Neuronal plasticity in hibernation and the proposed role of the microtubule-associated protein tau as a “master switch” regulating synaptic gain in neuronal networks

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Arendt T, Bullmann T. Neuronal plasticity in hibernation and the proposed role of the microtubule-associated protein tau as a “master switch” regulating synaptic gain in neuronal networks. Am J Physiol Regul Integr Comp Physiol 305: R478 –R489, 2013. First published July 13, 2013; doi:10.1152/ajpregu.00117.2013.—The present paper provides an overview of adaptive changes in brain structure and learning abilities during hibernation as a behavioral strategy used by several mammalian species to minimize energy expenditure under current or anticipated inhospitable environmental conditions. One cellular mechanism that contributes to the regulated suppression of metabolism and thermogenesis during hibernation is reversible phosphorylation of enzymes and proteins, which limits rates of flux through metabolic pathways. Reversible phosphorylation during hibernation also affects synaptic membrane proteins, a process known to be involved in synaptic plasticity. This mechanism of reversible protein phosphorylation also affects the microtubule-associated protein tau, thereby generating a condition that in the adult human brain is associated with aggregation of tau protein to paired helical filaments (PHFs), as observed in Alzheimer’s disease. Here, we put forward the concept that phosphorylation of tau is a neuroprotective mechanism to escape NMDA-mediated hyperexcitability of neurons that would otherwise occur during slow gradual cooling of the brain. Phosphorylation of tau and its subsequent targeting to subsynaptic sites might, thus, work as a kind of “master switch,” regulating NMDA receptor-mediated synaptic gain in a wide array of neuronal networks, thereby enabling entry into torpor. If this condition lasts too long, however, it may eventually turn into a pathological trigger, driving a cascade of events leading to neurodegeneration, as in Alzheimer’s disease or other “tauopathies”.

Alzheimer’s disease; neurodegeneration; neuroprotection; neuronal plasticity; protein phosphorylation; tauopathies

Seasonal Brain Plasticity Might Be a Widespread Phenomenon

Animals living in environments with daily or seasonal rhythms have developed different strategies to coordinate endogenous processes with ambient conditions. To optimize their energy balance under conditions of restricted availability of food and increased costs for temperature regulation, animals can either reduce the expense for foraging or the need of foraging (96). The expense for foraging can be reduced by food storing or escaping a cold climate by migration. Alternatively, hibernation is a powerful strategy to reduce the metabolic costs of daily activity. All of these aforementioned strategies to optimize energy expenditure are associated with plastic adaptive changes both at the level of behavior and brain structure.

The seasonal modulation of food-storing behavior (33, 177, 178, 182) or migration (17) in birds has been shown to require special spatial learning abilities, which are paralleled by seasonal changes in neuronal structure. These cyclic changes in size and morphology of brain circuitry in the bird’s brain represent one of the leading models of seasonal plasticity (for review, see Ref. 201) and might involve both structural neuronal plasticity and neurogenesis (10). It is most pronounced in the hippocampus, which is in direct agreement with the concept of the hippocampus to create mental representation of space (139, 142). Seasonal plasticity in learning ability and brain structure, however, may well be a more general phenomenon with a wide array of phenotypic manifestations that also occurs in mammals, including humans (101; for review, see Ref. 81), where it might even reach clinical significance (141).

Under natural conditions, cranial volume and brain weight show large seasonal fluctuations in several rodents and insec-tivores with larger brains in the summer compared with winter months (44, 90–93, 124, 155, 156). Most pronounced relative changes between seasons were found in the hippocampus (59, 92, 93, 147), clearly showing that brain structure is phenotypically flexible in response to seasonal changes in environment. In seasonally changing environments, photoperiod length is a good predictor to anticipate availability of food and costs for temperature regulation (12, 42). Accordingly, exposure to...
increased photoperiod produces significant increases in brain weight in small mammals (40, 41), while short photoperiods impair spatial learning, produce decreases in brain weight and hippocampal volume, and alter hippocampal dendritic complexity (157, 216).

In the present paper, we will focus on adaptive changes in learning abilities and brain structure during hibernation as a behavioral strategy used by several mammalian species to minimize energy expenditure under current or anticipated inhospitable environmental conditions. Endotherm animals usually maintain a constant body temperature by thermoregulation, which may require energetically expensive metabolic heat production in a cold environment. Some animals are able to restrict energy expenditure by temporarily readjusting the set point for the body temperature from around 37°C to much lower values. This state of torpor is marked by decreased overall metabolic rate and a reduced heart and respiratory rate (61, 62, 207), by tolerating a body temperature near the ambient temperature in a regulated hypothermic state. Hibernation is found in a variety of species in several different orders (75). This behavior is not restricted to specific geographical areas, and it is not a feature of a particular evolutionary stage of development. Rather, it can be observed in rodents inhabiting the Arctic, where core body temperature can decrease to below the freezing point (11) and in primates living in tropical regions (43) (for a recent review, including a history of terms, see Ref. 214).

Neuronal Plasticity During Hibernation

As the mammalian brain, relative to other tissues, is particularly energetically expensive (128), it represents a large resource to effectively balance the energetic budget during periods of limited availability of food and receptive breeding partners. So, in an environment that requires no processing of new information, modest reductions in brain activity and volume might be an adaptation that saves considerable energy (96). By far, most of the energy budget of the mammalian brain, which under normothermic conditions is remarkably constant over time (158), is needed for maintaining cellular integrity and neuronal resting potential (46). Accordingly, the low metabolic rate during torpor is accompanied by dramatically reduced neuronal activity (28, 71, 78, 94, 100, 106, 112, 120). EEG measurements of torpid hibernators have shown that almost no brain activity is present (39, 106, 206).

As neuronal activity is a measure of use and neuronal connections remain functional through regular use, this decrease may negatively affect the maintenance of neuronal connections (97). Evidence for a reduced neuronal connectivity during the hibernation cycle has been provided for different brain regions in a variety of hibernating animals (89, 116–119, 153). By the pioneering work of Popov and colleagues (152, 153), which prompted a number of subsequent studies (9, 115, 168, 192, 203, 204), a cycle of synaptic regression during torpor and subsequent reinnervation in phases of arousal has nicely been shown for synaptic contacts of the CA3 pyramidal neurons and their main excitatory afferents, the mossy fiber terminals, that originate in the dentate gyrus.

Quantitative Golgi study of hippocampal pyramidal neurons of ground squirrels and European hamsters showed rapid and profound transformation of their apical dendrites in the course of hibernation. The dendrites were significantly shorter, less branched, and had fewer dendritic spines in the middle of the hibernation bout than in the active euthermic ground squirrels between bouts (115, 152). After arousal from torpor, within 2 h, dendrites completely restored their structure (152). During hibernation, remodeling of the hippocampal dendrites occurs repeatedly during each torpor-activity cycle (152). Accordingly, synapses of mossy fibers, repeatedly undergo a striking structural transformation during the hibernation cycle (153). In the middle of the hibernation bout, the giant complex of mossy fiber synapses have a reduced number of dendritic spine infoldings that are smaller and have a decreased number of postsynaptic densities compared with mossy fiber synapses of active animals. Two hours after arousal, all of these parameters of mossy fiber synapses increase and significantly exceed their levels, not only in torpid, but in active, euthermic animals between bouts of torpor. The larger postsynaptic densities and the greater proportion of perforated postsynaptic densities were found soon after arousal (153). These plastic synaptic changes during the hibernation cycle are associated with cyclic changes of synaptic vesicle density and presynaptic and postsynaptic marker proteins that dissociate from the cytoskeletal active zone and postsynaptic density, potentially creating a reservoir of proteins that can be quickly mobilized for rapid rebuilding of dendritic spines and synapses during the return to euthermia (9, 115, 192, 204). Some of these changes of spine regression and translocation of postsynaptic proteins away from the synapse can be mimicked in vitro by cooling of brain slices (30, 102, 166). Still, the in vivo effects of hypothermia on spines in the hippocampus are rather small (154) or even absent in the neocortex (218).

Cyclic changes of the CA3 pyramidal cell afferentation by mossy fibers are associated with changes in PSA-NCAM expression (9). PSA-NCAM is the developmentally regulated polysialylated (PSA) form of the neural cell adhesion molecule (NCAM), a cell surface glycoprotein that is involved in neuronal migration and neurite outgrowth (37, 172). In the adult rodent brain, expression of PSA-NCAM is restricted to brain areas that retain a high neuroplastic potential, such as the hippocampus and olfactory areas, where it might fulfill an instructive function in brain plasticity (48, 171, 172). PSA-NCAM expression is related to bouton formation and remodeling, which accompany synapse formation (171), a process of critical importance for hippocampal plasticity in learning and memory (55, 137, 138) and for reestablishing neuronal connectivity during arousal in hibernating animals (9).

These rapid, reversible, and repeated changes indicate a cyclic process of partial denervation and reinnervation of hippocampal neurons by mossy fibers in the course of the innate, stereotyped behavior. Similar regressive adaptive changes during torpor in cell body area, dendritic arbor complexity, spine density, synaptic ultrastructure, presynaptic and postsynaptic proteins and its quick regrowth to euthermic values after arousal have been observed in brain areas other than the hippocampus, such as neocortex and thalamus, suggesting a global phenomenon (168, 203, 204, 221, 222).

Hibernation Affects Learning and Memory

Cyclic changes in brain circuitry relevant to processing of information and formation and consolidation of memory might
affect mechanisms of learning and memory during hibernation. Besides the neocortex, there are several brain areas particularly involved in learning and memory, e.g., the hippocampus for spatial and declarative learning, amygdala for fear conditioning, and the olfactory system and hypothalamus in social recognition. However, in any case, learning is followed by consolidation of memories, a process that can be further subdivided, according to the brain regions involved over time. Following a spatial learning task for instance, recent memories are formed by synaptic consolidation, which involves synaptic activation, second messenger signaling, activation of transcription factors, synthesis of new proteins, and structural changes. During the first minutes to hours after learning, these memories are prone to treatments that temporarily impair these processes but also to behavioral manipulation, e.g., retroactive interference. Remote memories are no longer afflicted by these treatments, indicating that they rely on other mechanisms. Indeed, during the first days to weeks, memories require an intact hippocampus, whereas, later they spread to other regions of the brain and become hippocampus-independent. This slow progress of reorganization integrates remote memories without interference on older memories. Furthermore, this establishment is accompanied with a decay of these memory traces in the hippocampus, which provides resources for the acquisition of new memories. Diminished neuronal activity during hypothermia or anesthesia and additional synaptic regressive changes during hibernation might affect different aspects related to the spatiotemporal pattern of memory traces. The effects of hibernation, hypothermia, and anesthesia on learning and memory have extensively been tested in different species, employing a variety of paradigms (see Table 1 and table footnote).

Table 1. Effects of hibernation and hypothermia on behavior

<table>
<thead>
<tr>
<th>Species</th>
<th>Hypothermia</th>
<th>Behavioral Test</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hibernating animals</strong>&lt;br&gt;Greater mouse-eared bat&lt;br&gt;(Myotis myotis)&lt;br&gt;Arctic ground squirrels&lt;br&gt;(Spermophilus parryii)&lt;br&gt;European ground squirrel&lt;br&gt;(Spermophilus citellus)&lt;br&gt;Golden mantled ground squirrel&lt;br&gt;(Spermophilus lateralis)&lt;br&gt;European ground squirrel&lt;br&gt;(Spermophilus citellus)&lt;br&gt;Alpine marmots&lt;br&gt;(Marmota marmota)&lt;br&gt;Beldings ground squirrel&lt;br&gt;(Spermophilus beldingi)&lt;br&gt;Syrian hamsters&lt;br&gt;(Mesocricetus auratus)&lt;br&gt;Nembutal&lt;br&gt;<strong>Nonhibernating animals</strong>&lt;br&gt;Laboratory mice&lt;br&gt;(Mus musculus)&lt;br&gt;Laboratory mice&lt;br&gt;(Mus musculus)&lt;br&gt;Laboratory mice&lt;br&gt;(Mus musculus)&lt;br&gt;Laboratory rat&lt;br&gt;(Rattus rattus)&lt;br&gt;Laboratory rat&lt;br&gt;(Rattus rattus)&lt;br&gt;Laboratory rat&lt;br&gt;(Rattus rattus)&lt;br&gt;Laboratory rat&lt;br&gt;(Rattus rattus)</td>
<td>hibernation</td>
<td>spatial (T-maze, food reward)</td>
<td>no effect on retention</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>hibernation</td>
<td>contextual fear conditioning (foot shock chamber) before and after hibernation</td>
<td>no effect on retention but better acquisition after arousal from torpor</td>
<td>212</td>
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<tr>
<td></td>
<td>hibernation</td>
<td>spatial (Hebb Williams maze, food reward)</td>
<td>no effect on retention</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>hibernation</td>
<td>spatial (water maze, escape)</td>
<td>improved retention</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>hibernation</td>
<td>a) spatial (complex maze, food rewards)&lt;br&gt;b) operant ( Skinner box, feeding machine)&lt;br&gt;c) social recognition (preference)&lt;br&gt;a) operant (jumping on box, food reward)&lt;br&gt;b) operant (walking trough tube, food reward)&lt;br&gt;c) habitat recognition (open field test for preference to familiar environment)</td>
<td>a,b,c) no effect on retention</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>hibernation</td>
<td>social recognition (odor preference)</td>
<td>impaired recognition</td>
<td>121</td>
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<tr>
<td></td>
<td>Nembutal</td>
<td>spatial (maze)</td>
<td>impairment in subsequent trial if cooling was within 1 min but not later than 5 min after each trial</td>
<td>161, 162</td>
</tr>
<tr>
<td></td>
<td>artificial</td>
<td>passive avoidance (foot shock chamber)</td>
<td>transitory impairment on acquisition by 15 min cooling; lasting effect by 30 min cooling; impaired retention after 30 min of cooling</td>
<td>13</td>
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<tr>
<td></td>
<td>artificial</td>
<td>operant ( Skinner box)</td>
<td>impairment if cooling was within 3 h after training reached criterion</td>
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<td></td>
<td>artificial</td>
<td>spatial (Morris Water maze)</td>
<td>shallow torpor preserves memories</td>
<td>140</td>
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<td></td>
<td>artificial</td>
<td>spatial (Morris Water maze)</td>
<td>no effect on retention</td>
<td>160</td>
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<tr>
<td></td>
<td>artificial</td>
<td>a) spatial (Lashley III maze as water maze)&lt;br&gt;b) spatial (Hebb Williams maze, food reward)</td>
<td>a) no effect on retention</td>
<td>4</td>
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<td></td>
<td>artificial</td>
<td>passive avoidance (foot shock chamber)</td>
<td>impairment of acquisition in deeply cooled rats; but only immediately after cooling</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>artificial</td>
<td>active avoidance (foot shock chamber)</td>
<td>impairment of retention by cooling ≤15 min after training</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>isoflurane</td>
<td>spatial (Y-maze, food shock)</td>
<td>no effect on memory impairment</td>
<td>198</td>
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</table>

Hebb Williams maze is a square arena with barriers at various positions (standardized by 159 from 74). Lashley maze consists of alleys interconnected at various positions (107). Morris water maze is a circular tank with a escape platform hidden in opaque water (132). Skinner box is an isolation box with a food dispenser operated by an lever (181). Active avoidance involves a cue preceding footshock, which evokes escape response (135). Passive avoidance involves a cue preceding a footshock, which evokes a freezing response (19).
About 50 years ago, advances in resuscitation of deeply cooled animals (5, 6, 67) allowed us to address the question of whether memory requires continuous activity and, thus, led to a series of experiments in mice (13, 184) and rats (3, 4, 136). Already, initial studies have failed to observe an impairment of remote memory, even after severe, near-freezing hypothermia (3, 4, 136), a finding that was basically confirmed and specified subsequently by more elaborate protocols. Tested by passive (13) or active (184) avoidance learning paradigms, hypothermia constantly failed to impair retention of remote memories, while it had a clear effect on recent memory. With other learning paradigms, such as a T-maze task reinforced by escape from water, as well as retention of remote memory, was found impaired by hypothermia (13). In addition, hypothermia can also induce anterograde amnesia (13), although this depends on the intensity of hypothermia, the length of time elapsed between hypothermia and learning, and the learning paradigm (4, 136). Taken together, the effects of hypothermia appear to be task-dependent with amygdala-dependent active and passive avoidance being less susceptible than hippocampus-dependent spatial learning. Furthermore, near-freezing hypothermia and subsequent resuscitation can have profound after-effects on learning abilities with similar task-dependency.

The influence of sustained neuronal activity on memory was also studied after anesthesia. Quite similar to hypothermia, anesthesia basically failed to affect remote memories, while it had clear effects on recent memory in an amygdala-dependent task (146).

Sequelae of hibernation on learning and memory might be more complex than after hypothermia as the synaptic regression in different brain circuits is caused by temperature, as well as hibernation-specific processes. It is, thus, not surprising that many of the initial behavioral studies suffer from inconsistency (Refs. 122 and 126; also, see comments in Refs. 1 and 127), and the more recent studies often have a complicated design (34, 121, 127). Perhaps one of the most convincing studies (127), performed on European ground squirrels (Spermophilus citellus), obtained clear evidence for impaired memory after hibernation in both spatial and operant tasks, while social memory remained unimpaired. This clearly shows that hibernation can deteriorate remote memory of a specific information type, whereas others are spared. Still, in another study, on greater mouse-eared bats (Myotis myotis), remote memory of a spatial task was not affected by hibernation (167). Thus, whether or not remote memory is affected during hibernation apparently depends on both the species and the learning paradigm. These differences in the type of memory impairment observed after hibernation might be related to species-specific preparedness, e.g., differences in memory consolidation, as required for specific behavior in the natural habitat.

Most instances of hibernation-related amnesia are reported for manipulations during a short interval after learning. After this short time, only engrams of selected hippocampus-dependent tasks are still vulnerable, while amygdala-dependent tasks seem to acquire resistance very quickly. This clearly indicates differences in the task-dependent speed of systemic consolidation. Amnesia due to hibernation is, thus, likely only observed for those engrams, which have long consolidation times. The preferential dependency of these engrams on the hippocampus coincides with pronounced synaptic regression in the hippocampus during hibernation. According to this hypothesis, synaptic regression should be negligible or even absent in other areas (neocortex, amygdala), although this has not been tested.

**Reversible Protein Phosphorylation and the Microtubule-Associated Protein Tau**

One cellular mechanism that contributes to the regulated suppression of metabolism and thermogenesis during hibernation is reversible phosphorylation of enzymes and other proteins, which limits rates of flux through metabolic pathways (14, 29, 80, 114, 189; for review, see Ref. 217). Reversible phosphorylation during hibernation also affects synaptic membrane proteins (175), a process known to be involved in synaptic plasticity (for review, see Ref. 205). As we observed recently in European ground squirrels (9, 186), Syrian hamsters (73, 186–188), Arctic ground squirrels, and Black bear (186–188), this mechanism of reversible protein phosphorylation also affects the microtubule-associated protein tau, a finding that was subsequently also replicated by another group (194).

Thereby, a condition is generated that in the adult human brain is associated with aggregation of tau protein into paired helical filaments (PHFs), as observed in Alzheimer’s disease and other tauopathies (20, 63, 64, 82, 83, 111, 169). During torpor phases of hibernation, tau is highly phosphorylated, containing a number of PHF-like epitopes. PHF-like phosphorylation of tau, however, appears to be well tolerated, is not associated with fibril formation, and is fully reversible after arousal. Interestingly, the formation of highly phosphorylated tau in CA3 neurons of the hippocampus was paralleled by the regression of synaptic contacts of mossy fiber terminals, suggesting a link between tau phosphorylation and synaptic plasticity in hibernation (Fig. 1).

The phosphorylation of tau leading to PHF-like epitopes is a phosphorylation of serine/threonine residues, which is regulated by several kinases, e.g., glycogen synthase kinase-3β (GSK-3β), cyclin-dependent kinase 5 (cdk5), MAPK (and its isoforms ERK1 and ERK2), and stress-activated kinases (SAPJ/JNK), as well as by phosphatases, mainly protein phosphatase 2A (8, 174). Tau phosphorylation can be induced by hypothermia, hypometabolism, or hibernation-specific regulation of enzyme kinetics. Each of these mechanisms might contribute to different degrees to the phosphorylation pattern of tau during hibernation.

Similar to hibernation, PHF-like hyperphosphorylation of tau has been observed in starving mice (219). A series of publications (219, 149–151) clearly demonstrated that starvation or anesthesia in mice at room temperature causes hypothermia. During hypothermia, enzyme activities of phosphatases and kinases are both diminished. Temperature-dependent reduction of enzyme activity, however, is more pronounced for phosphatases. Therefore, kinase activity overrides phosphatase activity, leading to a hyperphosphorylation of tau. The extent to which tau phosphorylation during torpor, might be caused by hypothermia, however, is difficult to estimate, and other mechanisms, such as hypometabolism and hibernation-specific regulation of enzyme kinetics, might contribute more directly.

Phosphorylation of tau can be influenced by hypoxia and hypometabolism. Although acute hypoxia might promote the dephosphorylation of tau in rat brain slices (110), a recent
The report indicates the contrary in mice. In vivo, acute hypoxia leads to phosphorylation of tau by the ERK pathway (51). Although this could be attributed to the hypothermia induced by hypoxia (for review, see Ref. 215), it was also present in SH-SY5Y and primary neuronal cell cultures under controlled temperature. In hibernation, this is unlikely the mechanism of tau phosphorylation because hypometabolism prevents hypoxia (128), even under conditions of greatly diminished breathing and blood flow. Consistently, the low oxygen-induced expression of the transcription factor HIF-1 (hypoxia-inducible factor 1) in hibernation is restricted to the organs involved in thermogenesis (brown adipose tissue, skeletal muscle) during arousal (131).

**Tau Phosphorylation During Hibernation Is a Regulated Process and Is Essentially Different From Effects Induced by Starvation, Energy Deprivation, or Hypothermia**

Hyperphosphorylation of tau during hibernation might not solely passively be driven by hypothermia. As we showed recently, at comparable temperatures, phosphate incorporation into tau is much better facilitated in tissue from torpid animals compared with euthermic animals (Ref. 188; see Fig. 2).

This indicates the existence of a specific component in the regulation of tau phosphorylation at early stages of hibernation when animals enter into torpor. Thus, a hibernation-specific regulation of enzyme kinetics, e.g., by altered expression or

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**Fig. 1. Cyclic changes in the hippocampal mossy fiber system during hibernation. Cyclic changes of disappearance and reappearance of PSA-NCAM and synaptophysin in mossy fiber terminals (stratum lucidum) coincide with phosphorylation and dephosphorylation of tau protein in postsynaptic CA3 pyramidal neurons (stratum pyramidale) (Note the red color over pyramidal neurons’ somata corresponding to hyperphosphorylated tau, detected by antibody AT8.).** [Modified with permission after Arendt and Brückner (7) and Arendt et al. (9).]
phosphorylation of regulatory subunits, might contribute actively to a complex regulation of major tau kinases with cdk5 and ERK1 positively, yet GSK-3β, SAPK/JNK, and ERK2 negatively regulated in torpid animals (186–188, 224).

These findings on hibernation-specific regulation of tau phosphorylation, which go beyond those observed after hypothermia, complement previous observations on differential effects of hypothermia and hibernation on synaptic plasticity. While hippocampal slices form hibernating animals (Turkish hamsters) show long-term potentiation (LTP) at lower temperature than those of euthermic animals (185), transient cooling does not affect LTP elicited in brain slices of both hibernators (Syrian hamsters; Ref. 105) and nonhibernators (rats; Ref. 18). By hibernation, animals may save up to 90% of the energy that would otherwise be required (62, 76, 207). In torpor, when the phosphorylation stage of tau is highest, the metabolic rate is strongly suppressed, often to below 5% of the normal eutherian rate. This situation reflects a “vita minima”, where energy supply and requirement, which eventually induces cell damage, result in reduced binding to microtubules and, thus, potentially higher plasticity. The phosphorylation state of tau also modulates its interactions with apolipoprotein E (193), Src kinases (15, 108, 109), and possibly other binding partners and regulates its subcellular distribution. Thus, hyperphosphorylated tau detaches from microtubules, allowing for translocation to the somatodendritic compartment and into spines where it might interfere with synaptic function (87, 195, 211).

During development, the shift in isoform expression (25, 197) and phosphorylation (220) from the embryonic to the adult pattern coincides with the formation of synapses and the appearance of stable microtubules, which represents the critical postnatal period for sensory and motor development of rat cortices (2, 180).

Besides its involvement in axonal microtubule binding, tau may serve additional functions in cytoskeletal organization and cell signaling at subsynaptic sites, where it might provide a protein scaffold for binding partners involved in synaptic plasticity (for review, see Ref. 133). Microtubules are also found in spines (70, 88), and a recent study suggests that microtubule dynamics might be critically involved in the formation and maintenance of memory (50). Furthermore, it has been shown that tau can bind to filamentous actin (F-actin) (36, 69, 130, 173). F-actin is a component of dendritic spines (52, 79), and the modulation of F-actin stability is of crucial importance for synaptic morphology and function (32). Therefore,
tau phosphorylation, through regulation of f-actin and dynamic regulation of microtubule stability within spines, might be indirectly involved in synaptic plasticity.

Another binding partner of tau is Fyn kinase (103), and a recent study showed that the postsynaptic targeting of Fyn is tau dependent. Furthermore, the expression of deletion mutants of tau that cannot bind to microtubules led to uncoupling of NMDA receptors and postsynaptic density protein PSD-95 (95). Thus, hyperphosphorylated tau with reduced affinity to microtubules might be mistargeted into spines, where it impairs synaptic function, probably through a global disruption of postsynaptic targeting or anchoring of NMDA receptors (87, 195). A similar mechanism might be active during hibernation, where phosphorylation of tau leads to detachment from microtubules. The impaired recruitment of NMDA receptors to the postsynaptic density then interferes with excitatory synaptic transmission and synaptic plasticity. In addition, the same mechanism might also serve for a lower vulnerability to excitotoxic insults observed during hibernation (95, 179, 223). Still our knowledge on regulation of synaptic plasticity during hibernation and the role of tau protein in this process is very limited, and further candidates might be involved, including tau-interacting proteins, such as 14–3-3 protein gamma, as recently identified by a proteomic approach (49).

In small mammals, torpor is interrupted regularly by arousal episodes, which are brief returns to normal levels of metabolic rate and body temperature. The energy expended for warming up and maintaining high body temperature during these arousal episodes is estimated to amount to up to 90% of the total energy spent during the entire hibernation season (38, 98, 208). This high degree of seemingly uneconomic energy expenditure clearly indicates an essential function for these brief returns to euthermia (213). Different theories have previously been proposed for the necessity of these periodic arousals, such as to eliminate metabolic waste products (47, 53), to replenish blood glucose levels (60), to restore cellular electrolyte balance (54), or to allow for the normal restorative function of sleep (39, 200, 190). Still, none of them has been validated, and the functional significance of these arousal episodes still remains unknown.

As hibernation elicits negative effects on memory retention in conditioned tasks (127), it could alternatively be hypothesized that because of these potentially deleterious effects, hibernators interrupt the torpor state regularly to return to euthermia. These regularly occurring euthermic phases might, thus, be necessary to protect against mechanisms that otherwise would lead to complete memory loss. They last 4–24 h, depending on species and size (56). Arousals, however, are expensive in terms of energy and require about 90% of the entire energy cost of the animal during the hibernation season (77, 99, 207). The reasons for the repeated arousals are still not understood, but they may allow for the repair of neuronal damage induced by prolonged hypometabolism and brain inactivity at low temperature. As we showed recently (188), hibernation in black bears is associated with conformational changes of highly phosphorylated tau protein that are typically related to neuropathological alterations. The particular hibernation characteristics of black bears with a continuous torpor period, which is not interrupted by spontaneous arousal and an only slightly decreased body temperature, therefore, potentially reflects the limitations of this adaptive reaction pattern if it is not regularly terminated by arousal episodes.

While it might intuitively be assumed that cooling of the brain leads to a gradual reduction of neuronal activity, systematic electrophysiological studies have shown that this is not correct. Gradual cooling of the mammalian brain, in fact, induces a biphasic response of neuronal activity. Moderate cooling leads first to an increased reactivity and hyperexcitability of nerve cells with a maximum around 20°C and only at temperatures below 10°C to the blockade of activity (16, 21, 22, 202). Rapid cooling of amphibians spinal cord even leads to “epileptiform” convulsions (143, 148). As shown for neocortical and hippocampal neurons in the mammalian brain, lowering the temperature by only a few degrees leads to depolarization and a corresponding increase of excitability, to an increase of the excitatory postsynaptic potential (EPSP) duration, and to an increase in both the amplitude and the width of action potential (202). In hippocampal slices of euthermic nonhibernating (rats), as well as hibernating species (Syrian hamsters, Mesocricetus auratus, and western chipmunks, Eutamias quadrivittatus), evoked electrical activity reaches a maximum between 20 and 30°C during both cooling and rewarming (86). Interestingly, the threshold at which a response can be elicited is lower for seasonal (chipmunks) than for facultative hibernators (hamsters), which are comparable to rats. But the threshold in cold-adapted, hibernating hamsters is reaching the threshold level of chipmunks (199). Thus, in both hibernating and nonhibernating species, entrance into and exit from hypothermia is accompanied with a phase of hyperexcitability. This means that neurons are closer to the spike threshold, and less excitatory drive is necessary to evoke their discharge (123, 125, 134). Accordingly, more cells may respond by spiking in response to a given stimulus. For the mammalian brain, this process of slow gradual cooling, resulting in brain regions with different temperatures along a temperature gradient, will have considerable consequences on the functional output of different brain regions, and thus, on functional connectivity. This temperature sensitivity of neuronal activity, apparently is a NMDA-dependent process (123). Functional inactivation of NMDA receptor signaling (reduction of NMDA-dependent synaptic gain; Refs. 27 and 209) as a likely consequence tau hyperphosphorylation (87, 95, 195) would, thus, not only be a great advantage for animals entering into torpor but perhaps would even be a prerequisite, necessary for a controlled shutdown of brain activity in the process of entering into torpor. This suggestion is in agreement with our observation on a very efficient tau phosphorylation in hibernators at the temperature range between 37°C and 30°C, i.e., just below normothermic body temperature (Ref. 188; also, see Fig. 2).

**Perspectives and Significance**

Phosphorylation of tau and its subsequent targeting to sub-synaptic sites might, thus, work as a kind of “master switch,” regulating neuronal activity and, more specifically, NMDA receptor-mediated neuronal plasticity in a wide array of neuronal networks, thereby enabling entry into torpor. Hyperphosphorylation of tau under conditions of controlled “hypometabolism” may, thus, represent a physiological reaction with a protective function (9). In a comparable fashion, hyperphos-
phorylation of tau in Alzheimer’s disease has recently been suggested to represent some compensatory attempt occurring initially during the course of the disease (133) to suppress excitatory and inhibitory imbalances and neuronal network dysrhythmias (129, 144, 145, 164, 165). Reversible deficits of memory might be the cost by which neuroprotection during hibernation is achieved.

In certain species, such as in black bears, hibernation, however, can be associated with conformational changes of highly phosphorylated tau protein that are typically related to neuropathological alterations (188). Aged bears also show permanent accumulations of highly phosphorylated tau in a kind of pretangle state (72). The particular hibernation characteristics of black bears with a long-lasting uninterrupted torpor period might, therefore, potentially reflect the limitations of this adaptive reaction pattern, which if it lasts too long, may eventually turn into a pathological trigger driving a cascade of events leading to neurodegeneration as in Alzheimer’s disease or other tauopathies.

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