Cognitive control of heart rate in diving harbor porpoises

Running title: Cognitive heart rate regulation in porpoises

Word count (main text): 982

Elmegaard, Siri L.[1], Johnson, Mark[2], Madsen, Peter T.[1, 3], and McDonald, Birgitte I.[4, 1]

1) Zoophysiology, Department of Bioscience, Aarhus University, 8000 Aarhus C, Denmark

2) Scottish Oceans Institute, University of St. Andrews, St. Andrews KY16 8LB, Scotland, UK

3) Murdoch University Cetacean Research Unit, School of Veterinary and Life Sciences, Murdoch University, Perth, WA 6150, Australia

4) Moss Landing Marine Laboratories, California State University, Moss Landing, CA 95039-9647, USA

*Corresponding author: sirielme@gmail.com

Marine mammals have adapted to forage while breath-holding in a suite of aquatic niches from shallow rivers to deep oceans. The key to tolerate such extensive apnea is the dive response, comprising bradycardia and peripheral vasoconstriction. Although initially considered an all-or-nothing reflex [1], studies on freely diving marine mammals have through time revealed substantial dynamics of the dive response, matching it to the impending dive demands of depth, duration and exercise [2]. Such adjustments are not only autonomic responses, but are under acute cognitive control in pinnipeds [3] living amphibiously on land and in water. The fully aquatic cetaceans would similarly benefit from cognitive cardiovascular control; however, even though they have exercise modulated diving bradycardia [2] and full voluntary control of their respiratory system to such extent that even mild anesthesia often leads to asphyxiation [4], a cognitive cardiovascular control has never been demonstrated for this large group of marine mammals. To address this, we tested the hypothesis that porpoises modulate bradycardia according to anticipated dive duration. Two harbor porpoises, instrumented with ECG recording tags, were trained to perform 20- and 80-second stationary dives, during which they adjusted bradycardia to the anticipated duration, demonstrating cognitive control of their dive response.

Data was collected from June through October 2015 from two captive female harbor porpoises (*Phocoena phocoena*), Freja (18 years, 54 kg) and Sif (11 years, 50 kg), at the Fjord & Belt Aquarium in Kerteminde, Denmark. ECG was measured with a modified Dtag3 (ECG-Dtag3) with two external differential chlorinated silver electrodes embedded in silicone suction cups (Figure 1 A). The porpoises performed stationary dives to a biteplate at 1 meter of depth for two extensively trained durations: 20 (S20) and 80 (S80) seconds. S80 was trained and data collected before S20 was trained and collected (see Supplemental information). A sound cue was played prior to each S20 to enforce the comprehension of a new shorter dive task compared to S80. Each dive was preceded by surface time in minimal activity for full recovery.

Heart rate, f_H, was compared between S20 and S80 (Figure 1 B). During the initial 15 seconds of the dives there was no difference in dive factors such as pressure or exercise. Still, the porpoises attained minimum f_H (Figure 1 B inset) that were 15% (Freja) and 26% (Sif) lower in S80 compared to S20 (*Freja min f_H*: *S80:* 45.5 ±0.9 beats min^{-1} *vs*. *S20*: 53.8±1.0 beats min^{-1}, $p<0.001$. *Sif min f_H: S80:* 41.9 ±1.9 beats min^{-1} *vs. S20:* 56.4±0.6 beats min^{-1}, $p<0.001$). We argue that this difference in f_H represents cognitive control rather than a conditioned reflex since gradual reinforcement of the dive response due to repetition would result in a stronger response in the latter S20 compared to the initial S80, contrary to the observed effect. Furthermore, we monitored the development of bradycardia during S20 training dives of one animal and found that after only a few dives a constant mild bradycardia was reached, indicating that the animal quickly anticipated the shorter dive duration. We thus conclude that harbor porpoises can cognitively modulate their diving bradycardia according to expectations of a dive. The cognitive modulation probably encompass cardiovascular responses in general, considering the strong correlation between vasoconstriction and heart rate exhibited in harbor seals [5].

Cetaceans vary both the duration and depth of their dives according to foraging conditions and information obtained on prior dives [6], and it is likely that all cetaceans incorporate experience in determining when, where and for how long to dive. Cognitive fine-tuning of the dive response would therefore allow matching of blood oxygen availability to the expected course of a dive. During dives, bradycardia and vasoconstriction combine to decrease cardiac output and organ perfusion, maintain blood pressure, conserve blood oxygen, and redistribute blood flow to the hypoxia-sensitive brain and heart. Meanwhile, low oxygen tensions in the muscles mobilize the large myoglobin-bound oxygen stores for local use minimizing anaerobic work [1]. For long dives, a strong dive response is needed to maintain brain and heart function; however for short dives, a mild dive response maintains organ functions and supplements myoglobin-bound oxygen stores in muscles, leading to minimal recovery time at the surface. A cognitively modulated dive response thus increases the overall dive-to-surface ratios, and therefore, ultimately the animal's foraging opportunities and fitness. This is likely to be an ecologically relevant and advantageous trait for porpoises, since the plasticity we report is manifested at dive durations well within the calculated aerobic dive limit for a harbor porpoise, as well as within mean observed dive times in the wild lasting about a minute [7]. The separate evolution of this faculty in both pinnipeds and cetaceans, the two major lineages of mammals returning to the sea, highlights the importance of fine-scale physiological control in achieving the remarkable underwater lifestyles of these air-breathing predators.

Interestingly, blood flow dynamics are not only important for management of oxygen stores, but may also influence tissue nitrogen levels [8]. We thus propose that cognitive control of the dive response may also be an important regulatory factor in avoiding decompression sickness for cetaceans and specifically for harbor porpoises that dive repetitively within lung collapse depth in a constant hunt for food [7]; a dive behavior known to cause decompression sickness in humans [9]. In consequence, abnormal cognitive physiological control may be an important contributing factor to the occasional mass strandings of whales following naval sonar exercises. It is well documented that some cetaceans react strongly to these sounds [10] and we propose, in the light of our results, that this stress may override, or divert attention from, cognitive control potentially causing unfavorable cardiovascular regulation which could be fatal due to oxygen and nitrogen mis-management. Our finding of cetacean cognitive dive response control is therefore an important step in understanding the physiology of these elusive animals, as well as the possible effects of the many human disturbances they face on a regular basis.

Supplemental information

Supplemental information including experimental procedures and one table can be found with this article online at XXX.

Author contributions

BIM and PTM conceived the experiment. SLE, BIM and PTM designed the experiment. MJ designed and created the ECG-Dtag3 and custom analysis software. SLE conducted the experiments, data analysis and interpretation, and wrote the manuscript with BIM, MJ and PTM. All authors have read and approved of the final manuscript.

Acknowledgements

This study was possible due to the skilled and dedicated effort by the animal trainers at the Fjord & Belt Centre: J.H. Kristensen, J. Larsson, C. Eriksson, and F. Johansson. Office of Naval research supported this research through grant # N000141210633 to PTM and BIM. The Danish Research Council (FNU) and Carlsberg funded training time and equipment through grants to PTM. BIM was supported by a National Science Foundation International Research Postdoctoral Fellowship. MJ was funded by the Marine Alliance for Science and Technology, Scotland, and by a Marie Curie Career Integration Grant. We thank two reveiwers for helpful critique on earlier versions of the manuscript. We declare no conflict of interests.

REFERENCES

1. Scholander, P.F. (1940). Experimental investigations on the respiratory function in diving mammals and birds. Hvalradets Skrifter *22*, 1–131.
2. Williams, T.M., Fuiman, L.A., Kendall, T., Berry, P., Richter, B., Noren, S.R., Thometz, N., Shattock, M.J., Farrell, E., Stamper, A.M., *et al.* (2015). Exercise at depth alters bradycardia and incidence of cardiac anomalies in deep-diving marine mammals. Nat. Commun. *6*, 6055.
3. Ridgway, S.H., Carder, D.A., and Clark, W. (1975). Conditioned bradycardia in the sea lion *Zalophus californianus*. Nature *256*, 1–2.
4. Higgins, J.L., and Hendrickson, D.A. (2013). Surgical procedures in pinniped and cetacean species. J. Zoo Wildl. Med. *44*, 817–36.
5. Grinnell, S.W., Irving, L., and Scholander, P.F. (1942). Experiments on the relation between blood flow and heart rate in the diving seal. J. Cell Comp. Physiol. *19*, 341–350.
6. Fais, A., Aguilar Soto, N., Johnson, M., Pérez-González, C., Miller, P.J.O., and Madsen, P.T. (2015). Sperm whale echolocation behaviour reveals a directed, prior-based search strategy informed by prey distribution. Behav. Ecol. Sociobiol., 1–12.
7. Reed, J.Z., Chambers, C., Hunter, C.J., Lockyer, C., Kastelein, R., Fedak, M.A., and Boutilier, R.G. (2000). Gas exchange and heart rate in the harbour porpoise, *Phocoena phocoena*. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. *170*, 1–10.
8. Fahlman, A., Olszowka, A., Bostrom, B., and Jones, D.R. (2006). Deep diving mammals: Dive behavior and circulatory adjustments contribute to bends avoidance. Respir. Physiol. Neurobiol. *153*, 66–77.
9. Paulev, P. (1965). Decompression sickness following repeated breath-hold dives. J. Appl. Physiol. *20*, 1028–1031.
10. DeRuiter, S.L., Southall, B.L., Calambokidis, J., Zimmer, M.X., Sadykova, D., Falcone, E.A., Friedlaender, A.S., Joseph, J.E., Moretti, D., Schorr, G.S., *et al.* (2013). First direct measurements of behavioural responses by Cuvier's beaked whales to mid-frequency active

sonar. Biol. Lett. *9*, 1–5.

FIGURE LEGENDS

Fig 1. Modulation of bradycardia according to expected dive duration. A). The placement of the ECG-Dtag3 is just caudal to the blowhole ensuring optimal sound recording of respirations, as well as depth measurements at the blowhole. The two ECG electrodes are placed on the sides of the porpoise (arrows): right rostral and left caudal to the heart. **B).** Heart rates of the two porpoises during anticipated 20 and 80 second stationary dives to 1-m depth. The start of the dive, time 0, is defined as the last breath before submergence. Heart rates are binned in one-second bins, and each point thus represents the mean instantaneous f_H of all recorded dives in that preceding second. Error bars reflect standard error of the mean (SEM). The number of dives recorded is stated in the legend-boxes. Arrows at the bottom of the plot indicate the approximate times of submersion and surfacing. The shaded area illustrates the 15 seconds from which minimum f_H are determined and compared between S20 and S80. **B inset).** The mean (± SEM) of the minimum instantaneous f_H obtained during the initial 15 seconds of dives reveal a significantly lower f_H during S80 dives for both porpoises. ** indicate that $p<0.001$ as tested with Welch's t-test. During the initial 15 seconds there is no difference in potential sensory triggers for bradycardia such as pressure or activity between S20 and S80. The difference observed in f_H during this interval thus suggests an anticipatory, i.e. cognitive regulation of f_H. See Supplemental Experimental Procedures and Table S1 for further details.