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 (Tb) 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 significant changes to dendritic structure, almost immediately rewarm, 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. If 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 and spine number until arousal. Importantly, and 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 Purkinje 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 (Roelands 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! This was observed in several brain regions of the hibernating ground squirrel, including the hippocampus, cerebral cortex, thalamus, and Purkinje cells in the cerebellum, suggesting a global brain adaptation. Importantly, this dendritic retraction was accompanied by synaptic plasticity. Perhaps future research could focus on identifying the phosphatases involved in alleviating taupathies in hibernator brains.

NMDAR signaling is re-wired during torpor: NMDAR 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 (Seikizawa et al., 2013), perhaps as a result of low Tb, during metabolic suppression, and could prevent excitotoxicity-mediated cell damage (Figure 1).

Furthermore, NMDAR signaling must be reduced to prevent dendritic growth until arousal, when stimulation of NMDA increases calcium influx and Ca2+/calmodulin-dependent protein kinase II (CaMKII) activation. Activation of CaMKII increases the synthesis of proteins that drive the synthesis of actin filaments, the main cytoskeletal element in dendrites that regulate spine 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 de monte (Dromiciops gliroides) brains (Lau et al., 2018). L. tridecimlineatus 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 taupathies in hibernator brains.

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 in intracellular processes and result in pathological neurofibrillary tangles, which cannot be removed, 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 brains of torpid hibernators, including hamsters, ground squirrels and bears, have 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, when stimulation of NMDA increases calcium influx and Ca2+/calmodulin-dependent protein kinase II (CaMKII) activation. Activation of CaMKII increases the synthesis of proteins that drive the synthesis of actin filaments, the main cytoskeletal element in dendrites that regulate spine 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.

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 re-grow their neurons faster than non-hibernators upon arousal from torpor. CaMKII: Calmodulin-dependent protein kinase II; NMDAR: N-methyl-D-aspartate receptor; ROS: reactive oxygen species.
Logan SM, Storey KB (2020) Regrowth and neuronal protection are key for mammalian hibernation: roles for metabolic suppression. *Neural Regen Res* 15(11):2027-2028. doi:10.4103/1673-5374.282242

...to control transcription? How do microRNAs and other non-coding RNAs of and exit from a torpor bout. Are certain genes methylated or acetylated thing from synaptogenesis to neuroprotection to the triggering of initiation NMDAR, CaMKII, translation activators and inhibitors, s-humanin, antioxidant enzymes, which serves as another major...While the mitochondria from animals capable of torpor are still...hormones including *Thalamic elegans* and *Myotis ricketti*, and may facilitate metabolic suppression by reduc...ing proteins involved in DNA repair (ATM, RAD50), antioxidant genes and the expression of select mRNA and proteins until more favourable conditions. Hibernators even have defenses against their defenses! They are able to manage their core T<sub>b</sub> 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 enzymes (heme oxygenase 1, oxidation reducing protein 1), and proteins oxid...heat shock protein 90 alpha family member A1, 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 *Thalamic elegans* and *Myotis ricketti*, and may facilitate metabolic suppression by reducing energy expenditure 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- , glycosyl- 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 via...ity is inhibited in the brain of a number of hibernators including *Thalamic elegans* and *Myotis ricketti*, and may facilitate metabolic suppression by reducing energy expenditure 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- , glycosyl- 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 via...hormone-dependent memory in hibernating golden hamsters. *Hippocampus* 26:301-318.

Cortes PA, Rozovnic J, Flieger PG (2018) mitochondrial phenotype during torpor: Modulation of mitochondrial electron transport system in the Chilomus musculus-opossum Thalamic elegans. Comp Biochem Physiol Part A Mol Integr Physiol 227:1-14.

Liu BE, Wijesinghe B, Zhang J, Tosier SN, Quintero-Galvan JF, Galan-Espitia JD, Nopolo RP, Storey KB (2018) Strategies of biochemical adaptation for hibernation in a South American marsupial, *Dromiciops gliroides*. J Control of the Atl 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...and apoptosis in the brain of hibernating thirteen-lined ground squirrels. *Biochem Cell Biol* 86:196-205.

Sekizawa S, Iriuchijima K, Horwitz BA (2013) Protein synthesis in the brain during hibernation. *J Neurochem* 125:229-243.

References

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

Aulston B, Liu Q, Mantis M, Ploin J, Rauscher FJ III, Horwitz BA (2011) Extracellular vesicles isolated from familial Alzheimer's disease neuronal cultures induce aberrant tau phosphorylation in the wild-type mouse brain. *J Alzheimer's Dis* 28:175-258.

Bullmann T, Seeger G, Stiefel J, Hanics K, Reimann K, Kretzschmann TP, Hillbich I, Holser M, Alpar A, Arendt T (2016) Tau phosphorylation-associated spine regression does not impact hippocam...hormone-dependent memory in hibernating golden hamsters. *Hippocampus* 26:301-318.

Cortes PA, Rozovinic J, Flieger PG (2018) mitochondrial phenotype during torpor: Modulation of mitochondrial electron transport system in the Chilomus musculus-opossum Thalamic elegans. Comp Biochem Physiol Part A Mol Integr Physiol 227:1-14.

Liu BE, Wijesinghe B, Zhang J, Tosier SN, Quintero-Galvan JF, Galan-Espitia JD, Nopolo RP, Storey KB (2018) Strategies of biochemical adaptation for hibernation in a South American marsupial, *Dromiciops gliroides*. J Control of the Atl 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. *Am J Physiol Integr Comp Physiol* 301:R438-447.

Sekizawa S, Iriuchijima K, Horwitz BA (2013) Protein synthesis in the brain during hibernation. *J Neurochem* 125:229-243.

References