Exploring molecular mechanisms of mouse hibernation for novel therapeutic angles in Alzheimer’s disease
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General Introduction
Neurodegeneration is the process of progressive degradation of cells in the brain and other parts of the nervous system. In the brain, neurodegeneration may affect a wide spectrum of functions impacting on daily life. For instance, how you process physiological body movement but also memories and a broad range of related emotions, such as joy over the birth of a child, sadness over painful life events, anger over injustice and love for the persons that stand by you. In dementias, the information processing by neuronal cells of the brain deteriorates causing cognitive decline and, depending on the type of dementia, preferentially does so in brain areas that produce memory. As such, memories, including self-defining memories, start fading and regression is eminent, until that what defines a person, the self, is lost. As this touches upon the core of being, countless efforts have been made to unravel what constitutes neurodegeneration and how to stop it. However, to provide an effective therapy, apart from putting neurodegeneration to a halt, restoring or better preventing the damage initially is essential, but diagnosis is currently only possible with certainty at advanced stages of disease (1). Also, current therapeutic designs in Alzheimer Disease mostly focus on the removal of the neuropathologically detected amyloid-beta or tau protein build-up. Though, whether these proteins are initially causative remains to be seen (2-5) and alternatively numerous other AD cascade hypotheses, such as the cholinergic-, neuroinflammation-, brain insulin resistance-, extracellular matrix-, and mitochondrial-cascade hypotheses have been put forward over the years (6). The multifaceted nature of Alzheimer’s disease makes capturing all aspects of the disease and finding a cure highly complex (Figure 1). Up till now, no effective cure has been found, in spite of decades of research. In this context there is a niche for new, more out of the box ideas in the fight against the devastating effects of neurodegeneration in dementias. Interestingly, hibernation, a process in which animals undergo bouts of extreme metabolic reduction with hypothermia to withstand harsh environmental conditions, embodies some fascinating adaptations that might be of use in this quest. In this introduction I will further introduce the principles of hibernation, and how hibernation-associated mechanisms might interfere with key pathophysiological principles as observed in Alzheimer’s disease.

HIBERNATION

Hibernation is the intriguing phenomenon in which animals undergo alternating bouts of hypometabolism and hypothermia (i.e. torpor) and reactivation of metabolism and rewarming (i.e. interbout arousals) to escape energetically challenging conditions such as extreme cold or food scarcity. In general, hibernators can reduce their metabolic rate to 2-4% of normal by active suppression of mitochondrial
function and can reach core body temperatures as low as −3°C during torpor (7). During interbout arousals, mitochondria are reactivated and produce thermogenic heat, most prominently in brown adipose tissue in a process called ‘non shivering thermogenesis’. This leads to the rapid rewarming of the body, typical for arousal from torpor. Hibernators are subdivided in seasonal hibernators, that generally have torpor and interbout arousals lasting up to weeks, and daily hibernators that enter a ~6-8 h single torpor bout and subsequent arousal on a daily basis (8). Whereas seasonal hibernators reach metabolic rates of ~3%, daily hibernators typically reduce metabolic rates to a lesser extent with a mean of ~35%. Some species, such as the edible dormouse, can even switch between daily torpor and seasonal hibernation, depending on what is energetically most favourable. Facultative hibernators on the other hand only exert torpor as an exit strategy in energetically challenging conditions. Collectively, different hibernation strategies create a continuum of metabolic plasticity (9-11).

The hibernating brain

Overall brain activity changes drastically during hibernation. Electroencephalograms and single-unit recordings in torpid arctic squirrel are isoelectric, indicating cessation of firing of neurons (12, 13), and REM (rapid-eye movement) sleep is absent (14). However, some brain areas remain at least in part active to facilitate basic functions, e.g. breathing, heart function, and to initiate arousal from torpor (15). Upon torpor entry, EEG activity remains measurable longest in the hippocampus, and it was therefore long thought that hippocampus controls hibernation (15, 16). More recently, a distinct subset of glutamatergic neurons in the hypothalamus has been implicated in torpor regulation (17, 18), as manipulation of these neurons was sufficient to induce or prevent torpor. Others have postulated that the glucose to

Figure 1: Alzheimer’s disease is multifaceted

The AD brain is characterized by tau and Aβ depositions (neurofibrillary tangles and Aβ plaques), extracellular matrix (ECM) changes, impaired synaptic plasticity, cholinergic deficits, genetic vulnerabilities such as carrying the ApoE4 isoform, neuroinflammation, mitochondrial dysfunction, insulin resistance and many more. The multifaceted nature of AD makes it difficult to delineate its aetiology and to define specific targets for treatment.

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glutamate pathway couples peripheral signals of low energy to brain wide torpor induction (19). However due to its complexity, the neuronal circuits regulating hibernation are still not fully delineated, complicating translation of the beneficial effects of this promising natural phenomenon to non-hibernators such as humans.

**Hibernation and Alzheimer’s Disease**

Alzheimer’s Disease (AD) is the most prevalent neurodegenerative dementia, constituting 60-80% of all cases and affecting an estimated 416 million people globally (20), with numbers only expected to rise as the population ages (21, 22). Neurodegeneration is the progressive loss of vulnerable neurons, and neurodegenerative diseases can further be specified by distinct pathological and clinical outcomes that depend on the anatomical location and the type of neurons affected and the pathological substrate of the disease. Clinically, AD diagnosis depends on progressive cognitive impairment, and neuropathologically on the presence of a mixed proteinopathy: β-amyloid (Aβ)-containing extracellular plaques and tau-containing intracellular neurofibrillary tangles (1, 23). Typically, the neuropathological detection of protein aggregates is performed post-mortem by Thal scoring, which describes 5 phases of β-amyloidosis, with the Aβ peptide derived from APP, spreading from the neocortex (phase 1) to the brainstem and cerebellum (phase 4/5) (24). Additionally, Braak staging scores tau pathology and its spread over 6 stages, with tau pathology first appearing in (trans)entorhinal regions (stage 1/2), spreading to limbic regions including the hippocampus (stage 3/4), and ending up in neocortical areas (stage 5/6) (25). Though Aβ staging correlates with tau staging, its correlation to antemortem clinical features of AD, such as cognitive impairment, is lower than that of tau pathology based Braak staging (26, 27). Also, Aβ may be present in the brain without the typical symptoms of Alzheimer’s being present, which has put the role of Aβ and its relationship to cognitive decline into question.

In addition to its two pathological hallmarks Aβ and tau aggregates, substantiating the amyloid cascade and tau hypothesis of AD disease aetiology, the disease displays countless other facets. For example, genetic risk factors have been found by genome wide association studies in the human population (28, 29). The most prevalent genetic risk factor for late-onset AD is the presence of the APOE4 isoform, rather than the common ApoE3, or the ‘protective’ ApoE2 isoform (30). ApoE is involved in lipid transport and cholesterol homeostasis, and consequently ApoE4 carriers show dysfunctional lipid metabolism (31). In addition, ApoE4 carriers have increased Aβ deposits and Aβ oligomers, tau-hyperphosphorylation and neuroinflammation. Moreover, ApoE4 carriers show mitochondrial dysfunction, probably due to downregulation of gene transcripts of mitochondrial respiratory complexes I, IV, and V, higher oxidative stress and decreased levels mitochondrial transport
proteins (30, 32). Furthermore, as mentioned previously, numerous other AD cascade hypotheses, supported by compelling evidence, have been put forward. This makes AD a highly complex disease to study, resulting in a lack of consensus and of effective treatment strategies so far (6) (Figure 1).

What makes hibernation so fascinating from an AD perspective is that the torpid brain undergoes particular changes that show remarkable similarities to those found in AD, with the distinction that hibernators have the capacity to completely reverse these changes in a matter of hours upon arousal. Below I discuss the most striking similarities between torpor and Alzheimer’s Disease and the reversibility of these during arousal.

Neuronal plasticity
Perhaps one of the most striking resemblances of hibernation and AD is that they meet in (the loss of) neuronal plasticity. Prior to the loss of neurons in AD, occurring throughout the end stages of neurodegeneration, synapse loss occurs (33-35) and there are strong indications this is preceded by a loss of their ability to change and adapt, i.e. synaptic plasticity impairment. Pathological Aβ leads to clear synaptic impairment as shown in vivo in Aβ based AD mouse models, and in vitro and ex vivo when studying effects of pathological Aβ in cell cultures and brain slices (36-40). Adaptation of synapses, such as spine rearrangements regulating connections in the neuronal circuitry and synaptic biochemical adaptations modulating the strength of synaptic transmission, are thought to be essential for memory formation. As such, loss of synaptic plasticity is thought to underlie memory impairment found in AD (41, 42). This has sparked interest in strategies stimulating neuroplasticity by brain stimulation techniques such as transcranial magnetic stimulation and deep brain stimulation (43). However, all brain stimulation efforts have failed to induce robust improvement of memory in AD patients (44). In addition, directly targeting the pathological protein accumulation of Aβ, using anti-Aβ monoclonal antibodies, has failed to significantly improve cognitive performance in AD patients (45-47).

During torpor (the hypometabolic and cold phase of hibernation) extensive, yet reversible, neuronal (48-50) and spinal (51-55) retraction is observed, with retraction of dendrites up to 25% and a 50–65% loss of synapses as seen in various hamster models and in ground squirrel (Figure 2) (50). This is paralleled by cessation of long-term potentiation (LTP), a widely used electrophysiological technique to quantify synaptic plasticity as the response of neurons to an electrical stimulus, and is thought to be key in memory formation (56, 57). It is hypothesized that retraction of dendrites and spines during torpor is an adaptation that ensures the disconnection of neurons and shutdown of communication in the torpid brain, as demonstrated by the flat EEG (12), to prevent toxic actions of excitatory amino
acids (excitotoxicity) upon rewarming (58). On a subcellular level, it has been shown that spine retraction and regeneration coincides with changes in the abundance of presynaptic synaptophysin and piccolo, and postsynaptic PSD-95 (53). It has been postulated that these proteins dissociate from the cytoskeletal active zone and the postsynaptic density during torpor, creating a reservoir of proteins that can be quickly mobilized for rapid rebuilding of dendritic spines and synapses during arousal (53, 59). In addition, hibernation also induces several cold-shock proteins in the brain, including RBM3, an RNA binding protein that mediates structural plasticity (60, 61). These targeted studies on synaptic protein dynamics during hibernation leave room for a broader approach of measuring overall synaptic protein regulation, to generate novel insights into the mechanisms of this extraordinary neuronal and synaptic plasticity in hibernation, which is of interest for understanding principles of modulating synaptic plasticity and maybe helpful in addressing affected synapse function in AD.

Figure 2: exceptional neuronal plasticity during hibernation. Example of Scholl analysis showing extensive dendritic retraction of a pyramidal neuron during torpor, which is completely reversed after arousal. Image adapted from Magariños et al. 2006 (49).

The extracellular matrix
AD is also characterized by extracellular matrix (ECM) changes in the brain, including higher numbers of perineuronal nets (PNNs) surrounding hippocampal neurons, most prominently hippocampal parvalbumin interneurons (62). The increase in PNN levels is thought to initially represent a protective mechanisms against Aβ and tau toxicity (63, 64), but in the long run to convey the pathological reduction of synaptic plasticity (65, 66). This has prompted studies to investigate chemically lowered ECM levels, using Chondroitinase ABC, and assess the effect of lower ECM levels on synaptic plasticity and memory. Indeed, Chondroitinase ABC treatment led to synaptic rearrangements and improved learning in wildtype mice and rescued both long-term potentiation and contextual memory performance in APP/PS1 AD mice (67, 68). However, Chondroitinase ABC treatment is not readily translatable to treatment in humans because homeostatic regulation of PNNs depends on the balance or ‘plasticity’ of the PNN system, and solely drastically reducing PNNs does
not represent a balanced system. Interestingly, the ECM system reverts back to its initial disease state after chondroitinase treatment is stopped.

There are some indications that hibernating animals also show ECM remodelling. For instance, in Syrian Hamster (a facultative hibernator), the lung ECM changes drastically with collagen levels increasing during torpor, and completely reversing back to normal upon arousal, under the regulation of matrix metalloproteases 2 and 9 (69). In addition, mRNA expression of aggrecan, an important component of PNNs, is significantly increased during hibernation in the hypothalamus of 13-lined ground squirrel (70), and significantly higher numbers of PNNs were found in the forebrain of arctic ground squirrel during torpor (71). This ECM regulation is thought to adjust synaptic plasticity during torpor and arousal to ensure vital functions are protected and excitotoxicity is prevented. In addition, increasing ECM levels in the brain during torpor may in theory be necessary to retain memories, as during torpor extensive neuronal and synaptic retraction occurs. The ECM might constitute a template for regenerating the original synaptic connectivity during arousal.

Studying hibernation might offer novel insight into the role of the ECM in healthy regulation of PNN levels and neuronal plasticity, which in turn is relevant for pathological ECM alterations in AD.

**Tau hyperphosphorylation**

Abnormal tau hyperphosphorylation and subsequent formation of pathological neurofibrillary tangles, is one of the two main neuropathological hallmarks of AD (1, 72). The tau hypothesis claims excessive or abnormal phosphorylation of tau as the causative agent of AD, which is supported by the fact that tau depositions correlate more closely with clinical AD severity and progression than Aβ plaque load. This makes Braak staging of tau aggregates the primary diagnostic tool for AD (1, 73). The tau protein (MAPT) exists in six isoforms in the human brain as a result of alternative mRNA splicing (three 3-repeat / 3R and three 4-repeat / 4R tau isoforms) (3). In Alzheimer’s disease (and other tauopathies), tau becomes abnormally hyperphosphorylated, dissociates from microtubules which renders tau unable to stabilize microtubules, which is eminent for healthy neuronal functioning (74). Moreover, it accumulates in the cytoplasm where it can form paired helical filaments and, eventually, neurofibrillary tangles (45). This results in a toxic action of the intracellular tau aggregates. Dysfunctional and toxic tau lead to synaptic impairment (75) and loss of mitochondrial integrity (76). Consequently, these cellular effects of tau aggregation may lead to the loss of function and ultimately death of nerve cells, i.e., cause neurodegeneration (77). Glutamate projection neurons, which are essential for memory formation and recall, are especially vulnerable to pathological tau hyperphosphorylation and it is hypothesized that the hyperphosphorylation of tau
in these neurons is responsible for aberrant Aβ cleavage and glutamate dependent Aβ toxicity, leading to the neurodegenerative cascade in Alzheimer's disease (78, 79).

During torpor, metabolism and thermogenesis are suppressed, and reversible phosphorylation of enzymes and other proteins have been implicated in its regulation. Remarkably, the tau protein is hyper-phosphorylated throughout the brain during torpor (Figure 3) (80, 81), with highest amounts of phosphorylation of the AD-typical phosphoepitopes, Ser202/Thr205, found in entorhinal cortex, hippocampus, cortex, and hypothalamic and epithalamic nuclei in ground squirrel, Syrian hamster and black bear (82-85). Strikingly, the pathological amount of paired helical filament-like hyperphosphorylation of tau in the brain of these hibernators is well tolerated and not associated with fibril formation, and is fully reversible after arousal (80). This has sparked the interest of Alzheimer researchers as hibernation might hold the key to valuable protective mechanisms against tau tangle formation and may yield mechanisms that promote the clearance of PHF-like hyperphosphorylated tau (83-85).

Figure 3: Reversible tau hyper-phosphorylation during hibernation. AT-8 (Ser202/Thr205) staining of hyperphosphorylated tau in coronal sections of laboratory mice (Mus musculus) brain during euthermia, torpor and arousal during daily torpor. While prominently present during torpor, tau hyperphosphorylation is completely reversed upon arousal (data from Chapter 3). Similar tau hyperphosphorylation patterns are found in hibernating ground squirrel, Syrian hamster and black bear.

Mitochondrial (dys)function

Apart from Aβ and tau aggregation in the AD patient’s brain, the mitochondrion represents a less obvious yet vital candidate underlying neurodegeneration. Mitochondrial dysfunction in AD is getting increased attention as a large body of evidence suggests that damaged mitochondria likely play fundamental roles in the causation of AD (86). One could argue that the mitochondrion is the organelle at the heart of life, as it generates the energy that cells need to exert basically all non-passive processes. But mitochondria are more than the powerhouses of the cell, as they also tightly regulate neuronal cell death via pro-apoptotic cytochrome c (87), produce reactive oxygen species (ROS) (88), regulate synaptic plasticity via local translation (89-91) and support synaptic transmission via calcium handling (92). Dysregulation of any of these processes can be the initiation of the disastrous downstream effects manifested as AD.
Mitochondria produce ATP that upon hydrolysis releases a phosphate group, generating free energy that can be transferred to other molecules wielding specific cellular functions. ATP is formed at the inner mitochondrial membrane by a process called oxidative phosphorylation that occurs in the electron transport chain (ETC). The ETC consists of four multimeric enzymes (complex 1; NADH oxidoreductase, complex 2; succinate dehydrogenase, complex 3; Q-cytochrome c oxidoreductase, complex 4; cytochrome c oxidase), two mobile electron carriers (coenzyme Q and cytochrome c) and an ATP synthase (complex 5) (Figure 4) (93). These complexes take reducing equivalents from Nicotinamide Adenine Dinucleotide (NADH) and Flavin adenine dinucleotide (FADH2), which are generated either directly from upstream catabolic pathways of nutrients (glycolysis or fatty acid degradation and oxidation) or via the tricyclic acid (TCA) cycle. The reducing equivalents are then used to generate a proton motive force over the mitochondrial membrane (93, 94), resulting in the end product ATP. Multiple studies in AD cell culture models, animal models and human post-mortem brain tissue link reduced mitochondrial complex activity and abundance (particularly of complex I/IV) to AD (95-99).

**Figure 4: Generation of energy by the mitochondrion.**

ATP is produced by the oxidative phosphorylation system (OXPHOS) or electron transport system (ETC) via a proton motive force over five complexes (complex 1; NADH oxidoreductase, complex 2; succinate dehydrogenase, complex 3; Q-cytochrome c oxidoreductase, complex 4; cytochrome c oxidase, and complex 5; ATP synthase) and coenzyme Q and Cytochrome C. Incomplete reduction of O₂ by complex 4 leads to the production of reactive oxygen species (ROS). The OXPHOS is driven by reducing NADH and FADH₂, provided by the tri-cyclic acid (TCA) cycle, pyruvate metabolism and fatty acid degradation and oxidation (FAD & FAO). The TCA gets input from Acetyl-CoA, glutamate, fatty acid beta oxidation and amino acid metabolism. Acetyl-CoA and glutamate are products of the three main metabolic pathways: Glycolysis, FAD & FAO and amino acid metabolism. Energy from ATP is released upon breaking its third, high energy phosphate bond, resulting in conversion to ADP and inorganic phosphate (Pi).
The generation of ATP by the ETC or oxidative phosphorylation (OXPHOS) is inherently coupled to ROS production as this is a by-product of incomplete reduction of O₂ naturally occurring in low amounts during OXPHOS. In healthy mitochondria ROS fulfils a signalling function and is tightly regulated via antioxidant systems. However, upon dysfunction of the mitochondrion (e.g. due to cell stress, aging or genetic mutations), an imbalance in ATP and ROS arises, with insufficient energy supply for neuronal demand and increased ROS amounts damaging lipids, proteins and DNA, leading to aberrant secondary signalling (e.g. Ca²⁺ signalling), and ultimately causing neurodegeneration (94, 100-102). All efforts to target mitochondria in the fight against AD, mostly via harnessing the anti-oxidant system by supplying antioxidants, have been unsuccessful so far (103).

During hibernation, a drastic (up to 98%) reduction in metabolic rate occurs, i.e. hypometabolism, prior to hypothermia onset (104). Though still poorly understood, it has been shown that during this hypometabolic state, the efficiency of complex I & II (105) of the ETC and of the mitochondrial H₂S oxidizing enzyme sulfide:quinone oxidoreductase (SQR) (106) is reduced, possibly due to their posttranslational modifications (105). During interbout arousals, mitochondria reverse this enzymatic efficiency which is instrumental to produce the initial thermogenic heat by ‘non shivering thermogenesis’ in uncoupled brown adipose tissue mitochondria and maintain normothermia a by-product of ATP production in other tissues such as skeletal muscle (‘shivering thermogenesis’). Brown adipose mitochondria are committed to thermogenesis rather than ATP production because of their relatively high levels of UCP1 (uncoupling protein 1), that uncouples respiration from ATP production, and low levels of ATPase (complex 5 of ETC) (104). Interestingly, this mitochondrial de- and re-activation during torpor and arousal occurs while maintaining efficient energy supply (107) and preventing ROS damage (108). This is of particular interest for the ischemia-reperfusion field as in humans the overproduction of reactive oxygen species (ROS) upon rewarming jeopardizes cellular integrity (108, 109). Consequently, exploiting the mechanisms that hibernators use to maintain mitochondrial ATP production without ROS damage during the extremes of physiology, might represent a promising therapeutic strategy in AD, given the observed mitochondrial dysfunction and subsequent ATP-ROS imbalance in AD brain.

**DAILY TORPOR IN MICE**

The potential of hibernation harbouring specific mechanisms to reverse pathological AD-like processes holds promise for the treatment of Alzheimer’s disease in humans (51, 80, 81). Obtaining such insights from routinely used hibernators is challenging,
largely because these non-standard laboratory animals lack proper genomic and proteomic annotation and genetic models are limited. In addition, the widely used Arctic ground squirrel, Syrian hamster and black bear are seasonal and facultative hibernators, with bouts of torpor and interbout arousals typically lasting several days to weeks (8). Though long overlooked, laboratory mice are also hibernating animals, exerting facultative daily torpor as response to energetic challenge, such as low food availability or higher energy expenditure (110, 111). Torpor in mice is generally induced using a fasting protocol (completely omitting food availability), or a work-for-food protocol, in which mice have so called high foraging-costs typically due to wheel running as requirement to access food (112). Both ultimately lead to a negative energy balance that is compensated for by daily torpor with body temperatures as low as 21 °C (111-113). Mouse daily torpor can even be accomplished in as little as one night of fasting, depending on torpor-sensitivity of the mouse (113). The notion that laboratory mice are also capable of daily torpor (18, 113, 114), showing bouts of torpor and arousal of several hours instead of days to weeks, offers the opportunity to explore hibernation and the mechanisms it employs to protect the brain in a standard animal model, and in a relatively short time-frame. Moreover, this offers the opportunity to study these mechanisms in numerous genetic neurodegenerative mouse models, such as the human tau mouse model or the commonly used APP/PS1 AD mouse model which contains transgenes of mutated APP and PSEN1 found in familial AD (115) and the humanized ApoE4 mouse model. All are used in this thesis.

**COMPOUNDS THAT MIMIC HIBERNATION**

Unfortunately, humans cannot hibernate. Efforts have been made to find ways of artificially inducing torpor (i.e. synthetic torpor) in non-hibernators, for example by using the gaseous transmitter H₂S that blocks mitochondrial activity via cytochrome c (116), inhibition of key neurons in central thermoregulation (Raphe Pallidus neurons) using muscimol (117) or activation of A1 adenosine receptors that are known to induce hypothermia and hypometabolism during torpor using N6-cyclohexyladenosine (118). However, translation to human hibernation therapies remains futuristic, not in the least due to ethical considerations of losing (control of) periods of time and possibly even losing memories formed prior to the torpor like state (119-121). Therefore, it is crucial to find hibernation-derived protective and regenerative mechanisms that can be specifically targeted without inducing an overall state of torpor.
SUL-138

Inspired by the effects of hibernation on mitochondria and the associated beneficial effects, small molecule compounds were developed that have the capacity to mimic the effect of endogenous mediators involved in protection from cell-damage in hibernation (122, 123). These novel compounds (SUL compounds) are (S) enantiomeric structural derivatives of 6-chromanol, the core scaffold of tocopherols (vitamin E) (124) and Trolox (125). SUL compounds preserve mitochondrial respiratory chain function via mitochondrial complexes I and IV activation, thereby preventing ROS formation while stimulating ATP production (122), particularly in cells and tissues under stress. So far, the compounds have proven successful in experimental research by protecting from a wide range of stressors such as cooling, diabetes mellitus, renal injury and airway inflammation (122, 126-130). SUL compounds do so without affecting basal mitochondrial membrane potential or causing apparent mitochondrial toxicity. Moreover, the physico-chemical properties of SUL compound 138 (SUL-138 or SUL-238 in its salt form) allows it to pass the blood-brain-barrier (131) (Figure 5), and preliminary toxicological assessment shows no toxic effects in dosages used in the animal studies (data not shown), making it a very interesting compound to test against brain diseases that display mitochondrial dysfunction, such as AD.

Figure 5: Chemical structure of SUL-138

AIM AND OUTLINE OF THIS THESIS

The aim of this thesis is to unravel hibernation-derived mechanisms against AD. I aimed to do so by exploring the use of daily torpor in mice to study AD related processes (neuronal plasticity and tau hyperphosphorylation) and to test hibernation and hibernation-derived mitochondrial activation (using a SUL-compound) in Alzheimer’s disease mouse models.

In Chapter 2 we set-up fasting-induced torpor in mice and studied arousal-associated structural, functional and molecular adaptations in the hippocampus and its effects on memory acquisition. We show that torpor in wildtype mice mainly acts on synaptic plasticity and mitochondria and is associated with increased long-term potentiation (LTP) and memory performance after arousal. In addition, one daily
torpor bout was sufficient to rescue memory retrieval capacity in an APP/PS1 mouse model of AD.

In Chapter 3 we established whether torpor in mouse tau (mtau) and human tau (htau) mice induce tau hyper-phosphorylation in the brain similar to that seen in seasonal hibernators, and assess its reversibility. We found that torpor induced robust and reversible tau-hyperphosphorylation in both mtau and htau mice, with highest levels of tau hyperphosphorylation in the hippocampus, posterior parietal cortex, piriform cortex and cortical amygdala. In addition, tau hyperphosphorylation was diffuse in mtau mice whereas in htau mice pretangle-like structures were formed which are cleared 24h after torpor. Lower levels of tau hyperphosphorylation 24h after torpor compared to baseline euthermia suggest that torpor prompts clearance pathways relevant for AD.

Next, we tested the hibernation-derived mitochondrial activating compound SUL-138 in two AD mouse models: APP/PS1 mice and ApoE4 targeted replacement (TR) mice. In the APP/PS1 mouse model of AD (and in healthy wildtype controls), we tested the effects of treatment at the molecular, physiological and behavioural level (Chapter 4). We show that 3 months of oral administration of SUL-138 increases synaptic transmission and memory performance in both APP/PS1 and wildtype controls. This was accompanied by a substantial decrease in amyloid plaque load and a partial rescue of AD-associated changes in protein expression in the brain of SUL-138-treated APP/PS1 mice. Treatment with SUL-138 induced a significant upregulation of mitochondrial metabolic proteins involved in fatty acid degradation and oxidation in particular, while having only a modest effect on synaptic protein levels.

In the ApoE4 TR mouse model of AD (Chapter 5) we tested the differences on the behavioural and molecular level between female ApoE3 and ApoE4 TR mice, and between female ApoE4 TR mice treated with vehicle or SUL-138 compound. In contrast to expectations, we found increased memory performance in ApoE4 TR mice. In addition, proteins involved in synaptic signalling and amino acid metabolism were upregulated. SUL-138 treatment in ApoE4 had no effect on memory and increased levels of proteins involved in synaptic vesicle cycle and glycolysis. These data point to early proteome dysregulation in ApoE4 mice and supported the mitochondrial effects of SUL treatment found previously.

Finally, the results of Chapter 2-5 are summarized, and challenges, implications and future perspectives of this work are discussed in Chapter 6 (general discussion) supported by a review on mitochondria in Alzheimer’s disease and the lessons learned from hibernation.
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Chapter 1  |  General introduction


Chapter 1 | General introduction


