Temperature 2:1, 1–2; January/February/March 2015; Published with license by Taylor & Francis Group, LLC

Hypoxic turtles keep their cool

Jesper G Madsen[1,*], Tobias Wang[2], and Peter T Madsen[2]

[1]Aarhus University Hospital Skejby; Aarhus University; Aarhus, Denmark; [2]Zoophysiology; Department of Bioscience; Aarhus University; Aarhus, Denmark

Several species of freshwater turtles spend the winter submerged in ice-covered lakes in a state of severe metabolic depression. It has been proposed that the hibernating turtles are comatose and entirely unresponsive, which raises the question of how they detect the arrival of spring and whether they respond to sensory information during hibernation. Using evoked potential studies in cold hypoxic turtles exposed to light and vibration, we show that hibernating turtles maintain neural responsiveness to light stimuli during prolonged hypoxia, while responsiveness to vibration is lost. This reveals a state of differential neural shutdown, in different sensory systems in the cold hypoxic turtle brain. In behavioral studies we show that turtles held for 14 days in hibernation increase locomotor activity in response to light or elevated temperatures, but not to vibration or increased oxygen. We conclude that hibernating freshwater turtles are not comatose, but remain vigilant during overwintering in cold hypoxia.

Northern species of freshwater turtles, as shown in **Figure 1**, face a physiological problem each winter; they cannot tolerate freezing, and must therefore overwinter submerged at the bottom of lakes where they can be trapped underwater until the ice thaws. In a recent study we examined to what extent cold and hypoxic turtles remain responsive under these conditions.[1] The turtles survive the cold anoxic winter by suppressing metabolism to less than 1% of normal metabolism at 20°C[2] and with their large capacity for anaerobic metabolism they endure an entire winter without breathing. In addition to the protective effects of hypometabolism, damage to the central nervous system is mitigated by channel arrest, where the density of ion channels in the central nervous system is dramatically decreased, allowing for ion homeostasis to be maintained at minimal energy expenditure, as well as spike arrest where activity of the nerve cells is markedly reduced.[3] This central nervous system depression during hibernation is mediated by release of inhibitory neurotransmitters, such as γ-aminobutyric acid,[4] and has led to the view that the cold and anoxic turtles are completely comatose and unresponsive during hibernation.[5] The self-imposed inhibition of the central nervous system, however, poses a conundrum; if the turtles are in an unresponsive coma, how do they know when spring has arrived and it is possible to resurface?

To address this puzzle, we studied the responsiveness of red eared slider turtles (Trachemys scripta elegans) to different sensory stimuli during cooling and hypoxia. Anesthetized turtles, warmed to a core body temperature of 25°C, were cooled and subjected to light or vibration stimuli until they reached a body temperature of 3°C. The evoked potentials from the stimuli were measured via electrodes placed subcutaneously on the turtles' heads.

To test for the effects of hypoxia per se, the animals underwent the same stimulation regime of light and vibration at a body temperature of 20°C while being ventilated with nitrogen gas to induce severe hypoxia. In this manner, we could separate the effects of cooling and hypoxia. We chose to study light and vibration stimuli based on the hypothesis that there might be a discrepancy between the reduction of light and vibration sensitivity, as increased light levels signal the arrival of spring, and the possibility to resurface, whereas vibration detection might be less relevant to a hibernating turtle.

Responsiveness to both light and vibration was gradually eliminated with cooling. However, as the animals reheated, light responsiveness recovered faster than to vibration. The neural response to vibration declined and was undetectable after 1 h of nitrogen gas ventilation; in contrast light responses were sustained throughout hypoxia.

We also performed a behavioral experiment, where the turtles were submitted to hibernation conditions for 2 weeks in a dark, closed water tank at 3°C, and then exposed to one of several stimuli while being tracked on video to investigate whether particular sensory stimuli would induce a behavioral response. The behavioral experiments confirmed the findings of the stimulation experiments; when exposed to light an immediate increase in movement was recorded. Increased activity was also recorded when the water temperature was increased as expected because of the positive effect on metabolism, whereas neither vibration nor increased water oxygenation stimulated activity.

We have therefore shown that cold anoxic freshwater turtles are not in a state of unresponsive coma, as previously hypothesized. Instead they retain slow vigilance, ready to respond in a coordinated fashion when adequate stimuli are received. This implies that the mechanisms entertained to explain how turtles save energy during hibernation, such as channel arrest and increasing γ-aminobutyric acid, are not universally applied in the central nervous system.

Keywords: coma, evoked potential, freshwater turtles, hibernation, responsiveness

*Correspondence to: Jesper G Madsen; Email: jesperguldsmedmadsen@gmail.com

Submitted: 07/31/2014

Revised: 10/08/2014

Accepted: 10/14/2014

http://dx.doi.org/10.4161/23328940.2014.978167

Figure 1. Freshwater turtle species living at northern latitudes, such as Trachemys scripta species, avoid freezing by overwintering at the bottom of frozen lakes and ponds. As they cannot surface to breath during winter, they enter a state of deep metabolic depression to conserve energy until the ice melts.

Our study provides an example of a vertebrate central nervous system that maintains critical functions during cold (3°C) without oxygen. Future experiments on how a vertebrate central nervous system continues to function during such conditions could provide valuable insights into the energy conserving effects of low temperature, and may have relevance to understand the central nervous system function during clinical hypothermia as commonly used in the immediate treatment after severe oxygen deprivation during stroke or cardiac arrest. Furthermore, we have revealed what appears to be a selective decrease in certain central nervous system activities, while others remains functional. The result is apparently a wake-up sequence where a selective response to a certain appropriate stimulus seems to be followed by an increase in the overall central nervous system activity. Understanding how such sequential shut down and activation of higher brain functions occur in a vertebrate central nervous system could further our understanding of hibernation and coma conditions in both animals and humans.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Madsen JG, et al. Biol Lett 2013; 9:20130602; PMID:24108677; http://dx.doi.org/10.1098/rsbl.2013.0602
2. Jackson DC. J Physiol 2002; 543:731-737; PMID:12231634
3. Hochachka P W, et al. Proc Natl Acad Sci USA 1996; 93:9493-9498; PMID:19039328; http://dx.doi.org/10.1073/pnas.93.18.9493
4. Nilsson GE, Lutz PL. Am J Physiol 1991; 261:R32-R37; PMID:1677540
5. Nilsson GE, Lutz PL. J Cer Blood Flow Me 2004; 24:475-486; PMID:15129179; http://dx.doi.org/10.1097/00004647-200405000-00001