PERSPECTIVE

Regrowth and neuronal protection are key for mammalian hibernation: roles for metabolic suppression

Thought experiment: you're starving, huddled in the fetal position in a hole in the ground, with no sense of the world around you, except that you are really, really cold. In fact, your internal temperature can go as low as –2.9°C, which is as dangerous as it sounds, and somehow, you are not freaking out. Actually, your heart rate is only two beats per minute, and you are breathing just a few shallow breaths every half hour or so. You're not dead, so what are you? You're hibernating. Hibernation is a form of torpor used by capable species to defend against the stressors of the winter months such as low ambient temperatures and low food availability. It is characterized by substantial decreases in metabolic rate, breathing and heart rates, and organ perfusion. For this reason, hibernator brains are unique and a little unusual, at least, unusual enough to tolerate and survive these inhospitable conditions. Despite brains being especially sensitive to changes in oxygen/nutrient availability and temperature, hibernators can withstand decreases in brain perfusion of ~90% compared to euthermic levels and changes in body temperatures (T_b) from ~37°C to as low as –2.9°C (Schwartz et al., 2013; Tessier et al., 2019). Yet, hibernators arise from their final torpor-arousal cycle in the spring with no signs of brain injury, almost immediately remembering how to forage for food and find summertime mates. How do hibernators prevent and reverse brain damage? We will describe the role of temperature and torpor in the preservation of hibernator brain integrity with a focus on the molecular aspects of dendritic reorganization.

Hibernators re-grow their neurons faster than non-hibernators: Dendritic spines sense excitatory signals from axons and relay those signals to neuronal cell bodies. It therefore makes sense to retract dendrites during torpor to suppress brain activity and even protect against cellular damage. Multiple reports dating back to the 80's have shown hibernators to have noticeable decreases in spine density, post-synaptic density, spine diameter, and spine length per mossy fibre synapse in the hippocampus (Popov and Bocharova, 1992). Arousal brings with it a complete reversal, and then some: neurons from recently aroused ground squirrels had significantly more dendritic spines than non-hibernated ground squirrels and aroused ground squirrels sampled a day later. This was observed in several brain regions of the hibernating ground squirrel, including the hippocampus, cerebral cortex, thalamus, and Purjinke cells in the cerebellum, suggesting a global brain adaptation.

Retracting dendritic spines upon cold exposure is not unique to hibernators and has been observed in mouse and rat brain slices exposed to temperatures as low as 2°C (Roelandse and Matus, 2004). Even spine density has been shown to be restored in non-hibernators upon rewarming. What makes hibernator brains so incredible is that they are able to restore their dendritic spine density to pre-hypothermia conditions only two hours after arousal is initiated when non-hibernators require more than a day to restore neural synapses (Popov and Bocharova, 1992; Roelandse and Matus, 2004). Whether synaptic regression results from a passive response to changes in core T_b, or if a temperature-independent molecular mechanism is in place to "turn on" spine retraction for neuroprotection via the inhibition of neurosynaptic transmission prolong limited oxygen and nutrient stores, remains unknown.

Hibernator brains can heal their taupathies: During torpor, hibernators exhibit a tau protein phenotype typically seen in the brains of individuals in the advanced stages of Alzheimer's disease. When phosphorylated, tau proteins associate with microtubules (polymers of tubulin) to promote cell cytoskeleton stability, but too much phospho-tau can accumulate as intracellular protein aggregates along with other plaque-forming proteins, that cannot be disposed of, creating neuroinflammation (Aulston et al., 2019). Indeed, the brains of torpid hibernators, including hamsters, ground squirrels and bears, accrue phosphorylated tau and paired-helical filaments, like humans with Alzheimer's disease (Su et al., 2008; Bullmann et al., 2016). Low T_b likely inhibits the phosphatases necessary for tau dephosphorylation, so tau remains phosphorylated until arousal from hibernation. The idea that tau phosphorylation was connected to synaptic regression originally stemmed from the observation that tau phosphorylation and dendrite spine retraction both occurred in the CA3 hippocampal neuron but tau phosphorylation and synaptic regression/regeneration have also been reported in the dentate gyrus and CA1 neurons of hibernators such as Syrian hamsters (Bullmann et al., 2016).

Propelled by the excitement that unique mammals could hold the secret to the reversal of "taupathies" and other neurological diseases, research on hibernators focused on determining the mechanisms that mediate the removal of phospho-tau and the regeneration of dendritic spines during arousal. Kinases in mitogen-activated protein kinase signaling cascades known to be involved in mainstream models of taupathies were suspected of regulating tau phosphorylation in hibernators. Syrian hamster CA3 neurons and dentate gyrus neurons were shown to have increased p-Tau at serine 396 (Bullmann et al., 2016), so glycogen synthase kinase 3 beta (GSK3β), a kinase known to phosphorylate S396, became an exciting prospective regulator of hibernator tau phosphorylation. GSK3β regulates N-methyl-D-aspartate (NMDA)-mediated α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor activation and long-term potentiation (LTP). LTP is a key process in synaptic plasticity that allows for rapid remodeling of the brain-scape for learning and memory, and as such, could play a role in the reestablishment of dendritic spines during arousal from torpor by strengthening the synapses in highly stimulated neurons.

However, antibody-based research by our lab and others discovered that GSK3β is less active in brain tissue from several hibernating species. Arctic ground squirrels (*Urocitellus parryii*) had less p-GSK3β (Y216), a marker for active GSK3β, and more p-GSK3β (S9), a marker for inactive GSK3β (Su et al., 2008). The levels of inhibited p-GSK3β (S9) increased over four-fold in hibernating Monito del monte (*Dromiciops gliroides*) brains (Luu et al., 2018). *I. tridecemlineatus* brain cortex, cerebellum and brainstem also had increased p-GSK3β (S9) during hibernation, and p-GSK3β levels decreased upon arousal to euthermic levels (Tessier et al., 2019). Furthermore, experiments have shown that GSK3β must be inhibited for LTP to occur but LTP cannot occur during the only window when GSK3β is inactive (torpor) since LTP is prevented at body temperatures below 15°C (Syrian hamster data) (Arant et al., 2011). With two independent groups confirming that neuronal GSK3β is inhibited during torpor in multiple hibernating species, there is strong suspicion that another kinase must be responsible for tau phosphorylation and any accompanying synaptic plasticity. Perhaps future research could focus on identifying the phosphatases involved in alleviating tauopathies in hibernator brains.

NMDA signaling is re-wired during torpor: NMDA receptor (NMDAR) signaling is important for learning and memory, which is why it makes sense that this process would be generally inhibited during hibernation. Interestingly, its complete inhibition rapidly arouses hibernating animals. As such, we know that NMDAR signaling is essential for hibernation, but its exact purpose has yet to be wholly defined. Experiments on brain slices from hibernating hamsters have shown that hibernation is associated with a decrease in calcium transport through NMDAR (Arant et al., 2011), which could effectively inhibit LTP until arousal. Importantly, long term depression requires low calcium influx, suggesting a mechanism promoting synaptic regression during torpor. Reduced NMDAR signaling was measured in Syrian hamster (Sekizawa et al., 2013), perhaps as a result of low T_b during metabolic suppression, and could prevent excitotoxicity-mediated cell damage (**Figure 1**).

Furthermore, NMDAR signaling must be reduced to prevent dendritic regrowth until arousal, when stimulation of NMDAR increases calcium influx and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activation. Activation of CaMKII increases the activity of proteins that drive the synthesis of actin filaments, the main cytoskeletal element in dendrites that regulate spine

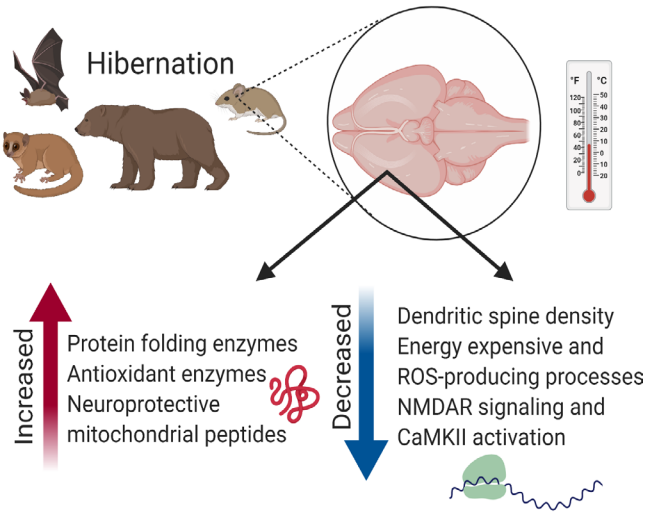

Figure 1 Hibernation in mammals typically involves a suppression of metabolic rate and body temperature.
Physical changes in brain structure include a retraction of dendrites and changes to the cytoskeletal matrix. Coordinated metabolic adaptations promoting neuronal viability include an increase in antioxidant, DNA damage repair and other neuroprotective pathways, while energy expensive processes are largely shut down, including transcription, translation, and NMDAR signaling. Incredibly, hibernators can rapidly reverse these changes upon arousal from torpor. CaMKII: Calmodulin-dependent protein kinase II; NMDAR: N-methyl-D-asparate receptor; ROS: reactive oxygen species.

length and diameter. Hiding in transcriptomic data for hypothalamic gene expression was the discovery that several genes involved in actin polymerization are differentially regulated (e.g., LIM domain kinase 2, Slingshot protein phosphatase 1, inverted formin-2) between groups of torpid and aroused ground squirrels (Schwartz et al., 2013). Future studies, perhaps involving transgenic hibernators or injection studies, should continue to explore how NMDA signaling might be regulated to promote synaptic plasticity as hibernators enter and exit torpor.

Hibernators use multiple lines of defense against cell damage: Hibernator brains have multiple lines of defense against cell death and tissue damage during torpor. The first line of defense includes metabolic suppression itself, which reduces excess consumption of oxygen and cellular ATP by matching metabolic output with metabolic demand. Metabolic suppression involves the shut-off of energy expensive processes like transcription, and translation as well as the shut-down of reactive reactive oxygen species-producing processes like oxidative phosphorylation. Translation is suppressed in whole brain and areas including the brainstem and the forebrain based on increases in mTOR inhibitor p-TSC (S939) and levels of inhibited eukaryotic initiation factor 2, decreases in the incorporation of radioactive leucine into nascent proteins during torpor, and a decrease in the formation of polysomes (Tessier et al., 2019). A reduction in core T_b may facilitate many of these changes: by reducing enzyme activity, altering the conformation of nascent proteins and mRNA, and prompting the sequestration of select mRNA and proteins until more favourable conditions. Hibernators even have defenses against their defenses! They are able to manage their core T_b by altering their thermogenic set-point. If their body temperature is reduced past this point, they initiate shivering and non-shivering thermogenesis to return to a more comfortable temperature.

Some genes are upregulated in hibernator brain during torpor, such as those encoding proteins involved in DNA repair (ATM, RAD50), antioxidant defenses (heme oxygenase 1, oxidation resistance protein 1), and protein folding (heat shock protein 90 alpha family class A member 1, heat shock 70 kDa protein 8) (Ni and Storey, 2010; Schwartz et al., 2013). Proteins encoded by these genes may protect against reactive oxygen species released from an inefficient electron transport chain. Indeed, electron transport chain enzyme activity is inhibited in the brain of a number of hibernators including *Thylamys elegans* and *Myotis ricketti*, and may facilitate metabolic suppression by reducing oxygen consumption and heat production (Zhang et al., 2014; Cortes et al., 2018). However, the mitochondria from animals capable of torpor are still poorly understood. Recently, novel post-translational modifications of mitochondrial enzymes like pyruvate dehydrogenase were discovered, but the mitochondrial methyl-, glucosyl- and acetyl-transferases that reversibly regulate these enzymes have yet to be investigated (Zhang et al., 2014). Further, our lab recently discovered a homologue of a human neuroprotective mitochondrial peptide in the brains of torpid ground squirrels that could promote cell viability during metabolic suppression (Szereszewski and Storey, 2019). Relative to euthermia, the levels of s-humanin increased at both the transcript and protein levels in the cerebral cortex during hibernation, suggesting that hibernators may use mitochondrial peptides as part of their defense core against neuronal cell stress. Humanin is neuroprotective in disease states such as Alzheimer's and can even reduce oxidative stress caused by protein aggregates by upregulating antioxidants and preventing cell death. In theory, s-humanin could upregulate the JAK2-STAT3 pathway, but recent research shows that p-STAT3 (Y705) is not increased during torpor (Tessier et al., 2019). Instead, s-humanin may provide neuroprotection by stimulating the expression of anti-apoptotic proteins or antioxidant enzymes, which serves as another major line of defense in the brains of hibernators (Ni and Storey, 2010).

Hibernation on our horizon? We have come a long way in our understanding of what makes hibernator brains so adaptable, but we have yet to determine what controls neuronal plasticity in hibernators. Scientists in the field have agreed for some time that hibernators are genetically no more special than other mammals, even humans. With some sequence variation, hibernators express all the same genes, resulting in many of the same proteins with highly similar functions. The major difference between hibernators and non-hibernators is thought to lie in the level of expression of these proteins in the face of stressful conditions, which could influence the power of each cell to surmount cell stress. Herein, we identified a few examples of genes whose mRNA/protein/post-translational modification levels change during torpor or arousal to promote neuronal regression/regrowth and neuroprotection in hibernators, including p-GSK3β, p-tau, actin cytoskeleton modifying proteins, NMDAR, CaMKII, translation activators and inhibitors, s-humanin, antiapoptotic proteins, and antioxidant enzymes.

The next step is investigating how the epigenome might control everything from synaptogenesis to neuroprotection to the triggering of initiation of and exit from a torpor bout. Are certain genes methylated or acetylated to control transcription? How do microRNAs and other non-coding RNAs contribute to neuronal plasticity? Could epigenetic tags on mRNA transcripts help cells determine which proteins to overproduce during stress? Molecular approaches have aided our understanding of what processes are occurring (or are inhibited) during torpor and upon arousal and they will continue to do so in our newest research programmes. For instance, RT-qPCR studies looking at microRNA expression in the brains of hibernating bats (*M. ricketti* and *Myotis lucifugus*) suggest that miRNAs like miR-29b could have roles in neuroprotection. Indeed, if miR-29b levels decrease in the brain, cellular death ensues. Thus, hibernators may upregulate miR-29b to promote neuronal cell viability. More comprehensive studies looking at the role of all microRNAs in neuroprotection would help identify more biomarkers of brain health in hibernators and could be useful in the treatment of human neuropathologies. As sequencing technologies continue to get less expensive and more widely accessible, bisulfite-sequencing, chromatin immunoprecipitation-sequencing and small non-coding RNA-sequencing tools will help us learn the answers to the above questions faster and at a deeper level than ever before. With each new study, we are getting closer to figuring out how the brains of these adaptable creatures are reorganized during stress, and less stressed out as we uncover medically relevant biomarkers that may help humans (or transplantable organs) get a little bit closer to being able to hibernate.

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References

Arant RJ, Goo MS, Gill PD, Nguyen Y, Watson KD, Hamilton JS, Horowitz JM, Horwitz BA (2011) Decreasing temperature shifts hippocampal function from memory formation to modulation of hibernation bout duration in Syrian hamsters. Am J Physiol Integr Comp Physiol 301:R438-447.

Aulston B, Liu Q, Mante M, Florio J, Rissman RA, Yuan SH (2019) Extracellular vesicles isolated from familial alzheimer's disease neuronal cultures induce aberrant tau phosphorylation in the wild-type mouse brain. J Alzheimers Dis 72:575-585.

Bullmann T, Seeger G, Stieler J, Hanics J, Reimann K, Kretzschmann TP, Hilbrich I, Holzer M, Alpár A, Arendt T (2016) Tau phosphorylation-associated spine regression does not impair hippocampal-dependent memory in hibernating golden hamsters. Hippocampus 26:301-318.

Cortes PA, Bozinovic F, Blier PU (2018) Mitochondrial phenotype during torpor: Modulation of mitochondrial electron transport system in the Chilean mouse–opossum Thylamys elegans. Comp Biochem Physiol Part A Mol Integr Physiol 221:7-14.

Luu BE, Wijenayake S, Zhang J, Tessier SN, Quintero-Galvis JF, Gaitán-Espitia JD, Nespolo RF, Storey KB (2018) Strategies of biochemical adaptation for hibernation in a South American marsupial, Dromiciops gliroides: 2. Control of the Akt pathway and protein translation machinery. Comp Biochem Physiol B Biochem Mol Biol 224:19-25.

Ni Z, Storey KB (2010) Heme oxygenase expression and Nrf2 signaling during hibernation in ground squirrels. Can J Physiol Pharmacol 88:379-387.

Popov VI, Bocharova LS (1992) Hibernation-induced structural changes in synaptic contacts between mossy fibres and hippocampal pyramidal neurons. Neuroscience 48:53-62.

Roelandse M, Matus A (2004) Hypothermia-associated loss of dendritic spines. J Neurosci 24:7843-7847.

Schwartz C, Hampton M, Andrews MT (2013) Seasonal and regional differences in gene expression in the brain of a hibernating mammal. PLoS One 8:e58427.

Sekizawa S, Horwitz BA, Horowitz JM, Chen CY (2013) Protection of signal processing at low temperature in baroreceptive neurons in the nucleus tractus solitarius of Syrian hamsters, a hibernating species. Am J Physiol Regul Integr Comp Physiol 305:R1153-1162.

Su B, Wang X, Drew KL, Perry G, Smith MA, Zhu X (2008) Physiological regulation of tau phosphorylation during hibernation. J Neurochem 105:2098-2108.

Szereszewski KE, Storey KB (2019) Identification of a prosurvival neuroprotective mitochondrial peptide in a mammalian hibernator. Cell Biochem Funct 37:494-503.

Tessier SN, Wu CW, Storey KB (2019) Molecular control of protein synthesis, glucose metabolism, and apoptosis in the brain of hibernating thirteen-lined ground squirrels. Biochem Cell Biol 97:536-544.

Zhang Y, Pan YH, Yin Q, Yang T, Dong D, Liao CC, Zhang S (2014) Critical roles of mitochondria in brain activities of torpid Myotis ricketti bats revealed by a proteomic approach. J Proteomics 105:266-284.

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