

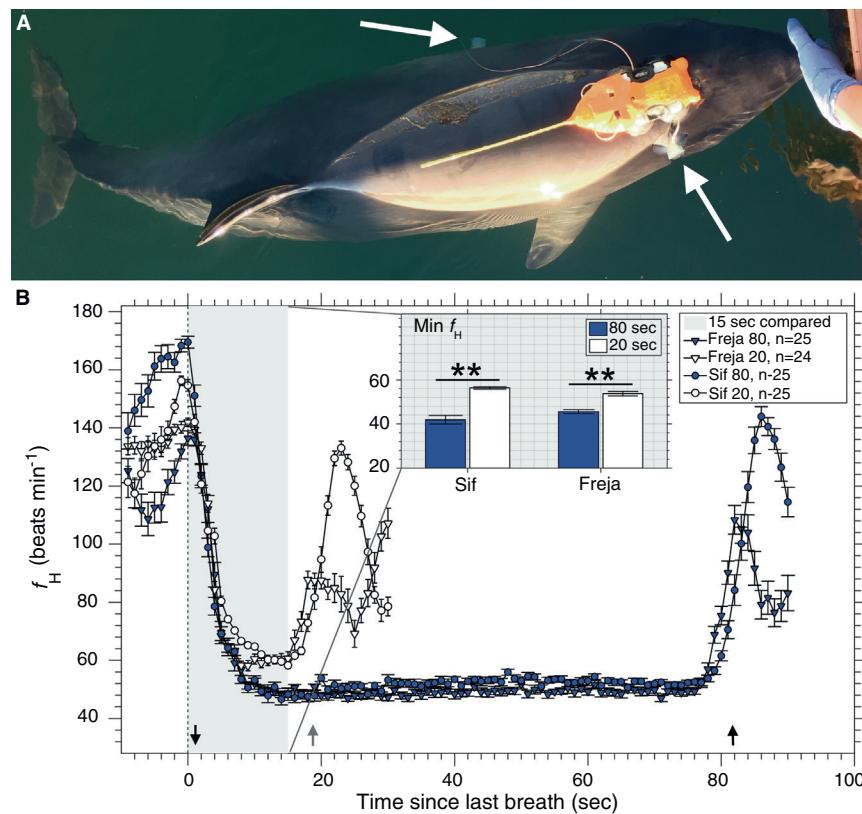
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**Cognitive control of heart rate in diving harbor porpoises**

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Marine mammals have adapted to forage while holding their breath in a suite of aquatic habitats from shallow rivers to deep oceans. The key to tolerate such extensive apnea is the dive response, which comprises bradycardia and peripheral vasoconstriction. Although initially considered an all-or-nothing reflex [1], numerous studies on freely diving marine mammals have revealed substantial dynamics of the dive response to meet 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], 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 were 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



**Figure 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 box. Arrows at the bottom of the plot indicate the approximate times of submergence and surfacing. The shaded area illustrates the 15 seconds from which minimum  $f_H$  are determined and compared between S20 and S80. Inset: The mean ( $\pm$  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 suggests an anticipatory, i.e. cognitive, regulation of  $f_H$ . See Supplemental information.

silver electrodes embedded in silicone suction cups (Figure 1A). The porpoises performed stationary dives to a biteplate at one 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 (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 one minute surface time in minimal activity for full recovery.

Heart rate ( $f_H$ ) was compared between S20 and S80 (Figure 1B). During the initial 15 seconds of the dives there was no difference in dive factors such as pressure or exercise. Yet, the porpoises attained minimum  $f_H$  (Figure 1B inset) that were 15% (Freja) and 26% (Sif) lower in S80 compared to S20 (Freja  $min f_H$ : S80:  $45.5 \pm 0.9$  beats  $min^{-1}$  vs. S20:  $53.8 \pm 1.0$  beats  $min^{-1}$ ,  $p < 0.001$ . Sif  $min f_H$ : S80:  $41.9 \pm 1.9$  beats  $min^{-1}$  vs. S20:  $56.4 \pm 0.6$  beats  $min^{-1}$ ,  $p < 0.001$ ). We argue that this difference in heart rate 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 in 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 of about one 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 to depths shallower than the depth of lung collapse 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 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 <http://dx.doi.org/10.1016/j.cub.2016.10.020>.

#### AUTHOR CONTRIBUTIONS

B.I.M. and P.T.M. conceived the experiment. S.L.E., B.I.M. and P.T.M. designed the experiment. M.J. designed and created the ECG-Dtag3 and custom analysis software. S.L.E. conducted the experiments, data analysis and interpretation, and wrote the manuscript with B.I.M., M.J. and P.T.M. All authors have read and approved of the final manuscript.

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