University of Groningen

Tau phosphorylation

In Hibernation and Alzheimer's

K.S.F. Klaver

1/20/2014

Abstract: Tau is a MAP found in neurons. Tau hyperphosphorylation, which detaches tau from microtubules is one of the hallmarks of PHF's observed in AD but is also observed in torpor where it is reversed after arousal without lasting neuronal damage. This review focusses on tau phosphorylation. We seek to understand the differences between tau phosphorylation in AD patients and tau phosphorylation in torpid animals in order to asses if reversal of tau phosphorylation, like during arousals, can treat tau pathology in AD. Human tau has an essential role in microtubule stability, intracellular transport and the release of neurotransmitters. When phosphorylated, tau detaches from microtubules. Phosphorylation is done by GSK3B, CDK5, CK1, PKA and many more kinases. PP2A is mostly responsible for the dephosphorylation of tau. AD is characterized by synaptic degeneration and extracellular SP's, consisting of AB and intracellular tangles consisting of hyperphosphorylated tau. Tau phosphorylation but also deficiency of glucose metabolism correlates with disease progression. In AD increased GSK3B, CDK5, CK1, PSK1 activity and decreased PP2A activity is found. *In vitro* they can cause the phosphorylation of all residues found in AD tau. Due to its complexity the process of tau phosphorylation is still not completely understood and it remains to be proven if these kinases are solely responsible *in vivo.* During torpor tau is hyperphosphorylated by increased PKA and GSK3B activity and decreased PP2A activity. This is likely caused by temperature dependent activity shifts. During arousals animals quickly rewarm to euthermic state. Neuronal connections reappear and phosphorylation sites within tau's MDB are rapidly dephosphorylated. Sites implicated in microtubule binding are phosphorylated in AD as well as during torpor. GSK3B activity is increased during AD and torpor possibly due to hypometabolism. Also PKA, CDK5, CK1 and PSK1 activity is increased in AD while in torpor only GSK3B and PKA are reported. Furthermore in AD the amount of phosphorylated tau slowly increases and is deposited into PHF's while in hibernators tau is dephosphorylated during arousals. During torpor metabolic challenge and decreased temperature cause hyperphosphorylated tau which protects neurons from damage. The neurons of torpid animals do not have AB deposition and clear phosphorylated tau before irreversible damage occurs. Removing AB has failed to show significant efficacy in AD but GSK3B inhibition with lithium was found to partially reverse tau pathology. Mimicking what we learned from torpid animal decreasing kinase activity and increasing phosphatase activity could reverse tau phosphorylation partially or even fully and stop disease progression. This could ultimately restore the natural balance between kinase and phosphatase activity and lead to normal functioning tau but will unfortunately not be able to reverse the existing damage.

1 Tau in hibernators and AD

1.1 Tau is a microtubule associated protein (MAP) found in all cell types but predominantly within axons of neurons in the central nervous system (CNS). Tau can bind and stabilize microtubules. Microtubules are involved in axonal transport and cell shape and are essential in neurons to maintain synaptic integrity. Tau's ability to bind and stabilize microtubules is decreased by its phosphorylation. Normally phosphorylation is tightly regulated by maintaining the balance between phosphorylation and dephosphorylation (1).

1.2 Abnormal hyperphosphorylation of tau is one of the hallmarks of Alzheimer's disease (AD) neurons in witch tau detaches from microtubules and forms intracellular aggregates called neurofibrillary tangles (NFT's) (2)(3). Hyperphosphorylation of tau is also observed in neurons of hibernating animals during torpor. Although there are several similarities in tau hyperphosphorylation between AD and torpid animals there are also differences. Importantly, phosphorylation of tau is reversed during arousal (fig.1) and there seems to be no lasting neuronal damage afterwards (4)(5).

1.3 The questions dealt with are: How do the mechanisms leading to phosphorylation of tau differ between AD and torpid animals? Why does tau phosphorylation cause neuronal damage in AD but not in torpid animals and can we reverse phosphorylated tau in AD patients to decrease neuronal damage.

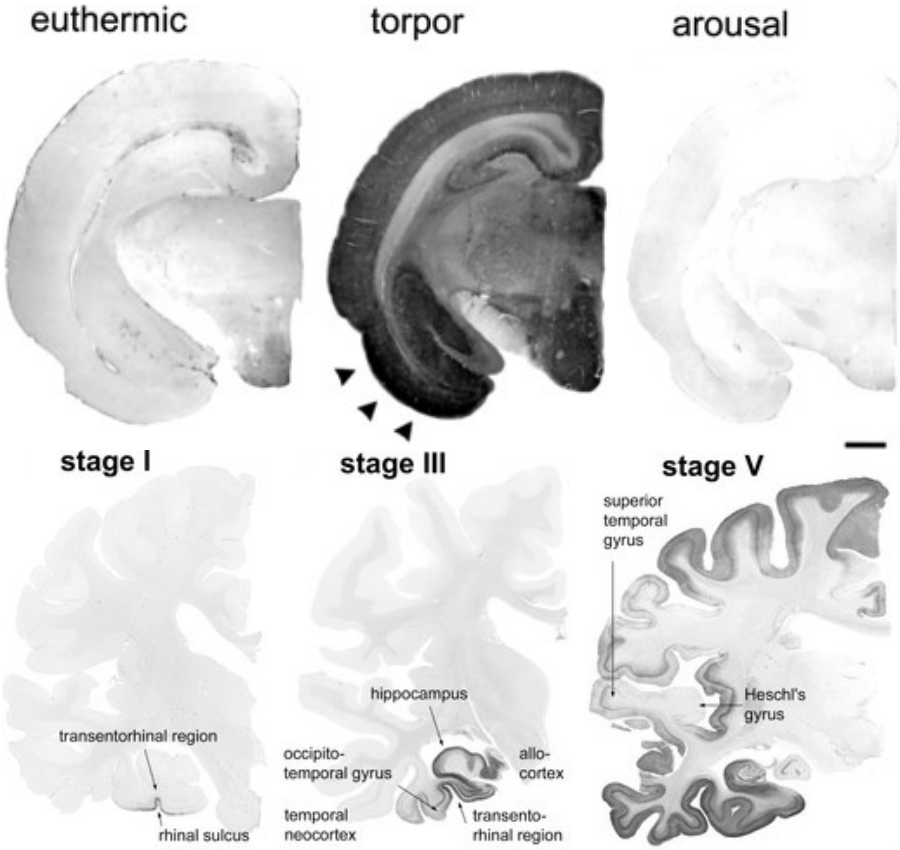

Figure 1: Staining of brain sections with antibody AT8 for Ser-202/Thr-205 found in PHF's. Upper panel: European ground squirrels during different moments of the hibernation cycle (5)*. Strong reactivity is seen around the entorhinal cortex (arrowhead). Lower panel: brain section from AD patients. The progression of Braak stages I-V: from transentorhinal region (I) to*

entorhinal region and hippocampus (III) and eventually to the frontal, parietal, and occipital neocortex (V) (3).

2 Tau function and phosphorylation

2.1 Human CNS-tau has 6 different isoform transcribed from a single gene, which are thus formed by alternative mRNA splicing. The longest isoform is 441 amino acids long of which approximately 45 can be found to be phosphorylated *in vivo* (6). Tau consists of an N-terminus, important in the association of tau with cell membranes, spanning residue 1 till 44. Residue 45 till 103 consists of 1 or 2 inclusions depending on the isoforms. A proline-rich domain (PRD) spans residue 151 till 243. Three or 4 (depending on the isoform) microtubule binding domains (MBD) span residues 244 till 368 in the longest human isoform and tau ends with a c-terminal region spanning residue 369 till 411(7)(fig.2).

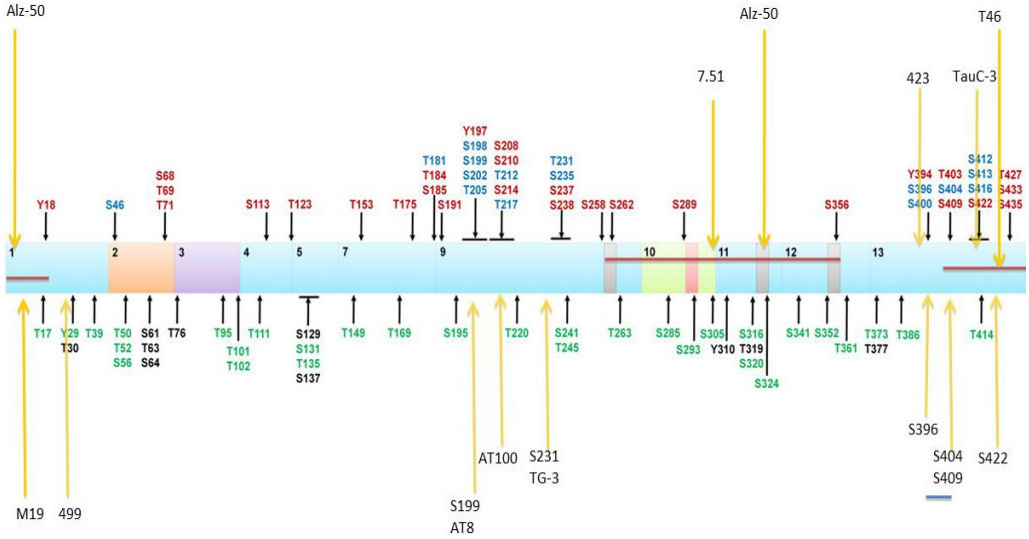

Figure 2: Phosphorylation sites found on the longest human tau splice variant (Tau40). Sites in red are phosphorylation sites found exclusively in AD patients. Phosphorylation sites in blue are found in control and AD patients. Sites in green and black are not phosphorylated in control and AD patients. Yellow arrows show possible epitopes for antibody staining. The numbers from left (N-terminus) to right (C-terminus) depict different regions of tau: 2 and 3 are inclusions; 10-13 are MBD's (8).

2.2 Tau has an essential role in neurons. It can bind tubulin, the smaller subunits of microtubules resulting in increased stability of the microtubules. In healthy mature neurons, practically all tau is bound to microtubules. The shape of neurons, in contrast to other cells is relatively long and its cytoskeleton needs to be flexible in order to form synapses and spines that are important for the communication between neurons. Furthermore, vesicles filled with essential materials and organelles such as mitochondria are transported to different parts of the neuron via the network of microtubules. Microtubules are also involved in the release of neurotransmitters.

2.3 Phosphorylation, the process of adding phosphate to another protein is catalyzed by kinases. Removal of phosphate, dephosphorylation, is catalyzed by phosphatases. Kinase and phosphatase activity are regulated. Extracellular signals can activate or inhibit them by activating intracellular cascades ultimate leading to increased or decreased protein phosphorylation. In healthy neurons phosphorylation is part of the way that the proteins are regulated. Tau can be phosphorylated at many residues (fig.2). Phosphorylation of tau decreases its binding to tubulin and by increasing phosphorylation of tau, microtubules become increasingly instable (1). Phosphorylation at different sites of tau can alter tau in different ways.

3 Regulation of normal tau phosphorylation

3.1 In healthy neurons, multiple kinases such as glucose synthase kinase 3B (GSK3B) (9), cycline dependent kinase 5 (CDK5) (10), Casein kinase 1 (CK1) (11) and protein kinase A (PKA) are present and responsible for the phosphorylation of tau (tab. 2). Although many other kinases have been found to phosphorylate tau *in vitro* their role *in vivo* remains to be elucidated.

Table 1: Sites found phosphorylated in healthy human tau. Important kinases able to phosphorylate the sites in vitro are shown. These sites are also phosphorylated in AD tau. No sites in the MBD's are found phosphorylated (http://cnr.iop.kcl.ac.uk/hangerlab/tautable).

Residue #	GSK-3	cdk 5	CK 1	PK A	MAP K	AMP K
S46	X		X		X	
T181	X	X			X	
S198	X		X	X		
S199	X	X		X	X	
S202	X	X		X	X	
T205	X	X		X	X	
T212	X	X	X	X	X	
T217	X			X	X	
T231	X	X		X	X	X
S235	X	X		X	X	X
S396	X	X	X		X	
S400	X					X
S404	X	X	X		X	
S412			X	X		
S413	X		X	X		
T414			X			
S416			X	X		

3.2 GSK3B is one of the major kinases found in neurons and is able to phosphorylate most of the serine and threonine residues in the proline rich and the c-terminal region (9) (table 1). GSK3B's kinase activity is increased when it is phosphorylated at tyr-216 and decreased when phosphorylated at ser-9. Activated GSK3B also phosphorylates glycogen synthase (GS) which is responsible for the formation of glycogen, the polymerized form of glucose. When GS is phosphorylated by GKS3B it stops glycogen production. GSK3B activity is negatively modulated by insulin and Wnt signaling (12). GSK3B is furthermore involved in many cell responses such as apoptosis, immune processes and migration. Substrates normally need to be primed by other kinases before they can be phosphorylated by GSK3B with the exception of specific residues e.g. ser-202.

3.3 Another kinase found in neurons is CDK5. CDK5 is normally involved in development, neuronal migration and plasticity of neurons (13). CDK5 is regulated by its activators, p35 and p39, which are expressed in mature, post mitotic neurons. CDK5's phosphorylation sites on tau overlap with those of GSK3B. In rat brain cortex CDK5 and GSK3B are found to form a complex is the presence of tau (14). CDK5 is important in priming tau for subsequent GSK3B phosphorylation. For instance, ser-400 phosphorylation and subsequent phosphorylation on ser-396 by GSK3B is preceded by ser-404 phosphorylation by CDK5. Likewise thr-231 phosphorylation by GSK3 requires tau to be primed on ser-235 by CDK5 (2)(14). While such relationships have been found *in vitro* it has been suggested that *in vivo*, p35 mediated CDK5 is activation is not involved in the phosphorylation of tau (15).

3.4 PKA is involved in the direct phosphorylation of several sites of tau but also in inhibition of subsequent phosphorylation of tau by GSK3B. Sequential phosphorylation of ser-199, ser-202 and thr-205 prime tau for thr-214 phosphorylation by PKA, blocking phosphorylation of thr-212 by GSK3 (16). CK1 can also phosphorylated tau on several residues *in vitro* (17).CK1 is normally involved in cell division, circadian rhythmicity and synaptic transmission (11). CK1 prefers substrates that are already phosphorylated and might therefore be important in the progression of normal phosphorylated to hyperphosphorylated tau. More kinases are present in healthy neurons although their contribution to normal tau phosphorylation is not well known. Mitogen activated protein kinases (MAPKs) such as extracellular signal-regulated kinases 1/2 (ERK1/2) are involved in the normal proliferation of neurons and their reaction to stress. 5'AMP activated protein kinase (AMPK)is involved in cellular energy homeostasis. When energy is low AMPK is activated and stimulates catabolic pathways while inhibiting anabolic pathways. Both are able to phosphorylate several tau residues *in vitro* but are not well studied *in vivo*. Several phosphatases are responsible for dephosphorylating tau of with protein phosphatase 2a (PP2A) is the most important in human neurons. PP2A is responsible for 71% of the dephosphorylation, PP1 for 11%, PP5 for 10% and PP2B for 7% (18).

4 Tau in Alzheimer's disease

4.1 AD is a progressive disease that causes synaptic degeneration (19). The disease is still not completely understood and to date medication can only slow disease progression but existing lesions are irreversible. NFT's and senile plaques (SP) are primary disease hallmarks seen in the dystrophic neurons of AD patients. AD can be divided into early onset familial (EOF) AD and late onset (LO) AD. LOAD is by far the most common form of AD accounting for 95% of the cases. EOFAD is characterized by its onset before the age of 65 and is linked to autosomal inheritance of genes involved in the production and processing of a protein called amyloid beta (AB). LOAD is mostly seen in elderly people above the age of 65 and is not characterized by these genes but displays the same disease hallmarks.

4.2 One hypothesis for the development of AD that has long been accepted is the amyloid cascade theory, which states that AD is caused by AB deposited extracellular into SP (20)(21). SP consist of toxic AB oligomers that are excreted to the extracellular space because neurons are unable to process and degrade them. The SP's accumulate extracellular and cause inflammation and stress to the neurons eventually causing the hyperphosphorylation that leads to tau dysfunction. Many people have challenged this hypothesis mainly because the distribution of SP's does not correlate with disease progression or severity and clinical trials focusses on removing AB show little efficacy (17)(22)(23). Therefore, people are currently focusing on hyperphosphorylated tau as treatment target in AD.

4.3 There is a clear hierarchy in the progression of AD (3)(fig. 1). The entorhinal cortex (EC) is the first region affected. The EC is involved in the formation of memory which requires the formation of synapses and spines and changes in the cytoskeleton. Brain region that normally have a high degree of neuroplastic potential seem to be more vulnerable to tau hyperphosphorylation and show most early and most severe degeneration in AD (24). Tau is also highly phosphorylated during neuronal synaptogenesis in early human neuronal development (25). Tau pathology has been found to be transmitted synaptically (26) when extracellular PHF's are taken up by cells (27). AD is also associated with a chronic inflammatory response in neurons. A correlation between NFT density and microglial activation has been shown (28). AB deposited into SP's possibly induces this microglial activation (29). MRI studies show that increased tau phosphorylation correlates with disease progression and deficiency of glucose metabolism in AD affected brain areas (30).

4.4 AD tau is phosphorylated on additional residues compared to normal brain tau (table 2). Phosphorylation at several of these residues in the MDB has been associated with decreased microtubule binding. Ser-262 and Ser-356 have been implied in modifying tau in this manner (31). Other phosphorylated residues such as Ser-214 and Thr-231 flanking the MBD have also been found to decrease microtubule binding while others such as ser-217 and ser-235 do not. (32)(33)(34)(34). Ser-214, ser-262 and ser-356 are the last residue of KXGS motifs that can be recognized by certain kinases. Phosphorylation can furthermore disrupt tau's ability to associate with plasma membranes and give tau the ability to aggregate into paired helical filaments (PHF) thereby gaining a toxic function (1). It has been suggested that phosphorylation of tau is a protective mechanism during AD by removing toxic tau species from the cytosol or protecting neurons from AB induced apoptosis (35)(8). In contrast, tau phosphorylation within the MBD has been shown to decrease its aggregation into PHF's (34).

4.5 GSK3B can directly phosphorylate several sites found exclusively in PHF tau such as Ser-214 and Ser-262 (32). GSK3B has been reported to become better at phosphorylating some sites after they have been primed by other kinases such as CDK5 (36). In AD tau phosphorylation by GSK3B is increased through interference of AB with insulin and Wnt pathways. CDK5 can modulate GSK3B activity (37) but can also phosphorylate several sites directly. Because of exposure to AB CDK5 activator p35 is cleaved to p25 with is degraded more slowly causing a more sustained activation. Elevated p25 has been found to be strongly correlated with AD (38).

4.6 CK1 has also been implicated in AD (6). Ser-113. Ser-238 and Ser-433 found in PHF tau are exclusively phosphorylated by CK1. Furthermore CK1 expression has been reported to be increased in AD brain (39). In the hippocampus some isoforms have been found to be elevated greater than 30-fold (40). CK1 is also co-localized with tangles (41).

Table 2: Sites found phosphorylated exclusively in AD tau. Important kinases able to phosphorylate the sites in vitro are listed. In contrast to normal tau, several sites in the MBD's (bold) are found phosphorylated (http://cnr.iop.kcl.ac.uk/hangerlab/tautable).

Residue #	GSK-3	CK1	AMPK	PSK1	MARK	PKA	MAPK	cdk5
Y18								
S68								
T69	X						X	
T71			X					
S113		X						
T123				X				
T153	X						X	X
T175	X						X	
S184	X	X		X				
S185				X				
S191				X				
Y197								
S208		X	X					
S210	X	X				X		
S214	X	X	X	X		X		X
S237	X	X		X				
S238		X						
S258	**X**	**X**	**X**	**X**		**X**		
S262	**X**	**X**	**X**	**X**	**X**	**X**		
S289	**X**	**X**	**X**	**X**				
S356	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Y394								
T403			X	X				
S409	X			X		X		
S422				X		X	X	
T427				X				
S433		X		X				
S435		X		X		X		

4.7 Prostate-derived sterile 20-like kinase 1 (PSK1) also known as thousand and one amino acid kinase 2 (TAOK2) also phosphorylates many residues found in PHF's. PSK1 is involved in the formation of the cytoskeleton and its reaction to stress. PSK1/TAOK2 has been found to be activated in NFT bearing neurons and to phosphorylate 41 tau sites of which 28 are found in PHF's (42).

4.8 Microtubule affinity regulating kinase (MARK) and PKA are also known to phosphorylate the KXGS motifs ser-214, ser-262, and ser-356 on tau (34). GKS3B phosphorylation of ser-212 is needed before phosphorylation at ser-214 by PKA in order to leads to the PHF epitope found in AD (16). β2-adrenergic receptor (β2AR) activation has also been implicated in AB mediated tau phosphorylation through the signaling pathways of PKA and c-jun terminal kinase (JNK) which phosphorylates tau at ser-214 and der-262 and thr-181 (2). Tyrosine residues can exclusively be phosphorylated by different tyrosine kinases (tyr-18 by Fyn and Syk, tyr-197 by Met and tyr-394 by c-Abl) and their involvement is not very well understood.

4.9 Not only increased kinase activity by also decreased PP2A activity has been found in AD. Both the activity and the expression of PP2A were found to be decreased in AD brain (18). On the contrary, in postmortem AD brain tissue a three-fold increase in PP2B activity was found compared to age-matched control brain tissue, but it did not counteract the increased tau phosphorylation found in AD (43).

4.10 Together, GSK3b, CK1 and PSK1/TAOK2 can phosphorylated all residues found on PHF tau although it remains to be proven if they are responsible *in vivo*. It is furthermore known that tau phosphorylation happens in a hierarchical and progressive manner with one kinase preceding another, which causes tau to become progressively phosphorylated. Transient activation of some kinases could prime tau for more sustained phosphorylation by others. Also many residues can be phosphorylated by many different kinases and the contribution of each kinase in respect to a specific residue remains to be elucidated. The effects that some kinases have on proteins other than tau and the interactions of kinases with each other make the process of tau phosphorylation even more complicated.

5 Tau in hibernation

5.1 During torpor tau is phosphorylated to a high extent and microtubules disintegrate but aggregates are not formed (5). Neurons become inactive and brain activity is brought to a minimum as neuronal endings retreat and spine density decreases (44). Tau hyperphosphorylation can also be induced by head trauma (45), starvation (46), hypothermia (47) and anesthesia (48). All of these situation cause stress to neurons in some degree. In these situations however phosphorylation of tau is reversed over time when the stress factors are removed. Hibernating animals alternate between active (aroused) and inactive (torpid) states. During aroused periods blood flow and body temperature are increased to normal. During this period of arousal tau is dephosphorylated within a short timeframe (5). Neuronal activity is increased while neuronal connections are strengthened and spines reappear (49)(50).

5.2 Increased PKA and GSK3B activity and decreased PP2A activity play key roles in tau phosphorylation during torpor. The activity of phosphatases and kinases change at temperatures reached during the immergence and emergence from torpor. In arctic ground squirrels kinetic studies found a 2.4-fold increased PKA activity at 4°C compared to 37°C and a 4-fold increase in GSK3B activity at 20°C compared to 30°C (fig. 4) (47). PKA could be involved in the direct phosphorylation of several sites but also in priming tau for GSK3B phosphorylation.
GSK3B is involved in altering metabolic flux (51) and in the regulation of protein translation through eukaryotic elongation factor-2 (eEF-2) during starvation. Together with CK1 and PP2A this system could be potentially neuroprotective during minimal energy availability. Furthermore PP2A activity was found to be decreased 5-fold at 5°C compared to 37°C (47). CDK5 is thought not to play a major role in phosphorylation during torpor. In summary hyperphosphorylation during torpor is likely caused by temperature dependent activity increase of PKA and GSK3B and simultaneous temperature dependent PP2A activity decrease.

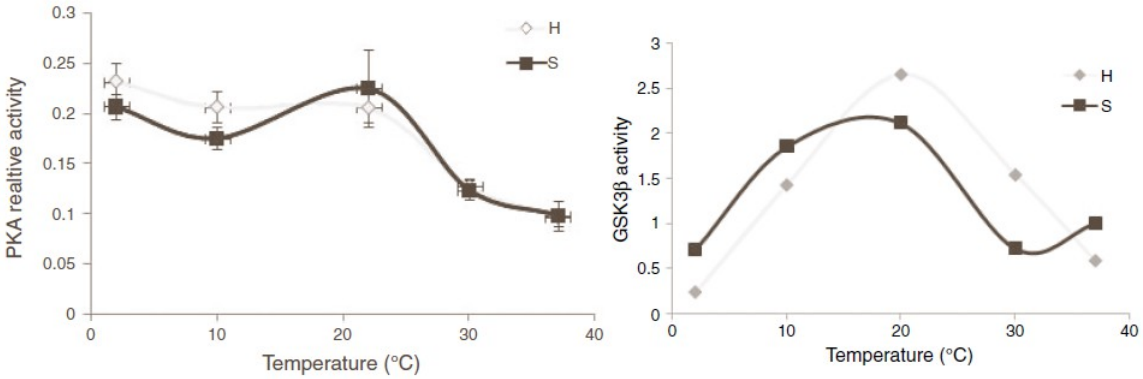

Figure 4: The relative kinetic activity of PKA and GSK3B at different temperatures. Brain homogenates of summer active (S) of topic (T) arctic ground squirrels were used. The activity is highest around 20°C in both kinases (47).

5.3 Ser-262, ser-199 and ser-404 become dephosphorylated in aroused arctic ground squirrels. These sites are all likely to play a role in tau's ability to bind microtubules. Phosphorylation of ser-205 and ser-214 is not reversed and are thought not to be crucial in tau's microtubule interaction but might be involved in protecting tau from degradation. Tau phosphorylation by PSK1/TAOK2 during hibernation has not been tested but. Like GSK3B, PKA and CK1 it can phosphorylate all sites relevant for tau's ability to bind microtubules *in vitro* including thr-231 which is not found in PHF tau but together with ser-262 can cause maximal inhibition tau's interaction with microtubules (33).

Table 3: Sites found phosphorylated in hibernation tau. Important kinases found to phosphorylate tau in vitro are shown. AD tau and hibernation tau have thr-214 outside the MDB and ser-262 and ser-356 in common (bold).

Residue #	(GSK-3)	(cdk 5)	(PKA)	(CK 1)	(PSK1/TAOK2)
T181	X	X			
S198	X		X	X	X
S199	X	X	X		
S202	X	X	X		
T205	X	X	X		X
T212	X	X	X	X	X
S214	**X**	**X**	**X**	**X**	**X**
T231	X	X	X		X
S262	**X**		**X**	**X**	**X**
S356	**X**		**X**	**X**	**X**
S404	X	X		X	

5.4 Comparison of the DNA sequence of human and rodent tau show several differences: In Syrian hamsters (*M. auratus*) 37 amino acids are substituted, 11 are deleted and 2 are inserted giving human tau 10 extra phosphorylatable residues compared to *M. auratus*. 35 of the substitutions were found in the N-terminal half (fig. 3). Many of these sites could be phosphorylated by CK1. One of these, ser-46, was found to have a significant decreasing effect on tau's ability to interact with cell membranes (52). Ser-46 phosphorylation causes mislocalization of tau into the cytoplasm in humans but hibernators are largely protected since ser-46 cannot be phosphorylated because a glutamic acid in humans has been substituted for an alanine in torpid animals (fig. 5)

Figure 5: Amino acid sequence of tau from rodents and human. Boxes indicate sequence similarities only between the hibernators M.auratus (Syrian hamster) and S. citelus (European ground squirrel).

6 Similarities and differences between tau in torpor and AD

6.1 Although phosphorylation of tau is not exactly the same during torpor and AD, specific residues are similar. Ser-214, ser-262 and ser-356 are phosphorylated in AD and during torpor but not in healthy neurons. These residues are known to affect microtubule binding negatively and indeed decreased binding of tau to microtubules is observed both during torpor and AD. A decrease in synaptic connectivity primarily in the most neuroplastic areas is observed in both cases. In torpid animals cholinergic neurons show frequent tau hyperphosphorylation whereas GABAergic neurons show almost none (53). Selective loss of cholinergic forebrain neurons is also an early event in AD related to hyperphosphorylated tau (54). Ser-202 and Ser-205 are responsible for the formation of the AT8 epitope that is found in hibernators. Although these sites are also found phosphorylated in healthy controls they are highly phosphorylated in PHF's of AD patients and during torpor. Ser-205 is known to prime tau for ser-214 phosphorylation by PKA exclusively seen during torpor and AD. Ser-212 and ser-214 are both phosphorylated during torpor and during AD forming the PHF-1 epitope. This implies a sequential involvement of GSK3B and PKA both during torpor and AD. Indeed a temperature dependent increase in GSK3B and PKA activity is observed at low temperatures only reached during immergence and emergence of torpor. Increased GSK3B activity is more susceptible to temperature decrease in early phases while PKA activity increase is more sustained (fig. 4). In AD, increased CDK5 activity plays a more prominent role than in torpor. This is possibly due to AB related p25 formation in AD, causing a more sustained CDK5 activation. During torpor no link between tau phosphorylation and p25 formation has been found (55). CK1 is found to exclusively phosphorylate several residues found during AD but none of these sites have been reported phosphorylated during torpor nor has increased CK1 activity been described. GSK3B can phosphorylate all residues found during torpor (table 3). In AD however, GSK3B, CK1 and PSK1 cover most sites. Also in AB tau different tyrosine residues are found phosphorylated while none are found in tau of torpid animals. So while increased GSK3B and PKA activity are similarities, increased CDK5, CK1 and PSK1 activity are not.

6.2 While during torpor the total amount of tau stays the same, in AD the amount of tau strongly increases over time as it is deposited into NFT's. Furthermore, increased tau phosphorylation in humans causes tau to accumulate in the cell body while hibernators seem more resistant, possibly due to the loss of ser-46 as a CK1 phosphorylation site. When in AD hyperphosphorylated tau can eventually no longer be cleared, the neurons die. During arousals tau is dephosphorylated well before NFT's start to form and neurons stay healthy. Arousals are necessary for the survival of neurons during hibernation. It is likely that increased body temperature causes the reversal of tau phosphorylation by the respective temperature dependent decrease in kinase activity and increase in phosphatase activity. This clears hyperphosphorylated tau and strengthens neuronal connections that would otherwise be lost. This is in line with findings that animals sleep most of the time during arousal (49). Sleep has been linked to the strengthening of neuronal connections. During torpor animals become increasingly deprived of sleep as normal sleeping brain activity halts. In contrast to small hibernators, such as arctic ground squirrels that show no tau pathology after arousal aged, American black bears have been reported to show AD-like tangle and SP formation in their cortex (56)(57) possibly showing a link between tangle formation and repeated long term phosphorylation of tau.

6.3 The maintenance of a proper phosphorylation balance in neurons costs a lot of energy (58). Furthermore the elongation of microtubules is done by the hydrolysis of GTP to GDP (59) which makes it very costly in energy. Energy supply and demand seems to be mismatched during torpor and in AD patients although there is a clear distinction. In AD hypometabolism or other stress factors like toxic AB cause the regulation of tau phosphorylation to be out of balance. Deposition of hyperphosphorylated tau into filaments might be an early defense to clear toxic phosphorylated tau and to try and maintain microtubule integrity but the mismatch between energy supply and demand remains. During hibernation decreased brain temperature and blood flow can also be seen as temporary stress factors for neurons. Decreased blood flow causes neurons of hibernators to be temporarily deprived of nutrients and decreased temperature affects proteins and enzyme properties. During torpor energy demand is decreased significantly to endure the period of low energy supply and tau phosphorylation can be seen as an adaptive process that helps decrease metabolic spending and protect neurons from damage.

6.4 Mouse models in which GSK3B is overexpressed show tau hyperphosphorylation, neuronal death and spatial learning deficits. Lithium, a GSK3B inhibitor is being used as a treatment strategy for AD. It was found to partially stop and reverse tau pathology but not remove NFT's that are already present (60). The gray mouse lemur (*M. murinus*) might be another useful primate model for human AD. Around 20% of the aged lemurs show neurodegeneration similar to humans displaying NFT's, SP and loss of cholinergic neurons but also loss of cognitive and social capabilities (61). *M. murinus* is known to occasionally undergo seasonal torpor. To date no studies have been done to see if torpor relieves the tau pathologies in *M. murinus*.

7 Conclusion

7.1 Although there seems to be a clear difference between the mechanisms leading to tau phosphorylation they show striking similarities like hypometabolism and increased GSK3B activation. A major difference is that the hypometabolism is transient during torpor but sustained during AD. AD always has AB as a hallmark of the disease and in EOFAD genetic predisposition in AB related genes plays a role. Although SP deposition does not correlate with disease progression and severity it is likely to be one of the causal factors in the disease. AB is known to interfere with insulin and wnt pathways and to increase GSK3 activity but clinical trials focused on removing AB have failed to show significant efficacy. The other hallmark of AD, hyperphosphorylated tau, is deposited into NFT's that correlates well with disease progression. Furthermore other diseases that show malfunctioning tau, and are therefore termed taopathies, such as progressive supranuclear palsy (PSP) and frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17), show clinical signs of cognitive impairment and dementia without signs of AB deposition. Tau is likely to be a downstream target for AB mediated neurodegeneration and although removing phosphorylated tau does not address the causes directly it might be of benefit to AD patients by slowing the disease progression as much as possible. In AD tau detaches from microtubule and form PHF's. NFT's consisting of multiple PHF's cause the internal compartments to be clogged and the cytoskeleton functions to become impaired. Eventually the neurons die from the progressive increase in AB buildup and related tau phosphorylation. After neurons die, the damage is irreversible.

7.2 The neurons of torpid animals do not form toxic AB and therefore do not have permanent, progressively increasing stress. The transient stress on neurons during torpor leads to increased tau phosphorylation and is likely caused by decreased blood flow and decreased body temperature. Neurons containing high amounts of phosphorylated tau were found to be protected against apoptosis (62) and many neuronal connections are lost during torpor. Phosphorylation of tau can therefore be seen as an adaptive process of pruning away connections that are not needed during hibernation or protecting the cells from apoptosis during times of low temperatures and energy availability (5) Arousal from torpor causes dephosphorylation by temporary reestablishment of blood flow and temperature. It does so before irreversible damage occurs.

7.3 Since clinical trials focused on removing AB have failed to show significant efficacy focusing on phosphorylated tau seems the other logical option. Since cytosolic hyperphosphorylated tau disrupts cytoskeleton dynamics, microtubule stabilizing agents would be one possible treatment strategy for AD. Phosphorylated tau is also open to several other possible treatments such as inhibition of tau kinases or activation of phosphatases, inhibition of tau aggregation, inhibition of tau propagation, inhibition of tau expression and immunization against tau (22). Removing phosphorylated tau by has been shown to be a promising treatment strategy. Possibly these treatment strategies could reverse tau phosphorylation partially or even fully and should stop disease progression but will not be able to reverse the damage already done.

7.4 Mimicking what we learned from torpid animals we might be able to relieve AD patients. Although reversible phosphorylation as seen during torpor does not address AB it does attenuate energy necessity of neurons. One possible treatment strategy would be supplying AD patients with alternative energy sources such as ketones that are independent of insulin signaling. Indeed a pilot study supplementing coconut oil to AB treated cortical neuron cultures showed improved survival although its relation to tau pathology was not assessed (63). Transgenic mice fed a ketone rich diet displayed decreased tau phosphorylation and deposition in the hippocampus amygdala and cortex and ameliorated memory and learning impairments (64). A strategy that wants to be effective in relieving the neurons of AD patients from stress should remove AB, restore neuronal metabolism and remove excess cytosolic tau. This would restore the natural balance between kinase and phosphatase activity and lead to normal functioning tau.

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