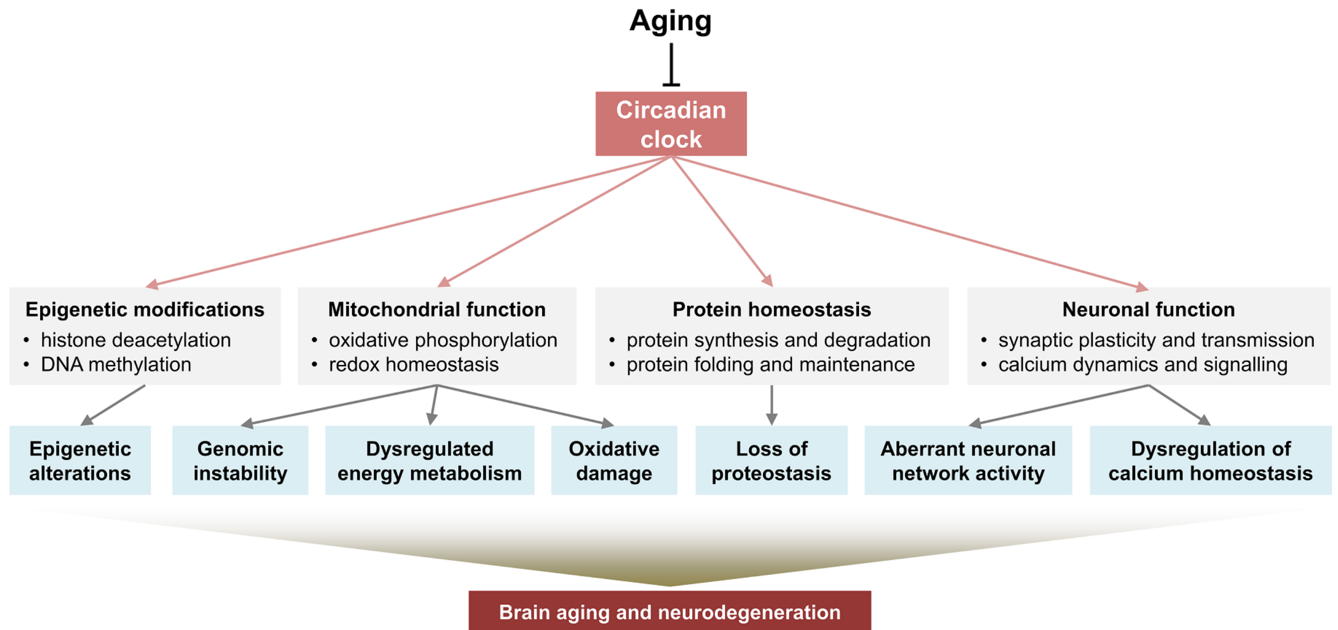

Aging
Circadian clock
Epigenetic modifications
• histone deacetylation
• DNA methylation
Mitochondrial function
• oxidative phosphorylation
• redox homeostasis
Protein homeostasis
• protein synthesis and degradation
• protein folding and maintenance
Neuronal function
• synaptic plasticity and transmission
• calcium dynamics and signalling
Epigenetic alterations
Genomic instability
Dysregulated energy metabolism
Oxidative damage
Loss of proteostasis
Aberrant neuronal network activity
Dysregulation of calcium homeostasis
Brain aging and neurodegeneration

Figure 1.3. Influence of the circadian clock on hallmarks of aging and brain aging.

Evidence suggests that the circadian system regulates several cellular and molecular processes involved in hallmarks of aging and brain aging, such as epigenetic alterations, genomic instability, dysregulated energy metabolism, oxidative damage, loss of proteostasis, aberrant neuronal network activity, and dysregulation of neuronal calcium homeostasis. These in turn may plays roles in the development of aging-associated neurodegenerative brain diseases.

Given evidence of the widespread regulation of cellular functions by the circadian clock and the importance of normal circadian function in health, insights into age-related changes in circadian rhythms at the molecular level may improve our understanding of aging as a risk factor for disease. Furthermore, if aging-associated weakening of the circadian system does in fact contribute to functional decline and increased likelihood of disease in older individuals, it is interesting to speculate that approaches to strengthen circadian rhythms might provide new opportunities to increase longevity, prevent age-related human pathologies, and promote healthy aging. Some proposed strategies to reduce age-related circadian disruption include non-pharmacological interventions such as caloric restriction and physical exercise (Froy, 2011; Cornelissen and Otsuka, 2017) as well as pharmacological circadian clock modulation (Gloston et al., 2017). These strategies might also be effective in preventing (and possibly reversing) age-associated cognitive impairment, through a combination of sleep-dependent and sleep-independent mechanisms (Kondratova and Kondratov, 2012).

1.3.4. Aging, the hippocampus, and memory

Although prevalence estimates vary considerably, age-related memory impairment may occur in around 40% of those aged 50 to 59 years and up to 85% of those aged greater than 80 years (Larrabee and Crook, 1994; Hanninen and Soininen, 1997). These estimates highlight the fact that not all older individuals exhibit age-related memory impairment, and there are significant inter-individual differences in how memory changes with aging (Nyberg et al., 2012). More broadly, cognitive impairment in older persons can be characterized as being an element of normal aging, MCI, or dementia based on various criteria, including severity of memory impairment and functional impairment (Hanninen and Soininen, 1997). While there is still debate as to whether these represent distinct entities or exist along a cognitive continuum (Brayne and Calloway, 1988; Petersen et al., 1997; Walters, 2010), a

variety of terms have been used in the past to describe cognitive impairment occurring during aging or those who exhibit these impairments, including: benign senescent forgetfulness (Kral, 1962), age-associated memory impairment (Crook et al., 1987), age-associated cognitive decline (Levy, 1994), cognitively impaired not demented (Ebly et al., 1995), MCI (Petersen, 2004), and memory impairment due to dementia or a major neurocognitive disorder (Hugo and Ganguli, 2014). Regardless of the specific terminology used, several studies have demonstrated that advanced age is generally associated with various cognitive changes, notably a decline in declarative memory (Ronnlund et al., 2005; Harada et al., 2013).

Given that the hippocampus has been shown to play important roles in declarative memory (see section 1.2.3 above), aging-associated hippocampal changes likely contribute to impairments in declarative memory exhibited by many older individuals. In support of this notion, various cellular, structural, and electrophysiological changes that can impact overall hippocampal function are correlated with decline in hippocampal-dependent learning and memory during aging (Lister and Barnes, 2009; Bettio et al., 2017; Fan et al., 2017). Importantly, many of these age-related changes are also seen in several neurodegenerative diseases that affect the hippocampus, highlighting mechanisms by which aging might predispose older individuals to neurodegeneration (see section 1.3.5 below).

(1) Cellular changes in the aging hippocampus. Mechanisms of brain aging in general include impaired glucose metabolism, impaired proteostasis, mitochondrial dysfunction, increased oxidative stress, and epigenetic changes (Bishop et al., 2010; Mattson and Arumugam, 2018). In the hippocampus, studies have shown that these involve alterations in the expression of proteins that participate in or regulate glycolysis and gluconeogenesis; dysregulation of proteins involved in protein folding, trafficking, and degradation; reductions in the activities of key enzymes involved in oxidative phosphorylation; decreased expression of antioxidant enzymes; and epigenetic

modifications affecting genes that have been shown to play roles in synaptic plasticity (Shetty et al., 2011; Vanguilder and Freeman, 2011; Bettio et al., 2017). Proteomic data also suggest that hippocampal aging involves changes in neuron-specific functions such as synaptic vesicle cycling, neurotransmitter release, and synaptic plasticity (Vanguilder and Freeman, 2011). Other age-related alterations in the hippocampus that have been proposed to contribute to functional decline include a reduction in neurotrophic support, an increase in neuroinflammation, and cellular senescence (Bettio et al., 2017).

(2) Structural changes in the aging hippocampus. Data from rodent and human studies indicate that structural changes in the aging hippocampus mainly occur at the cellular level, while findings regarding gross anatomical changes such as reduced hippocampal volume during aging have not been consistent across studies (Bettio et al., 2017). There is some evidence suggesting that there are age-related decreases in the number of synapses and dendritic spines, as well as alterations in dendritic morphology, although these changes have been observed in some subregions of the hippocampus but not others (Lister and Barnes, 2009; Bartsch and Wulff, 2015; Bettio et al., 2017). There is more consistent evidence of hippocampal vascular degeneration occurring during aging in both rodents and humans, and that this is associated with breakdown of the blood–brain barrier and impaired cognitive function (Montagne et al., 2015; Bettio et al., 2017).

(3) Electrophysiological changes in the aging hippocampus. Perhaps unexpectedly, most of the basic electrophysiological properties of neurons appear to be preserved in the aging hippocampus (Lister and Barnes, 2009). However, some specific changes have been reported and suggest that there are decreases in LTP and increases in long-term depression (LTD) at synapses in the CA1 field, as well as an elevated threshold for LTP induction at synapses in the dentate gyrus, in the hippocampus of aged rodents (Barnes et al., 2000; Lister and Barnes, 2009; Kumar, 2011). Age-related impairments in hippocampal LTP have been shown to correlate with poorer performance on hippocampal-dependent

memory tasks and suggest that it becomes more difficult for hippocampal neurons to encode and retain information during aging (Lister and Barnes, 2009; Bettio et al., 2017).

1.3.5. Aging and neurodegenerative diseases

Aging is a major risk factor for various neurodegenerative diseases, including AD, Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD) (van Ham et al., 2009; Niccoli et al., 2017). Consistent with this idea, substantial evidence indicates that there are multiple overlapping mechanisms not only among these neurodegenerative diseases, but also between these disorders and aging (Gan et al., 2018). In general, aged brains display many of the pathological features shared between these neurodegenerative diseases, including impaired proteostasis and associated accumulation of disease-linked protein aggregates (Powers et al., 2009; Douglas and Dillin, 2010), mitochondrial dysfunction and associated impairments in energy metabolism and redox homeostasis (Navarro and Boveris, 2010; Grimm and Eckert, 2017), and perturbations in calcium homeostasis implicated in neuronal dysfunction and death (Mattson, 2007). Circadian dysfunction is an additional shared feature of aging and several neurodegenerative diseases, suggesting that alterations in the circadian system might also contribute to their etiology and/or pathogenesis (Kondratova and Kondratov, 2012; Musiek and Holtzman, 2016). Despite sharing several underlying cellular and molecular mechanisms, these neurodegenerative diseases differ from normal brain aging and from one another in terms of the severity and specificity (regional and cell-type) of altered cellular functions and neuronal loss, leading to diverse clinical manifestations (Wyss-Coray, 2016; Fu et al., 2018). Nevertheless, therapies that target shared pathological mechanisms hold promise as potential approaches to prevent or slow the progression of these neurodegenerative diseases (Gan et al., 2018). This step is especially important given the lack of effective disease-modifying therapies (DMTs) for these diseases.

1.4. Alzheimer's disease

1.4.1. Terminology and history

Dementia (from the Latin *de-* meaning "without" and *mens* meaning "mind") refers to a clinical syndrome involving significant cognitive decline that substantially interferes with social and/or occupational functioning (Breitner, 2006). In people with dementia, these cognitive changes are often accompanied by **behavioural and psychological symptoms of dementia (BPSD**; also known as **neuropsychiatric symptoms**), a heterogeneous group of non-cognitive and behavioural signs and symptoms (Lawlor, 2002; Tible et al., 2017). The state intermediate between normal cognition and dementia is herein referred to as **mild cognitive impairment (MCI)**, which involves a decline in cognition that is more severe than that seen in normal aging but not sufficient to substantially interfere with social and/or occupational functioning (Winblad et al., 2004).

Our present understanding is that dementia and MCI each have multiple potential etiologies, with AD being one of these (Hugo and Ganguli, 2014). Defined in a variety of ways over the years for clinical and research purposes, **Alzheimer's disease (AD)** herein refers to a primary neurodegenerative disease characterized pathologically by β-amyloid (Aβ) plaques and neurofibrillary tau deposits and clinically by dementia in its late stages (McKhann et al., 1984; McKhann et al., 2011; Jack et al., 2018). Importantly, although current criteria state that these abnormal protein deposits are what differentiate AD from other diseases that can cause dementia, it remains unclear whether Aβ and tau actually play a causal role in the etiopathogenesis of AD (see section 1.4.6 below) (Pimplikar, 2009; Drachman, 2014; Jack et al., 2018). Dementia due to AD appears to be the most common form of dementia, and there is increasing evidence that brain pathologies associated with AD and other causes of dementia often coexist (a condition that has been called **mixed dementia**) (White et al., 2005; Jellinger, 2007).

In 1906, the German physician Alois Alzheimer reported the first documented case of AD, about a woman named Auguste Deter (Auguste D.) who displayed progressive cognitive and behavioural decline (Hippius and Neundorfer, 2003). Over the next five years, Alzheimer published reports of her case and other similar cases, describing both the main clinical features and neuropathological hallmarks of this newly discovered disease (Moller and Graeber, 1998). While the term "Alzheimer's disease" was introduced by the German physician Emil Kraepelin (a colleague of Alzheimer) in a psychiatry textbook in 1909 (Weber, 1997), it was not until the 1970s that AD was widely recognized as a main cause of dementia (Katzman, 1976). The mechanisms underlying the development of AD are still unclear, although several hypotheses have been proposed since then (see section 1.4.6 below). From 1993 to 2003, five neurotransmitter-based drugs became widely approved for the treatment of AD, specifically for symptomatic relief (see section 1.4.5 below).

Despite intense basic, translational, and clinical research in the field of AD in recent years, no new drugs have been widely approved for treating the disease since 2003 (Cummings et al., 2014). Major unmet clinical needs include DMTs that can prevent, halt, or reverse AD (Huang and Mucke, 2012; Winblad et al., 2016; Cummings and Fox, 2017) and effective treatment options for sleep and circadian disturbances in AD (McCleery et al., 2016; Urrestarazu and Iriarte, 2016). Clinical trials for pharmacologic interventions to treat AD are increasingly including the use of biomarkers as well as preclinical and prodromal populations, with the current drug development pipeline containing symptomatic and potential DMTs with a variety of mechanisms of action (Bachurin et al., 2017; Hung and Fu, 2017; Cummings et al., 2018). In light of the great challenges in developing new therapies for AD and the significant percentage of AD cases attributable to modifiable risk factors (see section 1.4.3 below), there is a critical need for public health measures that promote healthy aging and reduce the incidence of AD (Barnes and Yaffe, 2011; Norton et al., 2014; Livingston et al., 2017).

1.4.2. Epidemiology and societal impact of AD

Although the nosology of dementia has changed over the years and prevalence estimates vary, AD is widely viewed at the present time to be the leading cause of dementia, accounting for 50–75% of cases (Blennow et al., 2006; Qiu et al., 2009; Reitz et al., 2011; Winblad et al., 2016). The global prevalence of dementia was estimated to be around 47 million in 2015, and this number is projected to almost triple by 2050 (Prince et al., 2015). Results from an international consensus study suggest that the global incidence of dementia is approximately 4.6 million per year (Ferri et al., 2005). Advanced age is the main risk factor for dementia, such that the prevalence of dementia has been estimated to be ~1% at age 65 and ~30% at age 85 in developed countries (Ritchie and Lovestone, 2002; Blennow et al., 2006). Over 60% of people with dementia are women, due in part to the longer average lifespan of women at the present time (G.B.D. Dementia Collaborators, 2019). In Canada, approximately half a million people are living with dementia (Wong et al., 2016).

Dementia has been called one of the great global health challenges of the 21st century (Scheltens et al., 2016; Livingston et al., 2017), with the World Health Organization recently identifying AD and other causes of dementia as a global public health priority (Winblad et al., 2016). AD has enormous human and financial costs for individuals and their caregivers, with profound health, social, and economic consequences for communities and society as a whole (G.B.D. Dementia Collaborators, 2019). Globally, dementia is the 23rd largest cause of disability-adjusted life years (DALYs) and the fifth leading cause of death (G.B.D. Dementia Collaborators, 2019). The annual global economic cost of dementia was estimated at US$818 billion in 2016 and is projected to rise as the population continues to grow and age, leading to an increase in the number of people with dementia (Livingston et al., 2017). Notably, sleep and circadian disturbances can cause significant caregiver distress and are a major cause of institutionalization among people with AD, thereby contributing to the rising human and economic burden of dementia (Salami et al., 2011).

1.4.3. Classification and risk factors of AD

AD is a heterogeneous disease and can be classified in various ways, including: (*1*) genetically, with familial AD (cases with Mendelian inheritance) and sporadic AD (cases with no or less apparent familial aggregation) (Bertram et al., 2010; Piaceri et al., 2013); (*2*) according to age of onset, with early-onset AD (in people younger than an arbitrary cutoff of 65 years) and late-onset AD (in people ≥65 years old) (van der Flier et al., 2011; Panegyres and Chen, 2013); (*3*) by severity, according to the global Clinical Dementia Rating (CDR), with questionable, mild, moderate, and severe AD (Morris, 1997); (*4*) neuropathologically, according to neurofibrillary tangle pathology, with stages I–VI (Braak and Braak, 1991; Braak et al., 2006) or with typical and atypical (hippocampal sparing or limbic predominant) AD (Murray et al., 2011); and (*5*) according to clinical presentation, with typical and atypical AD (Galton et al., 2000). Most AD cases can be classified as sporadic (>95%) (Iqbal and Grundke-Iqbal, 2010) and late-onset (>95%) (Reitz et al., 2011; Balin and Hudson, 2014). A broader and deeper understanding of AD pathobiology may shed light on subtypes that would benefit from different treatments.

A large number of factors, either non-modifiable or modifiable, have been associated with an increased risk of developing AD. Non-modifiable risk factors for AD include advanced age, genetic factors, and having a first-degree relative with AD (Lindsay et al., 2002; Qiu et al., 2009). Familial AD, which is usually early-onset AD and accounts for <5% of all AD cases, is considered to be caused by autosomal dominant mutations in the amyloid precursor protein (APP) gene (*APP*), presenilin 1 gene (*PSEN1*), and presenilin 2 gene (*PSEN2*) (Qiu et al., 2009; Bertram et al., 2010). Moreover, studies have shown that sporadic AD might be up to 60–80% genetically determined, with the major susceptibility gene associated with sporadic and late-onset AD being the apolipoprotein E gene (*APOE*) (Gatz et al., 2006; Coon et al., 2007; Bertram et al., 2010). Potentially modifiable risk factors for AD include cardiovascular risk factors (e.g., diabetes, midlife hypertension, midlife

obesity), health behaviours (e.g., physical inactivity, poor diet, smoking, and cognitive inactivity or low educational attainment), and psychosocial factors (e.g., depression) (Qiu et al., 2009; Barnes and Yaffe, 2011; Reitz et al., 2011). It has been estimated that up to half of AD cases worldwide are potentially attributable to seven of these modifiable risk factors (Barnes and Yaffe, 2011) and that over 20% of the total global DALYs due to dementia can be attributed to four modifiable risk factors associated with obesity, diabetes, poor diet, and smoking (G.B.D. Dementia Collaborators, 2019). However, there is a lack of experimental evidence (largely due to practical considerations) from randomized controlled trials (RCTs) that preventative interventions to modify risk factors actually reduce the incidence of AD (Sosa-Ortiz et al., 2012). Nevertheless, population-wide interventions to reduce these risk factors and address the underlying social determinants could potentially have a profound impact on the future prevalence of not only AD, but also many other common diseases.

1.4.4. Diagnosis and clinical presentation of AD

There have been several diagnostic criteria for AD proposed over the years for use in clinical practice and research, and the current consensus is to typically use a combination of cognitive, functional, biological, and/or pathological criteria (Lopez et al., 2011; McKhann et al., 2011; Dubois et al., 2014). Despite differences between the diagnostic frameworks mentioned below, they generally recognize AD as a slowly progressive brain disorder with different stages. One way to conceptualize this is with a framework for AD that defines three successive stages in chronological fashion: (*1*) a preclinical (asymptomatic) stage; (*2*) a prodromal (early symptomatic, predementia) stage; and (*3*) a dementia stage (Dubois et al., 2010; Dubois, 2018). MCI can occur during the prodromal stage (Dubois, 2018) and studies have estimated that the conversion rate to AD dementia is 10–15% of people with MCI per year (Blennow et al., 2006). In general, the cognitive and functional criteria are derived from the core clinical phenotypic features of AD (see below) and their functional implications,

while the biological and pathological criteria are based on pathophysiological biomarkers and AD pathology confirmed post mortem (see section 1.4.6 below). In clinical practice, the diagnostic work-up for AD can include a medical history, a physical examination, cognitive testing, laboratory tests, neuropsychological assessment, and neuroimaging (Blennow et al., 2006; Feldman et al., 2008; Livingston et al., 2017). While not routinely used in clinical practice at the present time, biomarker evidence has been incorporated into diagnostic criteria for AD that are widely used in clinical trials and clinical research (McKhann et al., 2011; Dubois et al., 2014).

The prevailing criteria for the clinical diagnosis of AD dementia and MCI are currently the World Health Organization's International Classification of Diseases (ICD) criteria and the American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders* (DSM) criteria (Naik and Nygaard, 2008). ICD is the current international diagnostic standard for clinical purposes, although the DSM criteria are also frequently used for the diagnosis of AD. In the latest revision of the ICD (ICD-11), which will come into effect in 2022, AD is classified as a disease of the nervous system with neurocognitive impairment as a major feature, while dementia due to AD is classified as a neurocognitive disorder. In the latest edition of the DSM (DSM-5), published in 2013, AD is included as an etiological subtype of both major and mild neurocognitive disorders, with dementia generally corresponding to a diagnosis of a major neurocognitive disorder and MCI to a diagnosis of a mild neurocognitive disorder (Sachdev et al., 2014).

The prevailing criteria for the research diagnosis of AD and dementia due to AD are currently the International Working Group (IWG) criteria (Dubois et al., 2014), the National Institute of Neurological Disorders and Stroke–Alzheimer Disease and Related Disorders (NINCDS–ADRDA) criteria (McKhann et al., 2011), and the DSM criteria. The latest IWG framework (IWG-2), published in 2014, recognizes AD as a clinicobiological entity and proposes criteria for typical AD, atypical AD, mixed AD, and preclinical states of AD (Dubois

et al., 2014). The latest NINCDS–ADRDA framework, published in 2011, classifies individuals as having probable AD dementia, possible AD dementia, or probable/possible AD dementia with evidence of the AD pathophysiological process (McKhann et al., 2011). Recently, new guidelines were proposed that shift the definition of AD in living people to a strictly biological construct within the National Institute of Aging–Alzheimer's Association (NIA-AA) research framework (Jack et al., 2018), although it remains to be seen whether these guidelines will become a new international standard in the field of AD research.

The clinical presentation of AD can be described as either typical or atypical, with >85% of people having a typical presentation (Dubois, 2018). AD typically presents with an amnestic syndrome involving episodic memory decline, although it may present atypically with non-amnestic phenotypes such as posterior cortical atrophy, logopenic progressive aphasia, or frontal variant AD (Galton et al., 2000; McKhann et al., 2011; Dubois, 2018). Clinical features of AD include cognitive, behavioural, and psychological signs and symptoms, such as: progressive decline in episodic memory; deterioration of other cognitive functions such as working memory, decision-making, and judgement; and BPSD, such as sleep disturbances, apathy, depression, aggression, and agitation (McKhann et al., 1984; Blennow et al., 2006; Dubois et al., 2010; Cerejeira et al., 2012; Kirova et al., 2015; Tible et al., 2017). The vast majority of people with dementia will develop BPSD (Lyketsos et al., 2002), including sleep and circadian rhythm disturbances (see section 1.4.7.1 below). The presence and severity of BPSD can be systematically assessed with instruments such as the Neuropsychiatric Inventory (NPI) or Neuropsychiatric Inventory-Clinician rating scale (NPI-C) (Cummings et al., 1994; de Medeiros et al., 2010). An overview of current and emerging strategies to treat these symptoms, and potentially impact the underlying disease process, is provided in the following section. A deeper understanding of the mechanisms underlying the etiopathogenesis of AD, coupled with earlier and more accurate diagnosis, is a critical step towards developing rational treatment approaches for this disease.

1.4.5. Treatment of AD

People with AD often have complex problems resulting from multiple different symptoms and they can greatly benefit from interventions that address their physical, mental, and social needs (Livingston et al., 2017). Current treatment strategies to manage cognitive, behavioural, and psychological symptoms in people with AD include (*1*) pharmacological and (*2*) non-pharmacological interventions. Pharmacological interventions include the use of acetylcholinesterase inhibitors (AChEIs), the NMDAR antagonist memantine, antipsychotics, antidepressants, and mood stabilisers (Massoud and Gauthier, 2010; Wang et al., 2015; Livingston et al., 2017). Non-pharmacological interventions include cognitive, psychological, social, and environmental approaches (Olazaran et al., 2010; Ballard et al., 2011; Cammisuli et al., 2016; Livingston et al., 2017). Importantly, there are no treatments currently available for sleep and circadian rhythm disturbances in people with AD that have definitive evidence of effectiveness, underscoring a major unmet need (Urrestarazu and Iriarte, 2016; Livingston et al., 2017). Moreover, there are no DMTs currently available for AD, although several efforts to develop and test the efficacy of potential DMTs are currently underway (Bachurin et al., 2017; Graham et al., 2017; Cummings et al., 2018).

(1) Pharmacological interventions. Four drugs are currently routinely used to treat cognitive symptoms in AD: three AChEIs (donepezil, rivastigmine, and galantamine) and one NMDAR antagonist (memantine) (Livingston et al., 2017). Tacrine (another AChEI) was the first drug approved for the treatment of AD, but its use has been largely discontinued due to safety concerns (Mehta et al., 2012). GV-971 (Wang et al., 2019), a sodium oligomannate, recently received conditional approval in China for treating AD, although it remains to be seen whether it will be widely approved and used. Cochrane systematic reviews have concluded that donepezil, rivastigmine, and galantamine are efficacious for people with mild-to-moderate AD (Birks, 2006) and memantine is efficacious for people with moderate-to-severe AD (McShane et al., 2006). Although these drugs are mainly used to

treat cognitive symptoms, with AChEIs generally considered as first-line therapy, they have also been shown to have small beneficial effects on behavioural symptoms (Birks, 2006; Livingston et al., 2017). Pharmacological management of BPSD in AD (e.g., sleep disturbances, psychosis, depression, aggression, and agitation) is typically reversed for individuals with severe symptoms and can include the use of melatonin, antipsychotics, antidepressants, and mood stabilisers (Wang et al., 2015; Urrestarazu and Iriarte, 2016; Livingston et al., 2017; Tible et al., 2017). However, as many pharmacological interventions for BPSD carry the risk of serious side effects and offer limited benefits, non-pharmacological interventions are generally recommended as the first-line approach and can be complemented by pharmacological interventions if needed (Tible et al., 2017).

(2) Non-pharmacological interventions. The evidence base of non-pharmacological interventions is much more limited than that of pharmacological interventions for treating symptoms in people with AD. Nevertheless, there are some studies showing that non-pharmacological interventions can improve outcomes for people with AD and their caregivers, including cognitive function, autonomy and activities of daily living (ADL), institutionalization delay, behavioural symptoms, and mood (Olazaran et al., 2010; Cammisuli et al., 2016). While there is currently a lack of consensus guidelines on the use of non-pharmacological interventions in AD, it has been recommended that these be chosen on a case-by-case basis, taking into account individual needs and available services (Cammisuli et al., 2016). Non-pharmacological interventions for cognitive symptoms in AD include cognitive stimulation therapy (CST), cognitive training, and cognitive rehabilitation (Ballard et al., 2011; Livingston et al., 2017). Non-pharmacological interventions for BPSD in AD include physical activity, psychotherapeutic interventions, music therapy, reality orientation therapy, and caregiver training in behavioural management techniques (Nowrangi et al., 2015; Cammisuli et al., 2016; Tible et al., 2017). Non-pharmacological

interventions, used alone or in combination with pharmacological interventions, can improve the quality of life of people with AD as well as their caregivers (Olazaran et al., 2010).

1.4.6. Etiology and pathogenesis of AD

The etiopathogenesis of AD is presently unclear and appears to be multifactorial, with studies suggesting that familial AD can be caused by mutations in genes linked to Aβ metabolism and that sporadic AD results from the complex interactions of aging and multiple genetic and non-genetic risk factors (Blennow et al., 2006). Genetic factors influencing AD development include risk loci associated with pathways implicated in immunity, lipid metabolism, tau binding, and APP metabolism, as demonstrated by a recent genetic meta-analysis of diagnosed late-onset AD (Kunkle et al., 2019). These include several microglia-enriched genes (e.g., TREM2 and HLA-DRB1) that play critical roles in the immune system.

Several single-factor hypotheses have been proposed over the years, although none completely explain the etiopathogenesis of AD and it is being increasingly recognized that the disease is likely caused by interactions among multiple factors (Maccioni et al., 2010; Craig et al., 2011; Huang and Mucke, 2012; Sanabria-Castro et al., 2017). Two other notions add to this complexity: (*1*) that the set of causes of AD in one individual may not be the same as the set of causes of AD in another individual, especially in light of the heterogeneity of AD; and (*2*) that it is possible that there is no necessary cause of AD according to its current definition. Nevertheless, single-factor hypotheses have greatly impacted the allocation of resources to investigate the mechanisms underlying AD and to develop diagnostic and therapeutic strategies for the disease. Ongoing development of potential DMTs for AD based on many of these hypotheses has been reviewed elsewhere (Bachurin et al., 2017; Graham et al., 2017; Cummings et al., 2018). Hypotheses to explain the etiopathogenesis of AD that have received attention over the past four decades include:

1. **The cholinergic hypothesis**, which proposes that the degeneration of cholinergic neurons and associated reduction in cholinergic neurotransmission in certain brain regions contribute to the development of AD (Bartus et al., 1982; Francis et al., 1999). Three drugs widely used to treat AD are AChEIs (Livingston et al., 2017).
2. **The glutamatergic hypothesis**, which proposes that glutamatergic dysfunction and excitotoxicity contribute to the development of AD (Maragos et al., 1987). One drug widely used to treat AD is a NMDAR antagonist (Livingston et al., 2017).
3. **The amyloid hypothesis,** which proposes that accumulation of Aβ in the brain is a necessary cause of AD, including by driving tau hyperphosphorylation (see hypothesis #4 below) (Beyreuther and Masters, 1991; Hardy and Allsop, 1991; Hardy and Higgins, 1992). The amyloid hypothesis has dominated AD research for almost three decades, in spite of continuing debate over its applicability in cases of sporadic AD and the fact that it has yet to yield any effective treatments despite numerous clinical trials (Karran et al., 2011).
4. **The tau hypothesis**, which proposes that abnormal hyperphosphorylation of the microtubule-associated protein (MAP) tau protein in the brain is the main cause of AD, including through impairment of its ability to bind microtubules (Lee and Trojanowski, 2006). The progression of AD, including clinical presentation and neurodegeneration, is strongly associated with tau pathology (Kametani and Hasegawa, 2018).
5. **The *APOE* hypothesis**, which proposes that the ε4 allele of *APOE* (apoE4) is directly involved in the pathogenesis of AD, through a variety of mechanisms (Mahley and Huang, 2012). Given that apoE4 is by far the strongest genetic risk factor for AD, with 65–80% of all people with AD carrying at least one copy (cf. ~25% of all individuals), developing therapies that counter its neurotoxic effects could potentially have enormous value (Mahley and Huang, 2012).

6. **The pathogen hypothesis**, which proposes that infiltration of the brain by pathogens contributes to the etiopathogenesis of AD by acting as a trigger or co-factor (Itzhaki et al., 2004; Robinson et al., 2004; Itzhaki et al., 2016). Notably, several studies have implicated herpes simplex virus type 1 (HSV1), certain types of spirochetes, and *Chlamydia pneumoniae* in the etiology of AD (Maheshwari and Eslick, 2015; Itzhaki et al., 2016).
7. **The metabolism hypothesis**, which proposes that desensitization of neuronal insulin receptors in the brain plays an important role in the etiopathogenesis of AD (Hoyer, 1998).
8. **The mitochondrial cascade hypothesis**, which proposes that mitochondrial dysfunction in the brain, including low rates of mitochondrial oxidative phosphorylation and high rates of mitochondrial reactive oxygen species (ROS) production, is the main cause of AD (Swerdlow and Khan, 2004).
9. **The gut microbiota hypothesis**, which proposes that AD begins in the gut and involves dysbiosis of the gut microbiota that leads to changes in the brain via the microbiota-gut-brain axis (Hu et al., 2016; Jiang et al., 2017).
10. **The oxidative stress hypothesis**, which proposes that increased oxidative stress in the brain is the main cause of AD (Markesbery, 1997).
11. **The inflammation hypothesis**, which proposes that chronic neuroinflammation plays an important role in the etiopathogenesis of AD, including through exacerbation of tau and Aβ pathology (Krstic and Knuesel, 2013).
12. **The calcium hypothesis**, which proposes that sustained changes in cytosolic calcium concentration provide the final common pathway for explaining the etiopathogenesis of AD (Khachaturian, 1994).
13. **The cytoskeletal hypothesis**, which proposes that destabilization of the cytoskeleton is the central event in the etiopathogenesis of AD (Terry, 1996).

14. **The presenilin hypothesis**, which proposes that decreased presenilin function underlies memory impairment and neurodegeneration in the pathogenesis of AD (Shen and Kelleher, 2007).
15. **The "two-hit" hypothesis**, which proposes that oxidative stress and abnormal mitotic alterations together are necessary for the pathogenesis of AD (Zhu et al., 2001; Zhu et al., 2004).
16. **The GSK3 hypothesis**, which proposes that overactivity of GSK3 plays a leading role in the etiopathogenesis of AD, including through hyperphosphorylation of tau and increased Aβ production (Hooper et al., 2008).
17. **The "dual pathway" hypothesis**, which proposes that common upstream drivers can lead to both elevated Aβ levels and tau hyperphosphorylation through independent but parallel mechanisms, thereby contributing to the etiopathogenesis of late-onset AD (Small and Duff, 2008).

In addition, several lines of evidence suggest that circadian dysfunction contributes to the etiopathogenesis of AD (see section 1.4.7 below), which may lead us to put forward a **"circadian rhythm hypothesis of AD"**: this hypothesis proposes that circadian dysfunction, although neither a necessary nor a sufficient cause of AD, contributes to its etiopathogenesis in a substantial percentage of people with AD, including through dysregulation of sleep and multiple clock-regulated processes and pathways. Importantly, the circadian rhythm hypothesis of AD integrates multiple potential contributory causes, given evidence that the circadian system both regulates and is regulated by cellular and molecular processes involved in many of the single-factor hypotheses mentioned above (see section 1.4.7.2 below). Moreover, the age-related circadian decline (see section 1.3.3 above) is in accordance with the notion that aging is the main non-genetic risk factor for AD (see sections 1.4.2 and 1.4.3 above). Thus, the circadian rhythm hypothesis could provide

an integrated framework that links multiple interacting pathogenic components, and further studies are warranted to test this hypothesis as well as its clinical relevance.

While there is intense debate about the etiopathogenesis of AD, there is greater consensus regarding the common substrates of cognitive decline (Mattson, 2004; Huang and Mucke, 2012; Fu et al., 2018). Broadly, these include neuron dysfunction and degeneration in neuronal circuits critical for learning and memory. Loss of specific neuronal populations in the hippocampus and other cortical regions, synaptic dysfunction and loss, and alterations in neural network activities in the brains of people with AD have been consistently reported. The reasons underlying the selective neuronal and regional vulnerability in AD, a fundamental characteristic of the disease, remain unknown and might only become clearer once we have a better understanding of different neuronal subtypes and their physiology (Fu et al., 2018). The development of DMTs that can prevent—rather than attempt to reverse—these detrimental effects, along with early detection of AD and/or high-risk individuals, could greatly contribute to alleviating the impact of this disease.

1.4.7. Alzheimer's disease and the circadian system

As with aging, there exists a complex interplay between AD and the circadian system. A critical question that remains unanswered is whether circadian dysfunction in AD is a cause of neurodegeneration, a consequence of neurodegeneration, or both. As summarized below, there is increasing evidence suggesting that circadian dysfunction in AD is both a cause and consequence of neurodegeneration, and could thereby contribute to the pathogenesis and clinical manifestations of AD (**Figure 1.4**). On one hand, people with AD and various AD mouse models exhibit an overall dysfunction of the circadian system that is more severe than that which occurs in normal aging (Weldemichael and Grossberg, 2010; Coogan et al., 2013; Ju et al., 2014; Videnovic et al., 2014; Webster et al., 2014; Hood and Amir, 2017b; Duncan, 2019; Leng et al., 2019). On the other hand, accumulating evidence

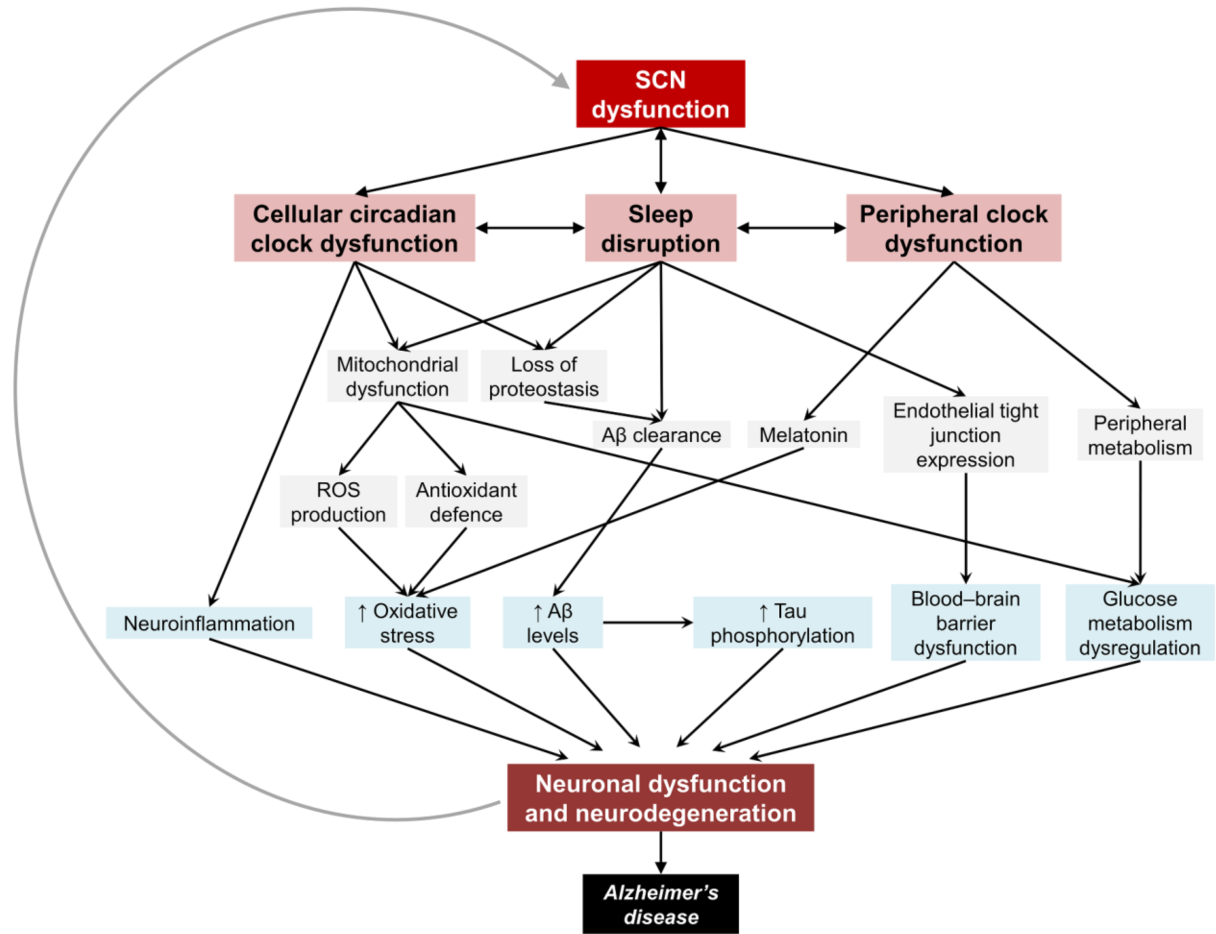

SCN dysfunction
Cellular circadian clock dysfunction
Sleep disruption
Peripheral clock dysfunction
Mitochondrial dysfunction
Loss of proteostasis
Aβ clearance
Melatonin
Endothelial tight junction expression
Peripheral metabolism
ROS production
Antioxidant defence
Neuroinflammation
↑ Oxidative stress
↑ Aβ levels
↑ Tau phosphorylation
Blood–brain barrier dysfunction
Glucose metabolism dysregulation
Neuronal dysfunction and neurodegeneration
Alzheimer's disease

Figure 1.4. Reciprocal interactions between the circadian system and Alzheimer's disease.

Impaired brain function in AD occurs largely as a result of neuronal dysfunction and neurodegeneration, which likely arise from multiple pathogenic mechanisms. Evidence suggests that circadian dysfunction can contribute to the pathogenesis of AD through a combination of sleep-dependent and sleep-independent pathways affecting brain homeostasis either directly or indirectly. Direct effects are mediated by weakened SCN outputs to neuronal and glial cellular clocks within the brain, while indirect effects result from alterations in peripheral oscillators outside the brain. Neuronal dysfunction and neurodegeneration can also affect the SCN and sleep/wake regulatory brain circuits, thereby exacerbating circadian dysfunction.

suggests that the circadian system—through both sleep-dependent and sleep-independent mechanisms—regulates several processes involved in the pathogenesis of AD, impaired hippocampal-dependent memory, and impaired behavioural circadian rhythms (Kondratova and Kondratov, 2012; Coogan et al., 2013; Ju et al., 2014; Musiek, 2015; Musiek et al., 2015; Musiek and Holtzman, 2016; Cedernaes et al., 2017; Chauhan et al., 2017; Leng et al., 2019), indicating that alterations in the circadian system could contribute to the development and clinical manifestations of AD.

1.4.7.1. Changes in the circadian system in AD

AD is associated with impaired circadian function that is characterized mainly by changes in the circadian system's multi-oscillator network and output rhythms, and it is believed that SCN dysfunction and degeneration is the main cause of circadian dysfunction in people with AD (Kondratova and Kondratov, 2012; Coogan et al., 2013; Musiek, 2015; Musiek et al., 2015; Musiek and Holtzman, 2016). Overt sleep and circadian rhythm disturbances have been shown to affect up to 39% of people with AD (Zhao et al., 2016), although the exact contribution of the circadian system is unclear, in part because other factors also regulate sleep. Nevertheless, studies have shown that AD is associated with several changes to the multi-oscillator network relative to normal aging, including loss of AVP- and VIP-expressing cells in the SCN (Swaab et al., 1985; Zhou et al., 1995; Stopa et al., 1999; Harper et al., 2008), alterations in the rhythmic methylation of *BMAL1* (Cronin et al., 2017), and alterations in the rhythmic expression of clock genes in various brain regions likely resulting in part from disrupted SCN outputs (Wu et al., 2006; Cermakian et al., 2011). Furthermore, evidence suggests that Aβ may contribute to AD-associated circadian clock disruption (Blake et al., 2015; Song et al., 2015; Schmitt et al., 2017).

Changes to output rhythms that have consistently been shown to occur in AD include changes to behavioural, physiological, and cellular circadian rhythms. Overall, AD is associated with alterations in the sleep/wake cycle, locomotor activity rhythms, body

temperature rhythms, neuroendocrine rhythms, and diurnal molecular rhythms in the brain (Weldemichael and Grossberg, 2010; Coogan et al., 2013; Videnovic et al., 2014; Hood and Amir, 2017b; Duncan, 2019). This includes sleep/wake cycle fragmentation leading to increases in daytime sleepiness and nocturnal awakenings and activity levels, which can have a major negative impact on quality of life of people with AD and their caregivers (Hood and Amir, 2017b). Moreover, studies have shown that locomotor activity rhythms have lower amplitudes and reduced inter-day stability, and the phases of body temperature and locomotor activity rhythms are delayed by ~4 hours, in people with AD relative to healthy elderly subjects (Duncan, 2019). There is also evidence that daily rhythms in cortisol and melatonin levels (Coogan et al., 2013) and gene expression in the brain (Lim et al., 2017) are altered in people with AD. In addition, sleep and circadian rhythm disturbances are a common feature in several AD mouse models (Wisor et al., 2005; Sterniczuk et al., 2010; Duncan et al., 2012; Videnovic et al., 2014; Zhou et al., 2016; Bellanti et al., 2017; Boggs et al., 2017; Stevanovic et al., 2017; Paul et al., 2018; Wu et al., 2018). Together, these studies have highlighted several changes in the circadian system associated with AD, which may in turn influence its pathogenesis and clinical manifestations.

1.4.7.2. Effects of the circadian system on Alzheimer's disease

There is indirect evidence that AD-related changes in the circadian system contribute to the pathogenesis and clinical manifestations of AD, including its occurrence early in the disease course, as well as shared mechanisms of AD pathogenesis and circadian dysfunction (see also sections 1.1.2.3, 1.1.3, and 1.3.3.2 above). Accumulating evidence suggests that sleep and circadian rhythm disruption occurs early in AD, is a risk factor for and often precedes the development of cognitive decline, and is associated with multiple characteristics of AD (e.g., sundowning, disease severity, and biomarker levels) (Volicer et al., 2001; Tranah et al., 2011; Schlosser Covell et al., 2012; Ju et al., 2013; Videnovic et al., 2014; Diem et al., 2016; Musiek et al., 2018; Shi et al., 2018).

The mechanisms underlying the links between circadian dysfunction, neurodegeneration, and impaired brain function in AD are not fully understood but might involve circadian regulation of proteostasis, including Aβ homeostasis; tau pathology and cytoskeletal function; glucose metabolism; mitochondrial function; gut microbiota composition; antioxidant defense; neuroinflammation; and levels of presenilin-2 and GSK3 (Kang et al., 2009; Huang et al., 2011; Perez et al., 2011; Kondratova and Kondratov, 2012; Masri and Sassone-Corsi, 2013; Mattis and Sehgal, 2016; Musiek and Holtzman, 2016; Cedernaes et al., 2017; Minakawa et al., 2017; Homolak et al., 2018; Kress et al., 2018; Vanderheyden et al., 2018; Greco and Sassone-Corsi, 2019; Holth et al., 2019; Leng et al., 2019; Lucey et al., 2019). Moreover, impaired circadian function in AD could negatively impact the regulation of other cellular and molecular processes that impact memory (see section 1.2.5 above) as well as the regulation of behavioural circadian rhythms, including the sleep/wake cycle (see section 1.1.2.3 above).

Direct pharmacological targeting of the circadian clock represents an emerging strategy to potentially treat neurodegenerative disorders (Gaikwad, 2018; Maiese, 2018). The CK1δ/ε isoforms of the CK1 family of serine/threonine protein kinases, which play critical roles in the mammalian circadian clock and are implicated in AD, have been proposed as new therapeutic targets for AD and other neurodegenerative diseases (Savage and Gingrich, 2009; Perez et al., 2011; Cozza and Pinna, 2016). This is partly based on studies demonstrating dramatic overexpression of CK1δ/ε in the brains of people with AD relative to healthy elderly subjects, including a >20-fold increase in CK1δ mRNA abundance in the hippocampus (Yasojima et al., 2000) and a 2.5-fold increase in CK1ε protein abundance in the cortex (Chen et al., 2017). CK1δ/ε might contribute to the pathogenesis of AD through their involvement in regulating circadian rhythms (see above), as well as through potentially clock-independent regulation of tau phosphorylation (Flaherty et al., 2000; Li et al., 2004; Hanger et al., 2007; Chen et al., 2017), Aβ production (Flajolet et al., 2007), and

glutamatergic synaptic transmission mediated by NMDARs (Chergui et al., 2005). Moreover, elevated expression and/or activity of CK1ε has been shown to impair working memory and result in behavioural circadian rhythm disturbances in mice (Meng et al., 2008; Chen et al., 2017), suggesting that CK1δ/ε overexpression might contribute to cognitive and sleep impairments in people with AD.

Together, these findings suggest that CK1δ/ε inhibition could potentially mitigate AD pathogenesis, enhance cognitive function, and treat sleep and circadian rhythm disturbances in people with AD. In support of this idea, pharmacological CK1δ/ε inhibition has previously been shown to reduce Aβ production and tau phosphorylation *in vitro* (Flajolet et al., 2007; Chen et al., 2017) as well as to normalize behavioural circadian rhythm disturbances in various mouse models of circadian dysfunction (Meng et al., 2010). Several small molecule CK1δ/ε inhibitors have been identified for use in basic and translational research (Chen et al., 2013; Chen et al., 2018), although none have yielded any marketed drug yet (Carles et al., 2018).

Interestingly, both acetylcholine and glutamate have been strongly implicated in regulation of the circadian system and display rhythmic levels in the brain (Mitsushima et al., 1996; Castaneda et al., 2004; Reghunandanan and Reghunandanan, 2006; Yamakawa et al., 2016; Hastings et al., 2018). Thus, it is tempting to speculate that the cholinergic and glutamatergic drugs widely used for treating AD might partially yield their beneficial effects through modulation of the circadian system and/or circadian rhythms in neurotransmitter levels. Although there is much that is still not understood with regard to how circadian dysfunction contributes to the pathogenesis and clinical manifestations of AD, strategies to mitigate AD-associated circadian dysfunction hold great potential to prevent neurodegeneration and mitigate symptoms by influencing multiple processes that are critical for brain function.

1.5. Rationale, hypotheses, and objectives

1.5.1. State of the art

In summary, evidence suggests that:

(1) the mammalian circadian system regulates several cellular and molecular processes that contribute to brain homeostasis and brain functions such as memory and sleep (sections 1.1.2.3, 1.1.3, 1.1.4, and 1.2.5);

(2) the mammalian hippocampus plays important roles in certain types of memory (sections 1.2.2, 1.2.3, and 1.2.4);

(3) the mammalian hippocampus displays circadian rhythms in several cellular and molecular processes that contribute to hippocampal function (section 1.2.5);

(4) in mammals, normal circadian regulation contributes to brain homeostasis and function and/or circadian disruption impairs these (sections 1.1.2.3 and 1.2.5);

(5) circadian disruption and impaired brain homeostasis result in deficits in hippocampal-dependent memory in mammals (sections 1.2.3 and 1.2.5);

(6) aging is a major risk factor for certain neurodegenerative brain diseases, including AD (sections 1.3.5 and 1.4.3);

(7) circadian dysfunction occurs during aging in mammals as well as in certain aging-associated neurodegenerative brain diseases, including AD (sections 1.3.3.1 and 1.4.7.1);

(8) impaired hippocampal-dependent memory occurs during aging in mammals as well as in certain aging-associated neurodegenerative brain diseases, including AD (sections 1.3.4, 1.4.4, and 1.4.6); and

(9) several aging-associated neurodegenerative brain diseases, including AD, involve dysregulation of cellular and molecular processes within the brain that are regulated by the circadian system (sections 1.4.6, and 1.4.7.2).

Therefore, it is conceivable that:

1. aging leads to deficits in hippocampal-dependent memory via aging-induced alterations in the circadian regulation of cellular and molecular processes within the hippocampus; and
2. aging increases the likelihood of developing certain neurodegenerative brain diseases via aging-induced alterations in the circadian regulation of cellular and molecular processes, which render the brain vulnerable to neurodegeneration.

Moreover, evidence suggests that:

(10) circadian dysfunction is more severe in AD than in normal aging and includes changes in output rhythms (sections 1.4.4 and 1.4.7.1);

(11) CK1δ/ε regulate core circadian clock proteins and are overexpressed in AD brains relative to brains of elderly subjects without AD (sections 1.1.2.2 and 1.4.7.2);

(12) the pathogenesis of AD is complex and several cellular and molecular processes within the brain that are involved in it are regulated by the circadian clock (sections 1.1.3, 1.4.6, and 1.4.7.2);

(13) several cellular and molecular processes within the brain that contribute to hippocampal function and are altered in AD, as well as hippocampal-dependent memory itself, are regulated by the circadian clock (sections 1.2.5 and 1.4.7.2);

(14) CK1ε overexpression in the hippocampus impairs working memory in mice (section 1.4.7.2);

(15) the sleep/wake cycle and locomotor activity rhythms are regulated by the circadian clock (sections 1.1.2.3 and 1.4.7.2); and

(16) CK1δ/ε inhibition can reduce Aβ production and tau phosphorylation *in vitro* as well as normalize impaired behavioural circadian rhythms in mouse models of circadian dysfunction (section 1.4.7.2).

Therefore, it is also conceivable that:

3. circadian dysfunction in AD (including alterations in the multi-oscillator network and output rhythms) contributes to the pathogenesis of AD, impaired hippocampal-dependent memory, and impaired behavioural circadian rhythms;
4. CK1δ/ε overexpression in AD brains is involved in this circadian dysfunction; and
5. CK1δ/ε inhibition *in vivo* might partially reverse AD-associated circadian dysfunction and thereby mitigate the pathogenesis of AD, impaired hippocampal-dependent memory, and impaired behavioural circadian rhythms.

1.5.2. Purpose

This work largely arises from a motivation to address the magnitude of the burden of age-related neurodegenerative brain diseases, the unmet clinical needs of people affected with these diseases, and the gaps in knowledge about the topics discussed in the sections above. Age-related cognitive impairment and AD are prevalent issues across the world, with dementia recently being identified as a global public health priority by the World Health Organization (see sections 1.3.4 and 1.4.2 above). Rational therapeutic interventions that can effectively address the etiopathogenesis of AD, as well as sleep and circadian disturbances in people with AD, remain major unmet clinical needs (see section 1.4.5 above). Notably, our understanding of how aging impacts the circadian regulation of biological processes in the brain is limited, as is our knowledge of the mechanisms underlying the association between circadian decline and cognitive decline during aging. Moreover, the applicability of pharmacological targeting of CK1δ/ε and the circadian clock for treating AD remains largely unexplored. Effectively addressing these knowledge gaps could enable the development of therapeutic interventions that foster healthy aging.

In order to address these gaps in our knowledge, the studies in this thesis utilize a combination of widely used methods and resources to investigate the hypotheses proposed

below. Mass spectrometry–based proteomics is a highly suitable approach to investigate the widespread alterations in biological processes in the brain associated with aging and CK1δ/ε inhibition, while rodent cognitive and behavioural tests provide complementary data regarding the functional effects of CK1δ/ε inhibition. PF-670462 (Badura et al., 2007), a small molecule CK1δ/ε inhibitor, has previously been shown to alter behavioural, physiological, and cellular circadian rhythms in rats (Badura et al., 2007; Sprouse et al., 2010; Perreau-Lenz et al., 2012; Kennaway et al., 2015), mice (Walton et al., 2009; Meng et al., 2010), and a non-human primate (Sprouse et al., 2009). 3xTg-AD mice (Oddo et al., 2003), which are frequently used as a model of AD, are the most studied animal model of AD exhibiting both amyloid and tau pathology (Esquerda-Canals et al., 2017). Notably, 3xTg-AD mice have been shown to display cognitive and behavioural changes similar to those seen in people with AD, such as memory impairments and behavioural circadian rhythm disturbances (Webster et al., 2014), allowing for evaluation of the effects of PF-670462 administration.

The main purpose of the novel work described in this thesis is twofold: (*1*) to improve our understanding of the links between the circadian system, aging, age-related cognitive impairment, and age-related neurodegenerative brain diseases; and (*2*) to evaluate the therapeutic potential of pharmacological circadian clock modulation *in vivo* in a preclinical model of AD. Knowledge of how circadian dysfunction during aging contributes to age-related cognitive impairment and neurodegenerative brain diseases could be harnessed to develop new strategies to prevent and treat these issues. In addition, *in vivo* evidence of the efficacy of CK1δ/ε inhibition, or pharmacological circadian clock modulation more broadly, could open new avenues for treating AD.

1.5.3. Hypothesis #1 and Objective #1

Hypothesis #1: Aging alters the circadian regulation of the murine hippocampal proteome, including the daily patterns of expression of proteins that have previously been shown to play roles in hippocampal-dependent memory and aging-associated neurodegenerative diseases involving the hippocampus.

Objective #1: Investigate the proteomic alterations in circadian rhythms of the hippocampus in middle-aged mice as compared to young mice.

1.5.4. Hypothesis #2 and Objectives #2a, 2b, and 2c

Hypothesis #2: CK1δ/ε inhibition reduces hippocampal proteomic alterations, deficits in hippocampal-dependent memory, and behavioural circadian rhythm disturbances associated with AD-like pathology in 3xTg-AD mice.

Objective #2: Determine the effects of administering the small molecule CK1δ/ε inhibitor PF-670462 in symptomatic 3xTg-AD mice on:

(2a) the hippocampal proteome;

(2b) hippocampal-dependent memory; and

(2c) behavioural circadian rhythms.

Chapter 2. Aging-Induced Alterations in the Murine Circadian Hippocampal Proteome

2.1. Introduction

Circadian regulation of various physiological and behavioural processes is critical to maintaining homeostasis and organismal health (Bass and Lazar, 2016; Chaix et al., 2016). In mammals, the SCN act as a central pacemaker to entrain an organism's internal clock using environmental cues such as the daily light/dark cycle, and in turn synchronizes peripheral oscillators located in other brain regions and organs (Liu et al., 2007; Dibner et al., 2010). Circadian disturbances occur during aging and are associated with cognitive decline and several brain disorders, including neurodegenerative diseases (Kondratov, 2007; Kondratova and Kondratov, 2012; Musiek and Holtzman, 2016). Moreover, the circadian clock influences processes involved in hallmarks of aging, underscoring its significance in maintaining physiological integrity (Kondratov, 2007; Lopez-Otin et al., 2013; Fonseca Costa and Ripperger, 2015; Chaix et al., 2016). Disruption of the temporal coordination of clock-controlled processes may therefore contribute to functional decline during aging (Kondratov, 2007; Hood and Amir, 2017a). Furthermore, given the interplay between the circadian system and the aging process, and that aging is a major risk factor for several neurodegenerative diseases, the effects of aging on circadian rhythms could have important implications for the pathogenesis of these disorders (Kondratov, 2007; Musiek and Holtzman, 2016). Yet, how aging affects the circadian orchestration of biological processes in the brain remains largely unexplored (Hatanaka et al., 2017).

Several molecular and cellular mechanisms underlying synaptic plasticity and hippocampal function, as well as hippocampal-dependent memory itself, have previously been shown to be clock-regulated and disrupted by clock dysregulation (Smarr et al., 2014; Hannou et al., 2018; Snider et al., 2018). Circadian clocks modulate the expression or activity of diverse proteins involved in processes contributing to hippocampal function, such as neurotransmission, LTP, and energy metabolism (Chiang et al., 2017; Hannou et al., 2018; Greco and Sassone-Corsi, 2019). These include proteins such as CaMKII, GSK3β, the GluA1 AMPAR subunit, synaptic vesicle glycoprotein 2A (SV2A), and isocitrate dehydrogenase (IDH) (Chiang et al., 2014; Neufeld-Cohen et al., 2016; Chiang et al., 2017; Hannou et al., 2018; Snider et al., 2018). Moreover, circadian dysfunction and cognitive decline are common features of aging and multiple age-related neurodegenerative disorders, and accumulating evidence suggests that aging- and disease-related circadian disruption contributes to memory impairment (Kondratova and Kondratov, 2012; Musiek and Holtzman, 2016). However, the mechanisms underlying the association between declines in circadian function and memory during aging remain elusive. The identification of age-related changes in the circadian regulation of processes underlying memory function in the hippocampus might therefore open new avenues to correct dysregulated rhythms and thereby prevent or reverse cognitive decline.

Systems biology approaches are uniquely positioned to provide insight into the ubiquitous role of the circadian clock in physiology and to identify links among diverse temporally regulated processes (Hughes et al., 2017; Millius and Ueda, 2017). Our lab has previously shown that the clock regulates key biological processes at the proteomic and phosphoproteomic levels in the SCN and hippocampus of young mice (Chiang et al., 2014; Chiang et al., 2017). However, the impact of aging on the hippocampus has not yet been investigated at the global proteomic level from a circadian perspective. In this study, we compared young and middle-aged mice using a quantitative MS-based approach to profile

the hippocampal circadian proteome across two consecutive days, in order to dissect changes in the temporal orchestration of biological pathways during aging. We show that aging disrupts the circadian regulation of proteins involved in cellular functions critical for hippocampal function and memory, notably energy metabolism, neurotransmission, and synaptic plasticity. Furthermore, aging altered the daily expression rhythms of proteins implicated in various processes linked to neurodegenerative diseases as well as hallmarks of aging, such as mitochondrial dysfunction and loss of proteostasis. Collectively, our findings provide further evidence supporting the contribution of the age-dependent circadian decline to the development of neurodegenerative diseases and cognitive deterioration over time.

2.2. Materials and Methods

2.2.1. Animals and tissue collection

Male C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA; Stock #000664) and aged to 9–10 weeks (young group) or 44–52 weeks (middle-aged group). Mice were group housed in polycarbonate cages with *ad libitum* access to food and water and entrained to a 12-h light:12-h dark (LD) schedule (lights on at 6:00 a.m., lights off at 6:00 p.m.) from 5 weeks of age (young group) or 28 weeks of age (middle-aged group) before being transferred to constant darkness (DD). After two days in constant darkness, mice were sacrificed at 4-hour intervals over two days starting at CT2 on the third day of DD, where CT was defined by the ZT of the previous LD schedule (Chiang et al., 2014; Chiang et al., 2017). Sample sizes were as follows: three mice per age group were sacrificed at each CT, except for CT18 and 22 (four mice per age group at each CT), CT42 (four young mice and two middle-aged mice), and CT46 (four young mice). Mice were sacrificed by cervical dislocation under dim red light and the hippocampi were quickly

excised. Tissues were immediately flash frozen in liquid nitrogen and stored at –80°C until further processing. All animal experiments were conducted at the Ottawa Hospital Research Institute and approved by the University of Ottawa Animal Care Committee in compliance with institutional and Canadian Council on Animal Care guidelines. Mouse colony maintenance was performed by Jasmine I. Moore. Drs. Chiang, Mayne, Zhang, Xu, and Cheng performed sample collection.

2.2.2. Proteomic analysis of hippocampal tissues

Protein extracts from hippocampal tissues of individual mice were obtained by homogenization in lysis buffer using a pellet pestle and sonication (three 10 s pulses with 30 s on ice between each pulse). The lysis buffer contained 4% (w/v) sodium dodecyl sulphate (SDS) in 50 mM ammonium bicarbonate (ABC; pH 8.2) supplemented with complete protease and phosphatase inhibitor cocktails (Roche; Mississauga, ON, Canada). Protein concentrations were determined using the DC Protein Assay (Bio-Rad; Mississauga, ON, Canada), and hippocampal lysates were loaded onto 30-kDa molecular weight cutoff Microcon filters (MilliporeSigma; Oakville, ON, Canada). Proteins were reduced by incubating samples with 20 mM dithiothreitol (DTT; MilliporeSigma) for 30 min at 37°C with agitation (245 rpm) and subsequently alkylated with 20 mM 2-iodoacetamide (IAA; MilliporeSigma) for 30 min in darkness at room temperature. Protein digestion was performed by incubation with 40:1 (w/w, protein:enzyme) trypsin (Worthington Biochemical Corporation; Lakewood, NJ, USA) overnight at 37°C with agitation (245 rpm). Prior to strong cation exchange (SCX) fractionation of hippocampal samples, peptides were diluted with 0.1% (v/v) formic acid (FA) and the pH adjusted with trifluoroacetic acid (TFA) to 3.0. Step elution of peptides was performed using in-house made SCX columns (10-µm SCX beads, Polymer Laboratories) and subsequent addition of buffers (20 mM boric acid, 20 mM phosphoric acid, and 20 mM acetic acid) at pH 5, 6, 8, 10, and 12. Samples were desalted

using in-house made C18 desalting cartridges (C18 beads: ReproSil-Pur C18-AQ, 10 μm; Dr. Maisch GmbH; Beim Brückle, Germany) and dessicated using a SpeedVac prior to being resuspended in 0.1% (v/v) FA for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Dr. Chiang contributed to sample preparation.

2.2.3. LC-MS/MS analysis

Four microlitres of resuspended peptides (equivalent to 2 μg of proteins) from each sample were analyzed by an online reverse-phase LC-MS/MS platform consisting of an Eksigent NanoLC 425 system (AB SCIEX) coupled with an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific; San Jose, CA, USA) via a nano-electrospray source. Prior to MS analysis, peptide mixtures were separated by reverse-phase chromatography using an in-house packed ReproSil-Pur C18-AQ column (75 μm internal diameter × 15 cm, 1.9 μm, 200 Å pore size; Dr. Maisch GmbH) over a 120-min gradient of 5–30% buffer B [acetonitrile (ACN) with 0.1% (v/v) FA] at a flow rate of 300 nL/min. The Orbitrap Elite instrument was operated in the data-dependent mode to simultaneously measure survey scan MS spectra (350–1,800 *m/z*, R = 60,000 defined at *m/z* 400). Up to the 20 most intense peaks were isolated and fragmented with collision-induced dissociation (CID). System controlling and data collection were carried out using Xcalibur software version 2.2 (Thermo Scientific). Dr. Ning contributed to ensuing mass spectrometry working conditions.

2.2.4. Mass spectrometry data processing

Mass spectrometry raw files were processed with MaxQuant (version 1.5.2.8) using the integrated Andromeda search engine and UniProt FASTA database from mouse (*Mus musculus;* 2013_05). The search included variable modifications for methionine oxidation (M) and acetylation (protein N-term) as well as fixed modification for carbamidomethylation (C). Trypsin/P was set as the cleavage specificity with up to two missed cleavages allowed. The false discovery rate (FDR) cutoffs were set at 0.01 at the peptide and protein levels and

the minimum peptide length was set at 7. Identification across different replicates and adjacent fractions was achieved by enabling the “match between runs” option with a matching time window of 5 min. Drs. Ning and Chiang contributed to mass spectrometry data analysis.

2.2.5. Bioinformatic and statistical analyses

Initial bioinformatic analysis was performed with Perseus (version 1.5.5.3). Following logarithmic (log_{10}) transformation of label-free quantification (LFQ) intensities, the raw proteomic dataset was filtered to include only proteins quantified in a minimum of two biological replicates per time point in either young or middle-aged mice (referred to as reliably quantified proteins in this chapter). Using these filtered datasets, circadian rhythmicity in protein abundance over the 12 CTs was determined using the Perseus periodicity algorithm (period = 23.6 h) (Robles et al., 2014) with q-value < 0.25 (Mauvoisin et al., 2014). Heatmaps displaying temporal expression profiles of circadian proteins ordered by phase were generated using the logarithmized LFQ intensities after z-score normalization. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway functional annotations and enrichment analyses were implemented using DAVID (version 6.8; one-sided Fisher’s exact test $p \leq 0.05$ relative to the backgrounds of reliably quantified proteins in our datasets was considered significant) in order to assess changes in GO biological processes, GO cellular components, and KEGG pathways. Protein–protein interaction networks were created using the STRING database (Szklarczyk et al., 2015) (confidence score cutoff = 70%) and visualized with Cytoscape (version 3.4.0) to include the phases and q-values of rhythmic proteins.

2.2.6. Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD013364. Note

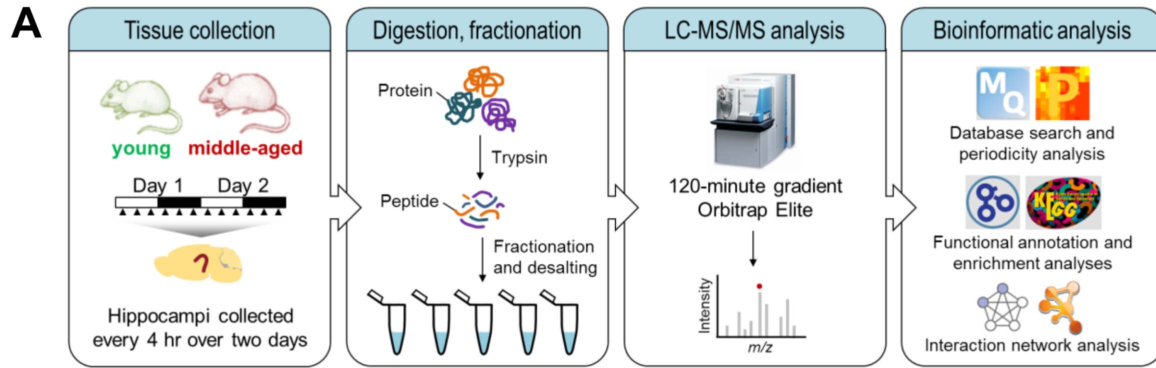
that a stable isotope labelling by amino acids in cell culture (SILAC) spike-in was introduced during sample preparation but not used for quantification.

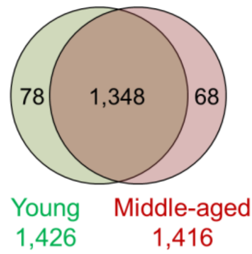
2.3. Results

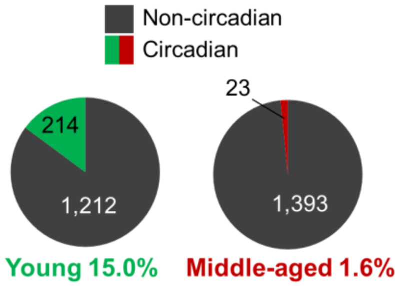
2.3.1. Aging disrupts the hippocampal circadian proteome

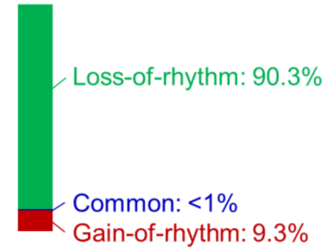
To examine how aging alters the regulation of rhythmic processes in the hippocampus, we used a quantitative MS-based approach to analyze total protein extracts from hippocampal tissues harvested from young and middle-aged mice over two consecutive circadian cycles (see the section “Materials and Methods”; **Figure 2.1 A**). Samples were processed individually to yield three to four biological replicates at each of the 12 time points for each age group, and relative protein abundances were determined using LFQ. This MS-based analysis identified a total of 4,433 proteins, of which 1,426 and 1,416 were quantified in a minimum of two biological replicates at each time point in the hippocampus of young mice and middle-aged mice, respectively (**Figure 2.1 B**). We used these stringently filtered datasets of reliably quantified proteins for downstream analysis.

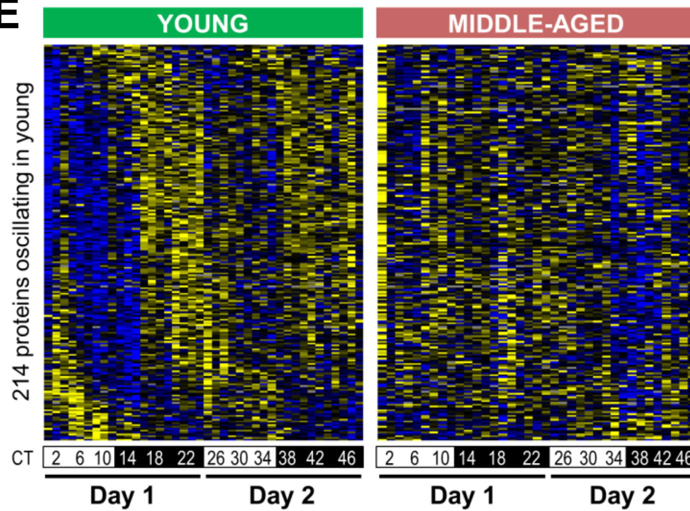
Proteins displaying circadian oscillations in their abundances were identified using the Perseus periodicity analysis algorithm (period = 23.6 h) (Robles et al., 2014) and a FDR value cutoff (Mauvoisin et al., 2014) of 0.25 in young and middle-aged mice. Abundance for 236 proteins changed as a function of circadian timing at either age (**Appendix A Tables 1** and **2**). As expected, the core circadian clock proteins were not reliably quantified in our datasets due to their low abundance in total protein extracts (Millius and Ueda, 2017). A total of 214 proteins displayed circadian rhythmicity in the hippocampus of young mice (15.0% of reliably quantified proteins), while in middle-aged mice a total of 23 rhythmic proteins were identified (1.6% of reliably quantified proteins) (**Figure 2.1 C**). Overall, there were fewer rhythmic proteins identified in the middle-aged group, reflective of the

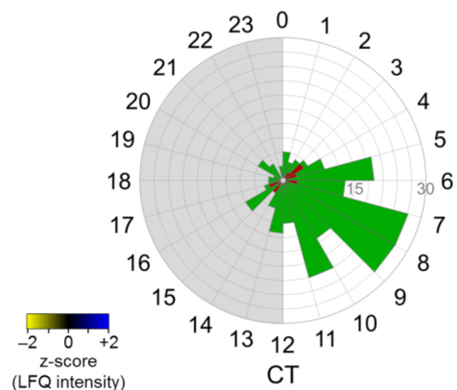

A
Tissue collection
young
middle-aged
Day 1
Day 2
Hippocampi collected every 4 hr over two days
Digestion, fractionation
Protein
Trypsin
Peptide
Fractionation and desalting
LC-MS/MS analysis
120-minute gradient Orbitrap Elite
Intensity
m/z
Bioinformatic analysis
Database search and periodicity analysis
Functional annotation and enrichment analyses
Interaction network analysis


B
Reliably quantified proteins
78
1,348
68
Young 1,426
Middle-aged 1,416


C
circadian proteins
Non-circadian
Circadian
214
1,212
23
1,393
Young 15.0%
Middle-aged 1.6%


D
% circadian proteins
Loss-of-rhythm: 90.3%
Common: <1%
Gain-of-rhythm: 9.3%


E
YOUNG
MIDDLE-AGED
214 proteins oscillating in young
CT
2 6 10 14 18 22 26 30 34 38 42 46
2 6 10 14 18 22 26 30 34 38 42 46
Day 1
Day 2
Day 1
Day 2
−2 0 +2
z-score
(LFQ intensity)


F
Phase distribution of circadian proteins
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
15
30
CT

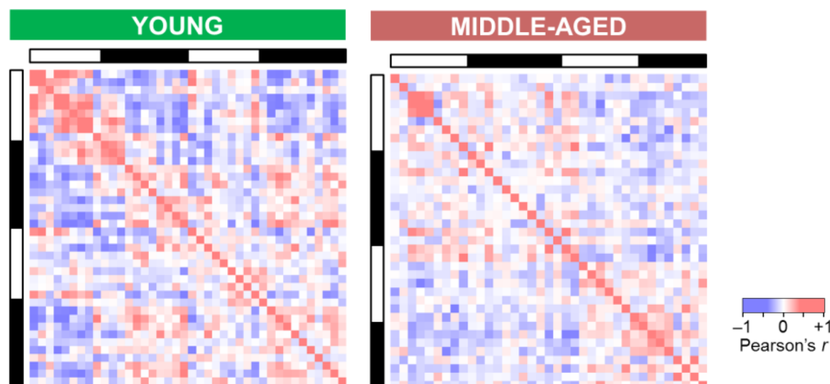

G
YOUNG
MIDDLE-AGED
−1 0 +1
Pearson's r

Figure 2.1. Aging disrupts the hippocampal circadian proteome.

(A) Experimental design and workflow of the MS-based analysis of proteins extracted from hippocampal tissues of young (9–10 weeks old) and middle-aged (44–52 weeks old) C57BL/6J mice. Samples were collected every 4 hours over two days, and proteins extracted from tissues of individual mice were digested with trypsin, fractionated, and analyzed by an Orbitrap Elite mass spectrometer.

(B) Proteome coverage: Venn diagram displaying the number of proteins quantified in at least two biological replicates per time point in young or middle-aged mice and overlap between ages.

(C) Circadian proteins detected in young or middle-aged mice using the Perseus periodicity algorithm (period = 23.6 h; q-value < 0.25).

(D) Percent distribution of circadian proteins based on changes in rhythmicity during aging.

(E) Heatmaps displaying z-score normalized abundances ($\log_{10}$ LFQ intensities) of circadian proteins detected in young mice and their temporal expression profiles in young mice (*Left*) and middle-aged mice (*Right*).

(F) Phase distribution of circadian proteins detected in young mice (green) or middle-aged mice (red).

(G) Correlation heatmaps across 48 h in young mice (*Left*) and middle-aged mice (*Right*) for circadian proteins detected in young mice. Pearson correlation coefficients are shown as red (positive) or blue (negative).

age-related decline in the circadian system. Strikingly, ~90% of circadian proteins detected at either age displayed a loss of rhythmicity during aging (i.e., oscillated exclusively in young mice) (**Figure 2.1 D**), suggesting that aging is associated with widespread disruption in the temporal regulation of cellular functions in the hippocampus.

Although most age-related changes involved a loss of rhythmicity in middle-aged mice, ~9% of all circadian proteins detected did not oscillate in young mice but gained rhythmicity during aging (**Figure 2.1 D**). These included proteins involved in specific processes known to play roles in aging, such as apoptosis and the cellular stress response (**Figure 2.2**). Consistent with our results, two previous studies have identified sets of transcripts that gained rhythmicity during aging in the human prefrontal cortex and in heads of *Drosophila melanogaster*, notably genes involved in stress response functions (Chen et al., 2016; Kuintzle et al., 2017). Moreover, proteins that have been linked to neurodegenerative diseases were among those displaying rhythmic abundances in the hippocampus of middle-aged mice (**Appendix A Table 3**). For instance, histone deacetylase 1 (HDAC1) as well as one of its target proteins, histone H3, gained rhythmicity during aging. Interestingly, histone acetylation regulates memory function as well as transcription of clock genes, and age-related changes in hippocampal-dependent memory have previously been linked to epigenetic modulation of the clock gene *Per1* through HDAC3 (Kwapis et al., 2018). Thus, aging might result in altered circadian epigenetic regulation of clock genes and other genes that affect hippocampal function.

Rhythmic proteins displayed a variety of temporal abundance profiles in the hippocampus of young mice (left panel in **Figure 2.1 E**), with the phases of peak expression clustering in the afternoon (**Figure 2.1 F**), while in middle-aged mice all but one of these proteins were no longer detected as oscillating (right panel in **Figure 2.1 E**). Furthermore, aging resulted in loss of positive and negative correlations among rhythmic proteins detected in young mice across the day/night cycle (**Figure 2.1 G**). Together, our findings

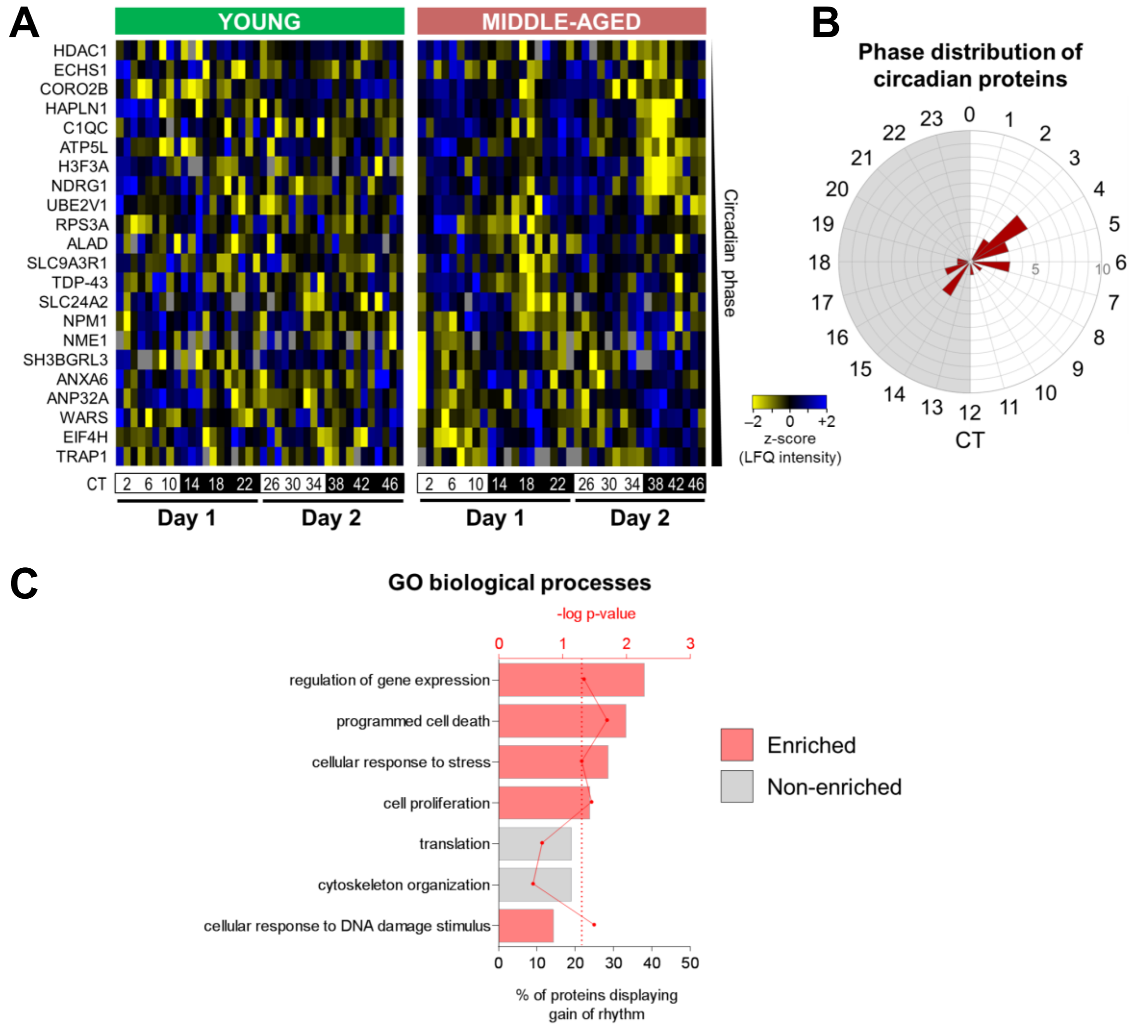

A
YOUNG
MIDDLE-AGED
HDAC1
ECHS1
CORO2B
HAPLN1
C1QC
ATP5L
H3F3A
NDRG1
UBE2V1
RPS3A
ALAD
SLC9A3R1
TDP-43
SLC24A2
NPM1
NME1
SH3BGRL3
ANXA6
ANP32A
WARS
EIF4H
TRAP1
CT
Day 1
Day 2
Circadian phase
z-score
(LFQ intensity)
B
Phase distribution of
circadian proteins
CT
C
GO biological processes
-log p-value
regulation of gene expression
programmed cell death
cellular response to stress
cell proliferation
translation
cytoskeleton organization
cellular response to DNA damage stimulus
Enriched
Non-enriched
% of proteins displaying
gain of rhythm

Figure 2.2. Proteins gaining circadian rhythmicity in the hippocampus of middle-aged mice.

(A) Heatmaps displaying z-score normalized abundances ($\log_{10}$ LFQ intensities) of circadian proteins specific to middle-aged mice, and their temporal expression profiles in young mice (*Left*) and middle-aged mice (*Right*). Circadian proteins were detected mice using the Perseus periodicity algorithm (period = 23.6 h; q-value < 0.25).

(B) Phase distribution (enlarged from Figure 2.1 F).

(C) Functional annotation and enrichment analysis. The vertical red line denotes the cutoff for significantly enriched biological processes ($p \leq 0.05$, Fisher's exact test relative to background of reliably quantified proteins in our dataset).

n = 2–4 mice per time point per age.

indicate that there are widespread aging-induced changes in the daily patterns of protein expression in the hippocampus.

2.3.2. Age-related changes in the circadian regulation of biological functions

To explore the functional relevance of these age-related changes, we examined biological pathways and processes using KEGG and GO analyses to identify functional terms over-represented among proteins displaying loss of rhythmicity in abundance during aging (**Figure 2.3**). Overall, pathways involved in basic cellular metabolism, circadian entrainment, synaptic function, and neurodegenerative diseases were among those enriched (**Figure 2.3 A**). Several enriched processes have previously been characterized as circadian in young mammalian models, including the tricarboxylic acid (TCA) cycle, dendritic spine organization, and regulation of DNA repair (Smarr et al., 2014; Chaix et al., 2016; Neufeld-Cohen et al., 2016). Biological processes involved in neurotransmission, synaptic plasticity, mitochondrial function, redox homeostasis, and proteostasis were also highly represented among proteins displaying age-related loss of circadian rhythmicity (**Figure 2.3 B**).

Furthermore, protein–protein interaction network analysis of proteins displaying loss of rhythmicity in the hippocampus during aging revealed interactions among proteins involved in energy metabolism, mitochondrial function, cytoskeletal organization, translation, and G-protein signalling (**Figure 2.4**). Given that many of these affected processes are critical for normal hippocampal function and have been implicated in neurodegenerative brain disorders, their altered circadian regulation might contribute to aging- and disease-associated decline in hippocampal function and memory. For instance, dysfunction of G-protein–coupled neurotransmission occurs in the brain during aging and may predispose older individuals to developing age-related neurodegenerative disorders (Mattson and Arumugam, 2018).

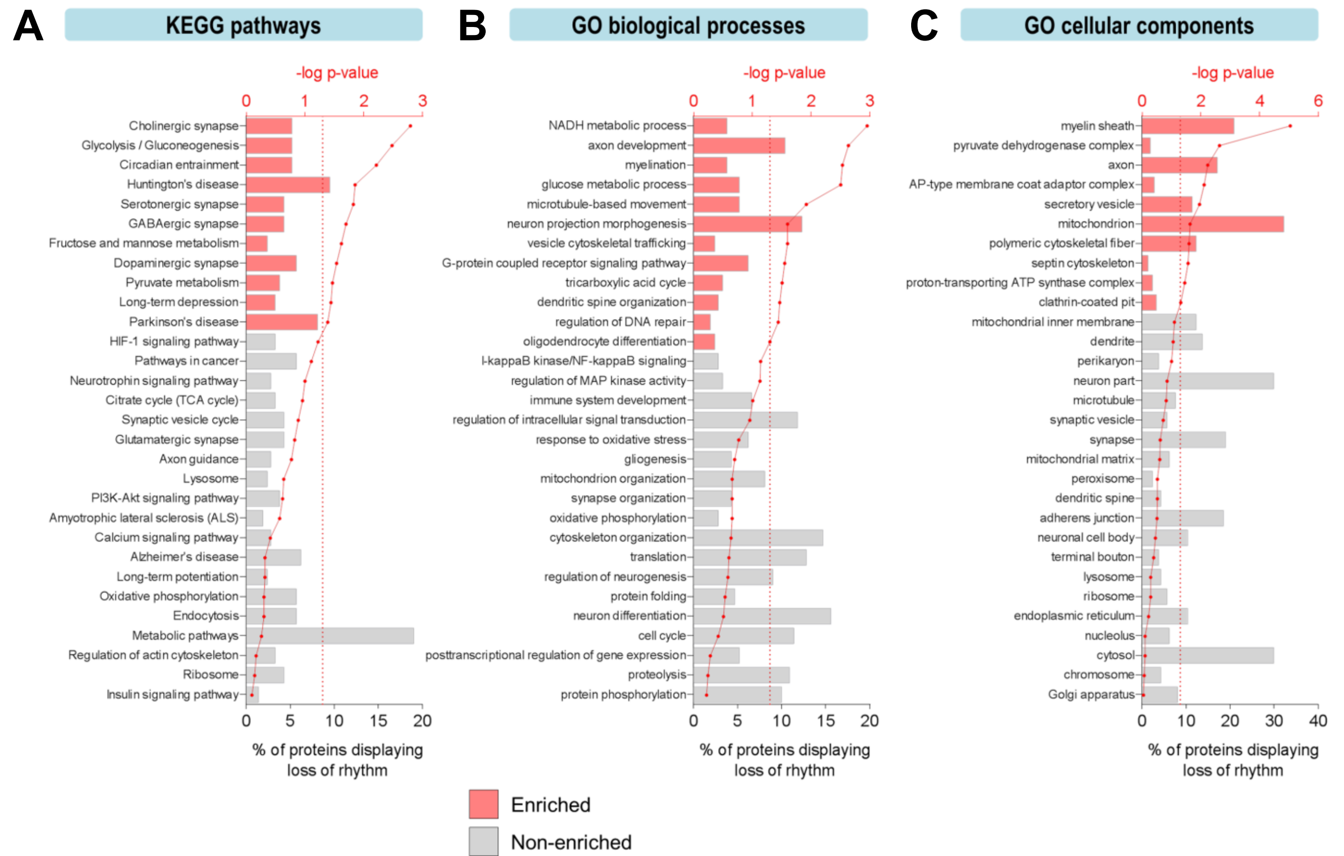

A
KEGG pathways
-log p-value
% of proteins displaying loss of rhythm
Cholinergic synapse
Glycolysis / Gluconeogenesis
Circadian entrainment
Huntington's disease
Serotonergic synapse
GABAergic synapse
Fructose and mannose metabolism
Dopaminergic synapse
Pyruvate metabolism
Long-term depression
Parkinson's disease
HIF-1 signaling pathway
Pathways in cancer
Neurotrophin signaling pathway
Citrate cycle (TCA cycle)
Synaptic vesicle cycle
Glutamatergic synapse
Axon guidance
Lysosome
PI3K-Akt signaling pathway
Amyotrophic lateral sclerosis (ALS)
Calcium signaling pathway
Alzheimer's disease
Long-term potentiation
Oxidative phosphorylation
Endocytosis
Metabolic pathways
Regulation of actin cytoskeleton
Ribosome
Insulin signaling pathway
B
GO biological processes
-log p-value
% of proteins displaying loss of rhythm
NADH metabolic process
axon development
myelination
glucose metabolic process
microtubule-based movement
neuron projection morphogenesis
vesicle cytoskeletal trafficking
G-protein coupled receptor signaling pathway
tricarboxylic acid cycle
dendritic spine organization
regulation of DNA repair
oligodendrocyte differentiation
I-kappaB kinase/NF-kappaB signaling
regulation of MAP kinase activity
immune system development
regulation of intracellular signal transduction
response to oxidative stress
gliogenesis
mitochondrion organization
synapse organization
oxidative phosphorylation
cytoskeleton organization
translation
regulation of neurogenesis
protein folding
neuron differentiation
cell cycle
posttranscriptional regulation of gene expression
proteolysis
protein phosphorylation
C
GO cellular components
-log p-value
% of proteins displaying loss of rhythm
myelin sheath
pyruvate dehydrogenase complex
axon
AP-type membrane coat adaptor complex
secretory vesicle
mitochondrion
polymeric cytoskeletal fiber
septin cytoskeleton
proton-transporting ATP synthase complex
clathrin-coated pit
mitochondrial inner membrane
dendrite
perikaryon
neuron part
microtubule
synaptic vesicle
synapse
mitochondrial matrix
peroxisome
dendritic spine
adherens junction
neuronal cell body
terminal bouton
lysosome
ribosome
endoplasmic reticulum
nucleolus
cytosol
chromosome
Golgi apparatus
Enriched
Non-enriched

Figure 2.3. Aging leads to loss of circadian oscillations in specific biological functions in the hippocampus.

(A–C) Functional analysis of proteins displaying loss of circadian rhythmicity with aging. Enriched ontology terms corresponding to (A) KEGG pathways, (B) GO biological processes, and (C) GO cellular components are shown with coloured bars ($p \leq 0.05$, Fisher's exact test relative to background of reliably quantified proteins in our dataset).

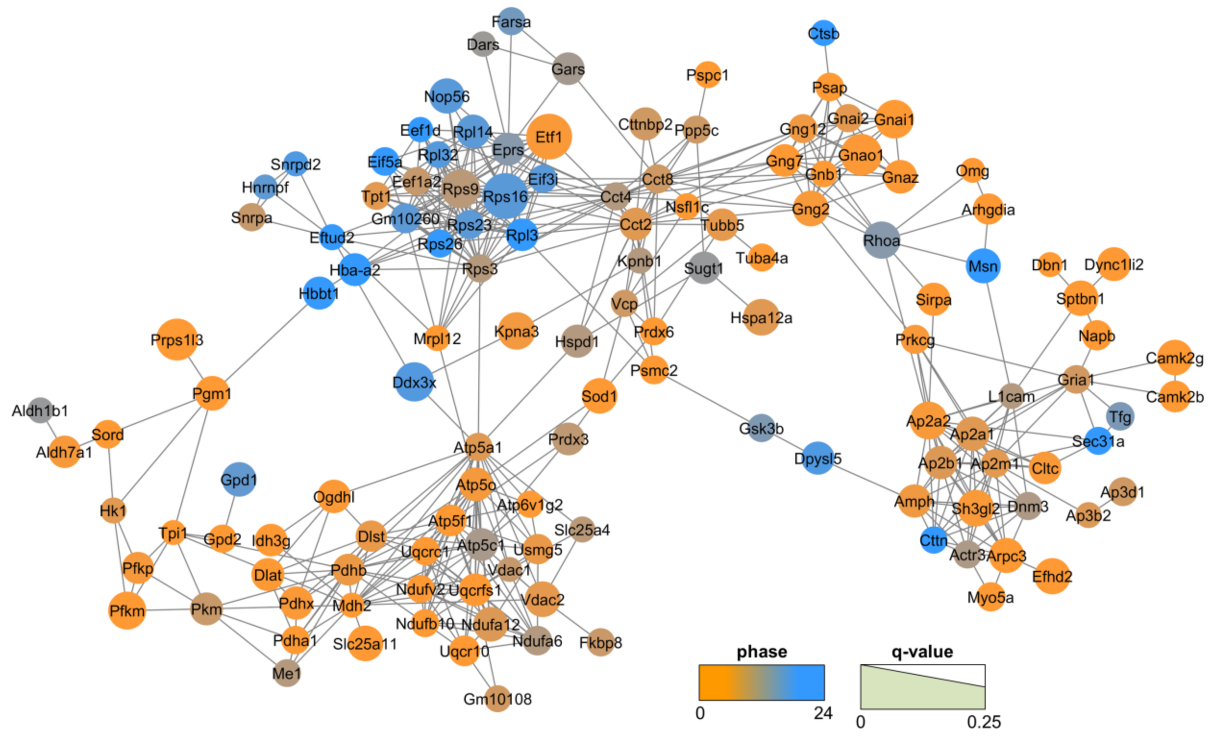

Farsa
Dars
Gars
Ctsb
Pspc1
Psap
Nop56
Eef1d
Rpl14
Etf1
Cttnbp2
Ppp5c
Gng12
Gnai2
Gnai1
Snrpd2
Eif5a
Rpl32
Eprs
Gng7
Gnao1
Hnrnpf
Tpt1
Eef1a2
Rps9
Rps16
Eif3i
Cct8
Gnb1
Gnaz
Omg
Snrpa
Gm10260
Cct4
Nsfl1c
Gng2
Arhgdia
Eftud2
Rps23
Rpl3
Cct2
Tubb5
Rhoa
Rps26
Tuba4a
Hba-a2
Rps3
Kpnb1
Sugt1
Msn
Dbn1
Dync1li2
Hbbt1
Sirpa
Sptbn1
Vcp
Hspa12a
Prps1l3
Mrpl12
Kpna3
Hspd1
Prdx6
Prkcg
Napb
Psmc2
Camk2g
Ddx3x
Gria1
Pgm1
Sod1
L1cam
Camk2b
Aldh1b1
Tfg
Sord
Gsk3b
Ap2a2
Ap2a1
Prdx3
Sec31a
Aldh7a1
Atp5a1
Dpysl5
Ap2b1
Ap2m1
Cltc
Gpd1
Ap3d1
Atp5o
Ogdhl
Atp6v1g2
Amph
Sh3gl2
Dnm3
Ap3b2
Hk1
Atp5f1
Slc25a4
Tpi1
Gpd2
Idh3g
Dlst
Atp5c1
Cttn
Uqcrc1
Usmg5
Actr3
Arpc3
Pfkp
Vdac1
Dlat
Pdhb
Efhd2
Ndufv2
Uqcrfs1
Myo5a
Pdhx
Vdac2
Pfkm
Pkm
Mdh2
Ndufb10
Ndufa12
Pdha1
Slc25a11
Ndufa6
Fkbp8
phase
q-value
Uqcr10
Me1
0
24
0
0.25
Gm10108

Figure 2.4. Protein interaction network analysis of proteins displaying loss of circadian rhythmicity in the hippocampus during aging.

Protein–protein interaction network of proteins displaying loss of circadian rhythmicity with aging. Protein interactions were determined using the STRING database (confidence cutoff = 0.7) and visualized using Cytoscape.

The effects of aging on rhythmic biological functions were complemented by results from a GO analysis of cellular components. In young mice, rhythmic proteins participated in diverse processes involved in the structure and function of various intracellular organelles, with the mitochondrion and cytoskeleton being two major sites of circadian regulation (**Figure 2.3 C**). Proteins localized to the mitochondrion (including the mitochondrial inner membrane, ATP synthase complex, and pyruvate dehydrogenase complex) and synapse (including synaptic vesicles, terminal boutons, and septin cytoskeleton) displayed loss of circadian oscillations in abundance, reflective of the pathways related to mitochondrial energy metabolism, neurotransmission, and synaptic plasticity (**Figure 2.3 A,B**).

Interestingly, some of the most highly enriched GO functional terms included myelination, myelin sheath, and oligodendrocyte differentiation (**Figure 2.3 B,C**), which might suggest another mechanism through which aging disrupts cognitive function. Consistent with this hypothesis and our results showing that proteins involved in these functions peak during the daytime in young mice, it has previously been reported that genes involved in myelination and promoting proliferation of oligodendrocyte precursor cells (OPCs) are transcribed preferentially during sleep in mice (Bellesi et al., 2013). Furthermore, OPCs demonstrate daily rhythms in proliferation in the hippocampus of young mice (Matsumoto et al., 2011) and sleep loss has been shown to disrupt myelin formation (Bellesi et al., 2018). While previous work has shown that myelination is compromised in the aged human brain and especially in people with cognitive deficits (Mattson and Arumugam, 2018), our results suggest that age-related changes in the circadian regulation of myelination may also contribute to cognitive decline during aging via neural circuit dysfunction and impaired communication between different brain regions.

We also noted age-related changes in rhythmic abundances of multiple proteins involved in regulating protein phosphorylation (**Figure 2.3 B**), indicating that temporal control of post-translational modifications such as phosphorylation may be altered and

associated with cognitive decline during aging. Interestingly, we found that the phases of peak expression of circadian proteins clustered in the afternoon, at the same time as the peak in rhythmic phosphoproteins our lab has previously reported (Chiang et al., 2017). Moreover, aging-associated changes in the daily expression profiles of kinases such as CaMKII and GSK3β (**Figure 2.5**) might contribute to the age-related declines in hippocampal-dependent memory and local clock function (Kon et al., 2014; Besing et al., 2017). GSK3β, which phosphorylates and regulates BMAL1 and REV-ERBα (Reischl and Kramer, 2011), has previously been shown to play important roles in modulating synaptic plasticity as well as the molecular clock in the hippocampus of young mice (Besing et al., 2017). Wang and colleagues previously reported that GSK3β peaks in nuclear activity/abundance in the livers of young mice during the day (Wang et al., 2017), consistent with our hippocampal study. In addition, other proteins involved in clock function displayed a loss of rhythmicity in the hippocampus of middle-aged mice (**Figure 2.5**), highlighting the age-dependent decline in local clock function at the molecular level. Taken together, our findings indicate that there is widespread disruption in the circadian regulation of biological processes and pathways critical for normal hippocampal function during aging.

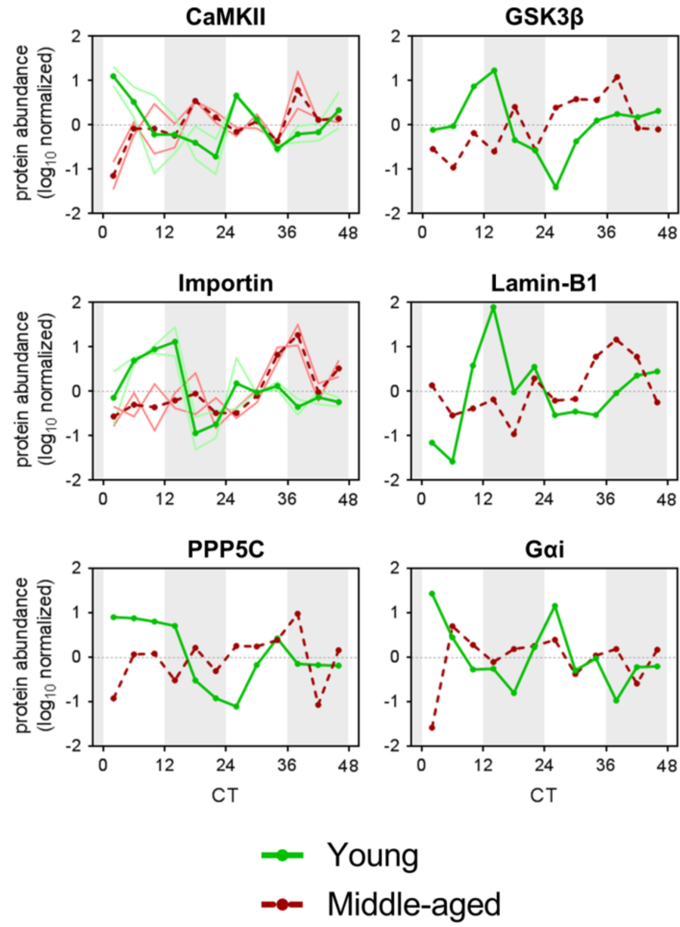

CaMKII
GSK3β
Importin
Lamin-B1
PPP5C
Gαi
protein abundance
(log10 normalized)
CT
Young
Middle-aged

Figure 2.5. Aging disrupts the circadian oscillations of proteins involved in clock regulation.

Individual examples of rhythmic proteins involved in clock regulation and losing their circadian rhythms in abundance in middle-aged mice.

2.3.3. Aging disrupts the circadian regulation of energy metabolism

Dysregulated energy metabolism is a hallmark of brain aging and its exacerbation underlies the molecular pathogenesis of several age-related neurodegenerative diseases, including AD, ALS, PD, and HD (Camandola and Mattson, 2017; Mattson and Arumugam, 2018). We therefore further examined the effects of aging on the circadian regulation of energy metabolism in the hippocampus, focusing on proteins involved in glucose metabolism as well as the downstream pyruvate metabolism, TCA cycle, and oxidative phosphorylation pathways.

The relative enrichment among proteins displaying loss of rhythmicity during aging of pathways such as glycolysis, pyruvate metabolism, and the TCA cycle (**Figure 2.3 A,B**) is consistent with previous studies from our lab and others demonstrating that several components of these pathways exhibit diurnal rhythms in protein abundance in the brain as well as peripheral organs of young mice (Chiang et al., 2014; Neufeld-Cohen et al., 2016; Chiang et al., 2017). We found over 20 proteins participating in critical energy metabolism pathways that displayed loss of rhythmicity in the hippocampus during aging (**Figure 2.6 A**). In young mice, these rhythmic proteins peaked in a coordinated manner during the day (**Figure 2.6 B**), and at the same time that circadian proteins involved in oxidative phosphorylation peak in the SCN of young mice (Chiang et al., 2014). Interestingly, many of the rhythmic proteins catalyzing steps in glycolysis and the TCA cycle in young mice are nicotinamide adenine dinucleotide (NAD^+)-dependent enzymes, suggesting a potential mechanism by which circadian oscillations in NAD^+ could couple energy production in the brain to the daily light/dark cycle (Peek et al., 2013). Importantly, phosphofructokinase (PFK) and the regulatory subunit of isocitrate dehydrogenase 3 (IDH3) were rhythmic in young mice but not middle-aged mice. Given that these enzymes catalyze the rate-limiting steps of glycolysis and the TCA cycle, respectively, aging may have a significant impact on the dynamics of these pathways and therefore energy production in the hippocampus.

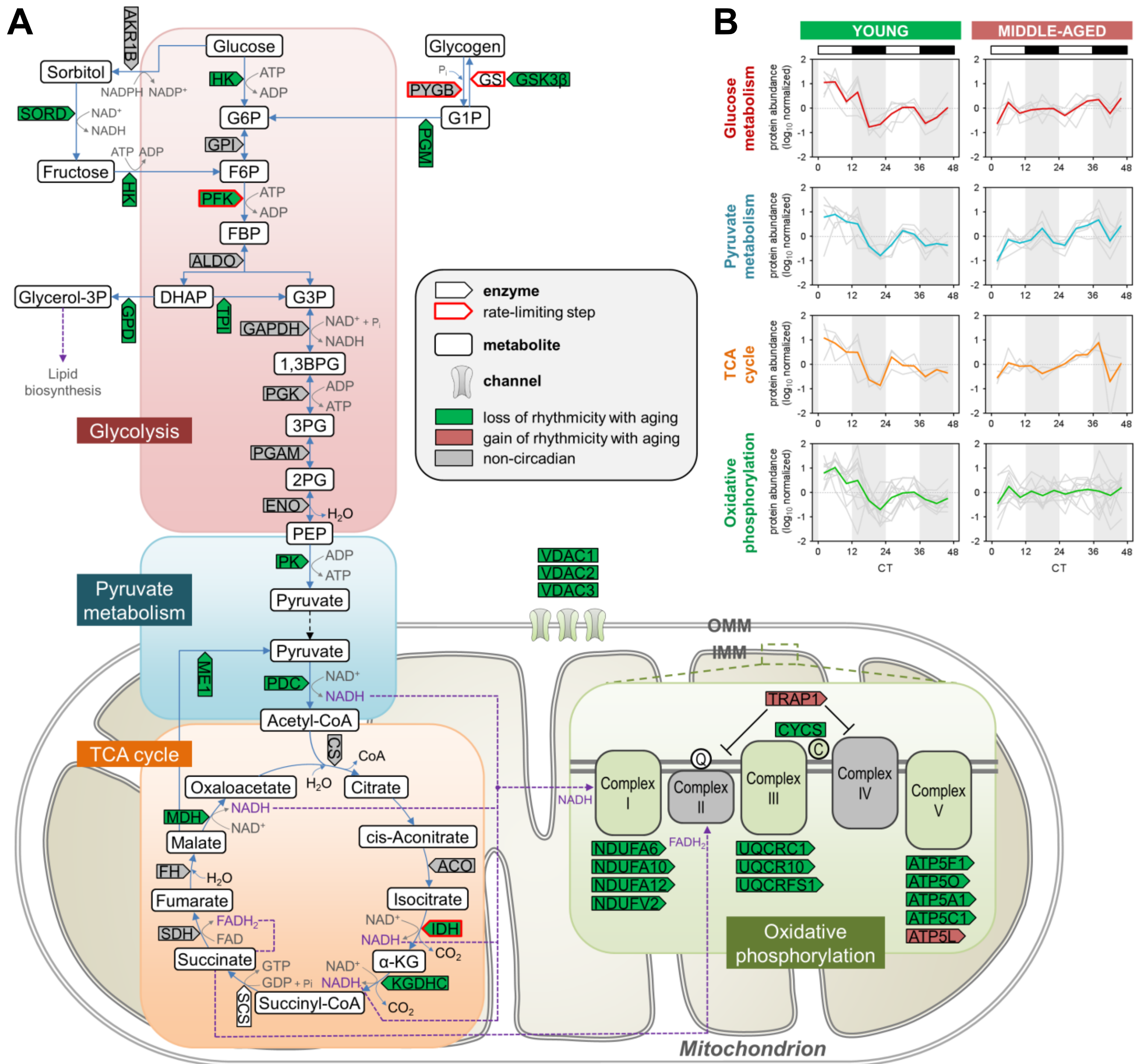

A
B
YOUNG
MIDDLE-AGED
Glucose metabolism
Pyruvate metabolism
TCA cycle
Oxidative phosphorylation
Glycolysis
Mitochondrion
enzyme
rate-limiting step
metabolite
channel
loss of rhythmicity with aging
gain of rhythmicity with aging
non-circadian
OMM
IMM
CT

Figure 2.6. Aging disrupts the circadian regulation of proteins involved in energy metabolism in the hippocampus.

(A) Schematic depiction of proteins involved in energy metabolism, including glycolysis, pyruvate metabolism, the TCA cycle, and oxidative phosphorylation. Proteins that displayed loss or gain of circadian rhythmicity in abundance during aging are highlighted in colour. Proteins that were reliably quantified, but not detected as circadian at either age, are shown in gray.

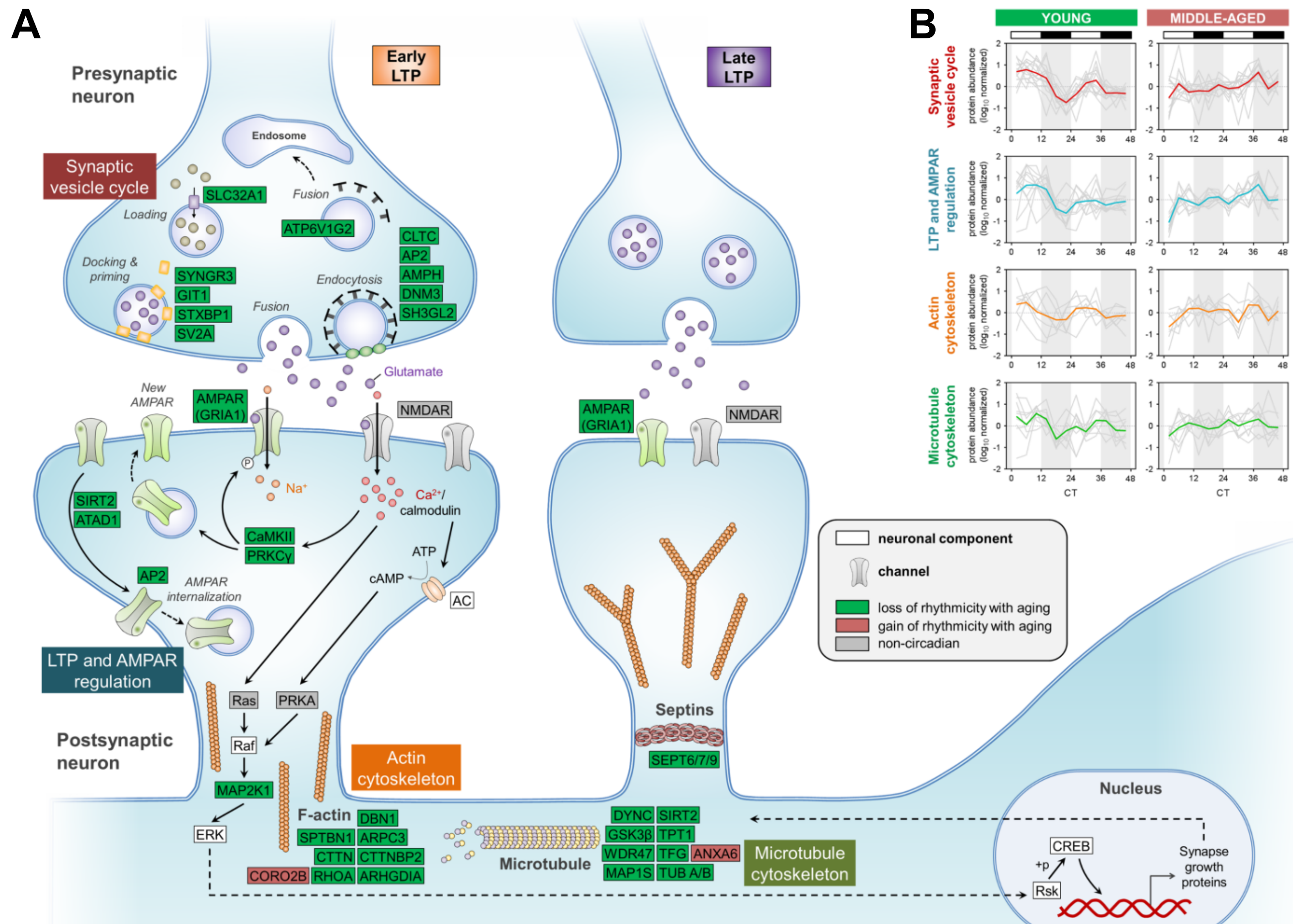
(B) Temporal abundance profiles of rhythmic proteins identified in young mice involved in energy metabolism.

These results suggest that the dysregulation of energy metabolism that occurs in the brain during aging extends to its temporal regulation, and disrupted circadian rhythms in energy metabolism pathways might contribute to age-related impairments in hippocampal function and memory.

2.3.4. Aging disrupts the circadian regulation of synaptic structure and function

Circadian modulation of hippocampal-dependent memory at the molecular level can occur through clock regulation of multiple neuronal and synaptic components, including synaptic vesicle proteins, receptors, transporters, and intracellular signalling cascades (Hannou et al., 2018; Rawashdeh et al., 2018; Snider et al., 2018). Thus, we were interested in examining the impact of aging on the circadian rhythmicity of proteins involved in synaptic plasticity and function in the hippocampus, given that changes in the regulation of these processes may contribute to age-related memory impairment.

We first examined the effects of aging on synaptic vesicle cycling, which has previously been shown to be rhythmic and important for circadian gene expression in the SCN (Deery et al., 2009). Strikingly, middle-aged mice displayed a loss of circadian oscillations in the abundances of over 10 proteins involved in this pathway, which were rhythmic in the hippocampus of young mice (**Figure 2.7 A**). These included syntaxin binding protein 1 (also known as Munc18-1), SV2A, and endophilin A1, which our lab and others have previously shown to be clock-regulated at the protein or mRNA level in the brains of young mice (Chiang et al., 2014; Hannou et al., 2018). Interestingly, we also found that the GluA1 AMPAR subunit, along with other proteins regulating AMPAR phosphorylation and trafficking, such as CaMKII and protein kinase C γ, displayed loss of rhythmicity in the hippocampus during aging. Previous work has demonstrated that glutamate receptor trafficking is regulated by clock-gated signaling pathways, in line with our results (Snider et al., 2018). In addition, multiple cytoskeletal components and regulators lost rhythmicity in



A
Presynaptic neuron
Early LTP
Late LTP
Endosome
Synaptic vesicle cycle
SLC32A1
Loading
Fusion
ATP6V1G2
CLTC
AP2
AMPH
DNM3
SH3GL2
Docking & priming
SYNGR3
GIT1
STXBP1
SV2A
Endocytosis
Glutamate
New AMPAR
AMPAR (GRIA1)
NMDAR
SIRT2
ATAD1
Na+
Ca2+/ calmodulin
CaMKII
PRKCγ
ATP
cAMP
AC
AP2
AMPAR internalization
LTP and AMPAR regulation
Ras
PRKA
Raf
MAP2K1
ERK
Postsynaptic neuron
Actin cytoskeleton
F-actin
DBN1
SPTBN1
ARPC3
CTTN
CTTNBP2
CORO2B
RHOA
ARHGDIA
Microtubule
Septins
SEPT6/7/9
DYNC
SIRT2
GSK3β
TPT1
WDR47
TFG
ANXA6
MAP1S
TUB A/B
Microtubule cytoskeleton
Nucleus
CREB
+p
Rsk
Synapse growth proteins
neuronal component
channel
loss of rhythmicity with aging
gain of rhythmicity with aging
non-circadian
B
YOUNG
MIDDLE-AGED
Synaptic vesicle cycle
LTP and AMPAR regulation
Actin cytoskeleton
Microtubule cytoskeleton
protein abundance (log10 normalized)
CT

Figure 2.7. Aging disrupts the circadian regulation of proteins involved in synaptic structure and function in the hippocampus.

(A) Schematic depiction of proteins involved in synaptic structure and function, including the synaptic vesicle cycle, LTP and AMPAR regulation, and the cytoskeleton. Proteins that displayed loss or gain of circadian rhythmicity in abundance during aging are highlighted in colour. Proteins that were reliably quantified, but not detected as circadian at either age, are shown in gray.

(B) Temporal abundance profiles of rhythmic proteins identified in young mice involved in synaptic structure and function.

middle-aged mice, including actin regulators such as RHOA and Rho GDP-dissociation inhibitor (RhoGDI), whose interaction is known to be clock-regulated (Ma et al., 2018). Several proteins involved in the organization of microtubules and the septin cytoskeleton were also found to display loss of circadian oscillations in the hippocampus of middle-aged mice, suggesting that aging could lead to disruption of synaptic plasticity through multiple neuronal cytoskeleton components given that synaptic plasticity is affected by cytoskeletal remodeling (Gordon-Weeks and Fournier, 2014).

While circadian rhythmicity of proteins involved in synaptic structure and function was largely lost in middle-aged mice, these proteins peaked in a coordinated manner during the day in the hippocampus of young mice (**Figure 2.7 B**), consistent with previous work demonstrating that processes supporting synaptic plasticity peak during sleep in mice (Eckel-Mahan et al., 2008). Thus, the age-related dampening in rhythmicity of proteins involved in the synaptic vesicle cycle, LTP and AMPAR regulation, and cytoskeleton organization might lead to compromised synaptic structure and function in the hippocampus of middle-aged mice, which could in turn result in impaired hippocampal-dependent memory during aging.

2.3.5. Aging disrupts rhythmic proteins involved in hallmarks of aging and neurodegenerative diseases

Mitochondrial dysfunction, a hallmark of aging, may contribute to age-associated damage and has been implicated in cognitive impairment as well as the pathogenesis of several neurodegenerative diseases (Lopez-Otin et al., 2013; Mattson and Arumugam, 2018). We were therefore interested in examining whether the age-related decline in efficiency of mitochondrial energy metabolism and antioxidant defense mechanisms extends to their circadian regulation in the hippocampus (Green et al., 2011). As discussed above, circadian rhythms of mitochondrial proteins involved in energy metabolism were particularly affected during aging, with over 20 proteins displaying age-related loss of rhythmicity (**Figure 2.6**).

These proteins included IDH3, which catalyzes the rate-limiting step of the TCA cycle, as well as several subunits belonging to electron transport chain complexes (**Figures 2.6** and **2.8**). In young mice, proteins involved in mitochondrial respiration peaked during the day along with many antioxidant enzymes, notably superoxide dismutase 1 (SOD1) and peroxiredoxins (PRDX3 and PRDX6). Interestingly, we found that these and other antioxidant enzymes displayed loss of rhythmic abundances during aging (**Figure 2.8**). We also found that all three voltage-dependent anion-selective channels (VDACs) lost their rhythmic expression in middle-aged mice, which could impact both mitochondrial function and synaptic plasticity in the hippocampus (Levy et al., 2003) (**Figure 2.8**). Thus, aging-induced changes in the circadian rhythms of proteins involved in mitochondrial function and reactive oxygen species (ROS) homeostasis provide additional potential mechanisms through which aging may lead to an increased susceptibility to brain disorders characterized by mitochondrial dysfunction, including neurodegenerative diseases such as AD and PD (Mattson and Arumugam, 2018).

Genomic instability and epigenetic alterations, two primary hallmarks of aging, are linked to clock function through circadian regulation of proteins involved in ROS homeostasis and epigenetic modification. Given that mitochondrial respiration is a major intracellular source of ROS (Mattson and Arumugam, 2018), and that both oxidative phosphorylation and the cellular response to oxidative stress display circadian rhythmicity (Chaix et al., 2016), age-related changes in the temporal control of proteins participating in these two processes (as discussed above) may lead to alterations in the accumulation of genetic damage. Genomic instability can also result from disruption of nuclear lamins, such as lamin B1 (Lopez-Otin et al., 2013). Levels of lamin B1 decline during aging (Lopez-Otin et al., 2013) and exhibited a loss of rhythmicity in the hippocampus of middle-aged mice (**Figure 2.8**), suggesting that this disruption could lead to aberrations in the nuclear lamina and contribute to genome instability during aging. We also found age-dependent changes in the rhythmicity of three

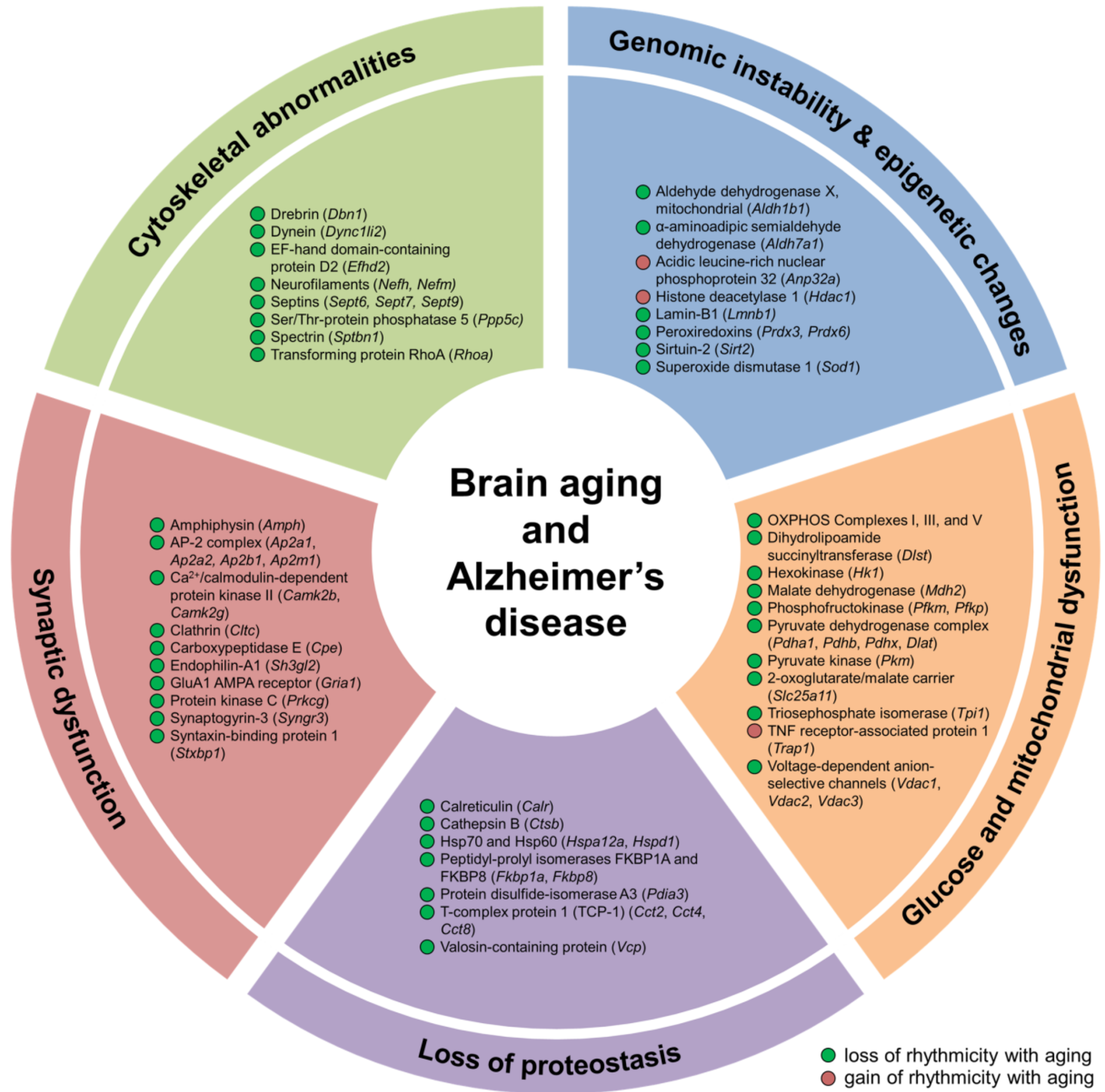

Cytoskeletal abnormalities
Drebrin (Dbn1)
Dynein (Dync1li2)
EF-hand domain-containing protein D2 (Efhd2)
Neurofilaments (Nefh, Nefm)
Septins (Sept6, Sept7, Sept9)
Ser/Thr-protein phosphatase 5 (Ppp5c)
Spectrin (Sptbn1)
Transforming protein RhoA (Rhoa)
Genomic instability & epigenetic changes
Aldehyde dehydrogenase X, mitochondrial (Aldh1b1)
α-aminoadipic semialdehyde dehydrogenase (Aldh7a1)
Acidic leucine-rich nuclear phosphoprotein 32 (Anp32a)
Histone deacetylase 1 (Hdac1)
Lamin-B1 (Lmnb1)
Peroxiredoxins (Prdx3, Prdx6)
Sirtuin-2 (Sirt2)
Superoxide dismutase 1 (Sod1)
Brain aging and Alzheimer's disease
Synaptic dysfunction
Amphiphysin (Amph)
AP-2 complex (Ap2a1, Ap2a2, Ap2b1, Ap2m1)
Ca2+/calmodulin-dependent protein kinase II (Camk2b, Camk2g)
Clathrin (Cltc)
Carboxypeptidase E (Cpe)
Endophilin-A1 (Sh3gl2)
GluA1 AMPA receptor (Gria1)
Protein kinase C (Prkcg)
Synaptogyrin-3 (Syngr3)
Syntaxin-binding protein 1 (Stxbp1)
Glucose and mitochondrial dysfunction
OXPHOS Complexes I, III, and V
Dihydrolipoamide succinyltransferase (Dlst)
Hexokinase (Hk1)
Malate dehydrogenase (Mdh2)
Phosphofructokinase (Pfkm, Pfkp)
Pyruvate dehydrogenase complex (Pdha1, Pdhb, Pdhx, Dlat)
Pyruvate kinase (Pkm)
2-oxoglutarate/malate carrier (Slc25a11)
Triosephosphate isomerase (Tpi1)
TNF receptor-associated protein 1 (Trap1)
Voltage-dependent anion-selective channels (Vdac1, Vdac2, Vdac3)
Loss of proteostasis
Calreticulin (Calr)
Cathepsin B (Ctsb)
Hsp70 and Hsp60 (Hspa12a, Hspd1)
Peptidyl-prolyl isomerases FKBP1A and FKBP8 (Fkbp1a, Fkbp8)
Protein disulfide-isomerase A3 (Pdia3)
T-complex protein 1 (TCP-1) (Cct2, Cct4, Cct8)
Valosin-containing protein (Vcp)
loss of rhythmicity with aging
gain of rhythmicity with aging

Figure 2.8. Aging alters hippocampal circadian oscillations of proteins implicated in Alzheimer's disease and hallmarks of aging.

Proteins displaying changes in rhythmicity during aging and involved in hallmarks of aging and Alzheimer's disease pathogenesis: genomic instability, epigenetic alterations, loss of proteostasis, glucose and mitochondrial dysfunction, synaptic dysfunction, and cytoskeletal abnormalities.

proteins involved in epigenetic modification, specifically post-translational modification of histones. These include HDAC1, sirtuin 2 (SIRT2), and acidic leucine-rich nuclear phosphoprotein 32 (ANP32A), which are all involved in regulating histone acetylation levels (**Figure 2.8**). Age-related changes in the circadian regulation of proteins involved in maintaining genomic and epigenetic stability might therefore result in transcriptional alterations and increased DNA damage, which could in turn stimulate accelerated aging and lead to increased disease risk.

Loss of protein homeostasis, another primary hallmark of aging, is linked to various age-related diseases (Lopez-Otin et al., 2013). While diurnal rhythms in protein synthesis, processing, and degradation have previously been described in several organs in young mammals (Panda et al., 2002; Robles et al., 2014), the impact of aging on the rhythmicity of these processes is less well characterized. We found that the daily abundance profiles of rhythmic proteins participating in protein synthesis, folding, and degradation were altered during aging (**Figure 2.8** and see **Figure 2.4**). These proteins included the molecular chaperones calreticulin, heat shock proteins 60 and 70 (HSP60 and HSP70), and T-complex protein 1 (TCP-1) subunits, as well as valosin-containing protein (VCP), which also regulates ubiquitin-dependent protein degradation (Dai and Li, 2001). Our results suggest that the temporal control of protein homeostasis in the hippocampus is altered during aging, which may in turn impact the core circadian clock transcriptional–translational feedback loop and thus affect oscillations in other local clock-controlled genes (Takahashi, 2017).

To further explore links between age-related disorders and changes in circadian rhythmicity of biological functions during aging, we examined disease-associated pathways containing proteins whose abundance was under circadian regulation in the hippocampus. We found rhythmic proteins associated with the pathogenesis of several age-related neurodegenerative diseases affecting the brain (**Appendix A Table 3**). In addition to proteins involved in the hallmarks of aging discussed above that are known to play roles in

the pathogenesis of AD, such as mitochondrial dysfunction and loss of proteostasis, several other proteins displaying age-related changes in rhythmicity in the hippocampus were also associated with AD. These included proteins involved in synaptic dysfunction and cytoskeletal abnormalities, such as synaptic vesicle proteins, cytoskeleton components and regulators, and enzymes that regulate protein phosphorylation (**Figure 2.8** and **Appendix A Table 3**). Several proteins linked to AD and involved in the synaptic vesicle cycle were found to display loss of rhythmicity in the hippocampus during aging, including amphiphysin, AP-2 complex subunits, clathrin heavy chain, endophilin-A1, synaptogyrin-3, and syntaxin-binding protein 1 (**Figures 2.7** and **2.8**; **Appendix A Table 3**). Additional proteins involved in synaptic function and dysregulated in AD included the GluA1 AMPAR subunit, CaMKII, and protein kinase C γ, which all displayed loss of rhythmicity during aging (**Figure 2.7** and **Appendix A Table 3**). Moreover, several cytoskeletal proteins and regulators known to be implicated in AD pathogenesis were found to lose rhythmicity during aging, including neurofilaments, septins, and actin regulators such as drebrin and RHOA (**Appendix A Table 3**; **Figures 2.7** and **2.8**). Our data indicate that age-dependent changes in circadian regulation of various processes are associated with and might contribute to AD pathogenesis.

While decline in hippocampal function is a prominent feature of AD, it also occurs in several other neurodegenerative disorders, namely PD (Camicioli et al., 2003), HD (Spargo et al., 1993), ALS (Takeda et al., 2009), and FTD (Laakso et al., 2000). We were therefore also interested in identifying age-related hippocampal alterations in the rhythmicity of proteins involved in these diseases. We found that several proteins believed to play major roles in the pathogenesis of these diseases displayed changes in circadian regulation in the hippocampus during aging, notably TDP-43, SOD1, and TNF receptor-associated protein 1 (TRAP1) (**Appendix A Table 3**). TDP-43, which gained rhythmicity during aging, has been identified as the major pathological protein in both ALS and FTD (Mackenzie and

Rademakers, 2008). Moreover, SOD1 displayed a loss of rhythmicity during aging, and mutations in *SOD1* and the TDP-43 gene *TARDBP* are known to occur in familial ALS (Millecamps et al., 2010). The mitochondrial chaperone TRAP1, also known as heat shock protein 75 (HSP75), gained rhythmicity in the hippocampus of middle-aged mice and has been linked to familial PD arising due to mutations in *PTEN induced putative kinase 1* (*PINK1*) (Pridgeon et al., 2007). Interestingly, the protective role of PINK1 against oxidative stress is mediated through phosphorylation of TRAP1 and inhibition of the mitochondrial release of cytochrome c (Pridgeon et al., 2007), another protein that displayed loss of rhythmicity in the hippocampus during aging (**Appendix A Table 3**). Taken together, these results provide further evidence for the association of age-related circadian disruption in the brain with the pathogenesis of neurodegenerative diseases. Furthermore, our findings highlight the widespread aging-associated alterations in temporal regulation of biological processes implicated in aging and age-related brain disorders.

2.4. Discussion

Given the regulation of hippocampal physiology and function by the circadian clock, the age-dependent decline of the circadian system may contribute to cognitive decline over time and development of aging-associated neurodegenerative disorders. Previous large-scale studies of circadian rhythms, while mainly restricted to examining rhythmic processes in young model organisms at the transcriptomic level, have greatly contributed to our understanding of the circadian system's role in modulating various processes in a tissue-specific manner (Zhang et al., 2014; Millius and Ueda, 2017). Proteomic analyses, which incorporate additional mechanisms of circadian regulation at the post-transcriptional level, have been performed on the brain and peripheral organs of young mice to reveal large-scale coordination of biological processes by the clock (Millius and Ueda, 2017). However, how aging modifies clock-controlled processes has only recently begun to be explored at the

transcriptomic level in the liver and stem cells of mice (Sato et al., 2017; Solanas et al., 2017), while the effects in the brain are largely unknown. A large-scale characterization of hippocampal circadian rhythms in protein abundances at different ages would therefore provide insight into age-related perturbations in the timing of cellular functions and could facilitate future studies on the molecular mechanisms of aging- and disease-associated cognitive impairment. Further studies are needed to identify age-related alterations in circadian rhythms of protein abundance in peripheral organs as well as other brain regions, particularly the central pacemaker in the SCN.

In this study, we used a quantitative MS-based approach to analyze hippocampal tissues from young and middle-aged mice and to explore the effects of aging on circadian regulation at the proteomic level. Middle-aged mice demonstrate impaired learning and memory (Shoji et al., 2016), indicating that age-dependent hippocampal proteomic alterations might contribute to cognitive decline at this age. We found that there is widespread disruption of the circadian orchestration of protein expression rhythms in the hippocampus during aging, reflective of the age-related decline in the circadian system. Notably, we have shown that aging leads to a loss of temporal coordination of pathways critical for normal hippocampal function, including energy metabolism, neurotransmission, and synaptic plasticity.

Rhythmic proteins involved in energy metabolism and synaptic vesicle cycling peaked in a coordinated manner during the day in young mice, complementing previous work showing that the synaptic vesicle cycle is the main source of activity-driven metabolic demand at synapses (Rangaraju et al., 2014). Importantly, these pathways lost rhythmicity in the hippocampus of middle-aged mice, suggesting that aging is associated with a disruption in the temporal coupling between energy demand and production, which could lead to impaired synaptic function. High rates of energy production in the brain are required to support neuronal and glial activities, with neurons relying on glucose as their main energy

source (Camandola and Mattson, 2017). Moreover, reduced hippocampal energy metabolism (particularly glucose metabolism) has been associated with cognitive impairment and AD (Mattson and Arumugam, 2018). Circadian regulation of energy metabolism and synchronization among local brain clocks might contribute to sustaining daily cycles in brain function, such as increases in memory consolidation and synaptic rewiring during sleep (Kyriacou and Hastings, 2010). Synaptic plasticity must be supported during sleep, when consolidation of memories is hypothesized to preferentially occur (Kondratova and Kondratov, 2012). In line with this hypothesis, circadian oscillations in signal transduction events underlying LTP in the hippocampus have previously been shown to peak during the day in young mice, and disruption of these rhythms results in impaired hippocampal-dependent memory (Eckel-Mahan et al., 2008). Thus, disruption of the temporal coordination of energy production, which supports these functions in the hippocampus, might contribute to age-related cognitive impairment.

The progressive functional deterioration that characterizes aging is a primary risk factor for various age-related disorders, including neurodegenerative diseases (Kondratova and Kondratov, 2012). Interestingly, sleep and circadian disturbances are shared clinical features of several neurodegenerative diseases, and a growing body of evidence indicates that disruption of circadian rhythms contributes directly to their pathogenesis (Kondratova and Kondratov, 2012; Musiek and Holtzman, 2016). Given that these disorders involve an exacerbation of aging-associated circadian dysfunction and hallmarks of aging (Musiek and Holtzman, 2016; Mattson and Arumugam, 2018), identifying changes in the daily cycles of biological functions in the brain during normal aging might contribute to a better understanding of the pathogenesis of these diseases. We found that proteins displaying age-related alterations in rhythmicity in the hippocampus of mice were involved in various hallmarks of aging, including mitochondrial dysfunction, genomic instability, epigenetic alterations, and loss of protein homeostasis. A recent study identified age-dependent

changes in dynamics of the oxidative stress response in the hippocampus of aged mice (Lacoste et al., 2017), consistent with our results. We also found rhythmic hippocampal proteins involved in the pathogenesis of several aging-associated neurodegenerative diseases affecting the brain, indicating that age-related alterations in circadian regulation are associated with and might contribute to the pathogenesis of these diseases.

Age-dependent changes in the temporal regulation of proteins involved in local hippocampal clock function, such as proteins involved in regulating protein phosphorylation and turnover, provide novel potential mechanisms through which aging may be associated with alterations in physiological circadian rhythms. Additional studies are needed to characterize the effects of aging on the circadian rhythms of post-translational modifications of proteins, given that our results implicate enzymes regulating protein acetylation and phosphorylation in hallmarks of aging and age-related functional decline. This is plausible given that our lab and others have previously shown the relevance of rhythmic protein phosphorylation in the hippocampus of young mice (Chiang et al., 2017; Snider et al., 2018).

Our study represents the first large-scale proteomic analysis of aging in mammals from a circadian perspective, and our findings provide a framework for understanding the links between age-related cognitive decline, neurodegenerative disorders, and the circadian clock. Circadian disruption is associated with hippocampal-dependent memory impairment, and it is conceivable that aging-induced alterations in the circadian regulation of processes critical for hippocampal function could contribute to this decline. Our results build upon previous studies examining the circadian proteomes of brain, liver, and heart tissues from young mice (Millius and Ueda, 2017) and the circadian transcriptomes of various central and peripheral tissues from young mice (Zhang et al., 2014) and baboons (Mure et al., 2018). Furthermore, a growing number of recent studies are exploring the effects of aging (Sato et al., 2017; Solanas et al., 2017), environmental and genetic circadian disruption (Archer et al., 2014; Martino and Young, 2015), and various diets (Sato et al., 2017; Solanas et al.,

2017; Tognini et al., 2017) on the circadian regulation of cellular functions in humans and mice. Our dataset may therefore serve as a resource to the circadian biology community for future studies investigating the effects of environmental and genetic modifications or potential therapeutic interventions on hippocampal function in mammals.

Chapter 3. Therapeutic Targeting of Casein Kinase 1δ/ε in an Alzheimer's Disease Mouse Model

3.1. Introduction

Alzheimer's disease (AD), the most common cause of dementia, is a progressive neurodegenerative disease with cognitive and behavioural symptoms (Livingston et al., 2017). The molecular pathogenesis of AD is complex, and several factors have been implicated in AD initiation and progression, including alterations in neurotransmission, β-amyloid (Aβ) levels, cytoskeletal proteins, mitochondrial function, and oxidative stress (Huang and Mucke, 2012; Sanabria-Castro et al., 2017). Given the multifactorial etiology of the disease, it is likely that therapies targeting multiple underlying mechanisms will be most effective. Moreover, disease-modifying approaches may be most beneficial early in the course of AD, before neurodegeneration has progressed and becomes irreversible (Huang and Mucke, 2012).

Memory impairment and disrupted sleep and circadian rhythms are symptomatic hallmarks of AD (Lyketsos et al., 2011; Musiek and Holtzman, 2016). Circadian disturbances, which range from altered sleep timing to severe sleep/wake cycle fragmentation, affect as many as half of people with AD and are a major reason for institutionalization (Lyketsos et al., 2011; Videnovic et al., 2014). Several lines of evidence suggest a bidirectional relationship between circadian dysfunction and AD (Coogan et al., 2013; Videnovic et al., 2014; Musiek and Holtzman, 2016). Disruption of circadian function results from neuropathological changes in the central circadian pacemaker, the SCN, and

may also directly contribute to neurodegeneration in other brain regions through clock-regulated processes such as oxidative stress, proteostasis, neuronal metabolism, and Aβ dynamics (Musiek and Holtzman, 2016). In addition, the circadian system plays critical roles in hippocampal synaptic plasticity and memory (Gerstner and Yin, 2010; Smarr et al., 2014) and circadian disturbances in AD correlate with lower cognitive function, suggesting that clock modulation might represent a novel approach for treating not only sleep problems but also cognitive impairment (Coogan et al., 2013). Given that clock genes throughout the brain have altered expression profiles in AD and regulate memory, sleep, and neurodegeneration, the circadian clock represents a viable therapeutic target against AD-related memory deficits and sleep disturbances (Coogan et al., 2013).

Casein kinase 1δ and 1ε (CK1δ/ε) are isoforms of the CK1 family of serine/threonine protein kinases that are implicated in AD and regulation of circadian rhythms (Lee et al., 2009; Perez et al., 2011). Degradation and subcellular localization of core clock proteins (notably PERIOD proteins) are regulated by CK1δ/ε, and mutations in the CK1δ and CK1ε genes in humans and rodents cause behavioural circadian rhythm disorders (Meng et al., 2010). Importantly, CK1δ/ε are highly overexpressed in AD brains (Perez et al., 2011), indicating that they are therapeutic targets against AD. A limited number of studies have examined how CK1 inhibition affects Aβ production and tau phosphorylation *in vitro* and shown that CK1ε overexpression in mouse hippocampus impairs working memory (Perez et al., 2011; Chen et al., 2017). However, to our knowledge, the global effects of CK1δ/ε inhibition on protein expression have not yet been investigated, and none of these previous reports have addressed whether CK1δ/ε inhibitors can improve cognitive function. Furthermore, whether circadian disturbances in AD can be normalized by inhibiting CK1δ/ε remains unclear.

Here, we assess the therapeutic potential of CK1δ/ε inhibition in an *in vitro* model of circadian clocks and in a triple transgenic mouse model of AD (3xTg-AD mice) with PF-

670462, a blood–brain barrier permeable small molecule CK1δ/ε inhibitor that can stabilize circadian rhythms in various mouse models of circadian dysfunction (Badura et al., 2007; Meng et al., 2010). 3xTg-AD mice display memory impairment and circadian abnormalities reminiscent of people with AD, allowing for evaluation of the effects of PF-670462 (Sterniczuk et al., 2010; Webster et al., 2014). Proteomic analyses using LC-MS/MS revealed that PF-670462 treatment *in vitro* altered the expression of various AD-related and clock-regulated proteins, while PF-670462 administration *in vivo* rescued hippocampal proteomic alterations in several AD-associated pathways. Moreover, PF-670462 administration restored working memory and normalized behavioural circadian rhythms in 3xTg-AD mice. Our findings provide proof of concept for pharmacological CK1δ/ε inhibition and, more broadly, for circadian clock modulation against AD-related proteomic alterations, cognitive deficits, and circadian disturbances.

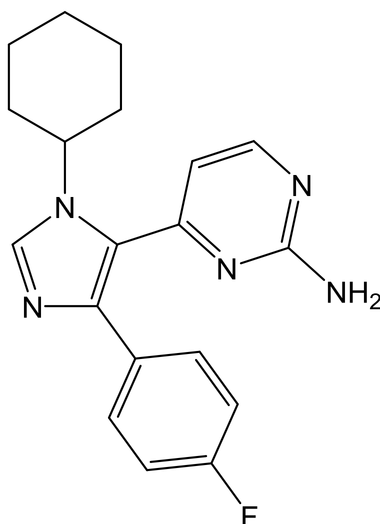


Figure 3.1. Chemical structure of PF-670462.

3.2. Materials and Methods

3.2.1. Animals

Homozygous 3xTg-AD mice [strain: B6;129-Tg(APPSwe,tauP301L)1Lfa *Psen1*^{tm1Mpm}/Mmjax] (Oddo et al., 2003) were compared to age- and sex-matched non-transgenic (NTg) B6129SF2/J control mice. Mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA; Stock #34830 for 3xTg-AD and #101045 for NTg; 3xTg-AD mice were a gift of Dr. Richard Bergeron, University of Ottawa) and maintained in-house by homozygous breeding. Mice were group housed in polycarbonate cages with *ad libitum* access to food and water and maintained on a 12-h light:12-h dark (LD) schedule from weaning. Mice from both genotypes were co-housed unless otherwise indicated. All animal experiments were conducted at the University of Ottawa and approved by the University of Ottawa Animal Care Committee in compliance with institutional and Canadian Council on Animal Care guidelines. Dr. Bergeron provided 3xTg-AD mice for breeding. Krystal Walker contributed to mouse colony maintenance.

3.2.2. Stable isotope labelling by amino acids in cell culture

To produce heavy isotope labelled reference proteins, Neuro-2a (N2a) cells (mouse neuroblastoma cell line; American Type Culture Collection [ATCC]; Manassas, VA, USA) were cultured in SILAC media at 37°C in a 5% CO2 humidified incubator as previously described (Chiang et al., 2017). N2a cells were cultured in customized Dulbecco's Modified Eagle Media (DMEM) (AthenaES; Baltimore, MD, USA) in which the natural arginine and lysine were replaced with heavy [$^{13}C_6$,$^{15}N_2$] L-arginine (Arg-10) and [$^{13}C_6$,$^{15}N_2$] L-lysine (Lys-8) and supplemented with 10% (v/v) dialyzed fetal bovine serum (FBS) (Gibco; Burlington, ON, Canada), 1 mM sodium pyruvate (Gibco), 28 µg/mL gentamicin (Gibco), and 5 µg/mL Plasmocin prophylactic (InvivoGen; San Diego, CA, USA). Cells were maintained in culture

with SILAC media for at least 10 doubling times to allow for complete (>98%) incorporation of the isotopically labelled amino acids into cells.

3.2.3. In vivo *CK1δ/ε inhibition and tissue collection*

Female 8-month-old 3xTg-AD and NTg mice group-housed by genotype from weaning were administered vehicle (20% [w/v] 2-hydroxypropyl-β-cyclodextrin buffered with 25 mM sodium citrate pH 6.0; MilliporeSigma) or the CK1δ/ε inhibitor PF-670462 (30 mg/kg body weight/day; Cayman Chemical; Ann Arbor, MI, USA) subcutaneously (s.c.) daily for 18 days at ZT11.5 in LD, then transferred to constant darkness (DD) and treated for another two days at CT11.5, where CT was defined by the ZT of the previous LD schedule. After two days in constant darkness, mice were sacrificed by cervical dislocation under dim red light at CT10 and CT14 on the third day of DD (Chiang et al., 2017). The hippocampi were quickly excised, immediately flash frozen in liquid nitrogen, and stored at –80°C until further processing. Krystal Walker contributed to *in vivo* treatments and sample collection. Dr. Mayne contributed to sample collection.

3.2.4. Proteomic analysis of hippocampal tissues

Protein extracts from hippocampal tissues of individual mice were obtained by mechanical homogenization in lysis buffer containing 4% (w/v) SDS in 50 mM ABC (pH 8.2) supplemented with complete protease and phosphatase inhibitor cocktails (Roche), followed by sonication (three 10 s pulses with 30 s on ice between each pulse). Proteins from the resulting supernatant were precipitated in 50% acetone/50% ethanol (EtOH)/0.1% acetic acid at a ratio of 1:5 by volume (supernatant to acidified acetone/EtOH buffer) at –20°C overnight. Proteins were pelleted by centrifugation at 16,000 × *g* for 20 min at 4°C and pellets were washed three times with ice-cold acetone. Proteins were resuspended in 8 M urea in 50 mM ABC (pH 8.2). Protein concentrations were determined using the DC Protein Assay (Bio-Rad). Proteins were reduced by incubating samples with 5 mM DTT

(MilliporeSigma) for 30 min at 56°C with agitation (245 rpm) and subsequently alkylated with 10 mM IAA (MilliporeSigma) for 30 min in darkness at room temperature. Protein digestion was performed by incubation with 40:1 (w/w, protein:enzyme) trypsin (Worthington Biochemical Corporation) overnight at 37°C with agitation (245 rpm). Samples were acidified using 10% (v/v) TFA (MilliporeSigma) and then desalted using in-house made C18 desalting cartridges (C18 beads: ReproSil-Pur C18-AQ, 10 µm; Dr. Maisch GmbH) and desiccated using a SpeedVac. Peptides were resuspended in 0.1% (v/v) FA for LC-MS/MS analysis.

3.2.5. In vitro *CK1δ/ε inhibition*

Light N2a cells (ATCC) were grown in DMEM (Gibco) supplemented with 10% (v/v) FBS (Gibco), 1 mM sodium pyruvate (Gibco), 28 µg/mL gentamicin (Gibco), and 5 µg/mL Plasmocin prophylactic (InvivoGen) at 37°C in a 5% CO2 humidified incubator. For CK1δ/ε inhibition, immediately following a media change for synchronization (Yeom et al., 2010), light N2a cells were treated with PF-670462 (5 µM) or dimethyl sulfoxide (DMSO) as a control for 24 h prior to being harvested for proteomic analysis. Heavy N2a cells were treated for 6 or 24 h with PF-670462 (5 µM) or DMSO prior to being harvested for use as a SILAC spike-in standard.

3.2.6. SILAC-based proteomic analysis of N2a cells

Protein extracts from light and heavy N2a cells were obtained by homogenization in lysis buffer containing 8 M urea in 50 mM ABC (pH 8.2) supplemented with complete protease and phosphatase inhibitor cocktails (Roche), followed by sonication (three 10 s pulses with 30 s on ice between each pulse). Protein concentrations were determined using the Bradford assay (Bio-Rad). Lysates from light and heavy N2a cells were mixed at a 1:1 ratio and loaded onto 30-kDa molecular weight cutoff Microcon filters (MilliporeSigma). Proteins were reduced by incubating samples with 20 mM DTT for 30 min at 37°C with agitation (245 rpm) and subsequently alkylated with 20 mM IAA for 30 min in darkness at room

temperature. Protein digestion was performed by incubation with 40:1 (w/w, protein:enzyme) trypsin (Worthington Biochemical Corporation) overnight at 37°C with agitation (245 rpm). Samples were acidified using 10% (v/v) FA, then desalted using in-house made C18 desalting cartridges (C18 beads: Dr. Maisch GmbH) and desiccated using a SpeedVac prior to being resuspended in 0.1% (v/v) FA for LC-MS/MS analysis.

3.2.7. LC-MS/MS analysis

A 4 µL portion of resuspended peptides (equivalent to 2 µg of proteins) from each sample was analyzed by an online reverse-phase LC-MS/MS platform consisting of an Eksigent NanoLC 425 system (AB SCIEX) coupled with an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific) via a nano-electrospray source. Prior to MS analysis, peptide mixtures were separated by reverse-phase chromatography using an in-house packed ReproSil-Pur C18-AQ column (75 µm internal diameter × 15 cm, 1.9 µm, 200 Å pore size; Dr. Maisch GmbH) over a 240-min gradient of 5–30% buffer B (ACN with 0.1% [v/v] FA) at a flow rate of 300 nL/min. The Orbitrap Elite instrument was operated in the data-dependent mode to simultaneously measure survey scan MS spectra (350–1,800 *m/z*, R = 60,000 defined at *m/z* 400). Up to 20 of the most intense peaks were isolated and fragmented with CID. System controlling and data collection were carried out using Xcalibur software version 2.2 (Thermo Scientific). Dr. Ning contributed to ensuing mass spectrometry working conditions.

3.2.8. Mass spectrometry data processing

Mass spectrometry raw files were processed for each experiment separately with MaxQuant (version 1.5.2.8) using the integrated Andromeda search engine and UniProt FASTA database from mouse (*Mus musculus*; 2013_05). The search included variable modifications for methionine oxidation (M) and acetylation (protein N-term) as well as fixed modification for carbamidomethylation (C). Trypsin/P was set as the cleavage specificity with up to two missed cleavages allowed. The FDR cutoffs were set at 0.01 at the peptide

and protein levels and the minimum peptide length was set at 7. Identification across different replicates was achieved by enabling the “match between runs” option with a matching time window of 5 min.

3.2.9. Cognitive testing

For cognitive function studies, 11.5-month-old female 3xTg-AD and NTg mice were administered vehicle (20% [w/v] 2-hydroxypropyl-β-cyclodextrin buffered with 25 mM sodium citrate pH 6.0; MilliporeSigma) or the CK1δ/ε inhibitor PF-670462 (20 mg/kg body weight/day; Cayman Chemical) s.c. daily at ZT11 in LD. Light levels during the course of the experiment were confirmed using a light sensor placed inside the housing room. Five days after the start of injections, mice underwent Y-maze and open field testing on days 6–9 of treatment. Mice were brought into the behavioural facility 30–60 min prior to testing for acclimatization to the testing environment.

3.2.9.1. Y-Maze

Hippocampal-dependent spatial working memory was assessed using the Y-maze spontaneous alternation behaviour test as previously described with minor modifications (Knight et al., 2014). The Y-maze apparatus consisted of three arms (38 cm long, 7.6 cm wide, and 13 cm tall) of opaque black plastic at a 120° angle from each other. Mice were placed into one of the arms of the maze and arm entries were recorded for 8 min as the animal freely explored all three arms. The maze was wiped clean with 70% EtOH between trials. An arm entry was defined as having all four paws in an arm. Spontaneous alternation behaviour was defined as successive entries into three different arms and expressed as a percentage of the maximum number of alternations (total number of arm entries minus 2). The total number of arm entries was also recorded as a measure of ambulatory activity and mice with fewer than six arm entries were excluded.

3.2.9.2. Open field

Anxiety-like behaviour was assessed using the open field test as previously described with minor modifications (Hebda-Bauer et al., 2013). Mice were placed in the centre of an empty open field box (44 cm × 44 cm × 44 cm) made of opaque white plastic, and their activity was recorded using Ethovision XT 11.5 video tracking software (Noldus Information Technology; Leesburg, VA, USA) for 5 min. The open field was wiped clean with 70% EtOH between trials. Time spent exploring the centre zone (24 cm × 24 cm) was recorded as a measure of anxiety level, while total distance travelled was recorded as a measure of ambulatory activity (Hebda-Bauer et al., 2013).

3.2.10. Behavioural circadian rhythm testing

3.2.10.1. Running wheels

For behavioural circadian rhythm studies, female 3xTg-AD and NTg mice entrained to a 12-h light:12-h dark schedule from weaning were individually housed with running wheels for two weeks in LD conditions and then released into DD at 10–11 months of age. Light levels during the course of the experiment were confirmed using a light sensor placed inside each housing room. After three weeks in DD, mice were administered vehicle (20% [w/v] 2-hydroxypropyl-β-cyclodextrin buffered with 25 mM sodium citrate pH 6.0; MilliporeSigma) or PF-670462 (20 mg/kg body weight/day; Cayman Chemical) s.c. daily for 10 days at CT12, where CT was defined by the ZT of the previous LD schedule. This time point was chosen to allow all mice to receive injections at a time when it had previously been shown that PF-670462 administration has robust effects on behavioural circadian rhythms in rats (Badura et al., 2007). Running wheel activity data were collected using Wheel Manager software version 2.2 (MED Associates, Inc.; Fairfax, VT, USA). Actograms were generated and chi-square periodogram analysis performed using circadian software available online (www.circadian.org) (Chang and Guarente, 2013). Krystal Walker contributed to treatments.

3.2.10.2. Dose–response study for PF-670462

Male 3xTg-AD mice entrained to a 12-h light:12-h dark schedule from weaning were individually housed with running wheels for two weeks in LD conditions and then released into DD at 7–8 months of age. After 16 days in DD, mice were administered vehicle (20% [w/v] 2-hydroxypropyl-β-cyclodextrin; MilliporeSigma) or PF-670462 (10 or 30 mg/kg body weight/day; Cayman Chemical) s.c. daily for 9 days at CT12, where CT was defined by the ZT of the previous LD schedule. Running wheel activity data were collected and analyzed for Figure 3.7 as described above. Krystal Walker contributed to treatments.

3.2.11. Bioinformatic analysis

Initial bioinformatic analysis was performed with Perseus (version 1.5.5.3). The raw proteomic dataset for each experiment was filtered to include only proteins quantified in at least half of samples (referred to as reliably quantified proteins or Q50 in this chapter). Hierarchical clustering analysis, using the median value of logarithmized values for the normalized light-to-heavy (L/H) ratio (for N2a samples) or LFQ intensity (for hippocampal samples) of each protein, was performed after z-score normalization of the data within Euclidean distances. Prior to principal component analysis (PCA) with MetaboAnalyst (version 4.0) (Chong et al., 2018), missing values were imputed by drawing random numbers from a normal distribution with a width parameter of 0.3 of the SD of all measured values and centre shifted towards low abundance by 1.8 times this SD. GO and KEGG pathway enrichment analyses were implemented using DAVID (version 6.8; Fisher's exact test $p \leq 0.01$ relative to the Q50 backgrounds in our datasets was considered significant) (Huang da et al., 2009). Protein–protein interaction (PPI) networks were created using the STRING database (Szklarczyk et al., 2015) (confidence score cutoff = 90%) and visualized with NetworkAnalyst (Xia et al., 2015).

3.2.12. Statistical analysis

In vitro CK1δ/ε inhibition proteomic data were analyzed using unpaired two-tailed Student's *t*-test (permutation-based FDR = 0.05; s0 = 0.1) for differential expression analysis. *In vivo* CK1δ/ε inhibition proteomic data were analyzed using unpaired two-tailed Student's *t*-test ($\alpha = 0.05$; $\log_{10}$ fold change [experimental/control] greater than $\log_{10}1.2$ or less than $-\log_{10}1.2$) for differential expression analysis. Y-maze and open field data were analyzed using unpaired two-tailed Student's *t*-test. Running wheel data were analyzed by chi-square periodogram analysis ($\alpha = 0.05$), and one-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) *post hoc* test or two-tailed unpaired or paired Student's *t*-tests (with Welch's correction where indicated) were performed to compare group differences as appropriate. Statistical analyses were carried out using Perseus (version 1.5.5.3) for proteomic data or Prism 6 (GraphPad; La Jolla, CA, USA) for behavioural data. Data in bar graphs are represented as means + SEM.

3.2.13. Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifiers PXD012281 and PXD012282.

3.3. Results

3.3.1. PF-670462 treatment alters expression of AD-related and clock-regulated proteins in vitro

To explore the global effects of CK1δ/ε inhibition on protein expression, we first conducted proteomic analysis using LC-MS/MS and N2a mouse neuroblastoma cells, which express multiple clock genes and demonstrate circadian oscillations in gene expression (Chang and Guarente, 2013). We treated N2a cells with either PF-670462 (5 μM) or DMSO (as a

control) for 24 h prior to harvesting cells for proteomic analysis. Protein extracts were mixed at a 1:1 ratio with a spike-in standard from heavy-labelled SILAC N2a cells to allow for accurate relative quantification. Using a 1% FDR threshold, a total of 1,595 proteins were identified, of which 1,173 and 1,169 were quantified in cells treated with PF-670462 and DMSO, respectively (**Figure 3.2 A**). Of these, 1,079 proteins yielded relative measurements in a minimum of half of samples (Q50). We used this stringently filtered dataset of reliably quantified proteins for downstream bioinformatic analysis.

Comparison of protein abundances (logarithmized L/H ratios) in N2a cells treated with PF-670462 or DMSO showed that the majority (89%, 147 proteins) of differentially expressed proteins were upregulated in response to PF-670462 treatment, with fewer showing downregulation (11%, 19 proteins) (**Figure 3.2 B**). Among these differentially expressed proteins were several proteins previously shown to be involved in circadian regulation and to exhibit diurnal rhythms in expression, including casein kinase 2 (Lee et al., 2009), prohibitin-2 (Kategaya et al., 2012), and calreticulin (Noguchi et al., 2017), suggesting that PF-670462 may exert effects on clock-regulated proteins through CK1δ/ε inhibition. To gain insight into the functions of the set of proteins upregulated with PF-670462 treatment (top cluster in **Figure 3.2 C**), we performed GO enrichment analysis and identified functional terms that were significantly overrepresented compared to a background of the reliably quantified proteins in our dataset. We found that several highly represented functional terms were related to AD pathogenesis, notably cytoskeletal and mitochondrial organization (**Figure 3.2 D**).

Given that these biological processes are not only pathologically altered in AD but also known to be regulated by the circadian clock (Chaix et al., 2016; Hoyle et al., 2017), we asked whether other clock-regulated processes and pathways were altered by PF-670462 treatment. Protein–protein interaction network analysis of differentially abundant proteins in response to PF-670462 treatment revealed that these proteins take part in a wide range of

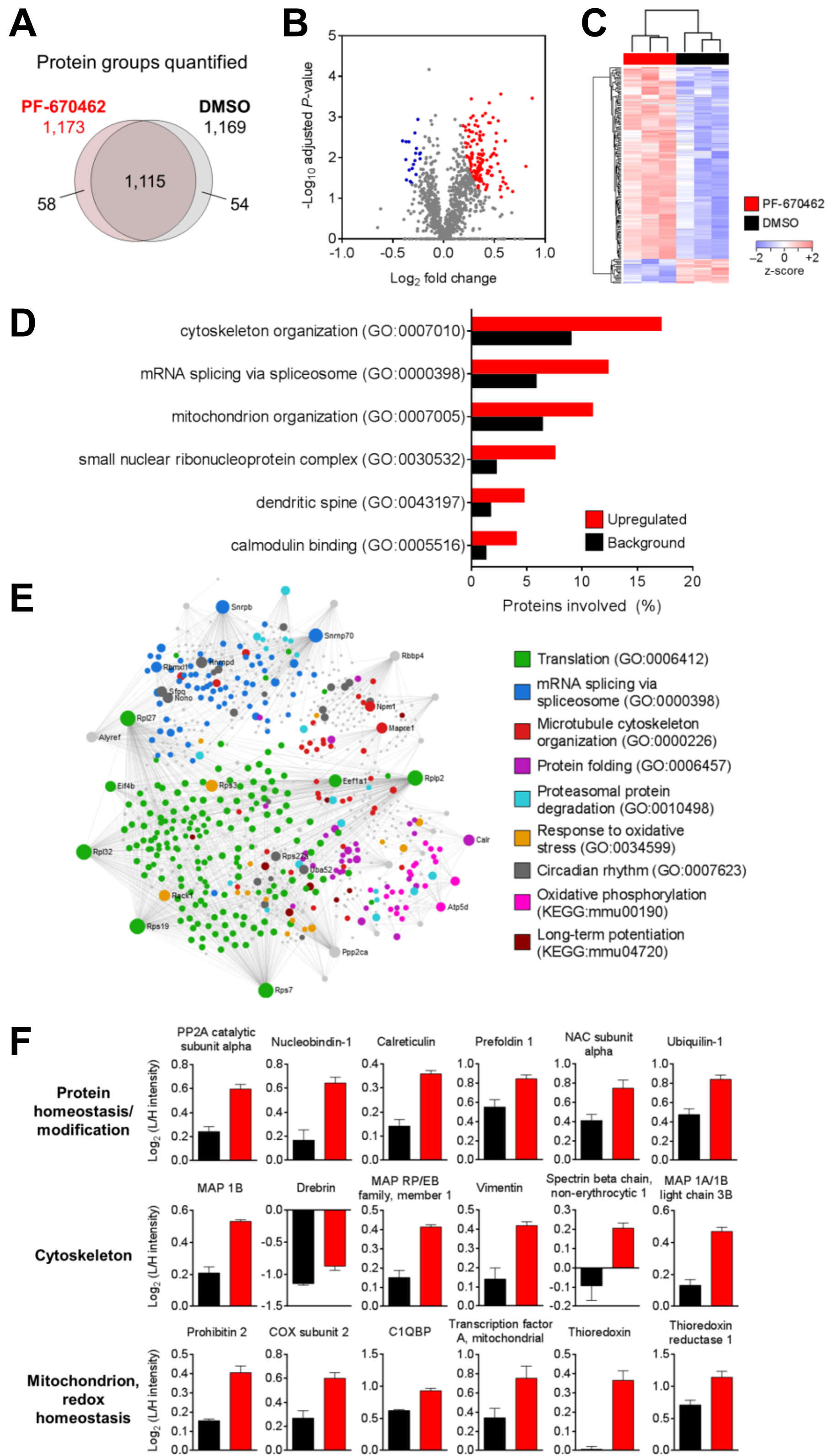

A
Protein groups quantified
PF-670462
1,173
DMSO
1,169
58
1,115
54
B
-Log10 adjusted P-value
Log2 fold change
C
PF-670462
DMSO
z-score
D
cytoskeleton organization (GO:0007010)
mRNA splicing via spliceosome (GO:0000398)
mitochondrion organization (GO:0007005)
small nuclear ribonucleoprotein complex (GO:0030532)
dendritic spine (GO:0043197)
calmodulin binding (GO:0005516)
Upregulated
Background
Proteins involved (%)
E
Translation (GO:0006412)
mRNA splicing via spliceosome (GO:0000398)
Microtubule cytoskeleton organization (GO:0000226)
Protein folding (GO:0006457)
Proteasomal protein degradation (GO:0010498)
Response to oxidative stress (GO:0034599)
Circadian rhythm (GO:0007623)
Oxidative phosphorylation (KEGG:mmu00190)
Long-term potentiation (KEGG:mmu04720)
F
Protein homeostasis/ modification
PP2A catalytic subunit alpha
Nucleobindin-1
Calreticulin
Prefoldin 1
NAC subunit alpha
Ubiquilin-1
Cytoskeleton
MAP 1B
Drebrin
MAP RP/EB family, member 1
Vimentin
Spectrin beta chain, non-erythrocytic 1
MAP 1A/1B light chain 3B
Mitochondrion, redox homeostasis
Prohibitin 2
COX subunit 2
C1QBP
Transcription factor A, mitochondrial
Thioredoxin
Thioredoxin reductase 1
Log2 (L/H intensity)

Figure 3.2. PF-670462 treatment alters expression of AD-related and clock-regulated proteins *in vitro*.

(A) Proteome coverage: Venn diagram displaying the number of proteins quantified in each treatment condition and overlap between conditions. N2a cells were treated with PF-670462 (5 μM) or DMSO (control) for 24 h and harvested for proteomic analysis. Protein extracts were mixed at a 1:1 ratio with a SILAC spike-in standard from heavy-labelled N2a cells prior to trypsin digestion and LC-MS/MS analysis. n = 3 biological replicates per group.

(B) Volcano plot highlighting differentially expressed proteins after PF-670462 treatment (blue = downregulation, red = upregulation; FDR-corrected $p < 0.05$, two-tailed Student's *t*-test; s0 = 0.1).

(C) Heatmap of z-score normalized abundances of differentially expressed proteins after PF-670462 treatment, showing unsupervised hierarchical clustering of proteins (rows) and samples (columns).

(D) GO enrichment analysis using DAVID of proteins upregulated with PF-670462 treatment. A selection of enriched ontology terms associated with AD pathogenesis is shown ($p \leq 0.01$, Fisher's exact test relative to Q50 background).

(E) First order PPI network of proteins differentially expressed after PF-670462 treatment created using the STRING database (interaction confidence score > 0.9). GO and KEGG functional annotations were performed using DAVID and network visualization using NetworkAnalyst. Proteins involved in AD-related and clock-regulated biological processes and pathways shown are represented as coloured nodes.

(F) Relative abundances of proteins associated with AD and upregulated in response to PF-670462 treatment.

C1QBP, complement C1q binding protein; COX, cytochrome c oxidase; MAP, microtubule-associated protein; NAC, nascent polypeptide-associated complex; PP2A, protein phosphatase 2A. Data are represented as means + SEM.

highly interconnected processes and KEGG pathways that have been implicated in AD pathogenesis, including proteasomal protein degradation, response to oxidative stress, LTP, oxidative phosphorylation, and circadian rhythm (Huang and Mucke, 2012; Sanabria-Castro et al., 2017) (**Figure 3.2 E**). Interestingly, these cellular processes also display circadian regulation in mammals (Chaudhury et al., 2005; Chaix et al., 2016), illustrating how the circadian clock and neurodegeneration are closely intertwined and suggesting that clock modulation might protect against neurodegenerative changes at the molecular level. Indeed, we found that PF-670462 treatment resulted in the upregulation of multiple proteins known to be dysfunctional or downregulated in AD and involved in diverse cellular functions, including protein homeostasis and modification, cytoskeletal organization, mitochondrial respiration, and redox homeostasis (**Figure 3.2 F**). Together, these findings suggest that CK1δ/ε inhibition might represent an approach to reverse AD-related protein expression changes across several pathways.

3.3.2. PF-670462 administration **in vivo** *normalizes hippocampal proteomic alterations associated with AD-like pathology*

Having established that PF-670462 treatment can alter the expression of AD-related proteins involved in a variety of biological processes *in vitro*, we asked whether PF-670462 administration *in vivo* would have similar effects on the hippocampus, which plays a critical role in memory and displays circadian rhythms in protein expression (Chiang et al., 2017). To examine this, we performed MS-based proteomic analyses using hippocampal tissues from 3xTg-AD mice treated with PF-670462 or vehicle, and NTg mice treated with vehicle. Mice were administered daily injections of PF-670462 (30 mg/kg/d) or vehicle for 20 days beginning at 8 months of age, then hippocampal tissues were harvested at two time points, CT10 and CT14, on the third day following transfer to DD (**Figure 3.3 A**). Samples were processed individually to yield three to five biological replicates per time point for each group, and relative protein abundances were determined by LFQ. This MS-based analysis

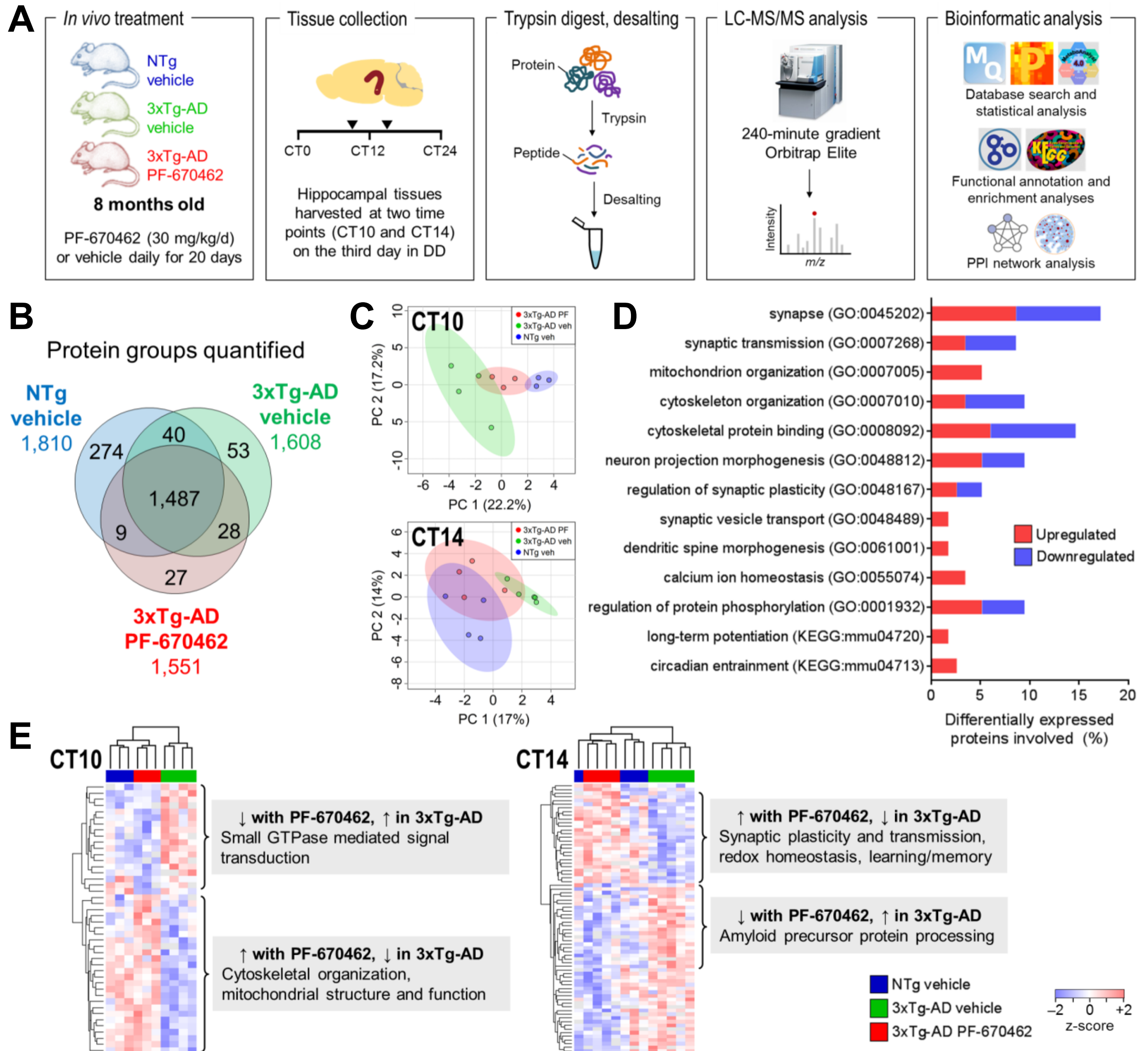

A
In vivo treatment
NTg vehicle
3xTg-AD vehicle
3xTg-AD PF-670462
8 months old
PF-670462 (30 mg/kg/d) or vehicle daily for 20 days
Tissue collection
CT0 CT12 CT24
Hippocampal tissues harvested at two time points (CT10 and CT14) on the third day in DD
Trypsin digest, desalting
Protein
Trypsin
Peptide
Desalting
LC-MS/MS analysis
240-minute gradient Orbitrap Elite
Intensity
m/z
Bioinformatic analysis
Database search and statistical analysis
Functional annotation and enrichment analyses
PPI network analysis
B
Protein groups quantified
NTg vehicle 1,810
3xTg-AD vehicle 1,608
3xTg-AD PF-670462 1,551
274
40
53
1,487
9
28
27
C
CT10
3xTg-AD PF
3xTg-AD veh
NTg veh
PC 2 (17.2%)
PC 1 (22.2%)
CT14
PC 2 (14%)
PC 1 (17%)
D
synapse (GO:0045202)
synaptic transmission (GO:0007268)
mitochondrion organization (GO:0007005)
cytoskeleton organization (GO:0007010)
cytoskeletal protein binding (GO:0008092)
neuron projection morphogenesis (GO:0048812)
regulation of synaptic plasticity (GO:0048167)
synaptic vesicle transport (GO:0048489)
dendritic spine morphogenesis (GO:0061001)
calcium ion homeostasis (GO:0055074)
regulation of protein phosphorylation (GO:0001932)
long-term potentiation (KEGG:mmu04720)
circadian entrainment (KEGG:mmu04713)
Upregulated
Downregulated
0 5 10 15 20
Differentially expressed proteins involved (%)
E
CT10
↓ with PF-670462, ↑ in 3xTg-AD
Small GTPase mediated signal transduction
↑ with PF-670462, ↓ in 3xTg-AD
Cytoskeletal organization, mitochondrial structure and function
CT14
↑ with PF-670462, ↓ in 3xTg-AD
Synaptic plasticity and transmission, redox homeostasis, learning/memory
↓ with PF-670462, ↑ in 3xTg-AD
Amyloid precursor protein processing
NTg vehicle
3xTg-AD vehicle
3xTg-AD PF-670462
−2 0 +2
z-score

Figure 3.3. PF-670462 administration shifts the hippocampal proteomic profile of 3xTg-AD mice towards that of NTg mice.

(A) Study design and workflow: 8-month-old NTg and 3xTg-AD mice were treated daily with PF-670462 (30 mg/kg/d) or vehicle for 20 days, then hippocampal tissues were collected at two time points for proteomic analysis. Protein extracts were digested with trypsin and analyzed by LC-MS/MS.

(B) Proteome coverage: Venn diagram displaying the number of proteins quantified in each experimental group and overlap between groups.

(C) PCA of reliably quantified proteins (Q50), showing clustering of samples by experimental group with 95% confidence ellipses.

(D) GO and KEGG functional annotations using DAVID of proteins upregulated or downregulated with PF-670462 administration ($p < 0.05$, two-tailed Student's *t*-test; $\log_{10}$ fold change > $\log_{10}1.2$ or < $-\log_{10}1.2$). A selection of ontology terms and pathways associated with AD pathogenesis is shown.

(E) Heatmaps of z-score normalized abundances of differentially expressed proteins after PF-670462 administration, showing unsupervised hierarchical clustering of proteins (rows) and samples (columns). Vehicle-treated NTg and 3xTg-AD mice have distinct hippocampal proteomic profiles, while the profiles of 3xTg-AD mice treated with PF-670462 are similar to those of NTg mice.

For CT10: n = 3 NTg vehicle; n = 4 3xTg-AD vehicle; n = 3 3xTg-AD PF-670462. For CT14: n = 4 NTg vehicle; n = 5 3xTg-AD vehicle; n = 4 3xTg-AD PF-670462.

identified a total of 1,929 proteins, of which 1,810, 1,608, and 1,551 were quantified in samples from vehicle-treated NTg mice, vehicle-treated 3xTg-AD mice, and PF-670462–treated 3xTg-AD mice, respectively (**Figure 3.3 B**). Of these, ~1,350 yielded relative measurements in a minimum of half of samples (Q50) at each time point, and we used these stringently filtered datasets of reliably quantified proteins for downstream bioinformatic analysis.

On the basis of the expression (logarithmized LFQ intensities) of proteins in these Q50-filtered lists, PCA showed that vehicle-treated 3xTg-AD mice can be separated from vehicle-treated NTg mice, while PF-670462 treatment partially reversed proteomic changes associated with AD-like pathology and induced a shift in the hippocampal protein expression profile of 3xTg-AD mice towards that of NTg mice at each time point (**Figure 3.3 C**). Comparison of the 3xTg-AD vehicle-treated and PF-670462–treated groups identified 117 proteins that were differentially expressed in response to PF-670462 administration (53 upregulated and 64 downregulated; **Appendix B Tables 1** and **2**). These proteins take part in a variety of biological processes and pathways, including a number involved in synaptic, cytoskeletal, and mitochondrial functions, as well as circadian entrainment and regulation of protein phosphorylation (**Figure 3.3 D**). Unsupervised hierarchical clustering performed on proteins differentially expressed with PF-670462 administration revealed distinct expression profiles between vehicle-treated 3xTg-AD and NTg mice, reflecting changes associated with AD-like pathology. Moreover, vehicle-treated NTg mice and PF-670462–treated 3xTg-AD mice clustered together, and apart from the vehicle-treated 3xTg-AD group (**Figure 3.3 E**), confirming that widespread alterations in the hippocampal proteome were associated with AD-like pathology and partially normalized by PF-670462 treatment.

Interestingly, PF-670462 administration induced changes in protein expression in distinct molecular pathways at each time point examined, suggesting that circadian regulation might underlie some of the hippocampal responses to CK1δ/ε inhibition and that PF-670462

administration might be restoring the normal rhythmic expression of some differentially expressed proteins. In line with this, we found that differentially expressed proteins were involved in several clock-regulated processes, including cytoskeletal organization and mitochondrial function at CT10, as well as synaptic plasticity and APP processing at CT14. Protein–protein interaction network analysis of differentially expressed proteins in the hippocampus of 3xTg-AD mice in response to PF-670462 treatment confirmed the effects of PF-670462 on multiple AD-related processes seen in our *in vitro* study, including cytoskeletal and mitochondrial organization, response to oxidative stress, and proteasomal protein degradation (**Figure 3.4 A**). Administration of PF-670462 in 3xTg-AD mice induced changes in the expression of a greater number of proteins involved in synaptic transmission, regulation of synaptic plasticity, and learning and memory than *in vitro* treatment, which might reflect differences in experimental models at the molecular level as well as contributions of systemic circadian signals *in vivo* (Smarr et al., 2014).

To gain further insight into the AD-related proteomic alterations in the hippocampus that were reversed by PF-670462 treatment in 3xTg-AD mice, we compared the lists of proteins differentially expressed in vehicle-treated versus PF-670462–treated 3xTg-AD mice, and vehicle-treated 3xTg-AD versus NTg mice at each time point. We found that a substantial proportion of proteins whose abundances were altered with PF-670462 were also differentially expressed with AD-like pathology (39% and 34% at CT10 and CT14, respectively). Strikingly, for all but one of these proteins, PF-670462 normalized their expression levels in the hippocampus of 3xTg-AD mice towards NTg levels (**Figure 3.4 B**; **Appendix B Tables 1** and **2**).

To further characterize the effects of PF-670462 on processes implicated in AD pathogenesis, we examined the expression of proteins involved in various functions critical for normal cellular homeostasis and hippocampal function, including cytoskeletal

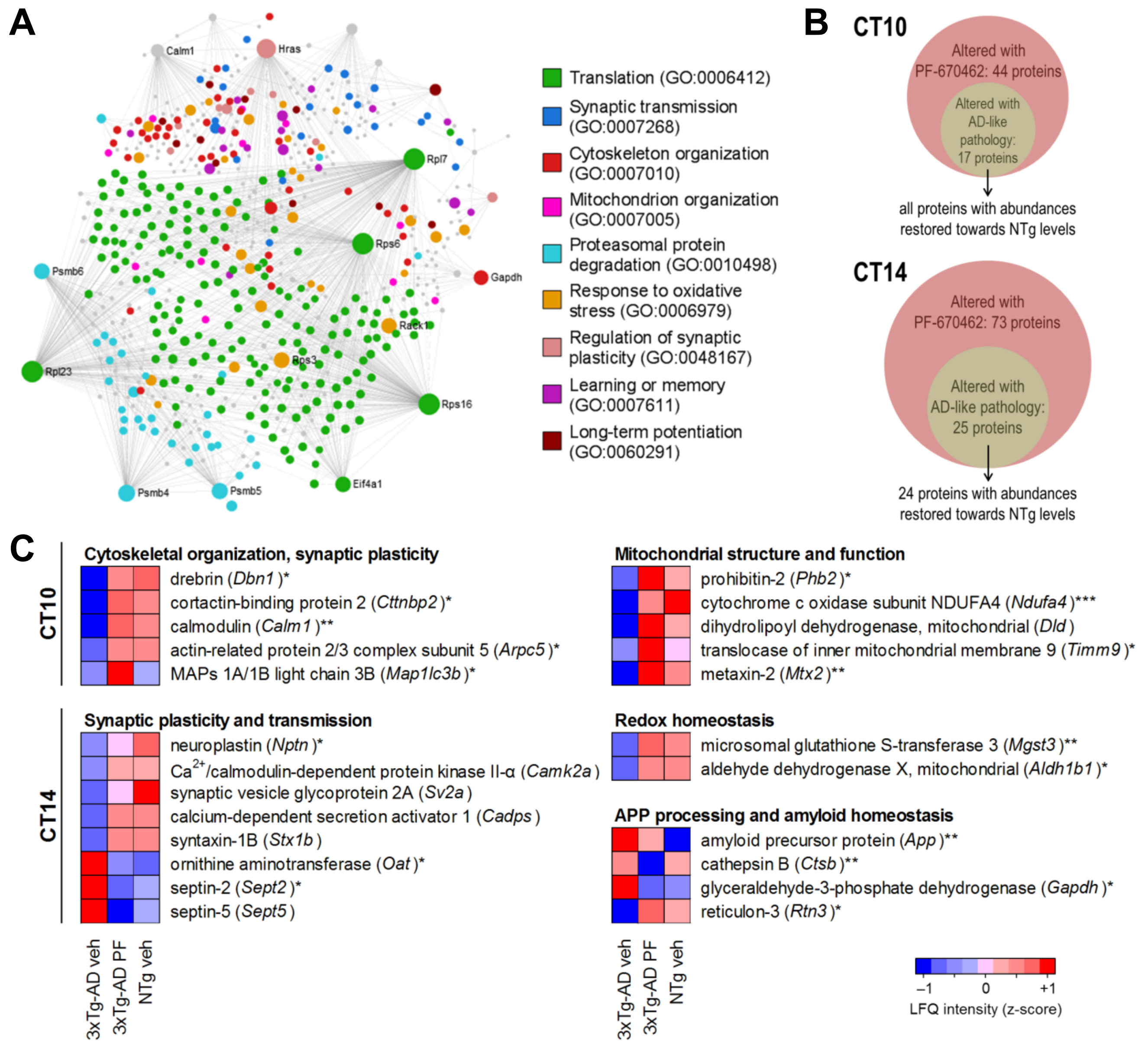

A
Calm1
Hras
Rpl7
Rps6
Gapdh
Psmb6
Rack1
Rps3
Rpl23
Rps16
Psmb4
Psmb5
Eif4a1
Translation (GO:0006412)
Synaptic transmission (GO:0007268)
Cytoskeleton organization (GO:0007010)
Mitochondrion organization (GO:0007005)
Proteasomal protein degradation (GO:0010498)
Response to oxidative stress (GO:0006979)
Regulation of synaptic plasticity (GO:0048167)
Learning or memory (GO:0007611)
Long-term potentiation (GO:0060291)
B
CT10
Altered with PF-670462: 44 proteins
Altered with AD-like pathology: 17 proteins
all proteins with abundances restored towards NTg levels
CT14
Altered with PF-670462: 73 proteins
Altered with AD-like pathology: 25 proteins
24 proteins with abundances restored towards NTg levels
C
CT10
Cytoskeletal organization, synaptic plasticity
drebrin (Dbn1)*
cortactin-binding protein 2 (Cttnbp2)*
calmodulin (Calm1)**
actin-related protein 2/3 complex subunit 5 (Arpc5)*
MAPs 1A/1B light chain 3B (Map1lc3b)*
Mitochondrial structure and function
prohibitin-2 (Phb2)*
cytochrome c oxidase subunit NDUFA4 (Ndufa4)***
dihydrolipoyl dehydrogenase, mitochondrial (Dld)
translocase of inner mitochondrial membrane 9 (Timm9)*
metaxin-2 (Mtx2)**
CT14
Synaptic plasticity and transmission
neuroplastin (Nptn)*
Ca2+/calmodulin-dependent protein kinase II-α (Camk2a)
synaptic vesicle glycoprotein 2A (Sv2a)
calcium-dependent secretion activator 1 (Cadps)
syntaxin-1B (Stx1b)
ornithine aminotransferase (Oat)*
septin-2 (Sept2)*
septin-5 (Sept5)
Redox homeostasis
microsomal glutathione S-transferase 3 (Mgst3)**
aldehyde dehydrogenase X, mitochondrial (Aldh1b1)*
APP processing and amyloid homeostasis
amyloid precursor protein (App)**
cathepsin B (Ctsb)**
glyceraldehyde-3-phosphate dehydrogenase (Gapdh)*
reticulon-3 (Rtn3)*
3xTg-AD veh
3xTg-AD PF
NTg veh
3xTg-AD veh
3xTg-AD PF
NTg veh
–1
0
+1
LFQ intensity (z-score)

Figure 3.4. PF-670462 administration rescues AD-related protein abundance changes in the hippocampus of 3xTg-AD mice.

(A) First order PPI network of proteins differentially expressed with PF-670462 administration created using the STRING database (interaction confidence score > 0.9) and visualized using NetworkAnalyst ($p < 0.05$, two-tailed Student's *t*-test; $\log_{10}$ fold change $> \log_{10}1.2$ or $< -\log_{10}1.2$). Proteins involved in AD-associated GO biological processes shown are represented as coloured nodes. The size of a given node represents the number of connections it has to other nodes.

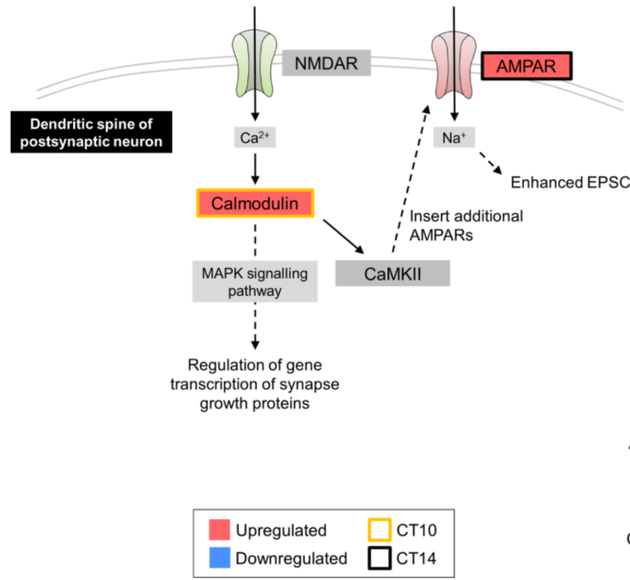
(B) The number of proteins differentially expressed after PF-670462 administration (3xTg-AD PF-670462 versus 3xTg-AD vehicle), as well as the number of these differentially expressed with AD-like pathology (3xTg-AD vehicle versus NTg vehicle).

(C) Relative abundances (mean z-score normalized $\log_{10}$ LFQ intensities) of proteins involved in various AD-related processes. Proteins differentially expressed with PF-670462: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, two-tailed Student's *t*-test; $\log_{10}$ fold change $> \log_{10}1.2$ or $< -\log_{10}1.2$. MAP, microtubule-associated protein.

For CT10: n = 3 NTg vehicle; n = 4 3xTg-AD vehicle; n = 3 3xTg-AD PF-670462. For CT14: n = 4 NTg vehicle; n = 5 3xTg-AD vehicle; n = 4 3xTg-AD PF-670462.

organization, mitochondrial structure and function, synaptic plasticity, and amyloid homeostasis (**Figure 3.4 C**). PF-670462 administration in 3xTg-AD mice upregulated the expression of several cytoskeleton-associated proteins, including drebrin and microtubule-associated proteins (MAPs) 1A/1B light chain 3B, as was seen in response to PF-670462 treatment *in vitro*. Hippocampal expression of drebrin, an actin-binding protein that regulates dendritic spine morphogenesis in neurons, is decreased in AD and associated with cognitive function in APP/PS1 mice (Liu et al., 2017). Vehicle-treated 3xTg-AD mice displayed decreased hippocampal levels of drebrin, which were normalized towards NTg levels by PF-670462. Cortactin-binding protein 2, another actin regulator that plays a role in dendritic arborization and synaptic plasticity (Chen and Hsueh, 2012), was also upregulated in response to PF-670462 treatment in 3xTg-AD mice. Moreover, PF-670462 increased the expression of proteins involved in the hippocampal LTP pathway, including calmodulin and the GluR1 AMPAR subunit (**Figure 3.5 A**). PF-670462 administration upregulated the expression of the calcium-buffering protein calmodulin, which was downregulated in the hippocampus of 3xTg-AD mice relative to NTg mice. Decreased expression of calmodulin in AD brains may contribute to dysregulated calcium homeostasis, which can lead to neurodegeneration, impaired synaptic plasticity, and memory deficits (Marambaud et al., 2009). PF-670462 also upregulated the levels of AMPAR, the trafficking of which is dysregulated by Aβ and represents a critical mechanism underlying LTP induction in the hippocampus (Guntupalli et al., 2016).

Furthermore, PF-670462 administration increased the expression of various proteins involved in mitochondrial function (**Figure 3.4 C**). These included prohibitin-2, which regulates mitophagy and maintenance of mitochondrial structure (Merkwirth et al., 2012; Wei et al., 2017), as well as translocase of inner mitochondrial membrane 9 (Tim9), part of the Tim9-Tim10 complex involved in mitochondrial protein import (Baker et al., 2009). In mice, loss of prohibitin-2 results in impaired mitochondrial respiration and defective


A Long-term potentiation (LTP)
NMDAR
AMPAR
Dendritic spine of postsynaptic neuron
Ca2+
Na+
Enhanced EPSC
Calmodulin
Insert additional AMPARs
MAPK signalling pathway
CaMKII
Regulation of gene transcription of synapse growth proteins
Upregulated
Downregulated
CT10
CT14

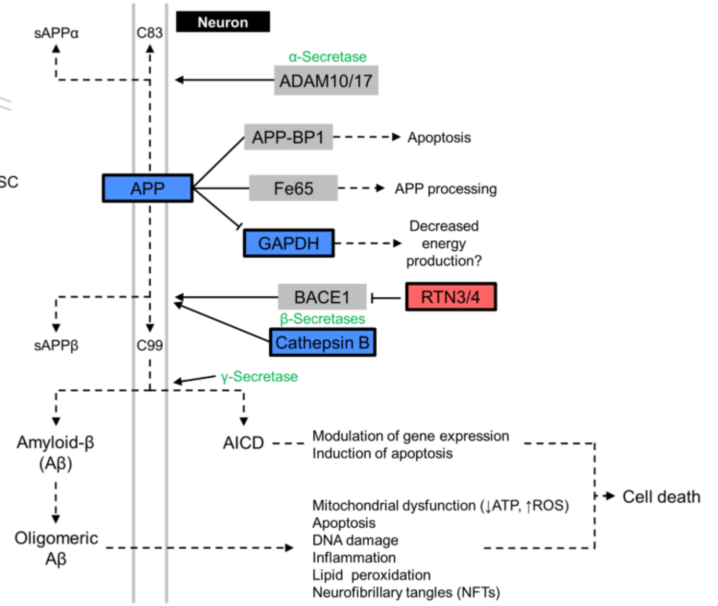

B Amyloid precursor protein (APP) processing
Neuron
sAPPα
C83
α-Secretase
ADAM10/17
APP-BP1
Apoptosis
APP
Fe65
APP processing
GAPDH
Decreased energy production?
BACE1
RTN3/4
β-Secretases
Cathepsin B
sAPPβ
C99
γ-Secretase
Amyloid-β (Aβ)
AICD
Modulation of gene expression
Induction of apoptosis
Cell death
Mitochondrial dysfunction (↓ATP, ↑ROS)
Apoptosis
DNA damage
Inflammation
Lipid peroxidation
Neurofibrillary tangles (NFTs)
Oligomeric Aβ

Figure 3.5. PF-670462 administration alters expression of proteins involved in LTP and APP processing in the hippocampus of 3xTg-AD mice.

(A and B) Schematic diagrams depicting the A) hippocampal LTP and B) APP processing pathways, highlighting proteins that were differentially expressed with PF-670462 administration ($p < 0.05$, two-tailed unpaired Student's *t*-test; $\log_{10}$ fold change > $\log_{10}1.2$ or < $-\log_{10}1.2$) Proteins depicted in gray were either not reliably quantified in our datasets or not differentially expressed with PF-670462. Adapted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways 04720 (long-term potentiation) and 05010 (Alzheimer disease).

8-month-old NTg and 3xTg-AD mice were treated daily with PF-670462 (30 mg/kg/d) or vehicle for 20 days, then hippocampal tissues were collected at two time points for proteomic analysis. Protein extracts were digested with trypsin and analyzed by LC-MS/MS. For CT10: n = 3 NTg vehicle; n = 4 3xTg-AD vehicle; n = 3 3xTg-AD PF-670462. For CT14: n = 4 NTg vehicle; n = 5 3xTg-AD vehicle; n = 4 3xTg-AD PF-670462.

ADAM, disintegrin and metalloproteinase domain-containing protein; AICD, APP intracellular domain; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; APP, amyloid precursor protein; APP-BP1, APP binding protein 1; BACE1, β-site APP cleaving enzyme; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; EPSC, excitatory postsynaptic current; Fe65, APP binding family B member 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase; NMDAR, N-methyl-D-aspartate receptor; ROS, reactive oxygen species; RTN, reticulon.

mitochondrial ultrastructure, and also leads to widespread neurodegeneration, notably in the hippocampus (Merkwirth et al., 2012). Furthermore, prohibitin-2 is regulated by CK1δ/ε and in turn modulates circadian gene expression (Kategaya et al., 2012), while cortactin-binding protein 2 regulates the actin-binding protein cofilin involved in circadian actin dynamics (Oser et al., 2009; Hoyle et al., 2017), highlighting the effects of PF-670462 on circadian regulation of diverse rhythmic processes. Together, these results suggest that PF-670462 administration *in vivo* normalizes hippocampal expression of multiple proteins linked to AD pathogenesis and implicated in cytoskeletal and mitochondrial functions, consistent with our *in vitro* findings.

We were also interested in examining the effects of PF-670462 treatment on other cellular processes, given that AD-related changes in a variety of processes contribute to neurodegeneration and impaired hippocampal function. In the hippocampus of 3xTg-AD mice, PF-670462 normalized the expression of several proteins implicated in synaptic dysfunction underlying memory impairment in AD, including neuroplastin and septin-2 (**Figure 3.4 C**). Neuroplastin plays an essential role in hippocampal LTP, and neuroplastin deficiency results in impaired associative learning and memory in mice (Bhattacharya et al., 2017). Septin-2, which is increased in AD brains and has been shown to interact with CK1δ (Kategaya et al., 2012; Musunuri et al., 2014), regulates cytoskeletal organization and may take part in the synaptic vesicle cycle (Burre and Volknandt, 2007). In addition, PF-670462 upregulated the expression of microsomal glutathione S-transferase 3 (MGST3) and aldehyde dehydrogenase 1B1, which play neuroprotective roles against oxidative stress (Singh et al., 2013; Ashbrook et al., 2014) (**Figure 3.4 C**). Interestingly, MGST3 is associated with hippocampal size and is downregulated in AD hippocampus (Ashbrook et al., 2014). PF-670462 also induced a decrease in the abundance of APP, which has previously been shown to be diurnally regulated (Dobrowolska et al., 2014) and is thought to play a central role in AD pathogenesis in the amyloid cascade hypothesis (Huang and

Mucke, 2012) (**Figure 3.5 B**). Moreover, PF-670462 altered the expression of other proteins involved in APP processing, notably reticulon-3 and cathepsin B. Reticulon-3 was upregulated by PF-670462 and has been shown to inhibit the activity of β-site APP cleaving enzyme (BACE1) (He et al., 2004), which is involved in Aβ production, while cathepsin B was downregulated by PF-670462 and exhibits β-secretase activity (Hook et al., 2008). Inhibitors of BACE1 and cathepsin B have been suggested as therapeutic approaches in AD given their efficacy in reducing Aβ levels and enhancing memory in preclinical models (Hook et al., 2008). Thus, treatment of 3xTg-AD mice with PF-670462 was associated with proteomic changes in hippocampal LTP and APP processing pathways that might be beneficial in AD. Taken together, these results demonstrate that PF-670462 administration *in vivo* can rescue diverse protein expression changes associated with AD in the hippocampus of 3xTg-AD mice, and suggest that CK1δ/ε represent promising therapeutic targets against AD-related hippocampal proteomic alterations.

3.3.3. PF-670462 administration rescues hippocampal-dependent working memory deficits in 3xTg-AD mice

Given that PF-670462 treatment normalized hippocampal proteomic alterations in 3xTg-AD mice, and since CK1ε overexpression has previously been shown to impair working memory in mice (Chen et al., 2017), we hypothesized that PF-670462 would improve hippocampal-dependent spatial working memory in 3xTg-AD mice. Moreover, given the associations between anxiety, working memory, and the circadian clock (Wall and Messier, 2000; Lamont et al., 2007), and the influence of anxiety on memory function in AD (Bierman et al., 2009), we were interested in examining the effects of CK1δ/ε inhibition on anxiety in 3xTg-AD mice. To determine if PF-670462 can reverse AD-associated cognitive deficits, 3xTg-AD and NTg mice were administered daily injections of PF-670462 (20 mg/kg/d) or vehicle starting at 11.5 months of age. Five days after starting treatment, mice underwent cognitive testing to

evaluate working memory and anxiety-like behaviour, with continued daily treatment over the course of testing (**Figure 3.6 A**).

To study the impact of PF-670462 on hippocampal-dependent working memory, we assessed spontaneous alternation behaviour in the Y-maze test, a widely used cognitive task to evaluate spatial working memory in rodents. The working memory of 11.5-month-old, vehicle-treated 3xTg-AD mice was significantly impaired compared with age-matched NTg control mice, in line with previous reports (Webster et al., 2014). 3xTg-AD mice treated with PF-670462 showed enhanced working memory relative to vehicle-treated 3xTg-AD mice, while the performance of NTg mice was unaffected by PF-670462 (**Figure 3.6 B**). To confirm that the improvement in working memory seen in 3xTg-AD mice treated with PF-670462 was not due to changes in activity levels, we measured the total number of arm entries and found that it was not altered by PF-670462, with 3xTg-AD mice in both treatment groups showing a similar number of arm entries and fewer than NTg controls (**Figure 3.6 C**). Thus, CK1δ/ε inhibition with PF-670462 rescued hippocampal-dependent working memory deficits in 3xTg-AD mice.

To determine whether the enhanced working memory resulting from PF-670462 treatment was associated with changes in anxiety, we also assessed anxiety-like behaviour in the open field test, using the time spent exploring the centre area of the open field as an index of anxiety. As expected, vehicle-treated 3xTg-AD mice spent less time in the centre compared with NTg control mice, indicating that 3xTg-AD mice exhibit increased anxiety (Webster et al., 2014). Although the time spent in the centre by 3xTg-AD mice treated with PF-670462 was not significantly different from that of vehicle-treated 3xTg-AD mice or NTg mice, PF-670462 treatment seemed to heighten anxiety levels in NTg mice (**Figure 3.6 D**). CK1δ/ε inhibition has previously been shown to alter anxiety-like behaviour in *Clock*Δ19 mice (Arey and McClung, 2012), suggesting that it might act through a similar mechanism to regulate anxiety levels in NTg mice but had no effect on anxiety in 3xTg-AD mice possibly

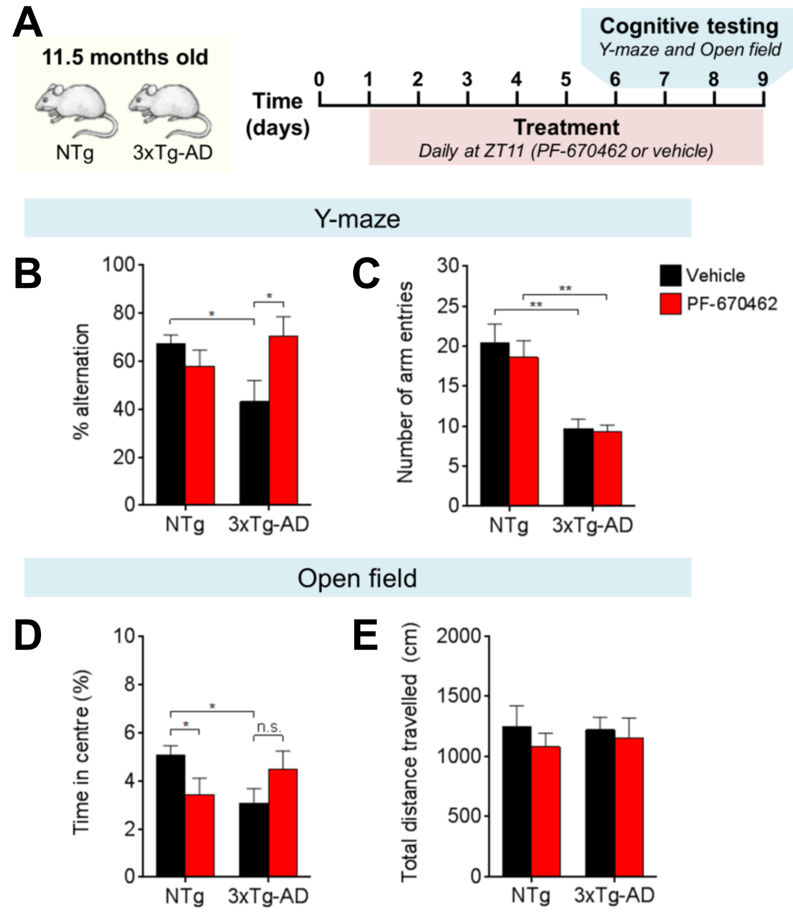

A
11.5 months old
NTg
3xTg-AD
Time
(days)
0 1 2 3 4 5 6 7 8 9
Cognitive testing
Y-maze and Open field
Treatment
Daily at ZT11 (PF-670462 or vehicle)
Y-maze
B
% alternation
NTg
3xTg-AD
C
Number of arm entries
Vehicle
PF-670462
Open field
D
Time in centre (%)
n.s.
E
Total distance travelled (cm)

Figure 3.6. PF-670462 administration rescues working memory deficits without altering anxiety-like behaviour in 3xTg-AD mice.

(A) Study design: NTg and 3xTg-AD mice were aged to 11.5 months and treatment was started 5 days prior to the beginning of behavioural testing. Mice received daily injections of PF-670462 (20 mg/kg/d) or vehicle, and the Y-maze and open field tests were conducted on days 6 to 9 of treatment. For Y-maze: n = 10 NTg vehicle; n = 11 NTg PF-670462; n = 6 3xTg-AD vehicle; n = 9 3xTg-AD PF-670462. For open field: n = 11 NTg vehicle; n = 11 NTg PF-670462; n = 9 3xTg-AD vehicle; n = 9 3xTg-AD PF-670462.

(B) Percent spontaneous alternation during the Y-maze test, showing improvement in spatial working memory in 3xTg-AD mice treated with PF-670462 (*$p < 0.05$, two-tailed Student’s *t*-test).

(C) Number of arm entries during the Y-maze test, showing no changes with PF-670462 treatment (**$p < 0.01$, two-tailed Student’s *t*-test versus NTg).

(D) Percent time spent in the centre during the open field test, showing no change in anxiety-like behaviour in 3xTg-AD mice treated with PF-670462 (*$p < 0.05$ versus NTg vehicle, two-tailed Student’s *t*-test).

(E) Distance travelled during the open field test, showing no differences in locomotion between any groups.

Data are represented as means + SEM. n.s., not significant.

due to pre-existing neurodegeneration in brain regions mediating anxiety. We also measured the total distance travelled during the open field test and did not find significant differences between any groups (**Figure 3.6 E**), indicating that genotype differences in anxiety-like behaviour were unrelated to changes in locomotion. These results suggest that the beneficial effect of PF-670462 on working memory in 3xTg-AD mice was not the result of altered anxiety levels, but might instead be linked to treatment-induced changes in the hippocampal proteome. Altogether, our findings provide support for a role of CK1δ/ε in regulating working memory and suggest that CK1δ/ε inhibition could represent a feasible approach to reverse AD-related memory impairment.

3.3.4. PF-670462 administration normalizes disrupted behavioural circadian rhythms in 3xTg-AD mice

Previous studies have shown that CK1δ/ε inhibition can restore defective circadian behaviour in various mouse models of circadian dysfunction (Meng et al., 2010), suggesting that daily treatment with PF-670462 might normalize altered behavioural circadian rhythms in 3xTg-AD mice. 3xTg-AD mice display loss of vasopressin- and vasoactive intestinal polypeptide–expressing neurons in the SCN, resulting in circadian disturbances in patterns of locomotor activity, notably a shortened free-running period under DD conditions (Sterniczuk et al., 2010).

To determine whether CK1δ/ε inhibition affects behavioural circadian rhythms in 3xTg-AD mice, we first conducted a preliminary study by administering PF-670462 at 10 and 30 mg/kg/d to allow for assessment of dose–response. At ~8 months of age, 3xTg-AD mice entrained to a 12-h light:12-h dark (LD) schedule were transferred to DD and their baseline free-running periods determined by monitoring wheel-running activity prior to beginning treatment. Mice were then administered daily injections of PF-670462 (10 or 30 mg/kg/d) or vehicle at CT12, where CT was defined by the ZT of the previous LD schedule (**Figure 3.7 A**). At baseline, 3xTg-AD mice exhibited free-running periods of 23.59 ± 0.1 h as seen on

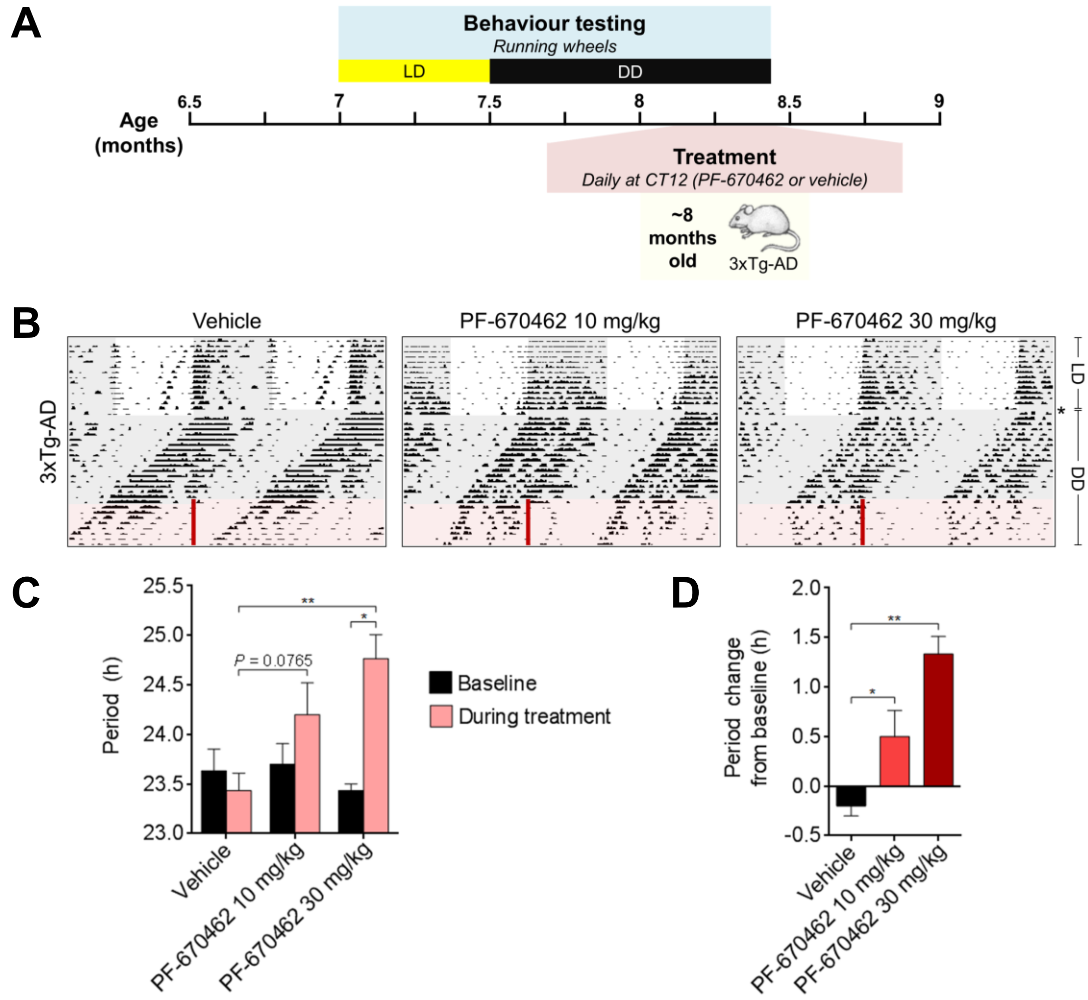

A
Behaviour testing
Running wheels
LD
DD
Age (months)
6.5
7
7.5
8
8.5
9
Treatment
Daily at CT12 (PF-670462 or vehicle)
~8 months old
3xTg-AD
B
Vehicle
PF-670462 10 mg/kg
PF-670462 30 mg/kg
3xTg-AD
LD
DD
C
Period (h)
P = 0.0765
Baseline
During treatment
Vehicle
PF-670462 10 mg/kg
PF-670462 30 mg/kg
D
Period change from baseline (h)
Vehicle
PF-670462 10 mg/kg
PF-670462 30 mg/kg

Figure 3.7. Dose determination for PF-670462 running wheel studies in 3xTg-AD mice.

(A) Study design: Wheel-running activities of 3xTg-AD mice were recorded for 2 weeks in LD, then mice were released into DD at ~7.5 months of age. After 16 days in DD, mice received daily s.c. injections of PF-670462 (10 or 30 mg/kg/d) or vehicle for 9 days in DD. n = 3 mice per treatment group.

(B) Representative actograms of wheel-running activities under different lighting conditions of ~8-month-old 3xTg-AD mice treated s.c. daily with vehicle, PF-670462 10 mg/kg/d, or PF-670462 30 mg/kg/d. The timing of treatment is indicated by red bars. Missing data due to computer failure are indicated by an asterisk.

(C) Free-running period under DD before and during treatment of 3xTg-AD mice with vehicle, PF-670462 10 mg/kg/d, or PF-670462 30 mg/kg/d (**$p < 0.01$ versus vehicle during treatment, one-way ANOVA with Fisher's LSD *post hoc* test; *$p < 0.05$ versus baseline, two-tailed paired Student's *t*-test).

(D) Change in free-running period under DD from pre-treatment baseline (*$p < 0.05$, **$p < 0.01$ versus vehicle, one-way ANOVA with Fisher's LSD).

Data are represented as means + SEM.

actograms of running wheel activity (**Figure 3.7 B**). Daily treatment with PF-670462, but not vehicle, resulted in lengthening of the free-running period by 0.5 ± 0.26 h and 1.3 ± 0.18 h in 3xTg-AD mice treated with 10 and 30 mg/kg/d PF-670462, respectively (**Figure 3.7 C,D**). Thus, PF-670462 treatment altered circadian locomotor activity patterns in 3xTg-AD mice, suggesting that CK1δ/ε inhibition might be capable of reversing AD-related circadian disturbances.

Next, we tested whether PF-670462 can re-establish normal behavioural circadian rhythms when administered daily at a moderate dose (20 mg/kg/d) in 3xTg-AD mice. At ~11 months of age, LD-entrained 3xTg-AD and NTg mice were transferred to DD and their baseline free-running periods determined prior to treatment. Mice were then administered PF-670462 (20 mg/kg/d) or vehicle daily at CT12, as described above (**Figure 3.8 A**). At baseline, 3xTg-AD mice had shortened free-running periods relative to NTg mice (NTg, 23.96 ± 0.02 h; 3xTg-AD, 23.65 ± 0.07 h; **Figure 3.8 B,C**), in line with previous studies (Sterniczuk et al., 2010). Middle-aged NTg mice had free-running periods similar to those seen in aged wild-type mice (Valentinuzzi et al., 1997), resulting in daily activity onsets occurring at approximately the same time each evening, while 3xTg-AD mice displayed irregular activity onsets (**Figure 3.8 D**). Treatment with PF-670462 significantly lengthened the free-running period of NTg mice, as expected (Meng et al., 2010), and also immediately reversed the shortened period in 3xTg-AD mice (**Figure 3.8 E–G**). Furthermore, 3xTg-AD mice treated with PF-670462, but not vehicle, exhibited rapid and stable entrainment of locomotor activity, with regular daily activity onset approximately 18 h after dosing time (**Figure 3.8 B,H**). Thus, daily treatment with PF-670462 normalized disrupted behavioural circadian rhythms associated with AD-like pathology in 3xTg-AD mice. Together, our findings suggest that CK1δ/ε inhibition and, more broadly, direct circadian clock modulation represent viable therapeutic approaches to treat AD-related sleep and circadian disturbances.

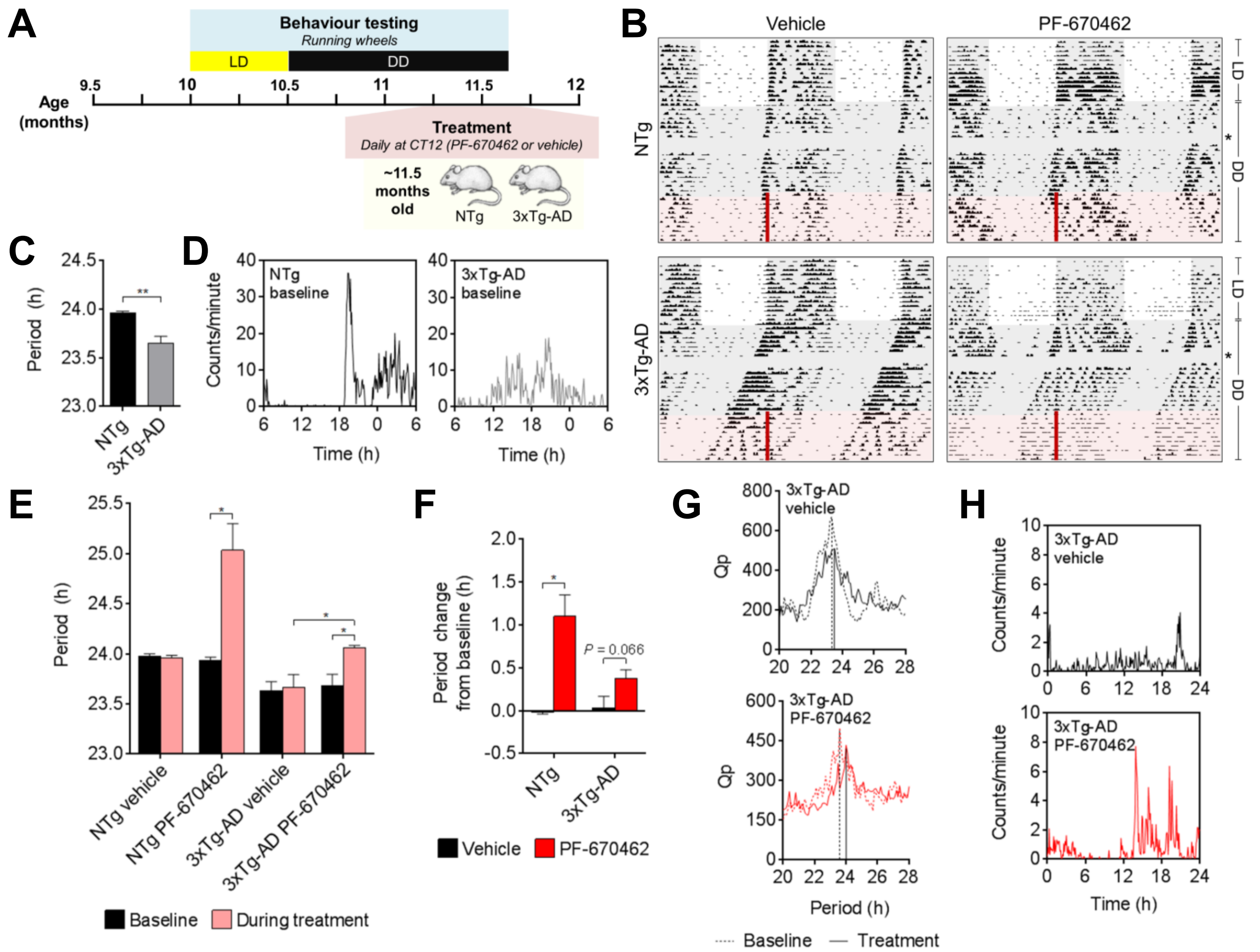

A
Behaviour testing
Running wheels
LD
DD
Age (months)
9.5
10
10.5
11
11.5
12
Treatment
Daily at CT12 (PF-670462 or vehicle)
~11.5 months old
NTg
3xTg-AD
B
Vehicle
PF-670462
NTg
3xTg-AD
LD
DD
C
Period (h)
NTg
3xTg-AD
D
Counts/minute
NTg baseline
3xTg-AD baseline
Time (h)
E
Period (h)
NTg vehicle
NTg PF-670462
3xTg-AD vehicle
3xTg-AD PF-670462
Baseline
During treatment
F
Period change from baseline (h)
P = 0.066
NTg
3xTg-AD
Vehicle
PF-670462
G
Qp
3xTg-AD vehicle
3xTg-AD PF-670462
Period (h)
Baseline
Treatment
H
Counts/minute
3xTg-AD vehicle
3xTg-AD PF-670462
Time (h)

Figure 3.8. PF-670462 administration normalizes disrupted behavioural circadian rhythms in 3xTg-AD mice.

(A) Study design: Wheel-running activities of NTg and 3xTg-AD mice were recorded for 2 weeks in LD, then mice were released into DD at ~10.5 months of age. After 3 weeks in DD, mice received daily injections of PF-670462 (20 mg/kg/d) or vehicle for 10 days in DD. n = 5 NTg vehicle; n = 3 NTg PF-670462; n = 6 3xTg-AD vehicle; n = 5 3xTg-AD PF-670462.

(B) Representative actograms of wheel-running activities under different lighting conditions of ~11-month-old NTg and 3xTg-AD mice treated with PF-670462 (20 mg/kg/d) or vehicle daily. The timing of treatment is indicated by red bars. Missing data due to computer failure are indicated by an asterisk.

(C) Baseline free-running period under DD of NTg (n = 8) and 3xTg-AD (n = 11) mice (**$p < 0.01$, two-tailed unpaired Student's *t*-test with Welch's correction).

(D) Baseline 24-hour activity profiles under DD of NTg (n = 8) and 3xTg-AD (n = 11) mice. Profiles reflect the mean daily activity across 11 days in DD prior to beginning treatment.

(E) Free-running period under DD before and during treatment of NTg and 3xTg-AD mice with PF-670462 or vehicle (*$p < 0.05$ baseline versus during treatment, two-tailed paired Student's *t*-test; *$p < 0.05$ vehicle versus PF-670462, two-tailed unpaired Student's *t*-test with Welch's correction).

(F) Change in free-running period under DD from pre-treatment baseline of NTg and 3xTg-AD mice treated with PF-670462 or vehicle (*$p < 0.05$, two-tailed unpaired Student's *t*-test with Welch's correction).

(G) Chi-square periodogram analysis of wheel-running activities of 3xTg-AD mice treated with PF-670462 or vehicle. Representative periodograms of 3xTg-AD mice under DD before and during treatment with vehicle or PF-670462, showing change in period with PF-670462 but not vehicle.

(H) 24-hour activity profiles under DD of 3xTg-AD mice treated with vehicle or PF-670462. Profiles reflect the mean daily activity across the entire dosing period.

Data in panels C, E, and F are represented as means + SEM.

3.4. Discussion

An estimated 47 million people worldwide are living with dementia, the most common cause of which is AD (Livingston et al., 2017). There is an urgent need for new therapies that address not only cognitive impairment but also behavioural symptoms commonly seen in AD, including sleep disturbances. However, drug development for AD has proven to be very challenging, evidenced by the fact that there have been no new drugs approved since 2003 for treatment of the disease (Cummings et al., 2018). The multifactorial basis of AD pathogenesis indicates that therapies targeting multiple causal factors will likely be most effective (Huang and Mucke, 2012). Memory, sleep, and neurodegeneration are regulated by the circadian clock (Musiek and Holtzman, 2016), suggesting that it is a viable therapeutic target against AD. In light of this, CK1δ/ε inhibition represents a feasible potential disease-modifying approach to treat AD, based on evidence that CK1δ/ε are markedly overexpressed in AD brains, play critical roles in regulating the circadian clock, and might also be involved in tau phosphorylation and controlling Aβ production (Lee et al., 2009; Perez et al., 2011).

In this study, daily administration of the small molecule CK1δ/ε inhibitor PF-670462 rescued hippocampal-dependent working memory deficits and normalized disrupted behavioural circadian rhythms in middle-aged 3xTg-AD mice. Our findings are consistent with recent work showing that CK1ε overexpression in mouse hippocampus impairs working memory (Chen et al., 2017) and build on previous studies demonstrating that PF-670462 can stabilize circadian rhythms in genetic and environmental models of circadian dysfunction in mice (Meng et al., 2010). PF-670462 administration enhanced working memory in 3xTg-AD mice and also resulted in significant proteomic reprogramming in the hippocampus, suggesting that PF-670462–induced changes in a number of pathways critical for neuronal function and cellular homeostasis might have contributed to improvements in hippocampal function. Additional studies are needed to explore the effects

of CK1δ/ε inhibition on long-term memory, which we were unable to evaluate with the cognitive tests used in the present study.

CK1δ/ε inhibition *in vitro* and *in vivo* altered the expression of proteins involved in diverse processes implicated in AD pathogenesis and hippocampal function, including synaptic plasticity and transmission, redox homeostasis, and APP processing. Notably, PF-670462 administration in 3xTg-AD mice restored the hippocampal expression of multiple proteins involved in these AD-related processes towards NTg levels, thereby partially rescuing protein abundance changes associated with AD-like pathology. The effects of PF-670462 on the hippocampal proteome are likely in part due to modulation of circadian regulation at the levels of the hippocampal clock as well as central SCN pacemaker (Smarr et al., 2014). In keeping with this, PF-670462 administration induced changes in distinct clock-regulated processes in the hippocampus of 3xTg-AD mice at different time points. Moreover, the circadian system is known to regulate hippocampal LTP and other processes that affect memory and cognitive function, including neurogenesis and epigenetic control of gene expression (Gerstner and Yin, 2010; Smarr et al., 2014). Further studies are needed to examine the effects of CK1δ/ε inhibition on hippocampal morphology and circadian regulation of hippocampal neurophysiology in the context of AD, as well as to determine the relative contributions of changes in local hippocampal clock function, systemic circadian control, and clock-independent regulation associated with CK1δ/ε inhibition (Gerstner and Yin, 2010).

There are currently no treatments available that have definitive evidence of effectiveness in treating AD-related sleep disturbances (Livingston et al., 2017). Given that sleep disturbances are a common symptom and major reason for institutionalization in AD (Coogan et al., 2013), there is a critical need for new therapies that act on underlying pathogenic mechanisms to treat these symptoms as well as prevent, halt, or reverse the disease. Multiple lines of evidence suggest that the circadian clock is a feasible therapeutic

target to treat sleep disturbances in AD, including the AD-related SCN neurodegeneration linked to circadian and sleep dysfunction (Musiek and Holtzman, 2016) as well as the finding that direct clock modulation can restore defective circadian behaviour in mice (Meng et al., 2010). The effectiveness of PF-670462 in normalizing behavioural circadian rhythm disturbances in middle-aged 3xTg-AD mice provides evidence in support of this hypothesis. Furthermore, PF-670462 has previously been shown to be well-tolerated and produce the same effects on behavioural circadian rhythms in a diurnal non-human primate (Sprouse et al., 2009) as in mice. Thus, this small molecule CK1δ/ε inhibitor represents a promising approach to normalize AD-related sleep and circadian disturbances in humans and could be further optimized to develop more highly potent, selective, and bioavailable drugs for the treatment of AD. Several efforts are already underway to synthesize and optimize different small molecule CK1δ/ε inhibitors for various therapeutic applications and use in basic research, including compounds with enhanced discrimination of δ and ε isoforms (Bibian et al., 2013; Halekotte et al., 2017; Monastyrskyi et al., 2018). Additional preclinical studies are needed to characterize the effects of these newer, more selective and potent CK1δ/ε inhibitors on circadian disturbances, as well as cognitive impairment, in the context of AD.

In summary, treatment of 3xTg-AD mice with the CK1δ/ε inhibitor PF-670462 rescued working memory deficits, normalized behavioural circadian rhythm disturbances, and reversed hippocampal proteomic alterations in diverse AD-related pathways. Collectively, our findings suggest that CK1δ/ε inhibition or, more broadly, direct circadian clock modulation has neuroprotective disease-modifying potential and represents a viable therapeutic avenue to treat cognitive impairment and sleep disturbances in people with AD.

Chapter 4. Conclusion

4.1. Key findings

- Global effects of aging on the circadian regulation of protein abundances in the hippocampus of C57BL/6J mice, suggesting that the aging-associated circadian decline contributes to cognitive deterioration over time and increased risk of developing several neurodegenerative diseases (section 2.3).
- Global proteomic effects of PF-670462 treatment *in vitro* on N2a cells and *in vivo* on the hippocampus of 3xTg-AD mice, suggesting that CK1δ/ε inhibition can reverse AD-associated proteomic alterations and has neuroprotective disease-modifying potential (sections 3.3.1 and 3.3.2). PF-670462 administration *in vivo* can alter the expression of multiple proteins displaying circadian expression profiles in the hippocampus (identified in section 2), suggesting that CK1δ/ε inhibition is likely exerting many of its beneficial effects via clock-dependent mechanisms.
- Effects of PF-670462 treatment on cognitive performance in 3xTg-AD mice, suggesting that CK1δ/ε inhibition can rescue working memory deficits associated with AD-like pathology and represents a new avenue to potentially treat cognitive impairment in people with AD (section 3.3.3).
- Effects of PF-670462 treatment on locomotor activity in 3xTg-AD mice, suggesting that CK1δ/ε inhibition can normalize behavioural circadian rhythm disturbances associated with AD-like pathology and represents a new avenue to potentially treat sleep and circadian rhythm disturbances in people with AD (section 3.3.4).

4.2. Significance and future directions

Altogether, these findings provide support for roles of the circadian clock in hippocampal aging and as a therapeutic target in AD. Moreover, these studies provide a framework for understanding the links between age-related memory decline, neurodegenerative brain diseases, and the circadian system. The aging-associated changes identified in the circadian regulation of biological processes underlying memory function in the hippocampus could open new avenues to restore dysregulated rhythms in the brain and thereby mitigate the impact of age-related cognitive decline. These findings also support the notion that interventions to maintain robust circadian rhythms throughout life, including by improving sleep (Sulli et al., 2018a), could promote healthy brain aging while helping to prevent the development of aging-associated neurodegenerative brain diseases such as AD. In addition, our findings regarding the beneficial effects of PF-670462 administration in a preclinical model of AD indicate that pharmacological CK1δ/ε inhibition or, more broadly, direct circadian clock modulation is a promising approach for treating AD, with the potential to both exert neuroprotective disease-modifying effects as well as provide symptomatic relief of cognitive symptoms and sleep disturbances in people with AD. It would be interesting for future work to examine how administration of other small molecule circadian clock modifiers in preclinical models of AD affects diverse AD-associated pathways, cognitive function, and circadian disturbances. Recent studies from others have provided evidence for the efficacy of targeting REV-ERBs to alleviate cognitive deficits in an accelerated aging mouse model (SAMP8), as well as to reduce the Aβ burden in these mice and in the 5XFAD mouse model of AD (Lee et al., 2019; Roby et al., 2019). Broadly, our results highlight the complex and dynamic nature of biological systems, as well as the potential consequences of disregarding time of day in biological and medical research (Smolensky and Peppas, 2007).

There are several limitations of these studies that could be addressed in future investigations. First, we may consider the generalizability of findings made in mice (a nocturnal species with a short lifespan) to humans (a diurnal species with a longer lifespan). The high degree of conservation of circadian mechanisms across mammalian species (Takahashi et al., 2008) supports the use of mice as a model for the human circadian system, although it would be interesting for future studies to examine how aging impacts circadian rhythms at the protein level in the human brain, similar to what Chen and colleagues have previously done at the RNA level in the human prefrontal cortex (Chen et al., 2016). Second, it has been shown that there are sex-related differences in aging-associated changes in the circadian system (Hood and Amir, 2017a) as well as in AD symptomatology, progression, and risk factor profiles (Ferretti et al., 2018). Moreover, 3xTg-AD mice have been shown to display sex-related differences in neuropathology, cognitive performance, and behaviour (Clinton et al., 2007; Sterniczuk et al., 2010; Belfiore et al., 2019). Although we used male C57BL/6J mice (Chapter 2) and mainly female 3xTg-AD mice (Chapter 3) in the studies we have reported here, future studies investigating whether our findings can be replicated in female C57BL/6J mice as well as male 3xTg-AD mice may help elucidate sex-related differences in AD treatment and how aging affects the brain. Third, although our hippocampal proteomic analyses involved examining the whole hippocampus, cell type–specific and subregion-specific molecular signatures have been previously described (Lein et al., 2004; Zeisel et al., 2015), suggesting that exploring more specific changes within the hippocampus in the context of the circadian system could be a future area for investigation. Furthermore, it has been shown that hippocampal subregions are differentially affected during aging (Bettio et al., 2017). Fourth, it would be interesting to conduct proteomic analyses on different subcellular fractions, especially given recent studies showing how subcellular fractionation can impact the detection of proteins displaying circadian rhythms in abundance in the mitochondria (Neufeld-Cohen et al., 2016) and

nucleus (Wang et al., 2017). Fifth, the inclusion of cognitive testing to enable us to associate alterations in the circadian hippocampal proteome more specifically with hippocampal-dependent memory impairment might have allowed us to provide stronger evidence regarding links between alterations at the proteomic level and age-related memory impairment, similar to what others have done in previous studies (Freeman et al., 2009; Neuner et al., 2017). Sixth, given that circadian phase can have significant impacts both on differential expression analysis (Hsu and Harmer, 2012) and performance on rodent hippocampal-dependent memory tasks (Snider et al., 2018), it would be interesting to examine the effects of PF-670462 administration on proteomic changes and cognitive function at a greater number of different time points. However, working memory assessed by spontaneous alternation behaviour in the Y-maze has previously been shown to not be modulated by time of day in mice (Snider and Obrietan, 2018), suggesting that the improvement in working memory associated with PF-670462 administration in 3xTg-AD mice we have reported is not specific to the time of day at which we tested our mice. Seventh, given studies from our lab and others highlighting the contributions of circadian rhythms in protein phosphorylation (Chiang et al., 2017; Robles et al., 2017) and that CK1δ/ε inhibition would also impact protein phosphorylation levels, exploring phosphoproteomic alterations in the brain with PF-670462 treatment or with aging over the circadian cycle would be interesting avenues for future investigation. Eighth, there is debate regarding the generalizability of findings made in 3xTg-AD mice (and indeed in models of disease in general) to AD in humans. Despite their current limitations and issues regarding translatability, animal models of AD remain, at least for the foreseeable future, instrumental in identifying potential therapies for the disease (Dawson et al., 2018). Lastly, future studies to validate with orthogonal methods some of the proteomic alterations we have identified in the hippocampus with PF-670462 administration and/or aging could be valuable, both in

supporting our findings as well as in focusing future investigations that may aim to translate some of our discoveries into clinical applications.

Aging-associated cognitive impairment and AD have profound global consequences, including significant health, social, and economic impacts. A better understanding of how age-related circadian dysfunction contributes to aging being a primary risk factor for several neurodegenerative diseases could help enable the rational design and evaluation of new therapies aimed at addressing the cognitive impairment as well as sleep and circadian rhythm disturbances in people living with these diseases. The first part of this thesis represents the first study to explore the global effects of aging on the mammalian hippocampal proteome from a circadian perspective. Future studies may build on our findings not only by investigating whether targeting dysregulated hippocampal rhythms could mitigate age-related cognitive impairment, but also by assessing the contribution of these dysregulated rhythms to impaired hippocampal function in normal aging as well as in age-related neurodegenerative diseases that affect the hippocampus, such as AD.

Similarly, additional studies examining the effects of targeting dysregulated circadian rhythms more broadly are also needed to further explore the therapeutic potential of circadian rhythm modulation in treating aging- and disease-associated cognitive impairment and sleep disturbances. The second part of this thesis provides *in vivo* proof of concept for the efficacy of direct circadian clock modulation in a transgenic mouse model of AD. Our focus here was on the effects of PF-670462 administration on behavioural circadian rhythms, the hippocampal proteome, and working memory in 3xTg-AD mice, with potential implications for addressing major unmet clinical needs in people with AD, namely alleviation of sleep disturbances, disease pathology, and cognitive impairment. PF-670462 administration in 3xTg-AD mice partially normalized the expression of multiple proteins that displayed loss of circadian rhythmicity in abundance in the hippocampus of middle-aged mice, underscoring the interplay between the circadian clock, aging, and AD.

In conclusion, these studies highlight emerging roles for the circadian clock in brain aging and as a therapeutic target for neurodegenerative brain disorders. Given the substantial contribution of age-related diseases and dementia towards the global burden of disease, as well as their significant social and economic impacts, there is a pressing need to address these complex and partly preventable public health issues. By simultaneously targeting multiple hallmarks of aging and potential contributory causes of neurodegeneration, circadian clock modulation may represent a promising new strategy to promote healthy brain aging and prevent, halt, or reverse neurodegenerative diseases and dementia. In addition to continued efforts aimed at discovering effective therapies for these problems, it will be critical to allocate resources towards developing, implementing, and evaluating a combination of preventative interventions guided by knowledge of these conditions' risk and protective factors as well as their underlying social determinants.

References

Abel, T. and Lattal, K.M. (2001). Molecular mechanisms of memory acquisition, consolidation and retrieval. Curr. Opin. Neurobiol. *11*, 180-187.

Akhtar, R.A., Reddy, A.B., Maywood, E.S., Clayton, J.D., King, V.M., Smith, A.G., Gant, T.W., Hastings, M.H. and Kyriacou, C.P. (2002). Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. Curr. Biol. *12*, 540-550.

Albrecht, U. (2012). Timing to perfection: the biology of central and peripheral circadian clocks. Neuron *74*, 246-260.

Amaral, D.G. (1993). Emerging principles of intrinsic hippocampal organization. Curr. Opin. Neurobiol. *3*, 225-229.

Amaral, D. and Lavenex, P. (2007). Hippocampal Neuroanatomy. In *The Hippocampus Book* (1st ed.), Andersen, P., Morris, R., Amaral, D., Bliss, T. and O'Keefe, J., eds. New York, USA: Oxford University Press.

Andersen, P., Morris, R., Amaral, D., Bliss, T. and O'Keefe, J. (2007). The Hippocampal Formation. In *The Hippocampus Book* (1st ed.), Andersen, P., Morris, R., Amaral, D., Bliss, T. and O'Keefe, J., eds. New York, USA: Oxford University Press.

Andersen, P., Morris, R., Amaral, D., Bliss, T. and O'Keefe, J. (2007). Historical Perspective: Proposed Functions, Biological Characteristics, and Neurobiological Models of the Hippocampus. In *The Hippocampus Book* (1st ed.), Andersen, P., Morris, R., Amaral, D., Bliss, T. and O'Keefe, J., eds. New York, USA: Oxford University Press.

Annese, J., Schenker-Ahmed, N.M., Bartsch, H., Maechler, P., Sheh, C., Thomas, N., Kayano, J., Ghatan, A., Bresler, N., Frosch, M.P.*, et al.* (2014). Postmortem examination of patient H.M.'s brain based on histological sectioning and digital 3D reconstruction. Nat. Commun. *5*, 3122.

Antoniadis, E.A., Ko, C.H., Ralph, M.R. and McDonald, R.J. (2000). Circadian rhythms, aging and memory. Behav. Brain Res. *114*, 221-233.

Archer, S.N., Laing, E.E., Moller-Levet, C.S., van der Veen, D.R., Bucca, G., Lazar, A.S., Santhi, N., Slak, A., Kabiljo, R., von Schantz, M.*, et al.* (2014). Mistimed sleep disrupts circadian regulation of the human transcriptome. Proc. Natl. Acad. Sci. U. S. A. *111*, E682-691.

Arellanes-Licea, E., Caldelas, I., De Ita-Perez, D. and Diaz-Munoz, M. (2014). The circadian timing system: a recent addition in the physiological mechanisms underlying pathological and aging processes. Aging and disease *5*, 406-418.

Arey, R. and McClung, C.A. (2012). An inhibitor of casein kinase 1 epsilon/delta partially normalizes the manic-like behaviors of the ClockDelta19 mouse. Behav. Pharmacol. *23*, 392-396.

Aristotle. Metaphysics. In *Aristotle I* (2nd ed.), M.J. Adler, ed. Chicago, USA: Encyclopaedia Britannica, Inc.

Asai, M., Yoshinobu, Y., Kaneko, S., Mori, A., Nikaido, T., Moriya, T., Akiyama, M. and Shibata, S. (2001). Circadian profile of Per gene mRNA expression in the suprachiasmatic nucleus, paraventricular nucleus, and pineal body of aged rats. J. Neurosci. Res. *66*, 1133-1139.

Ashbrook, D.G., Williams, R.W., Lu, L., Stein, J.L., Hibar, D.P., Nichols, T.E., Medland, S.E., Thompson, P.M. and Hager, R. (2014). Joint genetic analysis of hippocampal size in mouse and human identifies a novel gene linked to neurodegenerative disease. BMC Genomics *15*, 850.

Aujard, F., Herzog, E.D. and Block, G.D. (2001). Circadian rhythms in firing rate of individual suprachiasmatic nucleus neurons from adult and middle-aged mice. Neuroscience *106*, 255-261.

Bachurin, S.O., Bovina, E.V. and Ustyugov, A.A. (2017). Drugs in Clinical Trials for Alzheimer's Disease: The Major Trends. Med. Res. Rev. *37*, 1186-1225.

Badura, L., Swanson, T., Adamowicz, W., Adams, J., Cianfrogna, J., Fisher, K., Holland, J., Kleiman, R., Nelson, F., Reynolds, L.*, et al.* (2007). An inhibitor of casein kinase I epsilon induces phase delays in circadian rhythms under free-running and entrained conditions. J. Pharmacol. Exp. Ther. *322*, 730-738.

Baker, M.J., Webb, C.T., Stroud, D.A., Palmer, C.S., Frazier, A.E., Guiard, B., Chacinska, A., Gulbis, J.M. and Ryan, M.T. (2009). Structural and functional requirements for activity of the Tim9-Tim10 complex in mitochondrial protein import. Mol. Biol. Cell *20*, 769-779.

Balin, B.J. and Hudson, A.P. (2014). Etiology and pathogenesis of late-onset Alzheimer's disease. Curr. Allergy Asthma Rep. *14*, 417.

Ballard, C., Khan, Z., Clack, H. and Corbett, A. (2011). Nonpharmacological treatment of Alzheimer disease. Can. J. Psychiatry *56*, 589-595.

Barca-Mayo, O., Pons-Espinal, M., Follert, P., Armirotti, A., Berdondini, L. and De Pietri Tonelli, D. (2017). Astrocyte deletion of Bmal1 alters daily locomotor activity and cognitive functions via GABA signalling. Nat. Commun. *8*, 14336.

Barnes, C.A., Rao, G. and Houston, F.P. (2000). LTP induction threshold change in old rats at the perforant path--granule cell synapse. Neurobiol. Aging *21*, 613-620.

Barnes, D.E. and Yaffe, K. (2011). The projected effect of risk factor reduction on Alzheimer's disease prevalence. Lancet Neurol. *10*, 819-828.

Bartsch, T. and Wulff, P. (2015). The hippocampus in aging and disease: From plasticity to vulnerability. Neuroscience *309*, 1-16.

Bartus, R.T., Dean, R.L., 3rd, Beer, B. and Lippa, A.S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. Science *217*, 408-414.

Bass, J. and Lazar, M.A. (2016). Circadian time signatures of fitness and disease. Science *354*, 994-999.

Beard, J.R., Officer, A., de Carvalho, I.A., Sadana, R., Pot, A.M., Michel, J.P., Lloyd-Sherlock, P., Epping-Jordan, J.E., Peeters, G., Mahanani, W.R.*, et al.* (2016). The World report on ageing and health: a policy framework for healthy ageing. Lancet *387*, 2145-2154.

Belfiore, R., Rodin, A., Ferreira, E., Velazquez, R., Branca, C., Caccamo, A. and Oddo, S. (2019). Temporal and regional progression of Alzheimer's disease-like pathology in 3xTg-AD mice. Aging Cell *18*, e12873.

Bell-Pedersen, D., Cassone, V.M., Earnest, D.J., Golden, S.S., Hardin, P.E., Thomas, T.L. and Zoran, M.J. (2005). Circadian rhythms from multiple oscillators: lessons from diverse organisms. Nat. Rev. Genet. *6*, 544-556.

Bellanti, F., Iannelli, G., Blonda, M., Tamborra, R., Villani, R., Romano, A., Calcagnini, S., Mazzoccoli, G., Vinciguerra, M., Gaetani, S.*, et al.* (2017). Alterations of Clock Gene RNA Expression in Brain Regions of a Triple Transgenic Model of Alzheimer's Disease. J. Alzheimers Dis. *59*, 615-631.

Bellesi, M., Haswell, J.D., de Vivo, L., Marshall, W., Roseboom, P.H., Tononi, G. and Cirelli, C. (2018). Myelin modifications after chronic sleep loss in adolescent mice. Sleep *41*,

Bellesi, M., Pfister-Genskow, M., Maret, S., Keles, S., Tononi, G. and Cirelli, C. (2013). Effects of sleep and wake on oligodendrocytes and their precursors. J. Neurosci. *33*, 14288-14300.

Bertram, L., Lill, C.M. and Tanzi, R.E. (2010). The genetics of Alzheimer disease: back to the future. Neuron *68*, 270-281.

Besing, R.C., Rogers, C.O., Paul, J.R., Hablitz, L.M., Johnson, R.L., McMahon, L.L. and Gamble, K.L. (2017). GSK3 activity regulates rhythms in hippocampal clock gene expression and synaptic plasticity. Hippocampus *27*, 890-898.

Bettio, L.E.B., Rajendran, L. and Gil-Mohapel, J. (2017). The effects of aging in the hippocampus and cognitive decline. Neurosci. Biobehav. Rev. *79*, 66-86.

Beynon, A.L. and Coogan, A.N. (2010). Diurnal, age, and immune regulation of interleukin-1beta and interleukin-1 type 1 receptor in the mouse suprachiasmatic nucleus. Chronobiol. Int. *27*, 1546-1563.

Beynon, A.L., Thome, J. and Coogan, A.N. (2009). Age and time of day influences on the expression of transforming growth factor-beta and phosphorylated SMAD3 in the mouse suprachiasmatic and paraventricular nuclei. Neuroimmunomodulation *16*, 392-399.

Beyreuther, K. and Masters, C.L. (1991). Amyloid precursor protein (APP) and beta A4 amyloid in the etiology of Alzheimer's disease: precursor-product relationships in the derangement of neuronal function. Brain Pathol. *1*, 241-251.

Bhattacharya, S., Herrera-Molina, R., Sabanov, V., Ahmed, T., Iscru, E., Stober, F., Richter, K., Fischer, K.D., Angenstein, F., Goldschmidt, J.*, et al.* (2017). Genetically Induced Retrograde Amnesia of Associative Memories After Neuroplastin Ablation. Biol. Psychiatry *81*, 124-135.

Bibian, M., Rahaim, R.J., Choi, J.Y., Noguchi, Y., Schurer, S., Chen, W., Nakanishi, S., Licht, K., Rosenberg, L.H., Li, L.*, et al.* (2013). Development of highly selective casein kinase 1delta/1epsilon (CK1delta/epsilon) inhibitors with potent antiproliferative properties. Bioorg. Med. Chem. Lett. *23*, 4374-4380.

Bierman, E.J., Comijs, H.C., Jonker, C., Scheltens, P. and Beekman, A.T. (2009). The effect of anxiety and depression on decline of memory function in Alzheimer's disease. Int. Psychogeriatr. *21*, 1142-1147.

Bird, C.M. and Burgess, N. (2008). The hippocampus and memory: insights from spatial processing. Nat. Rev. Neurosci. *9*, 182-194.

Birks, J. (2006). Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst. Rev. CD005593.

Bishop, N.A., Lu, T. and Yankner, B.A. (2010). Neural mechanisms of ageing and cognitive decline. Nature *464*, 529-535.

Blake, M.R., Holbrook, S.D., Kotwica-Rolinska, J., Chow, E.S., Kretzschmar, D. and Giebultowicz, J.M. (2015). Manipulations of amyloid precursor protein cleavage disrupt the circadian clock in aging Drosophila. Neurobiol. Dis. *77*, 117-126.

Blatter, K. and Cajochen, C. (2007). Circadian rhythms in cognitive performance: methodological constraints, protocols, theoretical underpinnings. Physiol. Behav. *90*, 196-208.

Blennow, K., de Leon, M.J. and Zetterberg, H. (2006). Alzheimer's disease. Lancet *368*, 387-403.

Bliss, T.V. and Collingridge, G.L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. Nature *361*, 31-39.

Bliss, T.V. and Collingridge, G.L. (2013). Expression of NMDA receptor-dependent LTP in the hippocampus: bridging the divide. Mol. Brain *6*, 5.

Blundon, J.A. and Zakharenko, S.S. (2008). Dissecting the components of long-term potentiation. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry *14*, 598-608.

Boggs, K.N., Kakalec, P.A., Smith, M.L., Howell, S.N. and Flinn, J.M. (2017). Circadian wheel running behavior is altered in an APP/E4 mouse model of late onset Alzheimer's disease. Physiol. Behav. *182*, 137-142.

Bolk, N., Visser, T.J., Nijman, J., Jongste, I.J., Tijssen, J.G. and Berghout, A. (2010). Effects of evening vs morning levothyroxine intake: a randomized double-blind crossover trial. Arch. Intern. Med. *170*, 1996-2003.

Bollinger, T. and Schibler, U. (2014). Circadian rhythms - from genes to physiology and disease. Swiss Med. Wkly. *144*, w13984.

Bonaconsa, M., Malpeli, G., Montaruli, A., Carandente, F., Grassi-Zucconi, G. and Bentivoglio, M. (2014). Differential modulation of clock gene expression in the suprachiasmatic nucleus, liver and heart of aged mice. Exp. Gerontol. *55*, 70-79.

Borovok, N., Nesher, E., Levin, Y., Reichenstein, M., Pinhasov, A. and Michaelevski, I. (2016). Dynamics of Hippocampal Protein Expression During Long-term Spatial Memory Formation. Mol. Cell. Proteomics *15*, 523-541.

Bouchard-Cannon, P., Mendoza-Viveros, L., Yuen, A., Kaern, M. and Cheng, H.Y. (2013). The circadian molecular clock regulates adult hippocampal neurogenesis by controlling the timing of cell-cycle entry and exit. Cell Rep *5*, 961-973.

Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H. and Del Tredici, K. (2006). Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol *112*, 389-404.

Braak, H. and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol *82*, 239-259.

Brancaccio, M., Edwards, M.D., Patton, A.P., Smyllie, N.J., Chesham, J.E., Maywood, E.S. and Hastings, M.H. (2019). Cell-autonomous clock of astrocytes drives circadian behavior in mammals. Science *363*, 187-192.

Brancaccio, M., Patton, A.P., Chesham, J.E., Maywood, E.S. and Hastings, M.H. (2017). Astrocytes Control Circadian Timekeeping in the Suprachiasmatic Nucleus via Glutamatergic Signaling. Neuron

Brayne, C. and Calloway, P. (1988). Normal ageing, impaired cognitive function, and senile dementia of the Alzheimer's type: a continuum? Lancet *1*, 1265-1267.

Breitner, J.C. (2006). Dementia--epidemiological considerations, nomenclature, and a tacit consensus definition. J. Geriatr. Psychiatry Neurol. *19*, 129-136.

Broms, U., Pitkaniemi, J., Backmand, H., Heikkila, K., Koskenvuo, M., Peltonen, M., Sarna, S., Vartiainen, E., Kaprio, J. and Partonen, T. (2014). Long-term consistency of diurnal-type preferences among men. Chronobiol. Int. *31*, 182-188.

Bruel-Jungerman, E., Davis, S. and Laroche, S. (2007). Brain plasticity mechanisms and memory: a party of four. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry *13*, 492-505.

Bubu, O.M., Brannick, M., Mortimer, J., Umasabor-Bubu, O., Sebastiao, Y.V., Wen, Y., Schwartz, S., Borenstein, A.R., Wu, Y., Morgan, D.*, et al.* (2017). Sleep, Cognitive impairment, and Alzheimer's disease: A Systematic Review and Meta-Analysis. Sleep *40*,

Bunsey, M. and Eichenbaum, H. (1996). Conservation of hippocampal memory function in rats and humans. Nature *379*, 255-257.

Burgess, N., Maguire, E.A. and O'Keefe, J. (2002). The human hippocampus and spatial and episodic memory. Neuron *35*, 625-641.

Burre, J. and Volknandt, W. (2007). The synaptic vesicle proteome. J. Neurochem. *101*, 1448-1462.

Buscemi, N., Vandermeer, B., Hooton, N., Pandya, R., Tjosvold, L., Hartling, L., Baker, G., Klassen, T.P. and Vohra, S. (2005). The efficacy and safety of exogenous melatonin for primary sleep disorders. A meta-analysis. J. Gen. Intern. Med. *20*, 1151-1158.

Cajal, S.R. (1894). The Croonian Lecture: La fine structure des centres nerveux. Proc. R. Soc. London *55*, 444-467.

Cajochen, C., Krauchi, K. and Wirz-Justice, A. (2003). Role of melatonin in the regulation of human circadian rhythms and sleep. J. Neuroendocrinol. *15*, 432-437.

Camandola, S. and Mattson, M.P. (2017). Brain metabolism in health, aging, and neurodegeneration. EMBO J. *36*, 1474-1492.

Camicioli, R., Moore, M.M., Kinney, A., Corbridge, E., Glassberg, K. and Kaye, J.A. (2003). Parkinson's disease is associated with hippocampal atrophy. Mov. Disord. *18*, 784-790.

Cammisuli, D.M., Danti, S., Bosinelli, F. and Cipriani, G. (2016). Non-pharmacological interventions for people with Alzheimer’s Disease: A critical review of the scientific literature from the last ten years. Eur Geriatr Med *7*, 57-64.

Campisi, J. and d'Adda di Fagagna, F. (2007). Cellular senescence: when bad things happen to good cells. Nat. Rev. Mol. Cell Biol. *8*, 729-740.

Carles, F., Bourg, S., Meyer, C. and Bonnet, P. (2018). PKIDB: A Curated, Annotated and Updated Database of Protein Kinase Inhibitors in Clinical Trials. Molecules *23*,

Caroni, P., Donato, F. and Muller, D. (2012). Structural plasticity upon learning: regulation and functions. Nat. Rev. Neurosci. *13*, 478-490.

Castaneda, T.R., de Prado, B.M., Prieto, D. and Mora, F. (2004). Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. J. Pineal Res. *36*, 177-185.

Cedernaes, J., Osorio, R.S., Varga, A.W., Kam, K., Schioth, H.B. and Benedict, C. (2017). Candidate mechanisms underlying the association between sleep-wake disruptions and Alzheimer's disease. Sleep medicine reviews *31*, 102-111.

Cerejeira, J., Lagarto, L. and Mukaetova-Ladinska, E.B. (2012). Behavioral and psychological symptoms of dementia. Frontiers in neurology *3*, 73.

Cermakian, N., Lamont, E.W., Boudreau, P. and Boivin, D.B. (2011). Circadian clock gene expression in brain regions of Alzheimer 's disease patients and control subjects. J. Biol. Rhythms *26*, 160-170.

Chaix, A., Zarrinpar, A. and Panda, S. (2016). The circadian coordination of cell biology. J. Cell Biol. *215*, 15-25.

Chang, A.Y., Skirbekk, V.F., Tyrovolas, S., Kassebaum, N.J. and Dieleman, J.L. (2019). Measuring population ageing: an analysis of the Global Burden of Disease Study 2017. The Lancet. Public health *4*, e159-e167.

Chang, H.C. and Guarente, L. (2013). SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. Cell *153*, 1448-1460.

Chaudhury, D. and Colwell, C.S. (2002). Circadian modulation of learning and memory in fear-conditioned mice. Behav. Brain Res. *133*, 95-108.

Chaudhury, D., Loh, D.H., Dragich, J.M., Hagopian, A. and Colwell, C.S. (2008). Select cognitive deficits in vasoactive intestinal peptide deficient mice. BMC Neurosci. *9*, 63.

Chaudhury, D., Wang, L.M. and Colwell, C.S. (2005). Circadian regulation of hippocampal long-term potentiation. J. Biol. Rhythms *20*, 225-236.

Chauhan, R., Chen, K.F., Kent, B.A. and Crowther, D.C. (2017). Central and peripheral circadian clocks and their role in Alzheimer's disease. Dis. Model. Mech. *10*, 1187-1199.

Chee, C.A., Roozendaal, B., Swaab, D.F., Goudsmit, E. and Mirmiran, M. (1988). Vasoactive intestinal polypeptide neuron changes in the senile rat suprachiasmatic nucleus. Neurobiol. Aging *9*, 307-312.

Chen, C., Gu, J., Basurto-Islas, G., Jin, N., Wu, F., Gong, C.X., Iqbal, K. and Liu, F. (2017). Up-regulation of casein kinase 1epsilon is involved in tau pathogenesis in Alzheimer's disease. Sci Rep *7*, 13478.

Chen, C.Y., Logan, R.W., Ma, T., Lewis, D.A., Tseng, G.C., Sibille, E. and McClung, C.A. (2016). Effects of aging on circadian patterns of gene expression in the human prefrontal cortex. Proc. Natl. Acad. Sci. U. S. A. *113*, 206-211.

Chen, Y.K. and Hsueh, Y.P. (2012). Cortactin-binding protein 2 modulates the mobility of cortactin and regulates dendritic spine formation and maintenance. J. Neurosci. *32*, 1043-1055.

Chen, Z., Yoo, S.H. and Takahashi, J.S. (2013). Small molecule modifiers of circadian clocks. Cell. Mol. Life Sci. *70*, 2985-2998.

Chen, Z., Yoo, S.H. and Takahashi, J.S. (2018). Development and Therapeutic Potential of Small-Molecule Modulators of Circadian Systems. Annu. Rev. Pharmacol. Toxicol. *58*, 231-252.

Chergui, K., Svenningsson, P. and Greengard, P. (2005). Physiological role for casein kinase 1 in glutamatergic synaptic transmission. J. Neurosci. *25*, 6601-6609.

Chiang, C.K., Mehta, N., Patel, A., Zhang, P., Ning, Z., Mayne, J., Sun, W.Y., Cheng, H.Y. and Figeys, D. (2014). The proteomic landscape of the suprachiasmatic nucleus clock reveals large-scale coordination of key biological processes. PLoS Genet. *10*, e1004695.

Chiang, C.K., Xu, B., Mehta, N., Mayne, J., Sun, W.Y., Cheng, K., Ning, Z., Dong, J., Zou, H., Cheng, H.M.*, et al.* (2017). Phosphoproteome Profiling Reveals Circadian Clock Regulation of Posttranslational Modifications in the Murine Hippocampus. Frontiers in neurology *8*, 110.

Cho, K. (2001). Chronic 'jet lag' produces temporal lobe atrophy and spatial cognitive deficits. Nat. Neurosci. *4*, 567-568.

Cho, K., Ennaceur, A., Cole, J.C. and Suh, C.K. (2000). Chronic jet lag produces cognitive deficits. J. Neurosci. *20*, RC66.

Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., Wishart, D.S. and Xia, J. (2018). MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. *46*, W486-W494.

Chun, L.E., Woodruff, E.R., Morton, S., Hinds, L.R. and Spencer, R.L. (2015). Variations in Phase and Amplitude of Rhythmic Clock Gene Expression across Prefrontal Cortex, Hippocampus, Amygdala, and Hypothalamic Paraventricular and Suprachiasmatic Nuclei of Male and Female Rats. J. Biol. Rhythms *30*, 417-436.

Clinton, L.K., Billings, L.M., Green, K.N., Caccamo, A., Ngo, J., Oddo, S., McGaugh, J.L. and LaFerla, F.M. (2007). Age-dependent sexual dimorphism in cognition and stress response in the 3xTg-AD mice. Neurobiol. Dis. *28*, 76-82.

Cohen, M.C., Rohtla, K.M., Lavery, C.E., Muller, J.E. and Mittleman, M.A. (1997). Meta-analysis of the morning excess of acute myocardial infarction and sudden cardiac death. Am. J. Cardiol. *79*, 1512-1516.

Coogan, A.N., Schutova, B., Husung, S., Furczyk, K., Baune, B.T., Kropp, P., Hassler, F. and Thome, J. (2013). The circadian system in Alzheimer's disease: disturbances, mechanisms, and opportunities. Biol. Psychiatry *74*, 333-339.

Coon, K.D., Myers, A.J., Craig, D.W., Webster, J.A., Pearson, J.V., Lince, D.H., Zismann, V.L., Beach, T.G., Leung, D., Bryden, L.*, et al.* (2007). A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. J. Clin. Psychiatry *68*, 613-618.

Corkin, S., Amaral, D.G., Gonzalez, R.G., Johnson, K.A. and Hyman, B.T. (1997). H. M.'s medial temporal lobe lesion: findings from magnetic resonance imaging. J. Neurosci. *17*, 3964-3979.

Cornelissen, G. and Otsuka, K. (2017). Chronobiology of Aging: A Mini-Review. Gerontology *63*, 118-128.

Cortes-Mendoza, J., Diaz de Leon-Guerrero, S., Pedraza-Alva, G. and Perez-Martinez, L. (2013). Shaping synaptic plasticity: the role of activity-mediated epigenetic regulation on gene transcription. Int. J. Dev. Neurosci. *31*, 359-369.

Cowley, A.W., Jr. (2004). The elusive field of systems biology. Physiol. Genomics *16*, 285-286.

Cozza, G. and Pinna, L.A. (2016). Casein kinases as potential therapeutic targets. Expert Opin. Ther. Targets *20*, 319-340.

Craig, L.A., Hong, N.S. and McDonald, R.J. (2011). Revisiting the cholinergic hypothesis in the development of Alzheimer's disease. Neurosci. Biobehav. Rev. *35*, 1397-1409.

Craig, L.A. and McDonald, R.J. (2008). Chronic disruption of circadian rhythms impairs hippocampal memory in the rat. Brain Res. Bull. *76*, 141-151.

Crimmins, E.M. (2015). Lifespan and Healthspan: Past, Present, and Promise. Gerontologist *55*, 901-911.

Crnko, S., Du Pre, B.C., Sluijter, J.P.G. and Van Laake, L.W. (2019). Circadian rhythms and the molecular clock in cardiovascular biology and disease. Nat. Rev. Cardiol.

Cronin, P., McCarthy, M.J., Lim, A.S.P., Salmon, D.P., Galasko, D., Masliah, E., De Jager, P.L., Bennett, D.A. and Desplats, P. (2017). Circadian alterations during early stages of Alzheimer's disease are associated with aberrant cycles of DNA methylation in BMAL1. Alzheimer's & dementia : the journal of the Alzheimer's Association *13*, 689-700.

Crook, T., Bahar, H. and Sudilovsky, A. (1987). Age-associated memory impairment: diagnostic criteria and treatment strategies. Int. J. Neurol. *21-22*, 73-82.

Cummings, J. and Fox, N. (2017). Defining Disease Modifying Therapy for Alzheimer's Disease. The journal of prevention of Alzheimer's disease *4*, 109-115.

Cummings, J., Lee, G., Ritter, A. and Zhong, K. (2018). Alzheimer's disease drug development pipeline: 2018. Alzheimers Dement (N Y) *4*, 195-214.

Cummings, J.L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D.A. and Gornbein, J. (1994). The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. Neurology *44*, 2308-2314.

Cummings, J.L., Morstorf, T. and Zhong, K. (2014). Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimers Res. Ther. *6*, 37.

da Costa, J.P., Vitorino, R., Silva, G.M., Vogel, C., Duarte, A.C. and Rocha-Santos, T. (2016). A synopsis on aging-Theories, mechanisms and future prospects. Ageing Res. Rev. *29*, 90-112.

Dai, R.M. and Li, C.C. (2001). Valosin-containing protein is a multi-ubiquitin chain-targeting factor required in ubiquitin-proteasome degradation. Nat. Cell Biol. *3*, 740-744.

Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F. and Schibler, U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev. *14*, 2950-2961.

Daoudal, G. and Debanne, D. (2003). Long-term plasticity of intrinsic excitability: learning rules and mechanisms. Learn. Mem. *10*, 456-465.

Dardente, H. and Cermakian, N. (2007). Molecular circadian rhythms in central and peripheral clocks in mammals. Chronobiol. Int. *24*, 195-213.

Davidson, A.J., Yamazaki, S., Arble, D.M., Menaker, M. and Block, G.D. (2008). Resetting of central and peripheral circadian oscillators in aged rats. Neurobiol. Aging *29*, 471-477.

Davies, J.A., Navaratnam, V. and Redfern, P.H. (1973). A 24-hour rhythm in passive-avoidance behaviour in rats. Psychopharmacologia *32*, 211-214.

Dawson, T.M., Golde, T.E. and Lagier-Tourenne, C. (2018). Animal models of neurodegenerative diseases. Nat. Neurosci. *21*, 1370-1379.

de Jonghe, A., Korevaar, J.C., van Munster, B.C. and de Rooij, S.E. (2010). Effectiveness of melatonin treatment on circadian rhythm disturbances in dementia. Are there implications for delirium? A systematic review. Int. J. Geriatr. Psychiatry *25*, 1201-1208.

de Mairan, J. (1729). Observation botanique. Hist. Acad. Roy. Sci. 35-36.

de Medeiros, K., Robert, P., Gauthier, S., Stella, F., Politis, A., Leoutsakos, J., Taragano, F., Kremer, J., Brugnolo, A., Porsteinsson, A.P.*, et al.* (2010). The Neuropsychiatric Inventory-Clinician rating scale (NPI-C): reliability and validity of a revised assessment of neuropsychiatric symptoms in dementia. Int. Psychogeriatr. *22*, 984-994.

Deery, M.J., Maywood, E.S., Chesham, J.E., Sladek, M., Karp, N.A., Green, E.W., Charles, P.D., Reddy, A.B., Kyriacou, C.P., Lilley, K.S.*, et al.* (2009). Proteomic analysis reveals the role of synaptic vesicle cycling in sustaining the suprachiasmatic circadian clock. Curr. Biol. *19*, 2031-2036.

Deng, X.H., Bertini, G., Palomba, M., Xu, Y.Z., Bonaconsa, M., Nygard, M. and Bentivoglio, M. (2010). Glial transcripts and immune-challenged glia in the suprachiasmatic nucleus of young and aged mice. Chronobiol. Int. *27*, 742-767.

Depner, C.M., Melanson, E.L., McHill, A.W. and Wright, K.P., Jr. (2018). Mistimed food intake and sleep alters 24-hour time-of-day patterns of the human plasma proteome. Proc. Natl. Acad. Sci. U. S. A. *115*, E5390-E5399.

Devan, B.D., Goad, E.H., Petri, H.L., Antoniadis, E.A., Hong, N.S., Ko, C.H., Leblanc, L., Lebovic, S.S., Lo, Q., Ralph, M.R.*, et al.* (2001). Circadian phase-shifted rats show normal acquisition but impaired long-term retention of place information in the water task. Neurobiol. Learn. Mem. *75*, 51-62.

Dibner, C., Schibler, U. and Albrecht, U. (2010). The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu. Rev. Physiol. *72*, 517-549.

Diem, S.J., Blackwell, T.L., Stone, K.L., Yaffe, K., Tranah, G., Cauley, J.A., Ancoli-Israel, S., Redline, S., Spira, A.P., Hillier, T.A.*, et al.* (2016). Measures of Sleep-Wake Patterns and Risk of Mild Cognitive Impairment or Dementia in Older Women. Am. J. Geriatr. Psychiatry *24*, 248-258.

Dobrowolska, J.A., Kasten, T., Huang, Y., Benzinger, T.L., Sigurdson, W., Ovod, V., Morris, J.C. and Bateman, R.J. (2014). Diurnal patterns of soluble amyloid precursor protein metabolites in the human central nervous system. PLoS ONE *9*, e89998.

Dodson, E.R. and Zee, P.C. (2010). Therapeutics for Circadian Rhythm Sleep Disorders. Sleep Med Clin *5*, 701-715.

Douglas, P.M. and Dillin, A. (2010). Protein homeostasis and aging in neurodegeneration. J. Cell Biol. *190*, 719-729.

Dowling, G.A., Burr, R.L., Van Someren, E.J., Hubbard, E.M., Luxenberg, J.S., Mastick, J. and Cooper, B.A. (2008). Melatonin and bright-light treatment for rest-activity disruption in institutionalized patients with Alzheimer's disease. J. Am. Geriatr. Soc. *56*, 239-246.

Drachman, D.A. (2014). The amyloid hypothesis, time to move on: Amyloid is the downstream result, not cause, of Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association *10*, 372-380.

Dubois, B. (2018). The Emergence of a New Conceptual Framework for Alzheimer's Disease. J. Alzheimers Dis. *62*, 1059-1066.

Dubois, B., Feldman, H.H., Jacova, C., Cummings, J.L., Dekosky, S.T., Barberger-Gateau, P., Delacourte, A., Frisoni, G., Fox, N.C., Galasko, D.*, et al.* (2010). Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol. *9*, 1118-1127.

Dubois, B., Feldman, H.H., Jacova, C., Hampel, H., Molinuevo, J.L., Blennow, K., DeKosky, S.T., Gauthier, S., Selkoe, D., Bateman, R.*, et al.* (2014). Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol. *13*, 614-629.

Duffy, J.F., Zeitzer, J.M. and Czeisler, C.A. (2007). Decreased sensitivity to phase-delaying effects of moderate intensity light in older subjects. Neurobiol. Aging *28*, 799-807.

Duncan, M.J. (2019). Interacting influences of aging and Alzheimer's disease on circadian rhythms. Eur. J. Neurosci.

Duncan, M.J., Prochot, J.R., Cook, D.H., Tyler Smith, J. and Franklin, K.M. (2013). Influence of aging on Bmal1 and Per2 expression in extra-SCN oscillators in hamster brain. Brain Res. *1491*, 44-53.

Duncan, M.J., Smith, J.T., Franklin, K.M., Beckett, T.L., Murphy, M.P., St Clair, D.K., Donohue, K.D., Striz, M. and O'Hara, B.F. (2012). Effects of aging and genotype on circadian rhythms, sleep, and clock gene expression in APPxPS1 knock-in mice, a model for Alzheimer's disease. Exp. Neurol. *236*, 249-258.

Dunlap, J.C. (1999). Molecular bases for circadian clocks. Cell *96*, 271-290.

Dyar, K.A., Lutter, D., Artati, A., Ceglia, N.J., Liu, Y., Armenta, D., Jastroch, M., Schneider, S., de Mateo, S., Cervantes, M.*, et al.* (2018). Atlas of Circadian Metabolism Reveals System-wide Coordination and Communication between Clocks. Cell *174*, 1571-1585 e1511.

Ebly, E.M., Hogan, D.B. and Parhad, I.M. (1995). Cognitive impairment in the nondemented elderly. Results from the Canadian Study of Health and Aging. Arch. Neurol. *52*, 612-619.

Eckel-Mahan, K. and Sassone-Corsi, P. (2015). Phenotyping Circadian Rhythms in Mice. Curr Protoc Mouse Biol *5*, 271-281.

Eckel-Mahan, K.L., Phan, T., Han, S., Wang, H., Chan, G.C., Scheiner, Z.S. and Storm, D.R. (2008). Circadian oscillation of hippocampal MAPK activity and cAmp: implications for memory persistence. Nat. Neurosci. *11*, 1074-1082.

Eckel-Mahan, K.L. and Storm, D.R. (2009). Circadian rhythms and memory: not so simple as cogs and gears. EMBO Rep. *10*, 584-591.

Eichenbaum, H. (1999). The hippocampus and mechanisms of declarative memory. Behav. Brain Res. *103*, 123-133.

Eichenbaum, H. (2004). Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron *44*, 109-120.

Elliott, W.J. (1998). Circadian variation in the timing of stroke onset: a meta-analysis. Stroke *29*, 992-996.

Espiritu, J.R. (2008). Aging-related sleep changes. Clin. Geriatr. Med. *24*, 1-14, v.

Esquerda-Canals, G., Montoliu-Gaya, L., Guell-Bosch, J. and Villegas, S. (2017). Mouse Models of Alzheimer's Disease. J. Alzheimers Dis. *57*, 1171-1183.

Fan, X., Wheatley, E.G. and Villeda, S.A. (2017). Mechanisms of Hippocampal Aging and the Potential for Rejuvenation. Annu. Rev. Neurosci. *40*, 251-272.

Fekete, M., van Ree, J.M., Niesink, R.J. and de Wied, D. (1985). Disrupting circadian rhythms in rats induces retrograde amnesia. Physiol. Behav. *34*, 883-887.

Feldman, H.H., Jacova, C., Robillard, A., Garcia, A., Chow, T., Borrie, M., Schipper, H.M., Blair, M., Kertesz, A. and Chertkow, H. (2008). Diagnosis and treatment of dementia: 2. Diagnosis. CMAJ *178*, 825-836.

Ferretti, M.T., Iulita, M.F., Cavedo, E., Chiesa, P.A., Schumacher Dimech, A., Santuccione Chadha, A., Baracchi, F., Girouard, H., Misoch, S., Giacobini, E.*, et al.* (2018). Sex differences in Alzheimer disease - the gateway to precision medicine. Nature reviews. Neurology *14*, 457-469.

Ferri, C.P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., Hall, K., Hasegawa, K., Hendrie, H., Huang, Y.*, et al.* (2005). Global prevalence of dementia: a Delphi consensus study. Lancet *366*, 2112-2117.

Flaherty, D.B., Soria, J.P., Tomasiewicz, H.G. and Wood, J.G. (2000). Phosphorylation of human tau protein by microtubule-associated kinases: GSK3beta and cdk5 are key participants. J. Neurosci. Res. *62*, 463-472.

Flajolet, M., He, G., Heiman, M., Lin, A., Nairn, A.C. and Greengard, P. (2007). Regulation of Alzheimer's disease amyloid-beta formation by casein kinase I. Proc. Natl. Acad. Sci. U. S. A. *104*, 4159-4164.

Flatt, T. (2012). A new definition of aging? Frontiers in genetics *3*, 148.

Fonseca Costa, S.S. and Ripperger, J.A. (2015). Impact of the circadian clock on the aging process. Frontiers in neurology *6*, 43.

Franceschi, C., Garagnani, P., Morsiani, C., Conte, M., Santoro, A., Grignolio, A., Monti, D., Capri, M. and Salvioli, S. (2018). The Continuum of Aging and Age-Related Diseases: Common Mechanisms but Different Rates. Frontiers in medicine *5*, 61.

Francis, P.T., Palmer, A.M., Snape, M. and Wilcock, G.K. (1999). The cholinergic hypothesis of Alzheimer's disease: a review of progress. J. Neurol. Neurosurg. Psychiatry *66*, 137-147.

Frank, M.G. (2016). Circadian Regulation of Synaptic Plasticity. Biology (Basel) *5*,

Freeman, W.M., VanGuilder, H.D., Bennett, C. and Sonntag, W.E. (2009). Cognitive performance and age-related changes in the hippocampal proteome. Neuroscience *159*, 183-195.

Frey, U., Huang, Y.Y. and Kandel, E.R. (1993). Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. Science *260*, 1661-1664.

Friedman, L., Spira, A.P., Hernandez, B., Mather, C., Sheikh, J., Ancoli-Israel, S., Yesavage, J.A. and Zeitzer, J.M. (2012). Brief morning light treatment for sleep/wake disturbances in older memory-impaired individuals and their caregivers. Sleep Med *13*, 546-549.

Froy, O. (2011). Circadian rhythms, aging, and life span in mammals. Physiology (Bethesda) *26*, 225-235.

Fu, H., Hardy, J. and Duff, K.E. (2018). Selective vulnerability in neurodegenerative diseases. Nat. Neurosci. *21*, 1350-1358.

Gaikwad, S. (2018). The biological clock: Future of neurological disorders therapy. Neural regeneration research *13*, 567-568.

Gallego, M. and Virshup, D.M. (2007). Post-translational modifications regulate the ticking of the circadian clock. Nat. Rev. Mol. Cell Biol. *8*, 139-148.

Galton, C.J., Patterson, K., Xuereb, J.H. and Hodges, J.R. (2000). Atypical and typical presentations of Alzheimer's disease: a clinical, neuropsychological, neuroimaging and pathological study of 13 cases. Brain *123 Pt 3*, 484-498.

Gan, L., Cookson, M.R., Petrucelli, L. and La Spada, A.R. (2018). Converging pathways in neurodegeneration, from genetics to mechanisms. Nat. Neurosci. *21*, 1300-1309.

Garcia, J.A., Zhang, D., Estill, S.J., Michnoff, C., Rutter, J., Reick, M., Scott, K., Diaz-Arrastia, R. and McKnight, S.L. (2000). Impaired cued and contextual memory in NPAS2-deficient mice. Science *288*, 2226-2230.

Gatz, M., Reynolds, C.A., Fratiglioni, L., Johansson, B., Mortimer, J.A., Berg, S., Fiske, A. and Pedersen, N.L. (2006). Role of genes and environments for explaining Alzheimer disease. Arch. Gen. Psychiatry *63*, 168-174.

G.B.D. Dementia Collaborators (2019). Global, regional, and national burden of Alzheimer's disease and other dementias, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. *18*, 88-106.

Gerstner, J.R., Lyons, L.C., Wright, K.P., Jr., Loh, D.H., Rawashdeh, O., Eckel-Mahan, K.L. and Roman, G.W. (2009). Cycling behavior and memory formation. J. Neurosci. *29*, 12824-12830.

Gerstner, J.R. and Yin, J.C. (2010). Circadian rhythms and memory formation. Nat. Rev. Neurosci. *11*, 577-588.

Geschwind, D.H. and Konopka, G. (2009). Neuroscience in the era of functional genomics and systems biology. Nature *461*, 908-915.

Gibbs, J.E. and Ray, D.W. (2013). The role of the circadian clock in rheumatoid arthritis. Arthritis Res. Ther. *15*, 205.

Gibson, E.M., Wang, C., Tjho, S., Khattar, N. and Kriegsfeld, L.J. (2010). Experimental 'jet lag' inhibits adult neurogenesis and produces long-term cognitive deficits in female hamsters. PLoS ONE *5*, e15267.

Gilhooley, M.J., Pinnock, S.B. and Herbert, J. (2011). Rhythmic expression of per1 in the dentate gyrus is suppressed by corticosterone: implications for neurogenesis. Neurosci. Lett. *489*, 177-181.

Gloston, G.F., Yoo, S.H. and Chen, Z.J. (2017). Clock-Enhancing Small Molecules and Potential Applications in Chronic Diseases and Aging. Frontiers in neurology *8*, 100.

Golombek, D.A., Casiraghi, L.P., Agostino, P.V., Paladino, N., Duhart, J.M., Plano, S.A. and Chiesa, J.J. (2013). The times they're a-changing: effects of circadian desynchronization on physiology and disease. J. Physiol. Paris *107*, 310-322.

Gong, C., Li, C., Qi, X., Song, Z., Wu, J., Hughes, M.E. and Li, X. (2015). The daily rhythms of mitochondrial gene expression and oxidative stress regulation are altered by aging in the mouse liver. Chronobiol. Int. *32*, 1254-1263.

Gooley, J.J. (2008). Treatment of circadian rhythm sleep disorders with light. Ann. Acad. Med. Singapore *37*, 669-676.

Gordon-Weeks, P.R. and Fournier, A.E. (2014). Neuronal cytoskeleton in synaptic plasticity and regeneration. J. Neurochem. *129*, 206-212.

Graham, W.V., Bonito-Oliva, A. and Sakmar, T.P. (2017). Update on Alzheimer's Disease Therapy and Prevention Strategies. Annu. Rev. Med. *68*, 413-430.

Greco, C.M. and Sassone-Corsi, P. (2019). Circadian blueprint of metabolic pathways in the brain. Nat. Rev. Neurosci. *20*, 71-82.

Green, D.R., Galluzzi, L. and Kroemer, G. (2011). Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. Science *333*, 1109-1112.

Griffett, K. and Burris, T.P. (2013). The mammalian clock and chronopharmacology. Bioorg. Med. Chem. Lett. *23*, 1929-1934.

Grimm, A. and Eckert, A. (2017). Brain aging and neurodegeneration: from a mitochondrial point of view. J. Neurochem. *143*, 418-431.

Guntupalli, S., Widagdo, J. and Anggono, V. (2016). Amyloid-beta-Induced Dysregulation of AMPA Receptor Trafficking. Neural Plast. *2016*, 3204519.

Halekotte, J., Witt, L., Ianes, C., Kruger, M., Buhrmann, M., Rauh, D., Pichlo, C., Brunstein, E., Luxenburger, A., Baumann, U.*, et al.* (2017). Optimized 4,5-Diarylimidazoles as Potent/Selective Inhibitors of Protein Kinase CK1delta and Their Structural Relation to p38alpha MAPK. Molecules *22*,

Haley, G.E., Landauer, N., Renner, L., Weiss, A., Hooper, K., Urbanski, H.F., Kohama, S.G., Neuringer, M. and Raber, J. (2009). Circadian activity associated with spatial learning and memory in aging rhesus monkeys. Exp. Neurol. *217*, 55-62.

Han, X., Aslanian, A. and Yates, J.R., 3rd (2008). Mass spectrometry for proteomics. Curr. Opin. Chem. Biol. *12*, 483-490.

Hanford, N. and Figueiro, M. (2013). Light therapy and Alzheimer's disease and related dementia: past, present, and future. J. Alzheimers Dis. *33*, 913-922.

Hanger, D.P., Byers, H.L., Wray, S., Leung, K.Y., Saxton, M.J., Seereeram, A., Reynolds, C.H., Ward, M.A. and Anderton, B.H. (2007). Novel phosphorylation sites in tau from Alzheimer brain support a role for casein kinase 1 in disease pathogenesis. J. Biol. Chem. *282*, 23645-23654.

Hanninen, T. and Soininen, H. (1997). Age-associated memory impairment. Normal aging or warning of dementia? Drugs Aging *11*, 480-489.

Hannou, L., Roy, P.G., Ballester Roig, M.N. and Mongrain, V. (2018). Transcriptional control of synaptic components by the clock machinery. Eur. J. Neurosci.

Harada, C.N., Natelson Love, M.C. and Triebel, K.L. (2013). Normal cognitive aging. Clin. Geriatr. Med. *29*, 737-752.

Hardy, J. and Allsop, D. (1991). Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol. Sci. *12*, 383-388.

Hardy, J.A. and Higgins, G.A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. Science *256*, 184-185.

Harper, D.G., Stopa, E.G., Kuo-Leblanc, V., McKee, A.C., Asayama, K., Volicer, L., Kowall, N. and Satlin, A. (2008). Dorsomedial SCN neuronal subpopulations subserve different functions in human dementia. Brain *131*, 1609-1617.

Hastings, M.H., Maywood, E.S. and Brancaccio, M. (2018). Generation of circadian rhythms in the suprachiasmatic nucleus. Nat. Rev. Neurosci. *19*, 453-469.

Hastings, M.H., Reddy, A.B. and Maywood, E.S. (2003). A clockwork web: circadian timing in brain and periphery, in health and disease. Nat. Rev. Neurosci. *4*, 649-661.

Hatanaka, F., Ocampo, A. and Izpisua Belmonte, J.C. (2017). Keeping the Rhythm while Changing the Lyrics: Circadian Biology in Aging. Cell *170*, 599-600.

Haydon, P.G. and Nedergaard, M. (2014). How do astrocytes participate in neural plasticity? Cold Spring Harb. Perspect. Biol. *7*, a020438.

He, B., Nohara, K., Park, N., Park, Y.S., Guillory, B., Zhao, Z., Garcia, J.M., Koike, N., Lee, C.C., Takahashi, J.S.*, et al.* (2016). The Small Molecule Nobiletin Targets the Molecular Oscillator to Enhance Circadian Rhythms and Protect against Metabolic Syndrome. Cell Metab. *23*, 610-621.

He, W., Lu, Y., Qahwash, I., Hu, X.Y., Chang, A. and Yan, R. (2004). Reticulon family members modulate BACE1 activity and amyloid-beta peptide generation. Nat. Med. *10*, 959-965.

Hebb, D.O. (1949). The Organization of Behavior: A Neuropsychological Theory. New York, USA: John Wiley & Sons, Inc.

Hebda-Bauer, E.K., Simmons, T.A., Sugg, A., Ural, E., Stewart, J.A., Beals, J.L., Wei, Q., Watson, S.J. and Akil, H. (2013). 3xTg-AD mice exhibit an activated central stress axis during early-stage pathology. J. Alzheimers Dis. *33*, 407-422.

Heckers, S., Zalesak, M., Weiss, A.P., Ditman, T. and Titone, D. (2004). Hippocampal activation during transitive inference in humans. Hippocampus *14*, 153-162.

Hermida, R.C., Ayala, D.E., Fernandez, J.R. and Calvo, C. (2007). Comparison of the efficacy of morning versus evening administration of telmisartan in essential hypertension. Hypertension *50*, 715-722.

Hippius, H. and Neundorfer, G. (2003). The discovery of Alzheimer's disease. Dialogues Clin. Neurosci. *5*, 101-108.

Ho, V.M., Lee, J.A. and Martin, K.C. (2011). The cell biology of synaptic plasticity. Science *334*, 623-628.

Hoffmann, H.J. and Balschun, D. (1992). Circadian differences in maze performance of C57Bl/6 Ola mice. Behav. Processes *27*, 77-83.

Hofman, M.A., Fliers, E., Goudsmit, E. and Swaab, D.F. (1988). Morphometric analysis of the suprachiasmatic and paraventricular nuclei in the human brain: sex differences and age-dependent changes. J. Anat. *160*, 127-143.

Hofman, M.A. and Swaab, D.F. (1994). Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging. Brain Res. *651*, 134-142.

Hofman, M.A. and Swaab, D.F. (2006). Living by the clock: the circadian pacemaker in older people. Ageing Res. Rev. *5*, 33-51.

Hogenesch, J.B. and Ueda, H.R. (2011). Understanding systems-level properties: timely stories from the study of clocks. Nat. Rev. Genet. *12*, 407-416.

Holloway, F.A. and Wansley, R.A. (1973). Multiple retention deficits at periodic intervals after active and passive avoidance learning. Behav. Biol. *9*, 1-14.

Holth, J.K., Fritschi, S.K., Wang, C., Pedersen, N.P., Cirrito, J.R., Mahan, T.E., Finn, M.B., Manis, M., Geerling, J.C., Fuller, P.M.*, et al.* (2019). The sleep-wake cycle regulates brain interstitial fluid tau in mice and CSF tau in humans. Science

Homolak, J., Mudrovcic, M., Vukic, B. and Toljan, K. (2018). Circadian Rhythm and Alzheimer's Disease. Medical sciences *6*,

Hood, S. and Amir, S. (2017a). The aging clock: circadian rhythms and later life. J. Clin. Invest. *127*, 437-446.

Hood, S. and Amir, S. (2017b). Neurodegeneration and the Circadian Clock. Frontiers in aging neuroscience *9*, 170.

Hoogerwerf, W.A. (2009). Role of biological rhythms in gastrointestinal health and disease. Rev. Endocr. Metab. Disord. *10*, 293-300.

Hook, V.Y., Kindy, M. and Hook, G. (2008). Inhibitors of cathepsin B improve memory and reduce beta-amyloid in transgenic Alzheimer disease mice expressing the wild-type, but not the Swedish mutant, beta-secretase site of the amyloid precursor protein. J. Biol. Chem. *283*, 7745-7753.

Hooper, C., Killick, R. and Lovestone, S. (2008). The GSK3 hypothesis of Alzheimer's disease. J. Neurochem. *104*, 1433-1439.

Hoyer, S. (1998). Is sporadic Alzheimer disease the brain type of non-insulin dependent diabetes mellitus? A challenging hypothesis. J Neural Transm (Vienna) *105*, 415-422.

Hoyle, N.P., Seinkmane, E., Putker, M., Feeney, K.A., Krogager, T.P., Chesham, J.E., Bray, L.K., Thomas, J.M., Dunn, K., Blaikley, J.*, et al.* (2017). Circadian actin dynamics drive rhythmic fibroblast mobilization during wound healing. Sci. Transl. Med. *9*,

Hsu, P.Y. and Harmer, S.L. (2012). Circadian phase has profound effects on differential expression analysis. PLoS ONE *7*, e49853.

Hu, X., Wang, T. and Jin, F. (2016). Alzheimer's disease and gut microbiota. Science China. Life sciences *59*, 1006-1023.

Huang da, W., Sherman, B.T. and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. *4*, 44-57.

Huang, W., Ramsey, K.M., Marcheva, B. and Bass, J. (2011). Circadian rhythms, sleep, and metabolism. J. Clin. Invest. *121*, 2133-2141.

Huang, Y. and Mucke, L. (2012). Alzheimer mechanisms and therapeutic strategies. Cell *148*, 1204-1222.

Hughes, M.E., Abruzzi, K.C., Allada, R., Anafi, R., Arpat, A.B., Asher, G., Baldi, P., de Bekker, C., Bell-Pedersen, D., Blau, J.*, et al.* (2017). Guidelines for Genome-Scale Analysis of Biological Rhythms. J. Biol. Rhythms *32*, 380-393.

Hughes, M.E., DiTacchio, L., Hayes, K.R., Vollmers, C., Pulivarthy, S., Baggs, J.E., Panda, S. and Hogenesch, J.B. (2009). Harmonics of circadian gene transcription in mammals. PLoS Genet. *5*, e1000442.

Hugo, J. and Ganguli, M. (2014). Dementia and cognitive impairment: epidemiology, diagnosis, and treatment. Clin. Geriatr. Med. *30*, 421-442.

Hung, S.Y. and Fu, W.M. (2017). Drug candidates in clinical trials for Alzheimer's disease. J. Biomed. Sci. *24*, 47.

Ikeno, T., Weil, Z.M. and Nelson, R.J. (2013). Photoperiod affects the diurnal rhythm of hippocampal neuronal morphology of Siberian hamsters. Chronobiol. Int. *30*, 1089-1100.

Insausti, R., Munoz-Lopez, M., Insausti, A.M. and Artacho-Perula, E. (2017). The Human Periallocortex: Layer Pattern in Presubiculum, Parasubiculum and Entorhinal Cortex. A Review. Frontiers in neuroanatomy *11*, 84.

Iqbal, K. and Grundke-Iqbal, I. (2010). Alzheimer's disease, a multifactorial disorder seeking multitherapies. Alzheimer's & dementia : the journal of the Alzheimer's Association *6*, 420-424.

Itzhaki, R.F., Lathe, R., Balin, B.J., Ball, M.J., Bearer, E.L., Braak, H., Bullido, M.J., Carter, C., Clerici, M., Cosby, S.L., *et al.* (2016). Microbes and Alzheimer's Disease. J. Alzheimers Dis. *51*, 979-984.

Itzhaki, R.F., Wozniak, M.A., Appelt, D.M. and Balin, B.J. (2004). Infiltration of the brain by pathogens causes Alzheimer's disease. Neurobiol. Aging *25*, 619-627.

Jack, C.R., Jr., Bennett, D.A., Blennow, K., Carrillo, M.C., Dunn, B., Haeberlein, S.B., Holtzman, D.M., Jagust, W., Jessen, F., Karlawish, J., *et al.* (2018). NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association *14*, 535-562.

Jellinger, K.A. (2007). The enigma of mixed dementia. Alzheimer's & dementia : the journal of the Alzheimer's Association *3*, 40-53.

Jiang, C., Li, G., Huang, P., Liu, Z. and Zhao, B. (2017). The Gut Microbiota and Alzheimer's Disease. J. Alzheimers Dis. *58*, 1-15.

Jilg, A., Bechstein, P., Saade, A., Dick, M., Li, T.X., Tosini, G., Rami, A., Zemmar, A. and Stehle, J.H. (2019). Melatonin modulates daytime-dependent synaptic plasticity and learning efficiency. J. Pineal Res. e12553.

Jilg, A., Lesny, S., Peruzki, N., Schwegler, H., Selbach, O., Dehghani, F. and Stehle, J.H. (2010). Temporal dynamics of mouse hippocampal clock gene expression support memory processing. Hippocampus *20*, 377-388.

Jin, J. and Maren, S. (2015). Prefrontal-Hippocampal Interactions in Memory and Emotion. Frontiers in systems neuroscience *9*, 170.

Jin, K. (2010). Modern Biological Theories of Aging. Aging and disease *1*, 72-74.

Ju, Y.E., Lucey, B.P. and Holtzman, D.M. (2014). Sleep and Alzheimer disease pathology--a bidirectional relationship. Nature reviews. Neurology *10*, 115-119.

Ju, Y.E., McLeland, J.S., Toedebusch, C.D., Xiong, C., Fagan, A.M., Duntley, S.P., Morris, J.C. and Holtzman, D.M. (2013). Sleep quality and preclinical Alzheimer disease. JAMA Neurol *70*, 587-593.

Kalsbeek, A., la Fleur, S. and Fliers, E. (2014). Circadian control of glucose metabolism. Mol Metab *3*, 372-383.

Kametani, F. and Hasegawa, M. (2018). Reconsideration of Amyloid Hypothesis and Tau Hypothesis in Alzheimer's Disease. Front. Neurosci. *12*, 25.

Kandel, E.R., Dudai, Y. and Mayford, M.R. (2014). The molecular and systems biology of memory. Cell *157*, 163-186.

Kang, J.E., Lim, M.M., Bateman, R.J., Lee, J.J., Smyth, L.P., Cirrito, J.R., Fujiki, N., Nishino, S. and Holtzman, D.M. (2009). Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. Science *326*, 1005-1007.

Karran, E., Mercken, M. and De Strooper, B. (2011). The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat. Rev. Drug Discov. *10*, 698-712.

Kategaya, L.S., Hilliard, A., Zhang, L., Asara, J.M., Ptacek, L.J. and Fu, Y.H. (2012). Casein kinase 1 proteomics reveal prohibitin 2 function in molecular clock. PLoS ONE *7*, e31987.

Katzman, R. (1976). Editorial: The prevalence and malignancy of Alzheimer disease. A major killer. Arch. Neurol. *33*, 217-218.

Kaur, G., Phillips, C., Wong, K. and Saini, B. (2013). Timing is important in medication administration: a timely review of chronotherapy research. Int J Clin Pharm *35*, 344-358.

Kennaway, D.J., Varcoe, T.J., Voultsios, A., Salkeld, M.D., Rattanatray, L. and Boden, M.J. (2015). Acute inhibition of casein kinase 1delta/epsilon rapidly delays peripheral clock gene rhythms. Mol. Cell. Biochem. *398*, 195-206.

Kennedy, B.K., Berger, S.L., Brunet, A., Campisi, J., Cuervo, A.M., Epel, E.S., Franceschi, C., Lithgow, G.J., Morimoto, R.I., Pessin, J.E.*, et al.* (2014). Geroscience: linking aging to chronic disease. Cell *159*, 709-713.

Kervezee, L., Cuesta, M., Cermakian, N. and Boivin, D.B. (2019). The Phase-Shifting Effect of Bright Light Exposure on Circadian Rhythmicity in the Human Transcriptome. J. Biol. Rhythms 748730418821776.

Khachaturian, Z.S. (1994). Calcium hypothesis of Alzheimer's disease and brain aging. Ann. N. Y. Acad. Sci. *747*, 1-11.

Kinouchi, K., Magnan, C., Ceglia, N., Liu, Y., Cervantes, M., Pastore, N., Huynh, T., Ballabio, A., Baldi, P., Masri, S.*, et al.* (2018). Fasting Imparts a Switch to Alternative Daily Pathways in Liver and Muscle. Cell Rep *25*, 3299-3314 e3296.

Kirova, A.M., Bays, R.B. and Lagalwar, S. (2015). Working memory and executive function decline across normal aging, mild cognitive impairment, and Alzheimer's disease. BioMed research international *2015*, 748212.

Kirschner, M.W. (2005). The meaning of systems biology. Cell *121*, 503-504.

Knierim, J.J. (2015). The hippocampus. Curr. Biol. *25*, R1116-1121.

Knight, E.M., Martins, I.V., Gumusgoz, S., Allan, S.M. and Lawrence, C.B. (2014). High-fat diet-induced memory impairment in triple-transgenic Alzheimer's disease (3xTgAD) mice is independent of changes in amyloid and tau pathology. Neurobiol. Aging *35*, 1821-1832.

Kolker, D.E., Fukuyama, H., Huang, D.S., Takahashi, J.S., Horton, T.H. and Turek, F.W. (2003). Aging alters circadian and light-induced expression of clock genes in golden hamsters. J. Biol. Rhythms *18*, 159-169.

Kon, N., Yoshikawa, T., Honma, S., Yamagata, Y., Yoshitane, H., Shimizu, K., Sugiyama, Y., Hara, C., Kameshita, I., Honma, K.*, et al.* (2014). CaMKII is essential for the cellular clock and coupling between morning and evening behavioral rhythms. Genes Dev. *28*, 1101-1110.

Kondratov, R.V. (2007). A role of the circadian system and circadian proteins in aging. Ageing Res. Rev. *6*, 12-27.

Kondratova, A.A., Dubrovsky, Y.V., Antoch, M.P. and Kondratov, R.V. (2010). Circadian clock proteins control adaptation to novel environment and memory formation. Aging *2*, 285-297.

Kondratova, A.A. and Kondratov, R.V. (2012). The circadian clock and pathology of the ageing brain. Nat. Rev. Neurosci. *13*, 325-335.

Kral, V.A. (1962). Senescent forgetfulness: benign and malignant. Can. Med. Assoc. J. *86*, 257-260.

Kress, G.J., Liao, F., Dimitry, J., Cedeno, M.R., FitzGerald, G.A., Holtzman, D.M. and Musiek, E.S. (2018). Regulation of amyloid-beta dynamics and pathology by the circadian clock. J. Exp. Med. *215*, 1059-1068.

Krishnan, H.C. and Lyons, L.C. (2015). Synchrony and desynchrony in circadian clocks: impacts on learning and memory. Learn. Mem. *22*, 426-437.

Krstic, D. and Knuesel, I. (2013). Deciphering the mechanism underlying late-onset Alzheimer disease. Nature reviews. Neurology *9*, 25-34.

Kuintzle, R.C., Chow, E.S., Westby, T.N., Gvakharia, B.O., Giebultowicz, J.M. and Hendrix, D.A. (2017). Circadian deep sequencing reveals stress-response genes that adopt robust rhythmic expression during aging. Nat. Commun. *8*, 14529.

Kumar, A. (2011). Long-Term Potentiation at CA3-CA1 Hippocampal Synapses with Special Emphasis on Aging, Disease, and Stress. Frontiers in aging neuroscience *3*, 7.

Kunkle, B.W., Grenier-Boley, B., Sims, R., Bis, J.C., Damotte, V., Naj, A.C., Boland, A., Vronskaya, M., van der Lee, S.J., Amlie-Wolf, A.*, et al.* (2019). Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. Nat. Genet. *51*, 414-430.

Kwapis, J.L., Alaghband, Y., Kramar, E.A., Lopez, A.J., Vogel Ciernia, A., White, A.O., Shu, G., Rhee, D., Michael, C.M., Montellier, E.*, et al.* (2018). Epigenetic regulation of the circadian gene Per1 contributes to age-related changes in hippocampal memory. Nat. Commun. *9*, 3323.

Kyriacou, C.P. and Hastings, M.H. (2010). Circadian clocks: genes, sleep, and cognition. Trends in cognitive sciences *14*, 259-267.

Laakso, M.P., Frisoni, G.B., Kononen, M., Mikkonen, M., Beltramello, A., Geroldi, C., Bianchetti, A., Trabucchi, M., Soininen, H. and Aronen, H.J. (2000). Hippocampus and entorhinal cortex in frontotemporal dementia and Alzheimer's disease: a morphometric MRI study. Biol. Psychiatry *47*, 1056-1063.

Lacoste, M.G., Ponce, I.T., Golini, R.L., Delgado, S.M. and Anzulovich, A.C. (2017). Aging modifies daily variation of antioxidant enzymes and oxidative status in the hippocampus. Exp. Gerontol. *88*, 42-50.

Lamont, E.W., Legault-Coutu, D., Cermakian, N. and Boivin, D.B. (2007). The role of circadian clock genes in mental disorders. Dialogues Clin. Neurosci. *9*, 333-342.

Lamont, E.W., Robinson, B., Stewart, J. and Amir, S. (2005). The central and basolateral nuclei of the amygdala exhibit opposite diurnal rhythms of expression of the clock protein Period2. Proc. Natl. Acad. Sci. U. S. A. *102*, 4180-4184.

Lang, J.E., Anderson, L., LoGerfo, J., Sharkey, J., Belansky, E., Bryant, L., Prohaska, T., Altpeter, M., Marshall, V., Satariano, W.*, et al.* (2006). The Prevention Research Centers Healthy Aging Research Network. Prev. Chronic. Dis. *3*, A17.

Larrabee, G.J. and Crook, T.H., 3rd (1994). Estimated prevalence of age-associated memory impairment derived from standardized tests of memory function. Int. Psychogeriatr. *6*, 95-104.

Lawlor, B. (2002). Managing behavioural and psychological symptoms in dementia. Br. J. Psychiatry *181*, 463-465.

Lee, H., Chen, R., Lee, Y., Yoo, S. and Lee, C. (2009). Essential roles of CKIdelta and CKIepsilon in the mammalian circadian clock. Proc. Natl. Acad. Sci. U. S. A. *106*, 21359-21364.

Lee, J., Kim, D.E., Griffin, P., Sheehan, P.W., Kim, D.H., Musiek, E.S. and Yoon, S.Y. (2019). Inhibition of REV-ERBs stimulates microglial amyloid-beta clearance and reduces amyloid plaque deposition in the 5XFAD mouse model of Alzheimer's disease. Aging Cell e13078.

Lee, V.M. and Trojanowski, J.Q. (2006). Progress from Alzheimer's tangles to pathological tau points towards more effective therapies now. J. Alzheimers Dis. *9*, 257-262.

Lein, E.S., Zhao, X. and Gage, F.H. (2004). Defining a molecular atlas of the hippocampus using DNA microarrays and high-throughput in situ hybridization. J. Neurosci. *24*, 3879-3889.

Leng, Y., Musiek, E.S., Hu, K., Cappuccio, F.P. and Yaffe, K. (2019). Association between circadian rhythms and neurodegenerative diseases. Lancet Neurol. *18*, 307-318.

Leuner, B. and Gould, E. (2010). Structural plasticity and hippocampal function. Annu. Rev. Psychol. *61*, 111-140, C111-113.

Levenson, J.M. and Sweatt, J.D. (2005). Epigenetic mechanisms in memory formation. Nat. Rev. Neurosci. *6*, 108-118.

Levi, F. and Schibler, U. (2007). Circadian rhythms: mechanisms and therapeutic implications. Annu. Rev. Pharmacol. Toxicol. *47*, 593-628.

Levitis, D.A., Lidicker, W.Z. and Freund, G. (2009). Behavioural biologists don't agree on what constitutes behaviour. Anim. Behav. *78*, 103-110.

Levy, M., Faas, G.C., Saggau, P., Craigen, W.J. and Sweatt, J.D. (2003). Mitochondrial regulation of synaptic plasticity in the hippocampus. J. Biol. Chem. *278*, 17727-17734.

Levy, R. (1994). Aging-associated cognitive decline. Working Party of the International Psychogeriatric Association in collaboration with the World Health Organization. Int. Psychogeriatr. *6*, 63-68.

Li, G., Yin, H. and Kuret, J. (2004). Casein kinase 1 delta phosphorylates tau and disrupts its binding to microtubules. J. Biol. Chem. *279*, 15938-15945.

Lim, A.S., Klein, H.U., Yu, L., Chibnik, L.B., Ali, S., Xu, J., Bennett, D.A. and De Jager, P.L. (2017). Diurnal and seasonal molecular rhythms in human neocortex and their relation to Alzheimer's disease. Nat. Commun. *8*, 14931.

Lim, C. and Allada, R. (2013). Emerging roles for post-transcriptional regulation in circadian clocks. Nat. Neurosci. *16*, 1544-1550.

Lindsay, J., Laurin, D., Verreault, R., Hebert, R., Helliwell, B., Hill, G.B. and McDowell, I. (2002). Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging. Am. J. Epidemiol. *156*, 445-453.

Lisman, J. and Raghavachari, S. (2006). A unified model of the presynaptic and postsynaptic changes during LTP at CA1 synapses. Sci. STKE *2006*, re11.

Lisman, J. and Raghavachari, S. (2015). Biochemical principles underlying the stable maintenance of LTP by the CaMKII/NMDAR complex. Brain Res. *1621*, 51-61.

Lister, J.P. and Barnes, C.A. (2009). Neurobiological changes in the hippocampus during normative aging. Arch. Neurol. *66*, 829-833.

Liu, A.C., Lewis, W.G. and Kay, S.A. (2007). Mammalian circadian signaling networks and therapeutic targets. Nat. Chem. Biol. *3*, 630-639.

Liu, F. and Chang, H.C. (2017). Physiological links of circadian clock and biological clock of aging. Protein Cell

Liu, Y., Xu, Y.F., Zhang, L., Huang, L., Yu, P., Zhu, H., Deng, W. and Qin, C. (2017). Effective expression of Drebrin in hippocampus improves cognitive function and alleviates lesions of Alzheimer's disease in APP (swe)/PS1 (DeltaE9) mice. CNS neuroscience & therapeutics *23*, 590-604.

Livingston, G., Sommerlad, A., Orgeta, V., Costafreda, S.G., Huntley, J., Ames, D., Ballard, C., Banerjee, S., Burns, A., Cohen-Mansfield, J.*, et al.* (2017). Dementia prevention, intervention, and care. Lancet *390*, 2673-2734.

Loh, D.H., Navarro, J., Hagopian, A., Wang, L.M., Deboer, T. and Colwell, C.S. (2010). Rapid changes in the light/dark cycle disrupt memory of conditioned fear in mice. PLoS ONE *5*,

Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M. and Kroemer, G. (2013). The hallmarks of aging. Cell *153*, 1194-1217.

Lopez, O.L., McDade, E., Riverol, M. and Becker, J.T. (2011). Evolution of the diagnostic criteria for degenerative and cognitive disorders. Curr. Opin. Neurol. *24*, 532-541.

Lucey, B.P., McCullough, A., Landsness, E.C., Toedebusch, C.D., McLeland, J.S., Zaza, A.M., Fagan, A.M., McCue, L., Xiong, C., Morris, J.C.*, et al.* (2019). Reduced non-rapid eye movement sleep is associated with tau pathology in early Alzheimer's disease. Sci. Transl. Med. *11*,

Lund, T.M., Torsvik, H., Falch, D., Christophersen, B., Skardal, R. and Gullestad, L. (2002). Effect of morning versus evening intake of simvastatin on the serum cholesterol level in patients with coronary artery disease. Am. J. Cardiol. *90*, 784-786.

Luscher, C. and Malenka, R.C. (2012). NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). Cold Spring Harb. Perspect. Biol. *4*,

Lyketsos, C.G., Carrillo, M.C., Ryan, J.M., Khachaturian, A.S., Trzepacz, P., Amatniek, J., Cedarbaum, J., Brashear, R. and Miller, D.S. (2011). Neuropsychiatric symptoms in Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association *7*, 532-539.

Lyketsos, C.G., Lopez, O., Jones, B., Fitzpatrick, A.L., Breitner, J. and DeKosky, S. (2002). Prevalence of neuropsychiatric symptoms in dementia and mild cognitive impairment: results from the cardiovascular health study. JAMA *288*, 1475-1483.

Lynch, M.A. (2004). Long-term potentiation and memory. Physiol. Rev. *84*, 87-136.

Ma, T.J., Zhang, Z.W., Lu, Y.L., Zhang, Y.Y., Tao, D.C., Liu, Y.Q. and Ma, Y.X. (2018). CLOCK and BMAL1 stabilize and activate RHOA to promote F-actin formation in cancer cells. Exp. Mol. Med. *50*, 130.

Maccioni, R.B., Farias, G., Morales, I. and Navarrete, L. (2010). The revitalized tau hypothesis on Alzheimer's disease. Arch. Med. Res. *41*, 226-231.

Mackenzie, I.R. and Rademakers, R. (2008). The role of transactive response DNA-binding protein-43 in amyotrophic lateral sclerosis and frontotemporal dementia. Curr. Opin. Neurol. *21*, 693-700.

Madeira, M.D., Sousa, N., Santer, R.M., Paula-Barbosa, M.M. and Gundersen, H.J. (1995). Age and sex do not affect the volume, cell numbers, or cell size of the suprachiasmatic nucleus of the rat: an unbiased stereological study. J. Comp. Neurol. *361*, 585-601.

Magnusson, A. and Boivin, D. (2003). Seasonal affective disorder: an overview. Chronobiol. Int. *20*, 189-207.

Maheshwari, P. and Eslick, G.D. (2015). Bacterial infection and Alzheimer's disease: a meta-analysis. J. Alzheimers Dis. *43*, 957-966.

Mahley, R.W. and Huang, Y. (2012). Apolipoprotein e sets the stage: response to injury triggers neuropathology. Neuron *76*, 871-885.

Maiese, K. (2018). Novel Treatment Strategies for the Nervous System: Circadian Clock Genes, Non-coding RNAs, and Forkhead Transcription Factors. Curr. Neurovasc. Res. *15*, 81-91.

Malenka, R.C. and Bear, M.F. (2004). LTP and LTD: an embarrassment of riches. Neuron *44*, 5-21.

Mander, B.A., Rao, V., Lu, B., Saletin, J.M., Lindquist, J.R., Ancoli-Israel, S., Jagust, W. and Walker, M.P. (2013). Prefrontal atrophy, disrupted NREM slow waves and impaired hippocampal-dependent memory in aging. Nat. Neurosci. *16*, 357-364.

Maragos, W.F., Greenamyre, J.T., Penney Jr, J.B. and Young, A.B. (1987). Glutamate dysfunction in Alzheimer's disease: an hypothesis. Trends Neurosci. *10*, 65-68.

Marambaud, P., Dreses-Werringloer, U. and Vingtdeux, V. (2009). Calcium signaling in neurodegeneration. Mol. Neurodegener. *4*, 20.

Markesbery, W.R. (1997). Oxidative stress hypothesis in Alzheimer's disease. Free Radic. Biol. Med. *23*, 134-147.

Martin, S.J. and Clark, R.E. (2007). The rodent hippocampus and spatial memory: from synapses to systems. Cell. Mol. Life Sci. *64*, 401-431.

Martin, S.J., Grimwood, P.D. and Morris, R.G. (2000). Synaptic plasticity and memory: an evaluation of the hypothesis. Annu. Rev. Neurosci. *23*, 649-711.

Martino, T.A. and Young, M.E. (2015). Influence of the cardiomyocyte circadian clock on cardiac physiology and pathophysiology. J. Biol. Rhythms *30*, 183-205.

Masri, S., Patel, V.R., Eckel-Mahan, K.L., Peleg, S., Forne, I., Ladurner, A.G., Baldi, P., Imhof, A. and Sassone-Corsi, P. (2013). Circadian acetylome reveals regulation of mitochondrial metabolic pathways. Proc. Natl. Acad. Sci. U. S. A. *110*, 3339-3344.

Masri, S. and Sassone-Corsi, P. (2013). The circadian clock: a framework linking metabolism, epigenetics and neuronal function. Nat. Rev. Neurosci. *14*, 69-75.

Massoud, F. and Gauthier, S. (2010). Update on the pharmacological treatment of Alzheimer's disease. Current neuropharmacology *8*, 69-80.

Matsumoto, Y., Tsunekawa, Y., Nomura, T., Suto, F., Matsumata, M., Tsuchiya, S. and Osumi, N. (2011). Differential proliferation rhythm of neural progenitor and oligodendrocyte precursor cells in the young adult hippocampus. PLoS ONE *6*, e27628.

Mattis, J. and Sehgal, A. (2016). Circadian Rhythms, Sleep, and Disorders of Aging. Trends Endocrinol Metab *27*, 192-203.

Mattson, M.P. (2004). Pathways towards and away from Alzheimer's disease. Nature *430*, 631-639.

Mattson, M.P. (2007). Calcium and neurodegeneration. Aging Cell *6*, 337-350.

Mattson, M.P. and Arumugam, T.V. (2018). Hallmarks of Brain Aging: Adaptive and Pathological Modification by Metabolic States. Cell Metab. *27*, 1176-1199.

Maury, E., Ramsey, K.M. and Bass, J. (2010). Circadian rhythms and metabolic syndrome: from experimental genetics to human disease. Circ. Res. *106*, 447-462.

Mauvoisin, D., Wang, J., Jouffe, C., Martin, E., Atger, F., Waridel, P., Quadroni, M., Gachon, F. and Naef, F. (2014). Circadian clock-dependent and -independent rhythmic proteomes implement distinct diurnal functions in mouse liver. Proc. Natl. Acad. Sci. U. S. A. *111*, 167-172.

Mayford, M., Siegelbaum, S.A. and Kandel, E.R. (2012). Synapses and memory storage. Cold Spring Harb. Perspect. Biol. *4*,

McCarthy, J.J., Andrews, J.L., McDearmon, E.L., Campbell, K.S., Barber, B.K., Miller, B.H., Walker, J.R., Hogenesch, J.B., Takahashi, J.S. and Esser, K.A. (2007). Identification of the circadian transcriptome in adult mouse skeletal muscle. Physiol. Genomics *31*, 86-95.

McCleery, J., Cohen, D.A. and Sharpley, A.L. (2016). Pharmacotherapies for sleep disturbances in dementia. Cochrane Database Syst. Rev. *11*, CD009178.

McClung, C.A. (2007). Circadian genes, rhythms and the biology of mood disorders. Pharmacol. Ther. *114*, 222-232.

McHugh, D. and Gil, J. (2018). Senescence and aging: Causes, consequences, and therapeutic avenues. J. Cell Biol. *217*, 65-77.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D. and Stadlan, E.M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology *34*, 939-944.

McKhann, G.M., Knopman, D.S., Chertkow, H., Hyman, B.T., Jack, C.R., Jr., Kawas, C.H., Klunk, W.E., Koroshetz, W.J., Manly, J.J., Mayeux, R.*, et al.* (2011). The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association *7*, 263-269.

McShane, R., Areosa Sastre, A. and Minakaran, N. (2006). Memantine for dementia. Cochrane Database Syst. Rev. CD003154.

Medvedev, Z.A. (1990). An attempt at a rational classification of theories of ageing. Biol. Rev. Camb. Philos. Soc. *65*, 375-398.

Mehta, M., Adem, A. and Sabbagh, M. (2012). New acetylcholinesterase inhibitors for Alzheimer's disease. International journal of Alzheimer's disease *2012*, 728983.

Meng, Q.J., Logunova, L., Maywood, E.S., Gallego, M., Lebiecki, J., Brown, T.M., Sladek, M., Semikhodskii, A.S., Glossop, N.R., Piggins, H.D.*, et al.* (2008). Setting clock speed in mammals: the CK1 epsilon tau mutation in mice accelerates circadian pacemakers by selectively destabilizing PERIOD proteins. Neuron *58*, 78-88.

Meng, Q.J., Maywood, E.S., Bechtold, D.A., Lu, W.Q., Li, J., Gibbs, J.E., Dupre, S.M., Chesham, J.E., Rajamohan, F., Knafels, J.*, et al.* (2010). Entrainment of disrupted circadian behavior through inhibition of casein kinase 1 (CK1) enzymes. Proc. Natl. Acad. Sci. U. S. A. *107*, 15240-15245.

Merkwirth, C., Martinelli, P., Korwitz, A., Morbin, M., Bronneke, H.S., Jordan, S.D., Rugarli, E.I. and Langer, T. (2012). Loss of prohibitin membrane scaffolds impairs mitochondrial architecture and leads to tau hyperphosphorylation and neurodegeneration. PLoS Genet. *8*, e1003021.

Mermet, J., Yeung, J. and Naef, F. (2017). Systems Chronobiology: Global Analysis of Gene Regulation in a 24-Hour Periodic World. Cold Spring Harb. Perspect. Biol. *9*,

Millecamps, S., Salachas, F., Cazeneuve, C., Gordon, P., Bricka, B., Camuzat, A., Guillot-Noel, L., Russaouen, O., Bruneteau, G., Pradat, P.F.*, et al.* (2010). SOD1, ANG, VAPB, TARDBP, and FUS mutations in familial amyotrophic lateral sclerosis: genotype-phenotype correlations. J. Med. Genet. *47*, 554-560.

Miller, B.H., McDearmon, E.L., Panda, S., Hayes, K.R., Zhang, J., Andrews, J.L., Antoch, M.P., Walker, J.R., Esser, K.A., Hogenesch, J.B.*, et al.* (2007). Circadian and CLOCK-controlled regulation of the mouse transcriptome and cell proliferation. Proc. Natl. Acad. Sci. U. S. A. *104*, 3342-3347.

Millius, A. and Ueda, H.R. (2017). Systems Biology-Derived Discoveries of Intrinsic Clocks. Frontiers in neurology *8*, 25.

Minakawa, E.N., Miyazaki, K., Maruo, K., Yagihara, H., Fujita, H., Wada, K. and Nagai, Y. (2017). Chronic sleep fragmentation exacerbates amyloid beta deposition in Alzheimer's disease model mice. Neurosci. Lett. *653*, 362-369.

Mistlberger, R.E., Houpt, T.A. and Moore-Ede, M.C. (1990). Effects of aging on food-entrained circadian rhythms in the rat. Neurobiol. Aging *11*, 619-624.

Mitsushima, D., Mizuno, T. and Kimura, F. (1996). Age-related changes in diurnal acetylcholine release in the prefrontal cortex of male rats as measured by microdialysis. Neuroscience *72*, 429-434.

Miyamoto, E. (2006). Molecular mechanism of neuronal plasticity: induction and maintenance of long-term potentiation in the hippocampus. J. Pharmacol. Sci. *100*, 433-442.

Mohawk, J.A., Green, C.B. and Takahashi, J.S. (2012). Central and peripheral circadian clocks in mammals. Annu. Rev. Neurosci. *35*, 445-462.

Moller, H.J. and Graeber, M.B. (1998). The case described by Alois Alzheimer in 1911. Historical and conceptual perspectives based on the clinical record and neurohistological sections. Eur. Arch. Psychiatry Clin. Neurosci. *248*, 111-122.

Monastyrskyi, A., Nilchan, N., Quereda, V., Noguchi, Y., Ruiz, C., Grant, W., Cameron, M., Duckett, D. and Roush, W. (2018). Development of dual casein kinase 1delta/1epsilon (CK1delta/epsilon) inhibitors for treatment of breast cancer. Bioorg. Med. Chem. *26*, 590-602.

Monk, T.H. (2005). Aging human circadian rhythms: conventional wisdom may not always be right. J. Biol. Rhythms *20*, 366-374.

Monk, T.H., Buysse, D.J., Carrier, J. and Kupfer, D.J. (2000). Inducing jet-lag in older people: directional asymmetry. J. Sleep Res. *9*, 101-116.

Monk, T.H., Weitzman, E.D., Fookson, J.E., Moline, M.L., Kronauer, R.E. and Gander, P.H. (1983). Task variables determine which biological clock controls circadian rhythms in human performance. Nature *304*, 543-545.

Montagne, A., Barnes, S.R., Sweeney, M.D., Halliday, M.R., Sagare, A.P., Zhao, Z., Toga, A.W., Jacobs, R.E., Liu, C.Y., Amezcua, L.*, et al.* (2015). Blood-brain barrier breakdown in the aging human hippocampus. Neuron *85*, 296-302.

Moore, R.Y. and Eichler, V.B. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res. *42*, 201-206.

Morris, J.C. (1997). Clinical dementia rating: a reliable and valid diagnostic and staging measure for dementia of the Alzheimer type. Int. Psychogeriatr. *9 Suppl 1*, 173-176; discussion 177-178.

Morse, D. and Sassone-Corsi, P. (2002). Time after time: inputs to and outputs from the mammalian circadian oscillators. Trends Neurosci. *25*, 632-637.

Moyer, J.R., Jr., Thompson, L.T. and Disterhoft, J.F. (1996). Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. J. Neurosci. *16*, 5536-5546.

Mozzachiodi, R. and Byrne, J.H. (2010). More than synaptic plasticity: role of nonsynaptic plasticity in learning and memory. Trends Neurosci. *33*, 17-26.

Mure, L.S., Le, H.D., Benegiamo, G., Chang, M.W., Rios, L., Jillani, N., Ngotho, M., Kariuki, T., Dkhissi-Benyahya, O., Cooper, H.M.*, et al.* (2018). Diurnal transcriptome atlas of a primate across major neural and peripheral tissues. Science *359*,

Murray, M.E., Graff-Radford, N.R., Ross, O.A., Petersen, R.C., Duara, R. and Dickson, D.W. (2011). Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. Lancet Neurol. *10*, 785-796.

Murthy, V.N. (1998). Synaptic plasticity: step-wise strengthening. Curr. Biol. *8*, R650-653.

Musiek, E.S. (2015). Circadian clock disruption in neurodegenerative diseases: cause and effect? Front Pharmacol *6*, 29.

Musiek, E.S., Bhimasani, M., Zangrilli, M.A., Morris, J.C., Holtzman, D.M. and Ju, Y.S. (2018). Circadian Rest-Activity Pattern Changes in Aging and Preclinical Alzheimer Disease. JAMA Neurol *75*, 582-590.

Musiek, E.S. and Holtzman, D.M. (2016). Mechanisms linking circadian clocks, sleep, and neurodegeneration. Science *354*, 1004-1008.

Musiek, E.S., Xiong, D.D. and Holtzman, D.M. (2015). Sleep, circadian rhythms, and the pathogenesis of Alzheimer disease. Exp. Mol. Med. *47*, e148.

Musunuri, S., Wetterhall, M., Ingelsson, M., Lannfelt, L., Artemenko, K., Bergquist, J., Kultima, K. and Shevchenko, G. (2014). Quantification of the brain proteome in Alzheimer's disease using multiplexed mass spectrometry. J. Proteome Res. *13*, 2056-2068.

Naik, M. and Nygaard, H.A. (2008). Diagnosing dementia -- ICD-10 not so bad after all: a comparison between dementia criteria according to DSM-IV and ICD-10. Int. J. Geriatr. Psychiatry *23*, 279-282.

Nakamura, T.J., Nakamura, W., Tokuda, I.T., Ishikawa, T., Kudo, T., Colwell, C.S. and Block, G.D. (2015). Age-Related Changes in the Circadian System Unmasked by Constant Conditions(1,2,3). eNeuro *2*,

Nakamura, T.J., Nakamura, W., Yamazaki, S., Kudo, T., Cutler, T., Colwell, C.S. and Block, G.D. (2011). Age-related decline in circadian output. J. Neurosci. *31*, 10201-10205.

Naseri Kouzehgarani, G., Bothwell, M.Y. and Gillette, M.U. (2019). Circadian rhythm of redox state regulates membrane excitability in hippocampal CA1 neurons. Eur. J. Neurosci.

Navarro, A. and Boveris, A. (2010). Brain mitochondrial dysfunction in aging, neurodegeneration, and Parkinson's disease. Frontiers in aging neuroscience *2*,

Neufeld-Cohen, A., Robles, M.S., Aviram, R., Manella, G., Adamovich, Y., Ladeuix, B., Nir, D., Rousso-Noori, L., Kuperman, Y., Golik, M.*, et al.* (2016). Circadian control of oscillations in mitochondrial rate-limiting enzymes and nutrient utilization by PERIOD proteins. Proc. Natl. Acad. Sci. U. S. A. *113*, E1673-1682.

Neuner, S.M., Wilmott, L.A., Hoffmann, B.R., Mozhui, K. and Kaczorowski, C.C. (2017). Hippocampal proteomics defines pathways associated with memory decline and resilience in normal aging and Alzheimer's disease mouse models. Behav. Brain Res. *322*, 288-298.

Neves, G., Cooke, S.F. and Bliss, T.V. (2008). Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nat. Rev. Neurosci. *9*, 65-75.

Niccoli, T. and Partridge, L. (2012). Ageing as a risk factor for disease. Curr. Biol. *22*, R741-752.

Niccoli, T., Partridge, L. and Isaacs, A.M. (2017). Ageing as a risk factor for ALS/FTD. Hum. Mol. Genet. *26*, R105-R113.

Nicoll, R.A. and Schmitz, D. (2005). Synaptic plasticity at hippocampal mossy fibre synapses. Nat. Rev. Neurosci. *6*, 863-876.

Noguchi, T., Leise, T.L., Kingsbury, N.J., Diemer, T., Wang, L.L., Henson, M.A. and Welsh, D.K. (2017). Calcium Circadian Rhythmicity in the Suprachiasmatic Nucleus: Cell Autonomy and Network Modulation. eNeuro *4*,

Norton, S., Matthews, F.E., Barnes, D.E., Yaffe, K. and Brayne, C. (2014). Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol. *13*, 788-794.

Nowrangi, M.A., Lyketsos, C.G. and Rosenberg, P.B. (2015). Principles and management of neuropsychiatric symptoms in Alzheimer's dementia. Alzheimers Res. Ther. *7*, 12.

Nyberg, L., Lovden, M., Riklund, K., Lindenberger, U. and Backman, L. (2012). Memory aging and brain maintenance. Trends in cognitive sciences *16*, 292-305.

O'Sullivan, C., Duggan, J., Atkins, N. and O'Brien, E. (2003). Twenty-four-hour ambulatory blood pressure in community-dwelling elderly men and women, aged 60-102 years. J. Hypertens. *21*, 1641-1647.

Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kayed, R., Metherate, R., Mattson, M.P., Akbari, Y. and LaFerla, F.M. (2003). Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron *39*, 409-421.

Olazaran, J., Reisberg, B., Clare, L., Cruz, I., Pena-Casanova, J., Del Ser, T., Woods, B., Beck, C., Auer, S., Lai, C., *et al.* (2010). Nonpharmacological therapies in Alzheimer's disease: a systematic review of efficacy. Dement. Geriatr. Cogn. Disord. *30*, 161-178.

Oosterman, J.M., van Someren, E.J., Vogels, R.L., Van Harten, B. and Scherder, E.J. (2009). Fragmentation of the rest-activity rhythm correlates with age-related cognitive deficits. J. Sleep Res. *18*, 129-135.

Oser, M., Yamaguchi, H., Mader, C.C., Bravo-Cordero, J.J., Arias, M., Chen, X., Desmarais, V., van Rheenen, J., Koleske, A.J. and Condeelis, J. (2009). Cortactin regulates cofilin and N-WASp activities to control the stages of invadopodium assembly and maturation. J. Cell Biol. *186*, 571-587.

Ota, Y., Zanetti, A.T. and Hallock, R.M. (2013). The role of astrocytes in the regulation of synaptic plasticity and memory formation. Neural Plast. *2013*, 185463.

Pagani, L., Schmitt, K., Meier, F., Izakovic, J., Roemer, K., Viola, A., Cajochen, C., Wirz-Justice, A., Brown, S.A. and Eckert, A. (2011). Serum factors in older individuals change cellular clock properties. Proc. Natl. Acad. Sci. U. S. A. *108*, 7218-7223.

Palomba, M., Nygard, M., Florenzano, F., Bertini, G., Kristensson, K. and Bentivoglio, M. (2008). Decline of the presynaptic network, including GABAergic terminals, in the aging suprachiasmatic nucleus of the mouse. J. Biol. Rhythms *23*, 220-231.

Panda, S. (2019). The arrival of circadian medicine. Nat. Rev. Endocrinol. *15*, 67-69.

Panda, S., Antoch, M.P., Miller, B.H., Su, A.I., Schook, A.B., Straume, M., Schultz, P.G., Kay, S.A., Takahashi, J.S. and Hogenesch, J.B. (2002). Coordinated transcription of key pathways in the mouse by the circadian clock. Cell *109*, 307-320.

Panegyres, P.K. and Chen, H.Y. (2013). Differences between early and late onset Alzheimer's disease. American journal of neurodegenerative disease *2*, 300-306.

Paul, J.R., Munir, H.A., van Groen, T. and Gamble, K.L. (2018). Behavioral and SCN neurophysiological disruption in the Tg-SwDI mouse model of Alzheimer's disease. Neurobiol. Dis. *114*, 194-200.

Peek, C.B., Affinati, A.H., Ramsey, K.M., Kuo, H.Y., Yu, W., Sena, L.A., Ilkayeva, O., Marcheva, B., Kobayashi, Y., Omura, C., *et al.* (2013). Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. Science *342*, 1243417.

Peng, M.T., Chen, Y.T., Hung, S.H. and Yaung, C.L. (1990). Circadian rhythms of feeding and drinking behavior of rats aged from 3 to 21 months. Proc. Natl. Sci. Counc. Repub. China B *14*, 98-104.

Pennartz, C.M., Ito, R., Verschure, P.F., Battaglia, F.P. and Robbins, T.W. (2011). The hippocampal-striatal axis in learning, prediction and goal-directed behavior. Trends Neurosci. *34*, 548-559.

Pereira, A.C., Gray, J.D., Kogan, J.F., Davidson, R.L., Rubin, T.G., Okamoto, M., Morrison, J.H. and McEwen, B.S. (2017). Age and Alzheimer's disease gene expression profiles reversed by the glutamate modulator riluzole. Mol. Psychiatry *22*, 296-305.

Perez-Cruz, C., Simon, M., Flugge, G., Fuchs, E. and Czeh, B. (2009). Diurnal rhythm and stress regulate dendritic architecture and spine density of pyramidal neurons in the rat infralimbic cortex. Behav. Brain Res. *205*, 406-413.

Perez, D.I., Gil, C. and Martinez, A. (2011). Protein kinases CK1 and CK2 as new targets for neurodegenerative diseases. Med. Res. Rev. *31*, 924-954.

Perreau-Lenz, S., Vengeliene, V., Noori, H.R., Merlo-Pich, E.V., Corsi, M.A., Corti, C. and Spanagel, R. (2012). Inhibition of the casein-kinase-1-epsilon/delta/ prevents relapse-like alcohol drinking. Neuropsychopharmacology *37*, 2121-2131.

Petersen, R.C. (2004). Mild cognitive impairment as a diagnostic entity. J. Intern. Med. *256*, 183-194.

Petersen, R.C., Smith, G.E., Waring, S.C., Ivnik, R.J., Kokmen, E. and Tangelos, E.G. (1997). Aging, memory, and mild cognitive impairment. Int. Psychogeriatr. *9 Suppl 1*, 65-69.

Phan, T.X., Chan, G.C., Sindreu, C.B., Eckel-Mahan, K.L. and Storm, D.R. (2011). The diurnal oscillation of MAP (mitogen-activated protein) kinase and adenylyl cyclase activities in the hippocampus depends on the suprachiasmatic nucleus. J. Neurosci. *31*, 10640-10647.

Phelps, E.A. (2004). Human emotion and memory: interactions of the amygdala and hippocampal complex. Curr. Opin. Neurobiol. *14*, 198-202.

Piaceri, I., Nacmias, B. and Sorbi, S. (2013). Genetics of familial and sporadic Alzheimer's disease. Front Biosci (Elite Ed) *5*, 167-177.

Pimplikar, S.W. (2009). Reassessing the amyloid cascade hypothesis of Alzheimer's disease. Int. J. Biochem. Cell Biol. *41*, 1261-1268.

Pirttimaki, T.M. and Parri, H.R. (2013). Astrocyte plasticity: implications for synaptic and neuronal activity. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry *19*, 604-615.

Pontes, A.H. and de Sousa, M.V. (2016). Mass Spectrometry-Based Approaches to Understand the Molecular Basis of Memory. Front Chem *4*, 40.

Popa-Wagner, A., Buga, A.M., Dumitrascu, D.I., Uzoni, A., Thome, J. and Coogan, A.N. (2017). How does healthy aging impact on the circadian clock? J Neural Transm (Vienna) *124*, 89-97.

Powers, E.T., Morimoto, R.I., Dillin, A., Kelly, J.W. and Balch, W.E. (2009). Biological and chemical approaches to diseases of proteostasis deficiency. Annu. Rev. Biochem. *78*, 959-991.

Pridgeon, J.W., Olzmann, J.A., Chin, L.S. and Li, L. (2007). PINK1 protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1. PLoS Biol. *5*, e172.

Prince, M., Wimo, A., Guerchet, M., Ali, G.C., Wu, Y.T., and Prina, M. (2015). World Alzheimer Report 2015—The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends. London: Alzheimer’s Disease International.

Qiu, C., Kivipelto, M. and von Strauss, E. (2009). Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. Dialogues Clin. Neurosci. *11*, 111-128.

Rangaraju, V., Calloway, N. and Ryan, T.A. (2014). Activity-driven local ATP synthesis is required for synaptic function. Cell *156*, 825-835.

Rawashdeh, O. and Maronde, E. (2012). The hormonal Zeitgeber melatonin: role as a circadian modulator in memory processing. Front. Mol. Neurosci. *5*, 27.

Rawashdeh, O., Parsons, R. and Maronde, E. (2018). Clocking In Time to Gate Memory Processes: The Circadian Clock Is Part of the Ins and Outs of Memory. Neural Plast. *2018*, 6238989.

Raymond, C.R. (2007). LTP forms 1, 2 and 3: different mechanisms for the "long" in long-term potentiation. Trends Neurosci. *30*, 167-175.

Reghunandanan, V. and Reghunandanan, R. (2006). Neurotransmitters of the suprachiasmatic nuclei. Journal of circadian rhythms *4*, 2.

Reischl, S. and Kramer, A. (2011). Kinases and phosphatases in the mammalian circadian clock. FEBS Lett. *585*, 1393-1399.

Reitz, C., Brayne, C. and Mayeux, R. (2011). Epidemiology of Alzheimer disease. Nature reviews. Neurology *7*, 137-152.

Reppert, S.M. and Weaver, D.R. (2002). Coordination of circadian timing in mammals. Nature *418*, 935-941.

Resuehr, D., Wu, G., Johnson, R.L., Jr., Young, M.E., Hogenesch, J.B. and Gamble, K.L. (2019). Shift Work Disrupts Circadian Regulation of the Transcriptome in Hospital Nurses. J. Biol. Rhythms *34*, 167-177.

Richards, J. and Gumz, M.L. (2013). Mechanism of the circadian clock in physiology. Am. J. Physiol. Regul. Integr. Comp. Physiol. *304*, R1053-1064.

Ritchie, K. and Lovestone, S. (2002). The dementias. Lancet *360*, 1759-1766.

Robinson, S.R., Dobson, C. and Lyons, J. (2004). Challenges and directions for the pathogen hypothesis of Alzheimer's disease. Neurobiol. Aging *25*, 629-637.

Robles, M.S., Cox, J. and Mann, M. (2014). In-vivo quantitative proteomics reveals a key contribution of post-transcriptional mechanisms to the circadian regulation of liver metabolism. PLoS Genet. *10*, e1004047.

Robles, M.S., Humphrey, S.J. and Mann, M. (2017). Phosphorylation Is a Central Mechanism for Circadian Control of Metabolism and Physiology. Cell Metab. *25*, 118-127.

Roby, D.A., Ruiz, F., Kermath, B.A., Voorhees, J.R., Niehoff, M., Zhang, J., Morley, J.E., Musiek, E.S., Farr, S.A. and Burris, T.P. (2019). Pharmacological activation of the nuclear receptor REV-ERB reverses cognitive deficits and reduces amyloid-beta burden in a mouse model of Alzheimer's disease. PLoS ONE *14*, e0215004.

Rodriguez, F., Lopez, J.C., Vargas, J.P., Broglio, C., Gomez, Y. and Salas, C. (2002). Spatial memory and hippocampal pallium through vertebrate evolution: insights from reptiles and teleost fish. Brain Res. Bull. *57*, 499-503.

Roenneberg, T., Kuehnle, T., Juda, M., Kantermann, T., Allebrandt, K., Gordijn, M. and Merrow, M. (2007). Epidemiology of the human circadian clock. Sleep medicine reviews *11*, 429-438.

Ronnlund, M., Nyberg, L., Backman, L. and Nilsson, L.G. (2005). Stability, growth, and decline in adult life span development of declarative memory: cross-sectional and longitudinal data from a population-based study. Psychol. Aging *20*, 3-18.

Roozendaal, B., van Gool, W.A., Swaab, D.F., Hoogendijk, J.E. and Mirmiran, M. (1987). Changes in vasopressin cells of the rat suprachiasmatic nucleus with aging. Brain Res. *409*, 259-264.

Ruby, N.F., Fernandez, F., Garrett, A., Klima, J., Zhang, P., Sapolsky, R. and Heller, H.C. (2013). Spatial memory and long-term object recognition are impaired by circadian arrhythmia and restored by the GABAAAntagonist pentylenetetrazole. PLoS ONE *8*, e72433.

Ruby, N.F., Hwang, C.E., Wessells, C., Fernandez, F., Zhang, P., Sapolsky, R. and Heller, H.C. (2008). Hippocampal-dependent learning requires a functional circadian system. Proc. Natl. Acad. Sci. U. S. A. *105*, 15593-15598.

Rugg, M.D. and Vilberg, K.L. (2013). Brain networks underlying episodic memory retrieval. Curr. Opin. Neurobiol. *23*, 255-260.

Sabeti, J. and Gruol, D.L. (2008). Emergence of NMDAR-independent long-term potentiation at hippocampal CA1 synapses following early adolescent exposure to chronic intermittent ethanol: role for sigma-receptors. Hippocampus *18*, 148-168.

Sachdev, P.S., Blacker, D., Blazer, D.G., Ganguli, M., Jeste, D.V., Paulsen, J.S. and Petersen, R.C. (2014). Classifying neurocognitive disorders: the DSM-5 approach. Nature reviews. Neurology *10*, 634-642.

Sahar, S. and Sassone-Corsi, P. (2009). Metabolism and cancer: the circadian clock connection. Nat. Rev. Cancer *9*, 886-896.

Saito, Y., Yoshida, S., Nakaya, N., Hata, Y. and Goto, Y. (1991). Comparison between morning and evening doses of simvastatin in hyperlipidemic subjects. A double-blind comparative study. Arterioscler. Thromb. *11*, 816-826.

Salami, O., Lyketsos, C. and Rao, V. (2011). Treatment of sleep disturbance in Alzheimer's dementia. Int. J. Geriatr. Psychiatry *26*, 771-782.

Sanabria-Castro, A., Alvarado-Echeverria, I. and Monge-Bonilla, C. (2017). Molecular Pathogenesis of Alzheimer's Disease: An Update. Annals of neurosciences *24*, 46-54.

Satinoff, E., Li, H., Tcheng, T.K., Liu, C., McArthur, A.J., Medanic, M. and Gillette, M.U. (1993). Do the suprachiasmatic nuclei oscillate in old rats as they do in young ones? Am. J. Physiol. *265*, R1216-1222.

Sato, S., Solanas, G., Peixoto, F.O., Bee, L., Symeonidi, A., Schmidt, M.S., Brenner, C., Masri, S., Benitah, S.A. and Sassone-Corsi, P. (2017). Circadian Reprogramming in the Liver Identifies Metabolic Pathways of Aging. Cell *170*, 664-677 e611.

Savage, M.J. and Gingrich, D.E. (2009). Advances in the Development of Kinase Inhibitor Therapeutics for Alzheimer’s Disease. Drug Dev Res *70*, 125-144.

Scheltens, P., Blennow, K., Breteler, M.M., de Strooper, B., Frisoni, G.B., Salloway, S. and Van der Flier, W.M. (2016). Alzheimer's disease. Lancet *388*, 505-517.

Schlosser Covell, G.E., Dhawan, P.S., Lee Iannotti, J.K., Hoffman-Snyder, C.R., Wellik, K.E., Caselli, R.J., Woodruff, B.K., Wingerchuk, D.M. and Demaerschalk, B.M. (2012). Disrupted daytime activity and altered sleep-wake patterns may predict transition to mild cognitive impairment or dementia: a critically appraised topic. The neurologist *18*, 426-429.

Schmidt, C., Peigneux, P. and Cajochen, C. (2012). Age-related changes in sleep and circadian rhythms: impact on cognitive performance and underlying neuroanatomical networks. Frontiers in neurology *3*, 118.

Schmitt, K., Grimm, A. and Eckert, A. (2017). Amyloid-beta-Induced Changes in Molecular Clock Properties and Cellular Bioenergetics. Front. Neurosci. *11*, 124.

Scoville, W.B. and Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. J. Neurol. Neurosurg. Psychiatry *20*, 11-21.

Sellix, M.T., Evans, J.A., Leise, T.L., Castanon-Cervantes, O., Hill, D.D., DeLisser, P., Block, G.D., Menaker, M. and Davidson, A.J. (2012). Aging differentially affects the re-entrainment response of central and peripheral circadian oscillators. J. Neurosci. *32*, 16193-16202.

Shen, J. and Kelleher, R.J., 3rd (2007). The presenilin hypothesis of Alzheimer's disease: evidence for a loss-of-function pathogenic mechanism. Proc. Natl. Acad. Sci. U. S. A. *104*, 403-409.

Shetty, P.K., Galeffi, F. and Turner, D.A. (2011). Age-Induced Alterations in Hippocampal Function and Metabolism. Aging and disease *2*, 196-218.

Shi, L., Chen, S.J., Ma, M.Y., Bao, Y.P., Han, Y., Wang, Y.M., Shi, J., Vitiello, M.V. and Lu, L. (2018). Sleep disturbances increase the risk of dementia: A systematic review and meta-analysis. Sleep medicine reviews *40*, 4-16.

Shoji, H., Takao, K., Hattori, S. and Miyakawa, T. (2016). Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. Mol. Brain *9*, 11.

Singh, S., Brocker, C., Koppaka, V., Chen, Y., Jackson, B.C., Matsumoto, A., Thompson, D.C. and Vasiliou, V. (2013). Aldehyde dehydrogenases in cellular responses to oxidative/electrophilic stress. Free Radic. Biol. Med. *56*, 89-101.

Small, S.A. and Duff, K. (2008). Linking Abeta and tau in late-onset Alzheimer's disease: a dual pathway hypothesis. Neuron *60*, 534-542.

Smarr, B.L., Jennings, K.J., Driscoll, J.R. and Kriegsfeld, L.J. (2014). A time to remember: the role of circadian clocks in learning and memory. Behav. Neurosci. *128*, 283-303.

Smolensky, M.H. and Peppas, N.A. (2007). Chronobiology, drug delivery, and chronotherapeutics. Adv Drug Deliv Rev *59*, 828-851.

Snider, K.H., Dziema, H., Aten, S., Loeser, J., Norona, F.E., Hoyt, K. and Obrietan, K. (2016). Modulation of learning and memory by the targeted deletion of the circadian clock gene Bmal1 in forebrain circuits. Behav. Brain Res. *308*, 222-235.

Snider, K.H. and Obrietan, K. (2018). Modulation of learning and memory by the genetic disruption of circadian oscillator populations. Physiol. Behav. *194*, 387-393.

Snider, K.H., Sullivan, K.A. and Obrietan, K. (2018). Circadian Regulation of Hippocampal-Dependent Memory: Circuits, Synapses, and Molecular Mechanisms. Neural Plast. *2018*, 7292540.

Soderling, T.R. and Derkach, V.A. (2000). Postsynaptic protein phosphorylation and LTP. Trends Neurosci. *23*, 75-80.

Solanas, G., Peixoto, F.O., Perdiguero, E., Jardi, M., Ruiz-Bonilla, V., Datta, D., Symeonidi, A., Castellanos, A., Welz, P.S., Caballero, J.M.*, et al.* (2017). Aged Stem Cells Reprogram Their Daily Rhythmic Functions to Adapt to Stress. Cell *170*, 678-692 e620.

Solt, L.A., Wang, Y., Banerjee, S., Hughes, T., Kojetin, D.J., Lundasen, T., Shin, Y., Liu, J., Cameron, M.D., Noel, R.*, et al.* (2012). Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. Nature *485*, 62-68.

Song, H., Moon, M., Choe, H.K., Han, D.H., Jang, C., Kim, A., Cho, S., Kim, K. and Mook-Jung, I. (2015). Abeta-induced degradation of BMAL1 and CBP leads to circadian rhythm disruption in Alzheimer's disease. Mol. Neurodegener. *10*, 13.

Sosa-Ortiz, A.L., Acosta-Castillo, I. and Prince, M.J. (2012). Epidemiology of dementias and Alzheimer's disease. Arch. Med. Res. *43*, 600-608.

Spargo, E., Everall, I.P. and Lantos, P.L. (1993). Neuronal loss in the hippocampus in Huntington's disease: a comparison with HIV infection. J. Neurol. Neurosurg. Psychiatry *56*, 487-491.

Spiers, H.J., Maguire, E.A. and Burgess, N. (2001). Hippocampal amnesia. Neurocase *7*, 357-382.

Sprouse, J., Reynolds, L., Kleiman, R., Tate, B., Swanson, T.A. and Pickard, G.E. (2010). Chronic treatment with a selective inhibitor of casein kinase I delta/epsilon yields cumulative phase delays in circadian rhythms. Psychopharmacology (Berl.) *210*, 569-576.

Sprouse, J., Reynolds, L., Swanson, T.A. and Engwall, M. (2009). Inhibition of casein kinase I epsilon/delta produces phase shifts in the circadian rhythms of Cynomolgus monkeys. Psychopharmacology (Berl.) *204*, 735-742.

Squire, L.R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol. Rev. *99*, 195-231.

Squire, L.R. and Zola-Morgan, S. (1991). The medial temporal lobe memory system. Science *253*, 1380-1386.

Stephan, F.K. and Zucker, I. (1972). Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc. Natl. Acad. Sci. U. S. A. *69*, 1583-1586.

Sterniczuk, R., Dyck, R.H., Laferla, F.M. and Antle, M.C. (2010). Characterization of the 3xTg-AD mouse model of Alzheimer's disease: part 1. Circadian changes. Brain Res. *1348*, 139-148.

Stevanovic, K., Yunus, A., Joly-Amado, A., Gordon, M., Morgan, D., Gulick, D. and Gamsby, J. (2017). Disruption of normal circadian clock function in a mouse model of tauopathy. Exp. Neurol. *294*, 58-67.

Stopa, E.G., Volicer, L., Kuo-Leblanc, V., Harper, D., Lathi, D., Tate, B. and Satlin, A. (1999). Pathologic evaluation of the human suprachiasmatic nucleus in severe dementia. J. Neuropathol. Exp. Neurol. *58*, 29-39.

Storch, K.F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F.C., Wong, W.H. and Weitz, C.J. (2002). Extensive and divergent circadian gene expression in liver and heart. Nature *417*, 78-83.

Sulli, G., Manoogian, E.N.C., Taub, P.R. and Panda, S. (2018a). Training the Circadian Clock, Clocking the Drugs, and Drugging the Clock to Prevent, Manage, and Treat Chronic Diseases. Trends Pharmacol. Sci. *39*, 812-827.

Sulli, G., Rommel, A., Wang, X., Kolar, M.J., Puca, F., Saghatelian, A., Plikus, M.V., Verma, I.M. and Panda, S. (2018b). Pharmacological activation of REV-ERBs is lethal in cancer and oncogene-induced senescence. Nature *553*, 351-355.

Sundar, I.K., Yao, H., Sellix, M.T. and Rahman, I. (2015). Circadian molecular clock in lung pathophysiology. Am. J. Physiol. Lung Cell. Mol. Physiol. *309*, L1056-1075.

Swaab, D.F., Fliers, E. and Partiman, T.S. (1985). The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. Brain Res. *342*, 37-44.

Swerdlow, R.H. and Khan, S.M. (2004). A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. Med. Hypotheses *63*, 8-20.

Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K.P.*, et al.* (2015). STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res. *43*, D447-452.

Takahashi, J.S. (2017). Transcriptional architecture of the mammalian circadian clock. Nat. Rev. Genet. *18*, 164-179.

Takahashi, J.S., Hong, H.K., Ko, C.H. and McDearmon, E.L. (2008). The genetics of mammalian circadian order and disorder: implications for physiology and disease. Nat. Rev. Genet. *9*, 764-775.

Takeda, N. and Maemura, K. (2011). Circadian clock and cardiovascular disease. J. Cardiol. *57*, 249-256.

Takeda, T., Uchihara, T., Arai, N., Mizutani, T. and Iwata, M. (2009). Progression of hippocampal degeneration in amyotrophic lateral sclerosis with or without memory impairment: distinction from Alzheimer disease. Acta Neuropathol *117*, 35-44.

Tapp, W.N. and Holloway, F.A. (1981). Phase shifting circadian rhythms produces retrograde amnesia. Science *211*, 1056-1058.

Terry, R.D. (1996). The pathogenesis of Alzheimer disease: an alternative to the amyloid hypothesis. J. Neuropathol. Exp. Neurol. *55*, 1023-1025.

Tharavanij, T., Wongtanakarn, S., Lerdvuthisopon, N., Teeraaunkul, S., Youngsriphithak, P. and Sritipsukho, P. (2010). Lipid lowering efficacy between morning and evening simvastatin treatment: a randomized double-blind study. J. Med. Assoc. Thai. *93 Suppl 7*, S109-113.

Thosar, S.S., Butler, M.P. and Shea, S.A. (2018). Role of the circadian system in cardiovascular disease. J. Clin. Invest. *128*, 2157-2167.

Tible, O.P., Riese, F., Savaskan, E. and von Gunten, A. (2017). Best practice in the management of behavioural and psychological symptoms of dementia. Ther. Adv. Neurol. Disord. *10*, 297-309.

Tognini, P., Murakami, M., Liu, Y., Eckel-Mahan, K.L., Newman, J.C., Verdin, E., Baldi, P. and Sassone-Corsi, P. (2017). Distinct Circadian Signatures in Liver and Gut Clocks Revealed by Ketogenic Diet. Cell Metab. *26*, 523-538 e525.

Toh, K.L., Jones, C.R., He, Y., Eide, E.J., Hinz, W.A., Virshup, D.M., Ptacek, L.J. and Fu, Y.H. (2001). An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. Science *291*, 1040-1043.

Tonegawa, S., Liu, X., Ramirez, S. and Redondo, R. (2015). Memory Engram Cells Have Come of Age. Neuron *87*, 918-931.

Touitou, Y. and Haus, E. (2000). Alterations with aging of the endocrine and neuroendocrine circadian system in humans. Chronobiol. Int. *17*, 369-390.

Tracy, A.L., Jarrard, L.E. and Davidson, T.L. (2001). The hippocampus and motivation revisited: appetite and activity. Behav. Brain Res. *127*, 13-23.

Tranah, G.J., Blackwell, T., Stone, K.L., Ancoli-Israel, S., Paudel, M.L., Ensrud, K.E., Cauley, J.A., Redline, S., Hillier, T.A., Cummings, S.R.*, et al.* (2011). Circadian activity rhythms and risk of incident dementia and mild cognitive impairment in older women. Ann. Neurol. *70*, 722-732.

Tsukahara, S., Tanaka, S., Ishida, K., Hoshi, N. and Kitagawa, H. (2005). Age-related change and its sex differences in histoarchitecture of the hypothalamic suprachiasmatic nucleus of F344/N rats. Exp. Gerontol. *40*, 147-155.

Tulving, E. and Markowitsch, H.J. (1998). Episodic and declarative memory: role of the hippocampus. Hippocampus *8*, 198-204.

Urban, N.N. and Barrionuevo, G. (1996). Induction of hebbian and non-hebbian mossy fiber long-term potentiation by distinct patterns of high-frequency stimulation. J. Neurosci. *16*, 4293-4299.

Urrestarazu, E. and Iriarte, J. (2016). Clinical management of sleep disturbances in Alzheimer's disease: current and emerging strategies. Nat Sci Sleep *8*, 21-33.

Valentinuzzi, V.S., Scarbrough, K., Takahashi, J.S. and Turek, F.W. (1997). Effects of aging on the circadian rhythm of wheel-running activity in C57BL/6 mice. Am. J. Physiol. *273*, R1957-1964.

Valnegri, P., Khelfaoui, M., Dorseuil, O., Bassani, S., Lagneaux, C., Gianfelice, A., Benfante, R., Chelly, J., Billuart, P., Sala, C.*, et al.* (2011). A circadian clock in hippocampus is regulated by interaction between oligophrenin-1 and Rev-erbalpha. Nat. Neurosci. *14*, 1293-1301.

Valor, L.M. and Barco, A. (2012). Hippocampal gene profiling: toward a systems biology of the hippocampus. Hippocampus *22*, 929-941.

van der Flier, W.M., Pijnenburg, Y.A., Fox, N.C. and Scheltens, P. (2011). Early-onset versus late-onset Alzheimer's disease: the case of the missing APOE varepsilon4 allele. Lancet Neurol. *10*, 280-288.

van Ham, T.J., Breitling, R., Swertz, M.A. and Nollen, E.A. (2009). Neurodegenerative diseases: Lessons from genome-wide screens in small model organisms. EMBO Mol. Med. *1*, 360-370.

van Strien, N.M., Cappaert, N.L. and Witter, M.P. (2009). The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network. Nat. Rev. Neurosci. *10*, 272-282.

Vanderheyden, W.M., Lim, M.M., Musiek, E.S. and Gerstner, J.R. (2018). Alzheimer's Disease and Sleep-Wake Disturbances: Amyloid, Astrocytes, and Animal Models. J. Neurosci. *38*, 2901-2910.

Vanguilder, H.D. and Freeman, W.M. (2011). The hippocampal neuroproteome with aging and cognitive decline: past progress and future directions. Frontiers in aging neuroscience *3*, 8.

Videnovic, A., Lazar, A.S., Barker, R.A. and Overeem, S. (2014). 'The clocks that time us'--circadian rhythms in neurodegenerative disorders. Nature reviews. Neurology *10*, 683-693.

Vinogradova, O.S. (2001). Hippocampus as comparator: role of the two input and two output systems of the hippocampus in selection and registration of information. Hippocampus *11*, 578-598.

Voigt, R.M., Forsyth, C.B., Green, S.J., Mutlu, E., Engen, P., Vitaterna, M.H., Turek, F.W. and Keshavarzian, A. (2014). Circadian disorganization alters intestinal microbiota. PLoS ONE *9*, e97500.

Volicer, L., Harper, D.G., Manning, B.C., Goldstein, R. and Satlin, A. (2001). Sundowning and circadian rhythms in Alzheimer's disease. Am. J. Psychiatry *158*, 704-711.

Wakamatsu, H., Yoshinobu, Y., Aida, R., Moriya, T., Akiyama, M. and Shibata, S. (2001). Restricted-feeding-induced anticipatory activity rhythm is associated with a phase-shift of the expression of mPer1 and mPer2 mRNA in the cerebral cortex and hippocampus but not in the suprachiasmatic nucleus of mice. Eur. J. Neurosci. *13*, 1190-1196.

Walcott, E.C. and Tate, B.A. (1996). Entrainment of aged, dysrhythmic rats to a restricted feeding schedule. Physiol. Behav. *60*, 1205-1208.

Wall, P.M. and Messier, C. (2000). Concurrent modulation of anxiety and memory. Behav. Brain Res. *109*, 229-241.

Wallach, T. and Kramer, A. (2015). Chemical chronobiology: Toward drugs manipulating time. FEBS Lett. *589*, 1530-1538.

Walters, G.D. (2010). Dementia: continuum or distinct entity? Psychol. Aging *25*, 534-544.

Walton, K.M., Fisher, K., Rubitski, D., Marconi, M., Meng, Q.J., Sladek, M., Adams, J., Bass, M., Chandrasekaran, R., Butler, T.*, et al.* (2009). Selective inhibition of casein kinase 1 epsilon minimally alters circadian clock period. J. Pharmacol. Exp. Ther. *330*, 430-439.

Wang, H.B., Loh, D.H., Whittaker, D.S., Cutler, T., Howland, D. and Colwell, C.S. (2018). Time-Restricted Feeding Improves Circadian Dysfunction as well as Motor Symptoms in the Q175 Mouse Model of Huntington's Disease. eNeuro *5*,

Wang, J., Mauvoisin, D., Martin, E., Atger, F., Galindo, A.N., Dayon, L., Sizzano, F., Palini, A., Kussmann, M., Waridel, P.*, et al.* (2017). Nuclear Proteomics Uncovers Diurnal Regulatory Landscapes in Mouse Liver. Cell Metab. *25*, 102-117.

Wang, J., Yu, J.T., Wang, H.F., Meng, X.F., Wang, C., Tan, C.C. and Tan, L. (2015). Pharmacological treatment of neuropsychiatric symptoms in Alzheimer's disease: a systematic review and meta-analysis. J. Neurol. Neurosurg. Psychiatry *86*, 101-109.

Wang, L.M., Dragich, J.M., Kudo, T., Odom, I.H., Welsh, D.K., O'Dell, T.J. and Colwell, C.S. (2009). Expression of the circadian clock gene Period2 in the hippocampus: possible implications for synaptic plasticity and learned behaviour. ASN Neuro *1*,

Wang, X., Sun, G., Feng, T., Zhang, J., Huang, X., Wang, T., Xie, Z., Chu, X., Yang, J., Wang, H.*, et al.* (2019). Sodium oligomannate therapeutically remodels gut microbiota and suppresses gut bacterial amino acids-shaped neuroinflammation to inhibit Alzheimer's disease progression. Cell Res. *29*, 787-803.

Watanabe, A., Shibata, S. and Watanabe, S. (1995). Circadian rhythm of spontaneous neuronal activity in the suprachiasmatic nucleus of old hamster in vitro. Brain Res. *695*, 237-239.

Weber, M.M. (1997). Aloys Alzheimer, a coworker of Emil Kraepelin. J. Psychiatr. Res. *31*, 635-643.

Webster, S.J., Bachstetter, A.D., Nelson, P.T., Schmitt, F.A. and Van Eldik, L.J. (2014). Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. Frontiers in genetics *5*, 88.

Wei, Y., Chiang, W.C., Sumpter, R., Jr., Mishra, P. and Levine, B. (2017). Prohibitin 2 Is an Inner Mitochondrial Membrane Mitophagy Receptor. Cell *168*, 224-238 e210.

Weinert, D. (2010). Circadian temperature variation and ageing. Ageing Res. Rev. *9*, 51-60.

Weldemichael, D.A. and Grossberg, G.T. (2010). Circadian rhythm disturbances in patients with Alzheimer's disease: a review. International journal of Alzheimer's disease *2010*,

Welsh, D.K., Takahashi, J.S. and Kay, S.A. (2010). Suprachiasmatic nucleus: cell autonomy and network properties. Annu. Rev. Physiol. *72*, 551-577.

White, L., Small, B.J., Petrovitch, H., Ross, G.W., Masaki, K., Abbott, R.D., Hardman, J., Davis, D., Nelson, J. and Markesbery, W. (2005). Recent clinical-pathologic research on the causes of dementia in late life: update from the Honolulu-Asia Aging Study. J. Geriatr. Psychiatry Neurol. *18*, 224-227.

Winblad, B., Amouyel, P., Andrieu, S., Ballard, C., Brayne, C., Brodaty, H., Cedazo-Minguez, A., Dubois, B., Edvardsson, D., Feldman, H.*, et al.* (2016). Defeating Alzheimer's disease and other dementias: a priority for European science and society. Lancet Neurol. *15*, 455-532.

Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L.O., Nordberg, A., Backman, L., Albert, M., Almkvist, O.*, et al.* (2004). Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J. Intern. Med. *256*, 240-246.

Wirz-Justice, A., Terman, M., Oren, D.A., Goodwin, F.K., Kripke, D.F., Whybrow, P.C., Wisner, K.L., Wu, J.C., Lam, R.W., Berger, M.*, et al.* (2004). Brightening depression. Science *303*, 467-469.

Wisor, J.P., Edgar, D.M., Yesavage, J., Ryan, H.S., McCormick, C.M., Lapustea, N. and Murphy, G.M., Jr. (2005). Sleep and circadian abnormalities in a transgenic mouse model of Alzheimer's disease: a role for cholinergic transmission. Neuroscience *131*, 375-385.

Wong, S.L., Gilmour, H. and Ramage-Morin, P.L. (2016). Alzheimer's disease and other dementias in Canada. Health Rep. *27*, 11-16.

Wu, M., Zhou, F., Cao, X., Yang, J., Bai, Y., Yan, X., Cao, J. and Qi, J. (2018). Abnormal circadian locomotor rhythms and Per gene expression in six-month-old triple transgenic mice model of Alzheimer's disease. Neurosci. Lett. *676*, 13-18.

Wu, Y.H., Fischer, D.F., Kalsbeek, A., Garidou-Boof, M.L., van der Vliet, J., van Heijningen, C., Liu, R.Y., Zhou, J.N. and Swaab, D.F. (2006). Pineal clock gene oscillation is disturbed in Alzheimer's disease, due to functional disconnection from the "master clock". FASEB J. *20*, 1874-1876.

Wu, Y.H. and Swaab, D.F. (2007). Disturbance and strategies for reactivation of the circadian rhythm system in aging and Alzheimer's disease. Sleep Med *8*, 623-636.

Wu, Y.H., Zhou, J.N., Van Heerikhuize, J., Jockers, R. and Swaab, D.F. (2007). Decreased MT1 melatonin receptor expression in the suprachiasmatic nucleus in aging and Alzheimer's disease. Neurobiol. Aging *28*, 1239-1247.

Wyse, C.A. and Coogan, A.N. (2010). Impact of aging on diurnal expression patterns of CLOCK and BMAL1 in the mouse brain. Brain Res. *1337*, 21-31.

Wyss-Coray, T. (2016). Ageing, neurodegeneration and brain rejuvenation. Nature *539*, 180-186.

Xia, J., Gill, E.E. and Hancock, R.E. (2015). NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data. Nat. Protoc. *10*, 823-844.

Xu, Y., Padiath, Q.S., Shapiro, R.E., Jones, C.R., Wu, S.C., Saigoh, N., Saigoh, K., Ptacek, L.J. and Fu, Y.H. (2005). Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. Nature *434*, 640-644.

Yamakawa, G.R., Basu, P., Cortese, F., MacDonnell, J., Whalley, D., Smith, V.M. and Antle, M.C. (2016). The cholinergic forebrain arousal system acts directly on the circadian pacemaker. Proc. Natl. Acad. Sci. U. S. A. *113*, 13498-13503.

Yamazaki, S., Straume, M., Tei, H., Sakaki, Y., Menaker, M. and Block, G.D. (2002). Effects of aging on central and peripheral mammalian clocks. Proc. Natl. Acad. Sci. U. S. A. *99*, 10801-10806.

Yasojima, K., Kuret, J., DeMaggio, A.J., McGeer, E. and McGeer, P.L. (2000). Casein kinase 1 delta mRNA is upregulated in Alzheimer disease brain. Brain Res. *865*, 116-120.

Ye, Y., Xiang, Y., Ozguc, F.M., Kim, Y., Liu, C.J., Park, P.K., Hu, Q., Diao, L., Lou, Y., Lin, C.*, et al.* (2018). The Genomic Landscape and Pharmacogenomic Interactions of Clock Genes in Cancer Chronotherapy. Cell Syst *6*, 314-328 e312.

Yeom, M., Pendergast, J.S., Ohmiya, Y. and Yamazaki, S. (2010). Circadian-independent cell mitosis in immortalized fibroblasts. Proc. Natl. Acad. Sci. U. S. A. *107*, 9665-9670.

Young, M.W. and Kay, S.A. (2001). Time zones: a comparative genetics of circadian clocks. Nat. Rev. Genet. *2*, 702-715.

Yu, E.A. and Weaver, D.R. (2011). Disrupting the circadian clock: gene-specific effects on aging, cancer, and other phenotypes. Aging *3*, 479-493.

Zeisel, A., Munoz-Manchado, A.B., Codeluppi, S., Lonnerberg, P., La Manno, G., Jureus, A., Marques, S., Munguba, H., He, L., Betsholtz, C.*, et al.* (2015). Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. Science *347*, 1138-1142.

Zhang, E.E. and Kay, S.A. (2010). Clocks not winding down: unravelling circadian networks. Nat. Rev. Mol. Cell Biol. *11*, 764-776.

Zhang, E.E., Liu, A.C., Hirota, T., Miraglia, L.J., Welch, G., Pongsawakul, P.Y., Liu, X., Atwood, A., Huss, J.W., 3rd, Janes, J.*, et al.* (2009). A genome-wide RNAi screen for modifiers of the circadian clock in human cells. Cell *139*, 199-210.

Zhang, R., Lahens, N.F., Ballance, H.I., Hughes, M.E. and Hogenesch, J.B. (2014). A circadian gene expression atlas in mammals: implications for biology and medicine. Proc. Natl. Acad. Sci. U. S. A. *111*, 16219-16224.

Zhang, W. and Linden, D.J. (2003). The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nat. Rev. Neurosci. *4*, 885-900.

Zhang, Y., Kornhauser, J.M., Zee, P.C., Mayo, K.E., Takahashi, J.S. and Turek, F.W. (1996). Effects of aging on light-induced phase-shifting of circadian behavioral rhythms, fos expression and CREB phosphorylation in the hamster suprachiasmatic nucleus. Neuroscience *70*, 951-961.

Zhao, Q.F., Tan, L., Wang, H.F., Jiang, T., Tan, M.S., Tan, L., Xu, W., Li, J.Q., Wang, J., Lai, T.J.*, et al.* (2016). The prevalence of neuropsychiatric symptoms in Alzheimer's disease: Systematic review and meta-analysis. J. Affect. Disord. *190*, 264-271.

Zhou, J.N., Hofman, M.A. and Swaab, D.F. (1995). VIP neurons in the human SCN in relation to sex, age, and Alzheimer's disease. Neurobiol. Aging *16*, 571-576.

Zhou, L., Gao, Q., Nie, M., Gu, J.L., Hao, W., Wang, L. and Cao, J.M. (2016). Degeneration and energy shortage in the suprachiasmatic nucleus underlies the circadian rhythm disturbance in ApoE-/- mice: implications for Alzheimer's disease. Sci Rep *6*, 36335.

Zhou, X., Xiao, W., Su, Z., Cheng, J., Zheng, C., Zhang, Z., Wang, Y., Wang, L., Xu, B., Li, S.*, et al.* (2018). Hippocampal proteomic alteration in triple transgenic mouse model of Alzheimer's disease and implication of PINK 1 regulation in donepezil treatment. J. Proteome Res.

Zhu, X., Castellani, R.J., Takeda, A., Nunomura, A., Atwood, C.S., Perry, G. and Smith, M.A. (2001). Differential activation of neuronal ERK, JNK/SAPK and p38 in Alzheimer disease: the 'two hit' hypothesis. Mech. Ageing Dev. *123*, 39-46.

Zhu, X., Raina, A.K., Perry, G. and Smith, M.A. (2004). Alzheimer's disease: the two-hit hypothesis. Lancet Neurol. *3*, 219-226.

Zovkic, I.B., Guzman-Karlsson, M.C. and Sweatt, J.D. (2013). Epigenetic regulation of memory formation and maintenance. Learn. Mem. *20*, 61-74.

Contributions of Collaborators

1. **Dr. Daniel Figeys (University of Ottawa)**
   Contributed to the study conception for Chapter 2, as well as the study design and interpretation of results for Chapters 2 and 3. He also provided important edits to my thesis.

2. **Dr. Hai-Ying Mary Cheng (University of Toronto)**
   Contributed to the study conception, study design, and sample collection for Chapter 2.

3. **Dr. Richard Bergeron (University of Ottawa)**
   Provided 3xTg-AD mice for breeding for Chapter 3.

4. **Dr. Cheng-Kang Chiang (University of Ottawa; currently at National Dong Hwa University)**
   Contributed to the study design, sample collection, sample preparation, data analysis, and interpretation of results for Chapter 2.

5. **Dr. Janice Mayne (University of Ottawa)**
   Contributed to the study design, sample collection, and interpretation of results for Chapters 2 and 3. She also provided important edits to my thesis.

6. **Dr. Zhibin Ning (University of Ottawa)**
   Contributed to the interpretation and analysis of mass spectrometry data and ensured mass spectrometry working conditions for Chapters 2 and 3.

7. **Dr. Xu Zhang (University of Ottawa)**
   Contributed to the sample collection, data analysis, and interpretation of data for Chapter 2.

8. **Dr. Bo Xu (University of Ottawa)**
   Contributed to the sample collection and interpretation of data for Chapter 2.

9. **Krystal Walker (University of Ottawa)**
   Contributed to mouse colony maintenance, *in vivo* treatments, sample collection, and interpretation of data for Chapter 3.

10. **Jasmine I. Moore (University of Ottawa)**
    Contributed to the sample collection and performed mouse colony maintenance for Chapter 2.

Rights and Permissions

For Adler, P., Chiang, C.-K., Mayne, J., Ning, Z., Zhang, X., Xu, B., Cheng, H.-Y.M. and Figeys, D. (2019). Aging disrupts the circadian patterns of protein expression in the murine hippocampus. Frontiers in Aging Neuroscience. (In press).

This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.
To view a copy of this licence, visit https://creativecommons.org/licenses/by/4.0/.

For Adler, P., Mayne, J., Walker, K., Ning, Z. and Figeys, D. (2019). Therapeutic Targeting of Casein Kinase 1δ/ε in an Alzheimer's Disease Mouse Model. Journal of Proteome Research. *18*, 3383–3393.

Reprinted in part with permission from Adler, P., Mayne, J., Walker, K., Ning, Z. and Figeys, D. (2019). Therapeutic Targeting of Casein Kinase 1δ/ε in an Alzheimer's Disease Mouse Model. Journal of Proteome Research. *18*, 3383–3393. Copyright 2019 American Chemical Society.

Appendices

Appendix A

Table 1. List of circadian proteins in the hippocampus of young mice. All proteins in this list were quantified in at least two biological replicates per CT and identified as circadian using the Perseus periodicity algorithm (FDR cutoff = 0.25).

UniProt ID	Protein name	Gene name
P08553	Neurofilament medium polypeptide	*Nefm*
Q64442	Sorbitol dehydrogenase	*Sord*
Q9CZ04	COP9 signalosome complex subunit 7a	*Cops7a*
P28652	Calcium/calmodulin-dependent protein kinase type II subunit beta	*Camk2b*
P08228	Superoxide dismutase [Cu-Zn]	*Sod1*
A2AEG6	Glycoprotein m6b, isoform CRA_g	*Gpm6b*
Q8JZQ2	AFG3-like protein 2	*Afg3l2*
Q9JL62	Glycolipid transfer protein	*Gltp*
Q9CQQ7	ATP synthase F(0) complex subunit B1, mitochondrial	*Atp5f1*
P46660	Alpha-internexin	*Ina*
P19246	Neurofilament heavy polypeptide	*Nefh*
Q9JM76	Actin-related protein 2/3 complex subunit 3	*Arpc3*
Q9QYB1	Chloride intracellular channel protein 4	*Clic4*
P17751	Triosephosphate isomerase	*Tpi1*
Q6PDL0	Cytoplasmic dynein 1 light intermediate chain 2	*Dync1li2*
B2RSH2	Guanine nucleotide-binding protein G(i) subunit alpha-1	*Gnai1*
Q61016	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-7; Guanine nucleotide-binding protein subunit gamma	*Gng7*
Q9WTT4	V-type proton ATPase subunit G 2	*Atp6v1g2*
O70318	Band 4.1-like protein 2	*Epb41l2*
E9Q8N8	Electrogenic sodium bicarbonate cotransporter 1	*Slc4a4*
Q8BVQ5	Protein phosphatase methylesterase 1	*Ppme1*
G3UXL2	Ribose-phosphate pyrophosphokinase 1	*Prps1l3;Prps1*
Q3UI33	Methionine aminopeptidase 2	*Metap2*
Q8C845	EF-hand domain-containing protein D2	*Efhd2*
E9Q1G8	Septin-7	*Sept7*
Q6GT24	Peroxiredoxin-6	*Prdx6*
E9Q171	Neurofascin	*Nfasc*
Q9DCS9	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10	*Ndufb10*
O08599	Syntaxin-binding protein 1	*Stxbp1*
P70404	Isocitrate dehydrogenase [NAD] subunit gamma 1, mitochondrial	*Idh3g*
P35486	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	*Pdha1*
Q9CR62	Mitochondrial 2-oxoglutarate/malate carrier protein	*Slc25a11*
P68368	Tubulin alpha-4A chain	*Tuba4a*
Q9QXS6	Drebrin	*Dbn1*
Q9DB15	39S ribosomal protein L12, mitochondrial	*Mrpl12*
Q9CPY7-2	Cytosol aminopeptidase	*Lap3*

Q8K1Z0	Ubiquinone biosynthesis protein COQ9, mitochondrial	*Coq9*
Q62261	Spectrin beta chain, non-erythrocytic 1	*Sptbn1*
Q8BYI9	Tenascin-R	*Tnr*
P62874	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	*Gnb1*
Q8BVQ9	26S protease regulatory subunit 7	*Psmc2*
O54991	Contactin-associated protein 1	*Cntnap1*
Q62059	Versican core protein	*Vcan*
Q923T9	Calcium/calmodulin-dependent protein kinase type II subunit gamma	*Camk2g*
G3XA53	Oligodendrocyte-myelin glycoprotein	*Omg*
Q9CZ13	Cytochrome b-c1 complex subunit 1, mitochondrial	*Uqcrc1*
P10649	Glutathione S-transferase Mu 1	*Gstm1*
Q64521	Glycerol-3-phosphate dehydrogenase, mitochondrial	*Gpd2*
Q9CZ44	NSFL1 cofactor p47	*Nsfl1c*
Q9D0F9	Phosphoglucomutase-1	*Pgm1;Pgm2*
Q9R0P9	Ubiquitin carboxyl-terminal hydrolase isozyme L1	*Uchl1*
Q9CR68	Cytochrome b-c1 complex subunit Rieske, mitochondrial; Cytochrome b-c1 complex subunit 11	*Uqcrfs1*
B9EKR1	Receptor-type tyrosine-protein phosphatase zeta	*Ptprz1*
A1BN54	Alpha actinin 1a	*Actn1*
Q99PT1	Rho GDP-dissociation inhibitor 1	*Arhgdia*
Q6P6I8	Tyrosine-protein phosphatase non-receptor type substrate 1	*Sirpa*
E9PZ00	Sulfated glycoprotein 1	*Psap*
P28663	Beta-soluble NSF attachment protein	*Napb*
Q8R1I1	Cytochrome b-c1 complex subunit 9	*Uqcr10*
P47857	ATP-dependent 6-phosphofructokinase, muscle type	*Pfkm*
Q9D6J6	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	*Ndufv2*
D3YZ62	Unconventional myosin-Va	*Myo5a*
P08249	Malate dehydrogenase, mitochondrial	*Mdh2*
Q8BMF4	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	*Dlat*
Q8R326-2	Paraspeckle component 1	*Pspc1*
O70443	Guanine nucleotide-binding protein G(z) subunit alpha	*Gnaz*
Q9QZX7-2	Serine racemase	*Srr*
A2AMQ5	Phosphatidate cytidylyltransferase; Phosphatidate cytidylyltransferase 2	*Cds2*
E9Q7L0	Oxoglutarate dehydrogenase-like	*Ogdhl*
Q91V61	Sideroflexin-3	*Sfxn3*
Q8BHZ0	Protein FAM49A	*Fam49a*
P18872	Guanine nucleotide-binding protein G(o) subunit alpha	*Gnao1*
Q9JIS5	Synaptic vesicle glycoprotein 2A	*Sv2a*
Q8BKZ9	Pyruvate dehydrogenase protein X component, mitochondrial	*Pdhx*
Q9R1T4-2	Septin-6	*Sept6*
Q8BWY3	Eukaryotic peptide chain release factor subunit 1	*Etf1*
A2ALV3	Endophilin-A1	*Sh3gl2*
Q5SXR6	Clathrin heavy chain; Clathrin heavy chain 1	*Cltc*
P63318	Protein kinase C gamma type; Protein kinase C	*Prkcg*
P17427	AP-2 complex subunit alpha-2	*Ap2a2*
P63213	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2	*Gng2*
Q9DBF1-2	Alpha-aminoadipic semialdehyde dehydrogenase	*Aldh7a1*

Q8C605	6-phosphofructokinase; ATP-dependent 6-phosphofructokinase, platelet type	*Pfkp*
Q9DB20	ATP synthase subunit O, mitochondrial	*Atp5o*
O35344	Importin subunit alpha-4	*Kpna3*
Q7TQF7	Amphiphysin	*Amph*
Q9DAS9	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12	*Gng12*
Q9D051	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	*Pdhb*
Q8C052	Microtubule-associated protein 1S; MAP1S heavy chain; MAP1S light chain	*Map1s*
B1AWN6	Sodium channel protein	*Scn2a1;Scn3a*
Q9CWJ9	Bifunctional purine biosynthesis protein PURH; Phosphoribosylaminoimidazolecarboxamide formyltransferase; IMP cyclohydrolase	*Atic*
Q78IK2	Up-regulated during skeletal muscle growth protein 5	*Usmg5*
Q9D2G2	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	*Dlst*
Q80UG5-3	Septin-9	*Sept9*
Q3UHH4	Tripartite motif-containing protein 2	*Trim2*
P99024	Tubulin beta-5 chain	*Tubb5*
Q9Z0P4	Paralemmin-1	*Palm*
Q60930	Voltage-dependent anion-selective channel protein 2	*Vdac2*
P84091	AP-2 complex subunit mu	*Ap2m1*
P08752	Guanine nucleotide-binding protein G(i) subunit alpha-2	*Gnai2*
P17710-3	Hexokinase-1	*Hk1*
P63028	Translationally-controlled tumor protein	*Tpt1*
J3QPE8	Voltage-dependent anion-selective channel protein 3	*Vdac3*
Q03265	ATP synthase subunit alpha, mitochondrial; ATP synthase subunit alpha	*Atp5a1*
Q8K0U4	Heat shock 70 kDa protein 12A	*Hspa12a*
P80314	T-complex protein 1 subunit beta	*Cct2*
P17426-2	AP-2 complex subunit alpha-1	*Ap2a1*
Q11011	Puromycin-sensitive aminopeptidase	*Npepps*
O54983	Ketimine reductase mu-crystallin	*Crym*
Q6ZQ38	Cullin-associated NEDD8-dissociated protein 1	*Cand1*
Q9DBG3	AP-2 complex subunit beta	*Ap2b1*
Q7TMF3	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12	*Ndufa12*
D3Z396	Neurotrimin	*Ntm*
Q9JME5	AP-3 complex subunit beta-2	*Ap3b2*
Q68FF6	ARF GTPase-activating protein GIT1	*Git1*
Q3UUI3	Acyl-coenzyme A thioesterase THEM4	*Them4*
E9Q4G8	CD166 antigen	*Alcam*
Q7TNB5	Glutamate receptor 1	*Gria1*
G3UWG1	Cytochrome c, somatic	*Gm10108;Cycs*
B9EJA2-5	Cortactin-binding protein 2	*Cttnbp2*
O54774	AP-3 complex subunit delta-1	*Ap3d1*
P42932	T-complex protein 1 subunit theta	*Cct8*
Q01853	Transitional endoplasmic reticulum ATPase	*Vcp*
G5E819	Serine/threonine-protein phosphatase 5; Serine/threonine-protein phosphatase	*Ppp5c*
O35465-2	Peptidyl-prolyl cis-trans isomerase FKBP8	*Fkbp8*
P52480-2	Pyruvate kinase PKM	*Pkm*
Q62189	U1 small nuclear ribonucleoprotein A	*Snrpa*

P62631	Elongation factor 1-alpha 2	*Eef1a2*
Q8CGF6	WD repeat-containing protein 47	*Wdr47*
Q60932-2	Voltage-dependent anion-selective channel protein 1	*Vdac1*
P61028	Ras-related protein Rab-8B	*Rab8b*
P48758	Carbonyl reductase [NADPH] 1	*Cbr1*
Q8R191	Synaptogyrin-3	*Syngr3*
P20108	Thioredoxin-dependent peroxide reductase, mitochondrial	*Prdx3*
Q7M6W1	Reticulon	*Rtn1*
P48962	ADP/ATP translocase 1	*Slc25a4*
Q6ZWN5	40S ribosomal protein S9	*Rps9*
Q00493	Carboxypeptidase E	*Cpe*
P62908	40S ribosomal protein S3	*Rps3*
Q08331	Calretinin	*Calb2*
P06801	NADP-dependent malic enzyme	*Me1*
Q6ZPJ3	E2/E3 hybrid ubiquitin-protein ligase UBE2O	*Ube2o*
P80315	T-complex protein 1 subunit delta	*Cct4*
P63038	60 kDa heat shock protein, mitochondrial	*Hspd1*
A2AFG7	Neural cell adhesion molecule L1	*L1cam*
Q9CQZ5	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 6	*Ndufa6*
P61089	Ubiquitin-conjugating enzyme E2 N	*Ube2n*
P70168	Importin subunit beta-1	*Kpnb1*
Q8C1A5	Thimet oligopeptidase	*Thop1*
P26350	Prothymosin alpha; Prothymosin alpha, N-terminally processed; Thymosin alpha	*Ptma*
Q8C854-3	Myelin expression factor 2	*Myef2*
E9QLL2	Dynamin-3	*Dnm3*
Q9D5T0	ATPase family AAA domain-containing protein 1	*Atad1*
Q99JY9	Actin-related protein 3	*Actr3*
Q8C2Q8	ATP synthase subunit gamma; ATP synthase subunit gamma, mitochondrial	*Atp5c1*
Q9CZD3	Glycine--tRNA ligase	*Gars*
O08529	Calpain-2 catalytic subunit	*Capn2*
Q9D394	Protein RUFY3	*Rufy3*
P58389	Serine/threonine-protein phosphatase 2A activator	*Ppp2r4*
Q922B2	Aspartate--tRNA ligase, cytoplasmic	*Dars*
Q9CX34	Suppressor of G2 allele of SKP1 homolog	*Sugt1*
Q9CZS1	Aldehyde dehydrogenase X, mitochondrial	*Aldh1b1*
Q9QXY6	EH domain-containing protein 3	*Ehd3*
P31938	Dual specificity mitogen-activated protein kinase kinase 1	*Map2k1*
O35633-2	Vesicular inhibitory amino acid transporter	*Slc32a1*
Q80XN0	D-beta-hydroxybutyrate dehydrogenase, mitochondrial	*Bdh1*
P57759	Endoplasmic reticulum resident protein 29	*Erp29*
Q920I9	WD repeat-containing protein 7	*Wdr7*
Q6PB66	Leucine-rich PPR motif-containing protein, mitochondrial	*Lrpprc*
Q9QUI0	Transforming protein RhoA	*Rhoa;Rhoc*
Q8CGC7	Bifunctional glutamate/proline--tRNA ligase; Glutamate--tRNA ligase; Proline--tRNA ligase	*Eprs*
Q8K3H0	DCC-interacting protein 13-alpha	*Appl1*
E9QAQ5	Glycogen synthase kinase-3 beta	*Gsk3b*

Q80SW1	Putative adenosylhomocysteinase 2	*Ahcyl1*
Q9Z1A1	TFG protein	*Tfg*
Q8C0C7	Phenylalanine--tRNA ligase alpha subunit	*Farsa*
B0V2N1-6	Receptor-type tyrosine-protein phosphatase S	*Ptprs*
E0CXN5	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	*Gpd1*
Q9Z2X1	Heterogeneous nuclear ribonucleoprotein F; Heterogeneous nuclear ribonucleoprotein F, N-terminally processed	*Hnrnpf*
F6YVP7	40S ribosomal protein S18	*Gm10260;Rps18*
Q9CR57	60S ribosomal protein L14	*Rpl14*
P62267	40S ribosomal protein S23	*Rps23*
P14131	40S ribosomal protein S16	*Rps16*
Q9QZD9	Eukaryotic translation initiation factor 3 subunit I	*Eif3i*
Q9D6Z1	Nucleolar protein 56	*Nop56*
P62911	60S ribosomal protein L32	*Rpl32*
Q62167	ATP-dependent RNA helicase DDX3X; Putative ATP-dependent RNA helicase Pl10	*Ddx3x;D1Pas1*
P62317	Small nuclear ribonucleoprotein Sm D2	*Snrpd2;Gm5449*
Q9EQF6	Dihydropyrimidinase-related protein 5	*Dpysl5*
P27773	Protein disulfide-isomerase A3	*Pdia3*
P14733	Lamin-B1	*Lmnb1*
Q91VR5	ATP-dependent RNA helicase DDX1	*Ddx1*
A8DUK4	Hemoglobin subunit beta-1	*Hbbt1;Hbb-bs; Hbb-b1*
A2AH85	116 kDa U5 small nuclear ribonucleoprotein component	*Eftud2*
Q91VI7	Ribonuclease inhibitor	*Rnh1*
P57776-3	Elongation factor 1-delta	*Eef1d*
Q91VB8	Hemoglobin subunit alpha	*Hba-a2;Hba*
Q8VDQ8-2	NAD-dependent protein deacetylase sirtuin-2	*Sirt2*
P27659	60S ribosomal protein L3	*Rpl3*
P10605	Cathepsin B; Cathepsin B light chain; Cathepsin B heavy chain	*Ctsb*
Q3UPL0-2	Protein transport protein Sec31A	*Sec31a*
P68033	Actin, alpha cardiac muscle 1; Actin, alpha skeletal muscle	*Actc1;Acta1*
P63242	Eukaryotic translation initiation factor 5A-1; Eukaryotic translation initiation factor 5A-2	*Eif5a;Eif5a2*
P26041	Moesin	*Msn*
P63158	High mobility group protein B1	*Hmgb1*
P62855	40S ribosomal protein S26	*Rps26*
E9Q3B9	Monoglyceride lipase	*Mgll*
P26883	Peptidyl-prolyl cis-trans isomerase FKBP1A	*Fkbp1a*
P16330	2,3-cyclic-nucleotide 3-phosphodiesterase	*Cnp*
Q9CZC8	Secernin-1	*Scrn1*
P14211	Calreticulin	*Calr*
Q9D1X8	Tetraspanin-2	*Tspan2*
Q9D2P8	Myelin-associated oligodendrocyte basic protein	*Mobp*
Q60598	Src substrate cortactin	*Cttn*

Table 2. List of circadian proteins in the hippocampus of middle-aged mice. All proteins in this list were quantified in at least two biological replicates per CT and identified as circadian using the Perseus periodicity algorithm (FDR cutoff = 0.25).

UniProt ID	Protein name	Gene name
D3YYI8	Histone deacetylase; Histone deacetylase 1	*Gm10093;Hdac1*
Q8BH95	Enoyl-CoA hydratase, mitochondrial	*Echs1*
Q8BH44	Coronin-2B	*Coro2b*
Q9QUP5	Hyaluronan and proteoglycan link protein 1	*Hapln1*
Q02105	Complement C1q subcomponent subunit C	*C1qc*
Q9CPQ8	ATP synthase subunit g, mitochondrial	*Atp5l*
P84244	Histone H3.3; Histone H3.3C; Histone H3	*H3f3a;H3f3c*
Q62433	Protein NDRG1	*Ndrg1*
Q9CZY3	Ubiquitin-conjugating enzyme E2 variant 1	*Ube2v1*
D3Z6C3	40S ribosomal protein S3a	*Rps3a*
P10518	Delta-aminolevulinic acid dehydratase	*Alad*
P70441	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	*Slc9a3r1*
Q921F2	TAR DNA-binding protein 43	*Tardbp*
Q14BI1	Slc24a2 protein	*Slc24a2*
Q5SQB0	Nucleophosmin	*Npm1;Gm5611*
P15532	Nucleoside diphosphate kinase A; Nucleoside diphosphate kinase	*Nme1*
Q91VW3	SH3 domain-binding glutamic acid-rich-like protein 3	*Sh3bgrl3*
P14824	Annexin A6	*Anxa6*
D3YYE1	Acidic leucine-rich nuclear phosphoprotein 32 family member A	*Anp32a*
P32921-2	Tryptophan--tRNA ligase, cytoplasmic; T1-TrpRS; T2-TrpRS	*Wars*
Q8BHZ0	Protein FAM49A	*Fam49a*
Q9WUK2-2	Eukaryotic translation initiation factor 4H	*Eif4h*
Q9CQN1	Heat shock protein 75 kDa, mitochondrial	*Trap1*

Table 3. Proteins associated with neurodegenerative diseases and displaying changes in circadian rhythmicity in the hippocampus of mice during aging. All proteins in this list were quantified in at least two biological replicates per CT and identified as circadian using the Perseus periodicity algorithm (FDR cutoff = 0.25). AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; HD, Huntington's disease; PD, Parkinson's disease.

UniProt ID	Gene name	Protein name	Function	Associated age-related diseases	Change in rhythmicity with aging	Reference (PubMed ID)
Q9CZS1 Q9DBF1-2	*Aldh1b1* *Aldh7a1*	Aldehyde dehydrogenase X, mitochondrial; α-aminoadipic semialdehyde dehydrogenase	Antioxidant defense	AD, PD	Loss	23195683
Q7TQF7	*Amph*	Amphiphysin	Synaptic vesicle endocytosis	AD	Loss	11454453; 22975846
D3YYE1	*Anp32a*	Acidic leucine-rich nuclear phosphoprotein 32 family member A	Histone acetylation; PP2A inhibition	AD	Gain	28473768
P14824	*Anxa6*	Annexin A6	Various, including tau localization	AD	Gain	29636414
P17426-2 P17427 Q9DBG3 P84091	*Ap2a1* *Ap2a2* *Ap2b1* *Ap2m1*	AP-2 complex subunits α1, α2, β, μ	Synaptic vesicle endocytosis	AD, HD	Loss	11454453, 24067654, 12615650
Q8K3H0	*Appl1*	DCC-interacting protein 13-α	Multifunctional adaptor protein	AD	Loss	26194181
Q03265 Q8C2Q8 Q9CQQ7 Q9DB20	*Atp5a1* *Atp5c1* *Atp5f1* *Atp5o*	ATP synthase subunits α, γ, b, o	ATP synthesis	AD, PD, HD	Loss	24900954; 29316249; 29895861; 17726098
Q80XN0	*Bdh1*	D-β-hydroxybutyrate dehydrogenase	Ketone synthesis and catabolism	AD	Loss	28560309
Q08331	*Calb2*	Calretinin	Intracellular calcium buffering	AD, ALS, PD, HD	Loss	20413859; 28294153; 10650144; 8914086
P14211	*Calr*	Calreticulin	Molecular chaperone; intracellular calcium buffering	AD, ALS, FTD, PD	Loss	23585889; 22492046; 22416763; 24860980
P28652 Q923T9	*Camk2b* *Camk2g*	Ca^{2+}/calmodulin-dependent protein kinase II subunits β, γ	Long-term potentiation; tau phosphorylation; maintenance of circadian rhythms	AD	Loss	24831701; 26603284
O08529	*Capn2*	Calpain-2 catalytic subunit	Calcium-dependent protease; regulation of GSK3β activity	AD, ALS	Loss	26874794; 23250437
P80314 P80315 P42932	*Cct2* *Cct4* *Cct8*	T-complex protein 1 subunits β, Δ, θ	Molecular chaperone	AD, PD, HD	Loss	11441917; 27601642; 28102321; 19393652
Q5SXR6	*Cltc*	Clathrin heavy chain	Endocytosis	AD, HD	Loss	24606058; 28258398
Q00493	*Cpe*	Carboxypeptidase E	Neurotransmitter biosynthesis/release	AD	Loss	22402194
P10605	*Ctsb*	Cathepsin B	Protein degradation	AD, ALS, PD, HD	Loss	16982417; 12677446; 25466281; 21631942
P62897	*Cycs*	Cytochrome c, somatic	Electron transfer in mitochondrial electron transport chain	ALS, PD	Loss	17454840; 27884192
Q9QXS6	*Dbn1*	Drebrin	Actin-binding protein; synaptic plasticity	AD	Loss	12009525

Q9D2G2	*Dlst*	Dihydrolipoamide succinyltransferase	Tricarboxylic acid cycle	AD, PD, HD	Loss	22820180
Q6PDL0	*Dync1li2*	Cytoplasmic dynein 1 light intermediate chain 2	Movement of various cargoes along microtubules	AD, PD, HD	Loss	21420428
Q8C845	*Efhd2*	EF-hand domain-containing protein D2	Various, including regulation of calcium signalling, the actin cytoskeleton, and synapse formation	AD, ALS, PD, HD	Loss	27064956
Q9QXY6	*Ehd3*	EH domain-containing protein 3	Endocytic transport	AD	Loss	24373286
P26883 O35465-2	*Fkbp1a* *Fkbp8*	Peptidyl-prolyl isomerases FKBP1A and FKBP8	Molecular chaperone	AD	Loss	25628064
Q68FF6	*Git1*	ARF GTPase-activating protein GIT1	GTPase-activating protein	HD	Loss	15383276
Q7TNB5	*Gria1*	GluA1 AMPA receptor subunit	Ionotropic glutamate receptor	AD	Loss	24077616
E9QAQ5	*Gsk3b*	Glycogen synthase kinase-3β	Regulation of various cellular processes	AD, ALS, FTD, PD	Loss	21629738; 18852354; 21651477
P17710-3	*Hk1*	Hexokinase-1	Glycolysis	AD, PD	Loss	29167179; 28962651
P63158	*Hmgb1*	High mobility group protein B1	DNA repair; gene transcription; neuroinflammation	AD, ALS, PD, HD	Loss	22580958; 23639787
Q8K0U4 P63038	*Hspa12a* *Hspd1*	Heat shock 70 kDa protein 12A; 60 kDa heat shock protein, mitochondrial	Molecular chaperone	AD, ALS, PD, HD	Loss	18270320; 25386560; 28852382
P14733	*Lmnb1*	Lamin-B1	Maintenance of nucleoskeleton	AD	Loss	26725200
P08249	*Mdh2*	Malate dehydrogenase, mitochondrial	Tricarboxylic acid cycle	AD	Loss	26907355
E9Q3B9	*Mgll*	Monoglyceride lipase	Lipid metabolism and signalling	AD, ALS, PD	Loss	23142242; 28373073
Q62433	*Ndrg1*	N-Myc down-regulated gene 1	Various, including apoptosis and microtubule regulation	AD, PD	Gain	23363009; 23430405
Q7TMF3 Q9CQZ5 Q9DCS9 Q9D6J6	*Ndufa12* *Ndufa6* *Ndufb10* *Ndufv2*	NADH dehydrogenase subunits	Oxidative phosphorylation	AD, ALS, PD	Loss	24900954; 21078990
P19246 P08553	*Nefh* *Nefm*	Neurofilament heavy and medium polypeptides	Neuronal cytoskeleton maintenance	AD, ALS, PD	Loss	21915226
P15532	*Nme1*	Nucleoside diphosphate kinase A	Synthesis of nucleoside triphosphates	AD	Gain	12200143
Q11011	*Nsfl1c*	NSFL1 cofactor p47	Vesicle/membrane fusion	PD	Loss	30783609
P35486 Q9D051 Q8BKZ9 Q8BMF4	*Pdha1* *Pdhb* *Pdhx* *Dlat*	Pyruvate dehydrogenase complex components	Conversion of pyruvate into acetyl-CoA	AD, HD	Loss	6219611
P27773	*Pdia3*	Protein disulfide-isomerase A3	Molecular chaperone	AD, ALS, PD	Loss	26779479
P47857 Q8C605	*Pfkm* *Pfkp*	Phosphofructokinase	Glycolysis	AD	Loss	28438892
P52480-2	*Pkm*	Pyruvate kinase PKM	Glycolysis	AD	Loss	10443553
Q8BVQ5	*Ppme1*	Protein phosphatase methylesterase 1	PP2A regulation	AD	Loss	24653673
P58389	*Ppp2r4*	Serine/threonine-protein phosphatase 2A activator	Regulatory subunit of PP2A, which dephosphorylates tau and α-synuclein	AD, FTD	Loss	24653673; 11032905

G5E819	*Ppp5c*	Serine/threonine-protein phosphatase 5	Dephosphorylation of various proteins, including tau	AD	Loss	22340724
P20108	*Prdx3*	Peroxiredoxin-3	Antioxidant defense	ALS, PD	Loss	23732987; 27130805
Q6GT24	*Prdx6*	Peroxiredoxin-6	Antioxidant defense	AD, PD	Loss	27130805
P63318	*Prkcg*	Protein kinase C γ	Regulation of various signalling pathways; long-term potentiation	AD, ALS	Loss	27001133; 14559406
P61028	*Rab8b*	Ras-related protein Rab-8B	Intracellular membrane trafficking	AD, PD, HD	Loss	29689231
Q9QUI0	*Rhoa*	Transforming protein RhoA	Cytoskeleton organization	AD, PD	Loss	25339865
Q9CZC8	*Scrn1*	Secernin-1	Exocytosis	AD	Loss	27448508
Q9R1T4-2 E9Q1G8 Q80UG5-3	*Sept6* *Sept7* *Sept9*	Septins 6, 7, 9	Synaptic vesicle trafficking; cytoskeleton remodelling; synaptic plasticity	AD	Loss	29602250; 25888325
Q91V61	*Sfxn3*	Sideroflexin-3	Mitochondrial serine transporter; regulation of synaptic morphology	PD	Loss	28049716
A2ALV3	*Sh3gl2*	Endophilin-A1	Regulation of neurotransmitter release; endocytosis	AD, PD, HD	Loss	30061577
Q8VDQ8-2	*Sirt2*	NAD-dependent protein deacetylase sirtuin-2	Regulation of diverse biological processes	ALS, PD, HD	Loss	22796962; 22563317
Q9CR62	*Slc25a11*	2-oxoglutarate/malate carrier	Inner mitochondrial membrane transporter	AD	Loss	26837425
P62317	*Snrpd2*	Small nuclear ribonucleoprotein Sm D2	Pre-mRNA splicing	AD	Loss	27718298
P08228	*Sod1*	Superoxide dismutase 1	Antioxidant defense	AD, ALS, PD	Loss	15659387
Q62261	*Sptbn1*	Spectrin β chain, non-erythrocytic 1	Cytoskeletal structure	AD	Loss	28258398
Q9QZX7-2	*Srr*	Serine racemase	D-serine production	AD, ALS	Loss	25942042; 22117694
O08599	*Stxbp1*	Syntaxin-binding protein 1 / Munc18-1	Synaptic vesicle cycle	AD	Loss	28258398
Q8R191	*Syngr3*	Synaptogyrin-3	Synaptic vesicle cycle	AD	Loss	29398363
Q921F2	*Tardbp*	TAR DNA-binding protein 43 (TDP-43)	RNA biogenesis and processing	AD, ALS, FTD	Gain	18592312
P17751	*Tpi1*	Triosephosphate isomerase	Glycolysis/gluconeogenesis	AD	Loss	23233058
Q9CQN1	*Trap1*	TNF receptor-associated protein 1 / Heat shock protein 75	Molecular chaperone	PD	Gain	17579517
Q9R0P9	*Uchl1*	Ubiquitin carboxyl-terminal hydrolase L1	Deubiquitination	AD, FTD, PD	Loss	27515257; 16890190
Q8R1I1 Q9CZ13 Q9CR68	*Uqcr10* *Uqcrc1* *Uqcrfs1*	Cytochrome b-c1 complex subunits	Oxidative phosphorylation	AD, HD	Loss	24900954
Q01853	*Vcp*	Valosin-containing protein	Various, including ubiquitin-dependent protein degradation	AD, ALS, FTD	Loss	17622780; 22572540; 16783167
Q60932-2 Q60930 Q60931	*Vdac1* *Vdac2* *Vdac3*	Voltage-dependent anion-selective channel proteins 1, 2, 3	Ion transport through mitochondrial outer membrane	AD, ALS, PD	Loss	27116927; 28669745

Appendix B

Table 1. List of proteins differentially expressed with PF-670462 administration in the hippocampus of 3xTg-AD mice for CT10. All proteins in this list were quantified in at least half of samples (Q50) and differentially expressed in the hippocampus of 3xTg-AD mice treated for 20 days with PF-670462 (30 mg/kg/d) versus vehicle ($p < 0.05$, two-tailed unpaired Student's *t*-test; $\log_{10}$ fold change > $\log_{10}1.2$ or < $-\log_{10}1.2$).

UniProt ID	Protein name	Gene name	Also differentially expressed with genotype?	Expression restored towards NTg levels?
Q8VDP6	CDP-diacylglycerol--inositol 3-phosphatidyltransferase	*Cdipt*	Yes	Yes
P14131	40S ribosomal protein S16	*Rps16*		
Q9ER00	Syntaxin-12	*Stx12*	Yes	Yes
Q62425	Cytochrome c oxidase subunit NDUFA4	*Ndufa4*	Yes	Yes
O55137	Acyl-coenzyme A thioesterase 1; Acyl-coenzyme A thioesterase 2, mitochondrial	*Acot1;Acot2*		
Q60692	Proteasome subunit beta type-6	*Psmb6*		
Q9WUK2-2	Eukaryotic translation initiation factor 4H	*Eif4h*		
O88441	Metaxin-2	*Mtx2*	Yes	Yes
Q5NC80	Nucleoside diphosphate kinase; Nucleoside diphosphate kinase A	*Nme1*	Yes	Yes
O54829	Regulator of G-protein signaling 7	*Rgs7*	Yes	Yes
Q06890	Clusterin; Clusterin beta chain; Clusterin alpha chain	*Clu*		
O35129	Prohibitin-2	*Phb2*		
P50171	Estradiol 17-beta-dehydrogenase 8	*Hsd17b8;H2-Ke6*	Yes	Yes
A3KGQ6	Actin-related protein 2/3 complex subunit 5; Actin-related protein 2/3 complex subunit 5-like protein	*Arpc5l*		
Q9CQV6	Microtubule-associated proteins 1A/1B light chain 3B	*Map1lc3b;Gm5612*		
P60843	Eukaryotic initiation factor 4A-I	*Eif4a1*		
O55234	Proteasome subunit beta type-5	*Psmb5*	Yes	Yes
Q8R326-2	Paraspeckle component 1	*Pspc1*		
P63213	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2	*Gng2*		
O89112	LanC-like protein 1	*Lancl1*	Yes	Yes
Q9WV98	Mitochondrial import inner membrane translocase subunit Tim9	*Timm9*		
P62204	Calmodulin; Calmodulin-like protein 3	*Calm1;Calml3*	Yes	Yes
H7BX95	Serine/arginine-rich splicing factor 1	*Srsf1*		
P14152	Malate dehydrogenase, cytoplasmic	*Mdh1*	Yes	Yes
Q9QXS6	Drebrin	*Dbn1*	Yes	Yes
B9EJA2-5	Cortactin-binding protein 2	*Cttnbp2*		
Q9DCZ1	GMP reductase 1	*Gmpr*		
Q9DCL9	Multifunctional protein ADE2; Phosphoribosylaminoimidazole-succinocarboxamide synthase; Phosphoribosylaminoimidazole carboxylase	*Paics*		

D3YXH0	Purkinje cell protein 4	*Igsf5;Pcp4*		
P99026	Proteasome subunit beta type-4	*Psmb4*		
E9Q805	FERM, RhoGEF and pleckstrin domain-containing protein 1	*Farp1*		
Q9DBP5	UMP-CMP kinase	*Cmpk1*	Yes	Yes
P60202	Myelin proteolipid protein	*Plp1*	Yes	Yes
D3Z3A0	Protein phosphatase inhibitor 2	*Ppp1r2*	Yes	Yes
Q7TME0	Lipid phosphate phosphatase-related protein type 4	*Lppr4*		
Q3UY21	Myelin-oligodendrocyte glycoprotein	*Mog*		
Q9CQD1	Ras-related protein Rab-5A	*Rab5a*		
P62849-2	40S ribosomal protein S24	*Rps24*		
Q61411	GTPase HRas; GTPase HRas, N-terminally processed	*Hras*	Yes	Yes
P09528	Ferritin heavy chain; Ferritin heavy chain, N-terminally processed	*Fth1*	Yes	Yes
Q922D8	C-1-tetrahydrofolate synthase, cytoplasmic; Methylenetetrahydrofolate dehydrogenase; Methenyltetrahydrofolate cyclohydrolase; Formyltetrahydrofolate synthetase; C-1-tetrahydrofolate synthase, cytoplasmic, N-terminally processed	*Mthfd1*		
Q9Z0P5	Twinfilin-2	*Twf2*		
Q91ZZ3	Beta-synuclein	*Sncb*		
P21107-2	Tropomyosin alpha-3 chain	*Tpm3;Tpm3-rs7*		

Table 2. List of proteins differentially expressed with PF-670462 administration in the hippocampus of 3xTg-AD mice for CT14. All proteins in this list were quantified in at least half of samples (Q50) and differentially expressed in the hippocampus of 3xTg-AD mice treated for 20 days with PF-670462 (30 mg/kg/d) versus vehicle ($p < 0.05$, two-tailed unpaired Student's *t*-test; $\log_{10}$ fold change > $\log_{10}1.2$ or < $-\log_{10}1.2$).

UniProt ID	Protein name	Gene name	Also differentially expressed with genotype?	Expression restored towards NTg levels?
Q9CPU4	Microsomal glutathione S-transferase 3	*Mgst3*	Yes	Yes
Q8R574	Phosphoribosyl pyrophosphate synthase-associated protein 2	*Prpsap2*		
Q9D3A9	Protein tweety homolog 1	*Ttyh1*		
E9PV44	ATPase inhibitor, mitochondrial	*Atpif1*	Yes	Yes
P62754	40S ribosomal protein S6	*Rps6*		
O35682	Myeloid-associated differentiation marker	*Myadm*		
A2AUE1	DnaJ homolog subfamily C member 5	*Dnajc5*	Yes	Yes
Q9ES97-2	Reticulon-3	*Rtn3*	Yes	Yes
F6WYQ5	Phospholipid-transporting ATPase IA	*Atp8a1*	Yes	Yes
P14148	60S ribosomal protein L7	*Rpl7*		
Q9Z268	RasGAP-activating-like protein 1	*Rasal1*		
O35449	Proline-rich transmembrane protein 1	*Prrt1*	Yes	Yes
Q9CQD1	Ras-related protein Rab-5A	*Rab5a*	Yes	Yes
Q8R464	Cell adhesion molecule 4	*Cadm4*		
Q9Z0Y1	Dynactin subunit 3	*Dctn3*		
Q7TNB5	Glutamate receptor 1	*Gria1*	Yes	Yes
P21107-2	Tropomyosin alpha-3 chain	*Tpm3;Tpm3-rs7*	Yes	No
P23242	Gap junction alpha-1 protein	*Gja1*	Yes	Yes
Q9CQZ6	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 3	*Ndufb3*	Yes	Yes
Q03137-2	Ephrin type-A receptor 4	*Epha4*		
P97300-3	Neuroplastin	*Nptn*		
Q8BJI1	Sodium-dependent neutral amino acid transporter SLC6A17	*Slc6a17*	Yes	Yes
O54774	AP-3 complex subunit delta-1	*Ap3d1*	Yes	Yes
P01831	Thy-1 membrane glycoprotein	*Thy1*		
Q8BGT8	Phytanoyl-CoA hydroxylase-interacting protein-like	*Phyhipl*		
P51660	Peroxisomal multifunctional enzyme type 2; (3R)-hydroxyacyl-CoA dehydrogenase; Enoyl-CoA hydratase 2	*Hsd17b4*		
Q9CZS1	Aldehyde dehydrogenase X, mitochondrial	*Aldh1b1*	Yes	Yes
D3YYK8	Microtubule-associated protein RP/EB family member 2	*Mapre2*		
B1AUQ7	Centrin-2	*Cetn2*		
Q8BNW9	Kelch repeat and BTB domain-containing protein 11	*Kbtbd11*		
P70296	Phosphatidylethanolamine-binding protein 1; Hippocampal cholinergic neurostimulating peptide	*Pebp1*	Yes	Yes
Q9D2G2	Dihydrolipoyllysine-residue succinyltransferase	*Dlst*	Yes	Yes

	component of 2-oxoglutarate dehydrogenase complex, mitochondrial			
P97450	ATP synthase-coupling factor 6, mitochondrial	*Atp5j*		
P60843	Eukaryotic initiation factor 4A-I	*Eif4a1*		
P60840-2	Alpha-endosulfine	*Ensa*		
Q99M71	Mammalian ependymin-related protein 1	*Epdr1*		
Q91VN4	MICOS complex subunit Mic25	*Chchd6*		
P28738	Kinesin heavy chain isoform 5C	*Kif5c*		
Q80UW2	F-box only protein 2	*Fbxo2*	Yes	Yes
Q80TL4-2	Protein KIAA1045	*Kiaa1045;N28178*		
P16858	Glyceraldehyde-3-phosphate dehydrogenase	*Gapdh;Gm7293*	Yes	Yes
Q8BTS0	Probable ATP-dependent RNA helicase DDX5	*Ddx5*		
Q8CC35-3	Synaptopodin	*Synpo*		
Q3UIU2	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 6	*Ndufb6*		
P17742	Peptidyl-prolyl cis-trans isomerase A; Peptidyl-prolyl cis-trans isomerase A, N-terminally processed	*Ppia*		
E9Q9C5	V-type proton ATPase 16 kDa proteolipid subunit	*Atp6v0c*		
P42669	Transcriptional activator protein Pur-alpha	*Pura*		
O54829	Regulator of G-protein signaling 7	*Rgs7*		
P29758	Ornithine aminotransferase, mitochondrial	*Oat*	Yes	Yes
A2A9X5	5(3)-deoxyribonucleotidase, cytosolic type	*Nt5c*		
P10605	Cathepsin B; Cathepsin B light chain; Cathepsin B heavy chain	*Ctsb*		
P62320	Small nuclear ribonucleoprotein Sm D3	*Snrpd3*		
Q9JKB1	Ubiquitin carboxyl-terminal hydrolase isozyme L3; Ubiquitin carboxyl-terminal hydrolase isozyme L4	*Uchl3;Uchl4*	Yes	Yes
Q9QUR7	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1	*Pin1*		
Q62419	Endophilin-A2	*Sh3gl1*		
Q3MIA8	COP9 signalosome complex subunit 1	*Gps1*		
P24369	Peptidyl-prolyl cis-trans isomerase B	*Ppib*		
Q9WTL7	Acyl-protein thioesterase 2	*Lypla2*		
P61329-2	Fibroblast growth factor 12; Fibroblast growth factor; Fibroblast growth factor 14	*Fgf12;Fgf14*		
Q8R326-2	Paraspeckle component 1	*Pspc1*		
P42208	Septin-2	*Sept2*	Yes	Yes
D3Z0A8	Redox-regulatory protein FAM213A	*Fam213a*		
P97492	Regulator of G-protein signaling 14	*Rgs14*	Yes	Yes
P58389	Serine/threonine-protein phosphatase 2A activator	*Ppp2r4*	Yes	Yes
Q8BHN3	Neutral alpha-glucosidase AB	*Ganab*		
Q6ZWQ9	Myosin regulatory light chain 12B	*Myl12a;Myl12b*		
E9Q0W6	Actin-binding LIM protein 2	*Ablim2*		
Q8C854-3	Myelin expression factor 2	*Myef2*		
P12023-2	Amyloid beta A4 protein; N-APP; Soluble APP-alpha; Soluble APP-beta; C99;Beta-amyloid protein 42; Beta-amyloid protein 40; C83; P3(42); P3(40); C80; Gamma-secretase C-terminal fragment 59; Gamma-secretase C-terminal fragment 57; Gamma-secretase C-terminal	*App*	Yes	Yes

	fragment 50; C31			
Q9DCL9	Multifunctional protein ADE2; Phosphoribosylaminoimidazole-succinocarboxamide synthase; Phosphoribosylaminoimidazole carboxylase	*Paics*		
Q64133	Amine oxidase [flavin-containing] A	*Maoa*	Yes	Yes
B1AWV9	Sodium-driven chloride bicarbonate exchanger; Electroneutral sodium bicarbonate exchanger 1	*Slc4a10;Slc4a8*		
P62830	60S ribosomal protein L23	*Rpl23*		