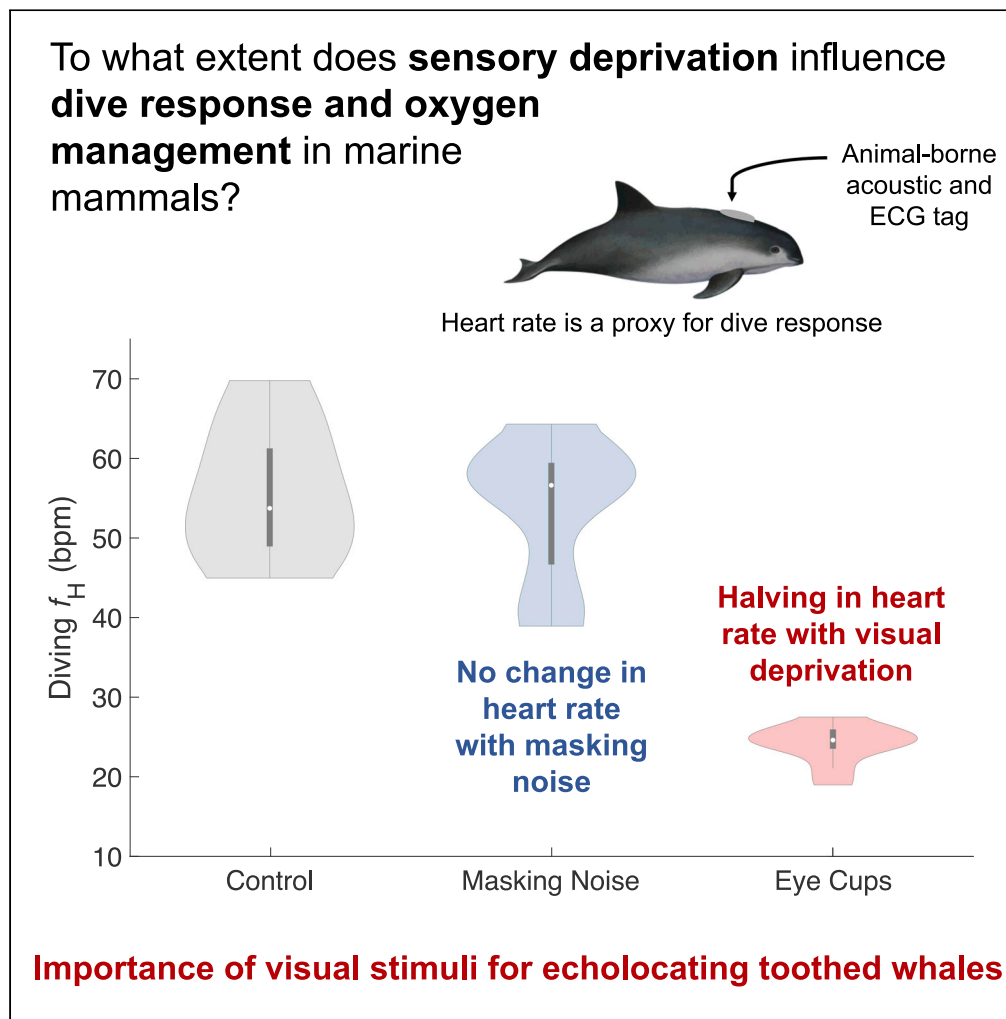


Article

Visual deprivation induces a stronger dive response in a harbor porpoise



Ciska Bakkeren, Michael Ladegaard, Kirstin Anderson Hansen, Magnus Wahlberg, Peter Teglberg Madsen, Laia Rojano-Doñate

peter.madsen@bio.au.dk

Highlights

It is unknown to what extent sensory deprivation influences oxygen management

Porpoises do not change diving heart rate during masking of their echolocation

Regardless of noise condition, blindfolding causes a halving in diving heart rate



Article

Visual deprivation induces a stronger dive response in a harbor porpoise

Ciska Bakkeren,¹ Michael Ladegaard,¹ Kirstin Anderson Hansen,^{2,3} Magnus Wahlberg,² Peter Teglbjerg Madsen,^{1,4,*} and Laia Rojano-Doñate¹

SUMMARY

The dive response allows marine mammals to perform prolonged breath-hold dives to access rich marine prey resources. Via dynamic adjustments of peripheral vasoconstriction and bradycardia, oxygen consumption can be tailored to breath-hold duration, depth, exercise, and even expectations during dives. By investigating the heart rate of a trained harbor porpoise during a two-alternative forced choice task, where the animal is either acoustically masked or blindfolded, we test the hypothesis that sensory deprivation will lead to a stronger dive response to conserve oxygen when facing a more uncertain and smaller sensory Umwelt. We show that the porpoise halves its diving heart rate (from 55 to 25 bpm) when blindfolded but presents no change in heart rate during masking of its echolocation. Therefore, visual stimuli may matter more to echolocating toothed whales than previously assumed, and sensory deprivation can be a major driver of the dive response, possibly as an anti-predator measure.

INTRODUCTION

The cornerstone of the impressive diving capabilities of marine mammals is the dive response, which in its most extreme form conserves blood oxygen for the hypoxia-sensitive brain and heart during prolonged breath-hold dives.¹ This is achieved by peripheral and visceral vasoconstriction, accompanied by a lowered heart rate to maintain steady blood pressure when diving.^{2–4} The tight connection between peripheral vasoconstriction and cardiac output makes heart rate a good proxy for the magnitude of the dive response. Oxygen conservation mechanisms during diving were previously considered parts of a simple on-off reflex,³ but later it was realized that for marine mammals, the intensity of the dive response is highly dynamic, depending on dive duration, depth, and exercise.^{5–10} Dive response plasticity is beneficial for marine mammals: for long dives, a strong dive response is needed to maintain the aerobic function of the brain and heart; however, for short dives, a mild dive response maintains some blood flow to muscles and organs, allowing for digestion while submerged and less use of myoglobin-bound oxygen stores in muscles, which leads to minimal recovery time at the surface.^{6,11,12}

Previous studies have identified that paranasal and blowhole wetting is an important driver to the onset of the dive response,^{2,13–15} and that colder water can initiate a stronger dive response.^{7,16} It has also been shown that cetaceans have some volitional control that can override peripheral vasoconstriction and bradycardia.^{12,13,17} This suggests a complex interplay between apnea, volitional planning, sensory feedback from facial and blowhole wetting, and direct cognitive control of the dive response. A previous study by McDonald et al (2018)⁸ noted a stronger decrease in heart rate when two captive harbor porpoises had eye cups placed over their eyes during simulated prey capture events. Harbor porpoises are known to have relatively good vision,^{18,19} but the degree to which it is used and for what is poorly understood. Consequently, animals may be more conservative with their oxygen usage when exposed to a more limited flow of sensory information (e.g., visual deprivation), but it is also possible that the observed cardiac response may stem from the increased drag of suction cups placed on the head.

To address if the dive response indeed is affected by sensory deprivation, we here studied the heart rate and sensory performance of a trained, echolocating harbor porpoise in a target discrimination task, where it was possible to make it challenging for the animal to either echolocate or use vision during active target selection. Specifically, we tested the hypothesis that sensory deprivation leads to a stronger dive response to conserve

¹Zoophysiology, Department of Biology, Aarhus University, Building 1131, C. F. Møllers Allé 3, DK-8000 Aarhus C, Denmark

²Department of Biology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark

³Fjord&Bælt, Margrethes Plads 1, 5300 Kerteminde, Denmark

⁴Lead contact

*Correspondence: peter.madsen@bio.au.dk
<https://doi.org/10.1016/j.isci.2023.106204>



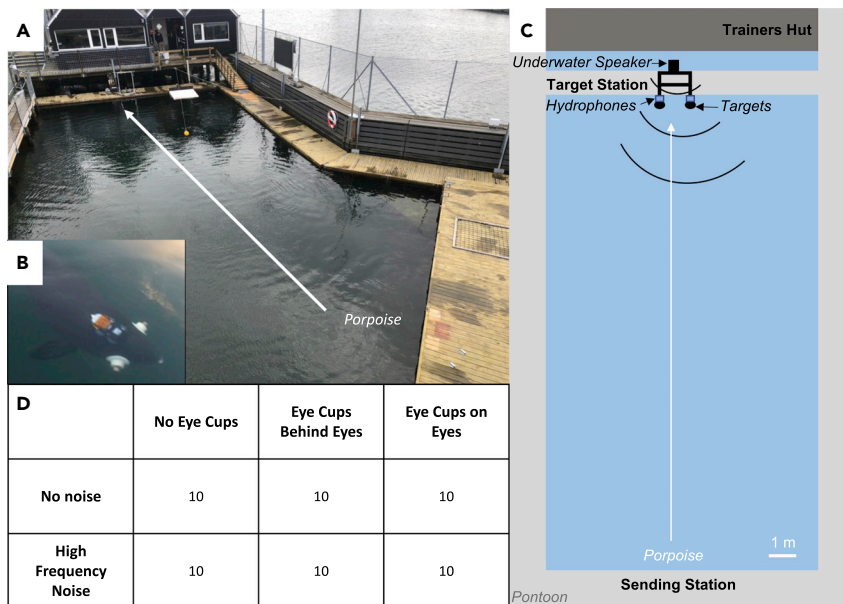


Figure 1. Experimental setup

(A) balcony view (B) porpoise with DTAG and eyecups on (C) schematic view (D) matrix of number of treatment conditions.

oxygen for a potentially longer dive, and that deprivation of what is considered its primary sense, echolocation, leads to a stronger response than deprivation of what is considered its secondary modality, vision.

RESULTS

Reduced diving heart rate when blindfolded

A total of 60 trials were collected: 10 sends for each treatment condition (Figure 1 and Table 1). The percentage of correct target selection was high, ranging from 90 to 100% correct selection during most treatment conditions regardless of noise. Success decreased slightly (80%) for trials with eye cups on both with and without noise (Table 1). Median diving f_H varied between 50 and 56 bpm in trials with no eye cups and eye cups behind the eyes, both with and without high-frequency noise (Table 1). However, median diving f_H significantly decreased, reaching 24 and 26 bpm, when eye cups were on the eyes during no noise and high-frequency noise, respectively ($\alpha = -28.9$ (CI -32.8 to -25.0), $p = <0.001$; Table 1, Figures 2, 3A and Table S1). Diving f_H during high-frequency noise trials did not significantly differ from no noise trials ($\alpha = 0.02$ (CI -3.1 – 3.1), $p = 0.992$; Figures 2, 3A, and Table S1).

Indicators of increased challenge when blindfolded and acoustically masked

Freja used a median of 20 scans during control conditions (no noise, no eye cups) that significantly increased to 24 when eyecups were on the eyes both with and without noise ($\alpha = 0.37$ (CI 0.2–0.5), $p = <0.001$; Figure 3B and Table S1). However, the total number of scans significantly decreased to 14 or 11, when high-frequency noise was played and eyecups were not placed on the eyes (no eyecups and eyecups behind eyes) ($\alpha = -0.25$ (CI -0.4 to -0.1), $p = <0.001$; Figure 3B and Table S1). The median click source level was 182 dB re 1 μ Pa during control treatments and significantly increased to 191 dB during noise trials for all eyecup conditions ($\alpha = 8.8$ (CI 7.4–10.1), $p = <0.001$; Figure 3C and Table S1). The duration of sends ranged from 18 to 22 s and did not significantly differ between most conditions (Tables 1 and S1). However, trials with eye cups on and no noise were significantly longer ($\alpha = 2.1$ (CI 0.5–3.7), $p = 0.010$; Figure 3D and Table S1). Median buzz start ranges for all conditions ranged from 22 to 38 cm and were not significantly different from control trials (Table 1, Figure 3E, and Table S1). The post-hoc analysis of the median percent change in diving f_H compared to median diving f_H following the buzz demonstrated an increase of 5–10% around 3 s following the target touch for all conditions with and without noise, except for those with eye cups on eyes that demonstrated a larger increase of 30–40% (Figure 4A). Minimum specific action (MSA) indicated maximal swimming effort around 0–1 s following the target touch for all treatment conditions (Figure 4B). During high-frequency noise trials, the received noise level

Table 1. Summary heart rate, correct target percentage, number of scans, click source level, duration of send, and buzz range

| Noise level | Treatment | N | Median diving f_H (bpm) | Correct target (%) | Median number of scans | Median Click Source Level (dB re 1 μ Pa) | Median duration of send (s) | Median buzz range (cm) |
|----------------------|----------------------|----|---------------------------|--------------------|------------------------|--|-----------------------------|------------------------|
| No noise | No eye cups | 10 | 53 \pm 12 | 100 | 20 \pm 5 | 182 \pm 4 | 19 \pm 4 | 24 \pm 25 |
| | Eye cups behind eyes | 10 | 50 \pm 12 | 100 | 21 \pm 4 | 182 \pm 6 | 18 \pm 4 | 38 \pm 14 |
| | Eye cups | 10 | 24 \pm 2 | 80 | 24 \pm 7 | 181 \pm 2 | 22 \pm 6 | 30 \pm 11 |
| High frequency noise | No eye cups | 10 | 56 \pm 13 | 100 | 14 \pm 3 | 190 \pm 7 | 19 \pm 3 | 35 \pm 113 |
| | Eye cups behind eyes | 10 | 52 \pm 8 | 90 | 11 \pm 7 | 192 \pm 5 | 19 \pm 3 | 22 \pm 20 |
| | Eye cups | 10 | 26 \pm 6 | 80 | 24 \pm 7 | 189 \pm 4 | 20 \pm 2 | 38 \pm 7 |

Number of sends (N), median across trials of median diving instantaneous heart rate (f_H), correct target percentage, median number of scans on both targets, median click source level, median duration of sends, and median start of buzz range for no noise and high frequency noise with no eye cups, eye cups behind eyes, and eye cups on eyes. Interquartile ranges (IQR) are given for median diving f_H , number of scans, click source level, duration of sends, and buzz range.

consistently increased during the approach (Figure 5C), and the playback noise dominated the frequency spectrum (Figures 5A and 5B). The median echo-to-noise ratio during the approach was 42 dB for control trials, but reduced to values ranging from 8 to 17 dB during noise trials (Figure 6).

DISCUSSION

Marine mammals manage their oxygen use and body nitrogen tensions via the dive response modulated according to exercise, dive duration, and expectation.^{8,10,20} Here we test if the dive response, as assessed by changes in heart rate, may also be modulated by the complexity of the dynamic sensory Umwelt underwater. As a model system to answer that question, we used a trained echolocating harbor porpoise carrying a sound and ECG recording DTAG in an active target discrimination task. Harbor porpoises find and capture prey using echolocation, commonly considered their main sensory modality^{21–24} despite their relatively good vision.^{18,19} As these marine predators are often considered obligate echolocators, we hypothesized that acoustic masking would lead to a stronger dive response compared to visual deprivation.

We reject that hypothesis by showing that sensory deprivation via visual blindfolding caused a significantly stronger dive response than during controls or when echolocation was masked (Table 1 and Figure 3). By also running trials with the eye cups behind the eyes, we show that the increased bradycardia during blind-

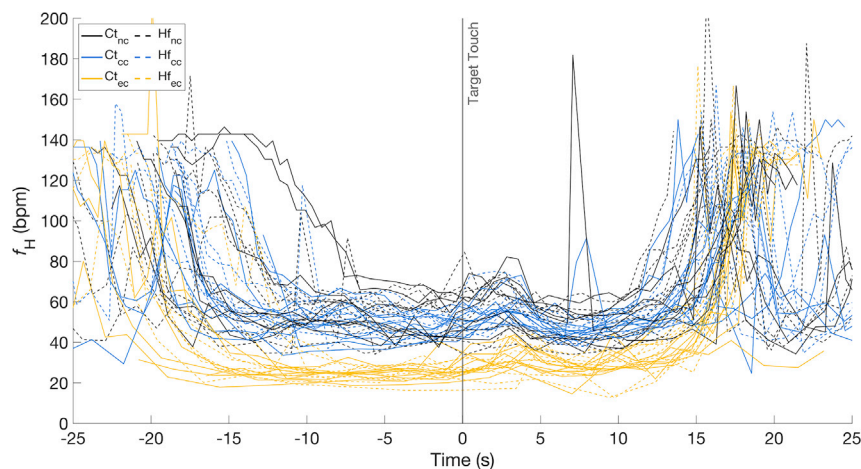


Figure 2. Instantaneous heart rate (f_H , bpm) over time during each dive

The solid vertical line indicates the time when the porpoise touches the target. Different trial conditions are indicated by colored lines, whereas noise conditions are indicated by dashed lines. Different conditions are: Ct_{nc} (no noise, no eye cups), Ct_{cc} (no noise, eye cups behind eyes), Ct_{ec} (no noise, eye cups on eyes), Hf_{nc} (high frequency noise, no eye cups), Hf_{cc} (high frequency noise, eye cups behind eyes), Hf_{ec} (high frequency noise, eye cups on eyes).

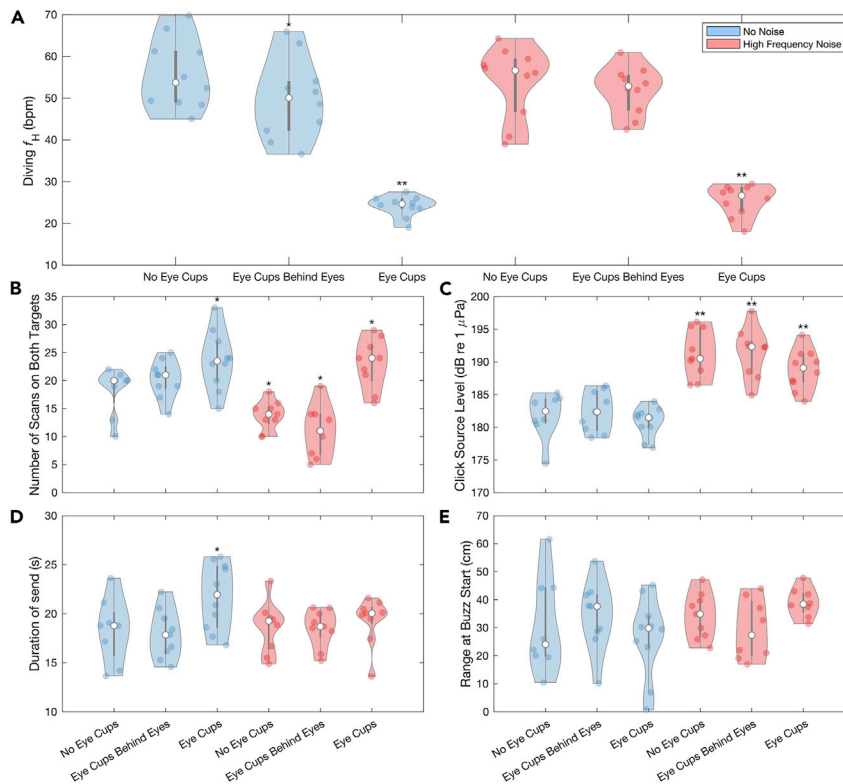


Figure 3. Heart rate and echolocation parameters as a function of sensory deprivation condition

(A) Diving instantaneous heart rate (f_H , bpm), (B) number of scans from porpoise on both correct and alternate target, (C) click source level of scans (dB re $1 \mu\text{Pa}$), (D) duration of send (s) from start of dive until target touch, (E) range from targets at which the buzz begins (cm). Conditions are: no eye cups, eye cups behind eyes, and eye cups on eyes, and noise level is indicated by the color. Violin plots indicate the distribution of values, the white point shows the median value, and the thick line shows the interquartile range. Significant differences from the no noise no eye cups (control) treatment are indicated by * with $p < 0.05$ and ** with $p < 0.001$.

folded trials is not the result of increased drag from the cups, but due to the blindfolding (Table 1 and Figure 3). These results are surprising to us as we predicted that masking of the primary sensory modality, echolocation, would evoke more conservative gas management via a deeper bradycardia than deprivation of what is considered its secondary modality, vision. However, in the blindfolded trials we completely removed visual cues for both the discrimination task and navigation at large, whereas the masking trials only made echolocation more difficult in the direction of the two targets, and not for navigation at large, owing to the highly directional nature of the toothed whale biosonar system.²⁵

In situations where the echolocating porpoise was masked by interfering noise but not blindfolded, the number of performed scans to successfully locate the target was reduced compared to control trials, and the number of scans equally increased when blindfolded or masked and blindfolded (Table 1 and Figure 3). Additionally, the duration of sends was longer for trials with eyecups on and the source levels (SL) of the clicks were higher (Table 1 and Figure 3), demonstrating increased difficulty in the echolocation task with noise. Harbor porpoises have been shown to increase the number of clicks, and thus sampling effort, in detection and discrimination experiments when the task becomes more difficult.^{26,27} However, during auditory masking, the reduction in the number of scans is likely related to Freja relying more on visual inputs when not blindfolded. Despite the reduction in scans, an increased click SL was necessary to solve the task when acoustically masked with and without blindfolding. Thus, despite that masking was successful as parametrized by higher error rates, more scans, longer dives, higher SL in noise, and decreased echo-to-noise ratio (Figures 3 and 6), we found no significant changes to the dive response (Figure 3A).

The stronger dive response during visual deprivation may reveal that, when possible, porpoises may use their eyes for navigation or predator avoidance much more than previously believed, indicating that

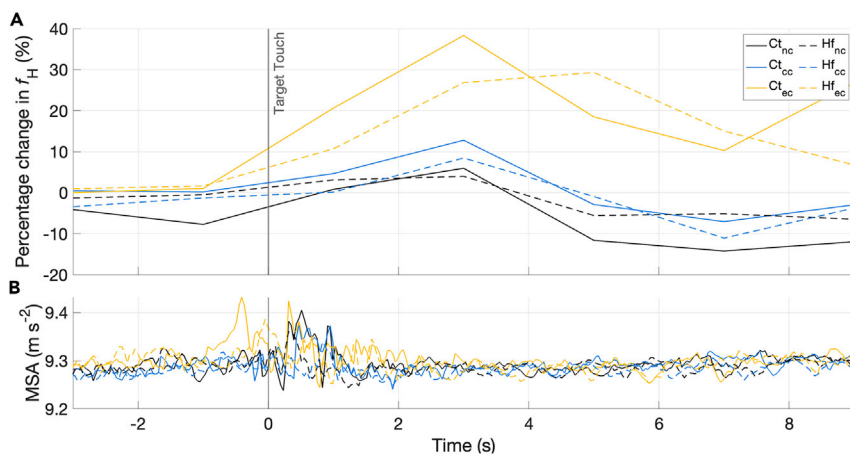


Figure 4. Percentage change in heart rate after target touch with MSA

(A) Median percent change in diving instantaneous heart rate (f_H) after target touch (solid line) compared to median diving heart rate, and (B) median minimum specific action (MSA; $m s^{-2}$) across conditions. Different conditions are: Ct_{nc} (no noise, no eye cups), Ct_{cc} (no noise, eye cups behind eyes), Ct_{ec} (no noise, eye cups on eyes), Hf_{nc} (high frequency noise, no eye cups), Hf_{cc} (high frequency noise, eye cups behind eyes), Hf_{ec} (high frequency noise, eye cups on eyes).

eyesight may be very useful for porpoises and other toothed whales despite their acute biosonar capabilities. We speculate that the stronger dive response when blindfolded could reflect an increased predation sensitivity resulting from facing a smaller information flow from the environment, whereby the animal employs stronger oxygen-conserving measures to potentially allow for prolonged dives as a counter-predation measure. Importantly, the biosonar system supplies ample information directly ahead of the animal in a narrow cone, whereas vision offers a much broader sensory field to detect and respond to predators.

Results from the post-hoc analysis, where we investigated diving f_H upon the completion of the task, revealed that following the target touch, a temporary increase in f_H was detected before returning to pre-buzz diving f_H (Figures 2 and 4). Such an increase in diving f_H could be caused by the increased effort of turning around following target selection. It has been shown that increases in exercise cause a corresponding increase in f_H .^{10,28} However, the peak in MSA, a proxy for swimming effort, and the increase in f_H did not coincide in time (Figure 4). MSA between conditions peaked approximately at the same time, in all cases shortly before the increase in f_H was detected 2 s later (Figure 4). Thus, we posit that increases in exercise may not be the cause of the post-target touch increase in f_H . It has also been shown that many marine mammals incite pre-surface tachycardia in anticipation of surfacing to maximize CO_2 offload and O_2 uptake and minimize time spent at the surface.^{11,28,29} However, this ascent tachycardia is often gradual as ascent begins with a sudden increase in the seconds before surfacing. This increase in f_H found with Freja is likely not due to ascent tachycardia as the increase in f_H following the target touch is very brief, and Freja swam back to the trainer underwater taking between 10 and 15 s before the ascent began. During this return trip to the trainer, f_H returned to pre-target touch values. It has previously been suggested that trained dolphins and belugas will produce a victory squeal following the completion of a task.³⁰ The increase in f_H observed in this study could therefore be an excitement response following a joy reaction to completing the task.

In conclusion, we find that for the same dive duration, task and depth, a marine mammal may evoke very different dive responses depending on the availability of sensory information. With such a strong regulation of gas exchange from visual blindfolding employed as a potential anti-collision or predator avoidance strategy, we demonstrate the importance of visual sensory information for this previously considered obligate echolocator. This link between the regulation of diving physiology and sensory inputs should continue to be studied and compared with other species, including deep divers where this physiological interplay may explain mass strandings in relation to sonar exercises.

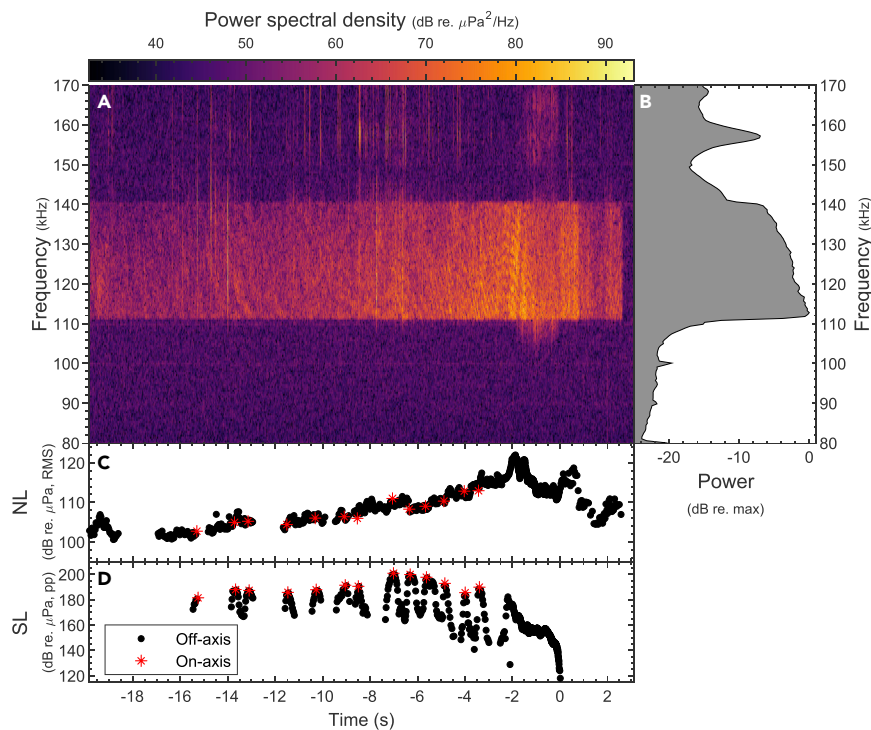


Figure 5. Target approach example for a trial with masking noise in the one third-octave band centered at 125 kHz

(A) Spectrogram of the on-animal recording showing the increase in masking noise as the porpoise approaches the target. The x axis shows time relative to when the porpoise touches the target sphere. Note that the on-animal recording contains considerable click energy well above 150 kHz, which is not observed in recordings made from the targets ahead of the porpoise. Note also that there are no obvious signs of the masking noise level suddenly dropping and increasing, showing that the masking noise was not being shadowed by the target sphere.

(B) Mean power spectrum of the same recording as in (A).

(C) The median noise level measured before each click in ten 1 ms time bins over the 10 ms window preceding each click detection.

(D) The back-calculated peak-to-peak apparent source level (ASL) estimated from the aluminum target recordings by adding $20\log_{10}(\text{range})$ to the received levels. The red asterisks in (C) and (D) indicate on-axis clicks where $\text{ASL} = \text{SL}$.

Limitations of the study

One of the limitations to consider is that the visual and auditory deprivations were not of equal strength. Freja was fully deprived of vision when blindfolded; however, acoustically she could still hear even though she was partially masked in the direction of the targets. It is therefore difficult to directly compare the effect of different types of sensory deprivation; if Freja was fully deprived of hearing, she would perhaps have reacted with a stronger dive response. Another potential limitation is that the conclusion of this study is based on the reaction of dive response to sensory deprivation on a single trained harbor porpoise living long-term in captivity. It is possible that Freja has adjusted to a lifetime of safety in a well-known environment and therefore, she could be less dependent on echolocation compared to visual cues than her wild counterparts when acoustically masked. Nonetheless, Freja has been shown to have maintained the same diel and seasonal variations in clicking activity as wild porpoises.³¹ It is also important to mention that there can be large variations in behavioral and physiological responses to disturbance between individuals in a population and hence, results from this study could be specific to Freja. However, in the previous study by McDonald et al (2018),⁸ Freja was studied along with another porpoise who also displayed a near halving in f_H during blindfolded trials suggesting a consistency in reaction to visual deprivation at least across long-term captive animals.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

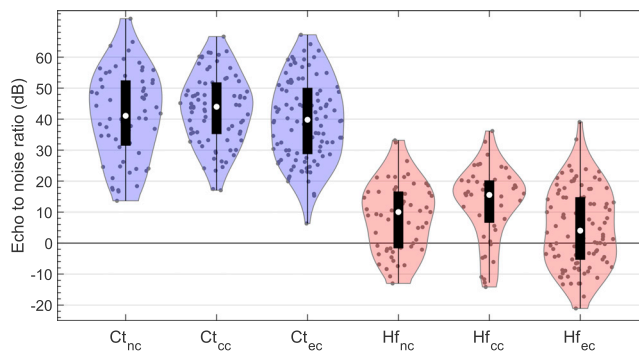


Figure 6. Echo-to-noise ratio (dB) of porpoise clicks that are on-axis relative to the aluminum target across varying conditions

Different conditions are: Ct_{nc} (no noise, no eye cups), Ct_{cc} (no noise, eye cups behind eyes), Ct_{ec} (no noise, eye cups on eyes), Hf_{nc} (high frequency noise, no eye cups), Hf_{cc} (high frequency noise, eye cups behind eyes), Hf_{ec} (high frequency noise, eye cups on eyes).

- [KEY RESOURCES TABLE](#)
- [RESOURCE AVAILABILITY](#)
 - Lead contact
 - Materials availability
 - Data and code availability
- [EXPERIMENTAL MODEL AND SUBJECT DETAILS](#)
 - Research facility and animal
- [METHOD DETAILS](#)
 - Experimental setup and equipment
 - Experimental conditions
- [QUANTIFICATION AND STATISTICAL ANALYSIS](#)
 - Data processing
 - Statistical analysis

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2023.106204>.

ACKNOWLEDGMENTS

We thank J. van der Hoop for bringing the possibility of drag contribution of the cups to our attention, K. Beedholm for creating the program for data collection, the trainers at Fjord&Bælt for all the work with the animals, and the Danish National Research Council (FNU) and the Office of Naval Research award nr. N00014-20-1-2748 for financial support.

AUTHOR CONTRIBUTIONS

Conceptualization, C.B., L.R.D., M.W., P.T.M.; Methodology, C.B., L.R.D., M.L., M.W., P.T.M.; Investigation, C.B., K.A.H.; Formal Analysis, C.B., L.R.D., M.L.; Writing – Original Draft, C.B., L.R.D., P.T.M.; Writing – Review & Editing, C.B., L.R.D., M.L., K.A.H., M.W., P.T.M.; Supervision, L.R.D., P.T.M.; Funding Acquisition, P.T.M.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: July 14, 2022

Revised: November 15, 2022

Accepted: February 10, 2023

Published: February 14, 2023

REFERENCES

1. Davis, R.W. (2014). A review of the multi-level adaptations for maximizing aerobic dive duration in marine mammals: from biochemistry to behavior. *J. Comp. Physiol. B* 184, 23–53. <https://doi.org/10.1007/s00360-013-0782-z>.
2. Panneton, W.M., and Gan, Q. (2020). The mammalian diving response: inroads to its neural control. *Front. Neurosci.* 14, 524. <https://doi.org/10.3389/FNINS.2020.00524>.
3. Scholander, P.F. (1940). *Experimental Investigations on the Respiratory Function in Diving Mammals and Birds*, 22 (Oslo: I kommissjon hos Jacob Dybwad), pp. 1–131.
4. Scholander, P.F. (1963). The master switch of life. *Sci. Am.* 209, 92–106. <https://doi.org/10.1038/SCIENTIFICAMERICAN1263-92>.
5. Hill, R.D., Schneider, R.C., Liggins, G.C., Schuette, A.H., Elliott, R.L., Guppy, M., Hochachka, P.W., Qvist, J., Falke, K.J., and Zapol, W.M. (1987). Heart rate and body temperature during free diving of Weddell seals. *Am. J. Physiol.* 253, R344–R351. <https://doi.org/10.1152/AJPREGU.1987.253.2.R344>.
6. Jobsis, P.D., Ponganis, P.J., and Kooyman, G.L. (2001). Effects of training on forced submersion responses in harbor seals. *J. Exp. Biol.* 204, 3877–3885. <https://doi.org/10.1242/JEB.204.22.3877>.
7. Kaczmarek, J., Reichmuth, C., McDonald, B.I., Kristensen, J.H., Larson, J., Johansson, F., Sullivan, J.L., and Madsen, P.T. (2018). Drivers of the dive response in pinnipeds; apnea, submergence or temperature? *J. Exp. Biol.* 221, jeb176545. <https://doi.org/10.1242/jeb.176545>.
8. McDonald, B.I., Johnson, M., and Madsen, P.T. (2018). Dive heart rate in harbour porpoises is influenced by exercise and expectations. *J. Exp. Biol.* 221, jeb168740. <https://doi.org/10.1242/jeb.168740>.
9. Williams, T.M., Blackwell, S.B., Richter, B., Sinding, M.H.S., and Heide-Jørgensen, M.P. (2017). Paradoxical escape responses by narwhals (*Monodon monoceros*). *Science* 358, 1328–1331. <https://doi.org/10.1126/SCIENCE.AAO2740>.
10. 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. <https://doi.org/10.1038/ncomms7055>.
11. McDonald, B.I., and Ponganis, P.J. (2014). Deep-diving sea lions exhibit extreme bradycardia in long-duration dives. *J. Exp. Biol.* 217, 1525–1534. <https://doi.org/10.1242/JEB.098558>.
12. Elmegaard, S.L., Johnson, M., Madsen, P.T., and McDonald, B.I. (2016). Cognitive control of heart rate in diving harbor porpoises. *Curr. Biol.* 26, R1175–R1176. <https://doi.org/10.1016/j.cub.2016.10.020>.
13. Elmegaard, S.L., McDonald, B.I., and Madsen, P.T. (2019). Drivers of the dive response in trained harbour porpoises (*Phocoena phocoena*). *J. Exp. Biol.* 222, jeb208637. <https://doi.org/10.1242/jeb.208637>.
14. Dykes, R.W. (1974). Factors related to the dive reflex in harbor seals: sensory contributions from the trigeminal region. *Can. J. Physiol. Pharmacol.* 52, 259–265. <https://doi.org/10.1139/Y74-035>.
15. Drummond, P.C., and Jones, D.R. (1979). The initiation and maintenance of bradycardia in a diving mammal, the muskrat, *Ondatra zibethica*. *J. Physiol.* 290, 253–271. <https://doi.org/10.1113/JPHYSIOL.1979.SP012770>.
16. Schagatay, E., and Holm, B. (1996). Effects of water and ambient air temperatures on human diving bradycardia. *Eur. J. Appl. Physiol. Occup. Physiol.* 73, 1–6. <https://doi.org/10.1007/BF00262802>.
17. Fahlman, A., Miedler, S., Rocho-Levine, J., Jabois, A., Arenarez, J., Marti-Bonmati, L., García-Párraga, D., and Cature, F. (2019). Re-evaluating the significance of the dive response during voluntary surface apnea in the bottlenose dolphin. *Sci. Rep.* 9, 8613. <https://doi.org/10.1038/s41598-019-45064-8>.
18. Mass, A.M., and Supin, A.Y. (2009). Vision. In *Encyclopedia of Marine Mammals*, W.F. Perrin, B. Würsig, and J.G.M. Thewissen, eds. (San Deigo, CA: Academic Press).
19. Kastelein, R.A., Zweypfenning, R.C.V.J., and Spekrijse, H. (1990). Anatomical and histological characteristics of the eyes of a month-old and an adult harbor porpoise (*phocoena phocoena*). *Sens. Abil. Cetaceans*, 463–480. https://doi.org/10.1007/978-1-4899-0858-2_33.
20. Fahlman, A., Cozzi, B., Manley, M., Jabas, S., Malik, M., Blawas, A., and Janik, V.M. (2020). Conditioned variation in heart rate during static breath-holds in the bottlenose dolphin (*Tursiops truncatus*). *Front. Physiol.* 11, 604018. <https://doi.org/10.3389/FPHYS.2020.604018>.
21. Johnson, M., Hickmott, L.S., Aguilar Soto, N., and Madsen, P.T. (2008). Echolocation behaviour adapted to prey in foraging Blainville's beaked whale (*Mesoplodon densirostris*). *Proc. Biol. Sci.* 275, 133–139. <https://doi.org/10.1098/RSPB.2007.1190>.
22. Au, W.W. (1993). *The Sonar of Dolphins* (New York: Springer Verlag).
23. Jensen, F.H., Bejder, L., Wahlberg, M., and Madsen, P.T. (2009). Biosonar adjustments to target range of echolocating bottlenose dolphins (*Tursiops sp.*) in the wild. *J. Exp. Biol.* 212, 1078–1086. <https://doi.org/10.1242/JEB.025619>.
24. Møhl, B., and Andersen, S. (1973). Echolocation: high-frequency component in the click of the Harbour Porpoise (*Phocoena ph. L.*). *J. Acoust. Soc. Am.* 54, 1368–1379. <https://doi.org/10.1121/1.1914435>.
25. Jensen, F.H., Johnson, M., Ladegaard, M., Wisniewska, D.M., and Madsen, P.T. (2018). Narrow acoustic field of view drives frequency scaling in toothed whale biosonar. *Curr. Biol.* 28, 3878–3885.e3. <https://doi.org/10.1016/J.CUB.2018.10.037>.
26. Teilmann, J., Miller, L.A., Kirketerp, T., Kastelein, R.A., Madsen, P.T., Nielsen, B.K., and Au, W.W. (2002). Characteristics of echolocation signals used by a harbour porpoise (*Phocoena phocoena*) in a target detection experiment. *Aquat. Mamm.* 28, 275–284.
27. Kastelein, R.A., Verlaan, M., and Jennings, N. (2008). Number and duration of echolocation click trains produced by a harbor porpoise (*Phocoena phocoena*) in relation to target and performance. *J. Acoust. Soc. Am.* 124, 40–43. <https://doi.org/10.1121/1.2924132>.
28. Noren, S.R., Kendall, T., Cuccurullo, V., and Williams, T.M. (2012). The dive response redefined: underwater behavior influences cardiac variability in freely diving dolphins. *J. Exp. Biol.* 215, 2735–2741. <https://doi.org/10.1242/JEB.069583>.
29. Thompson, D., and Fedak, M.A. (1993). Cardiac responses of grey seals during diving at sea. *J. Exp. Biol.* 174, 139–154. <https://doi.org/10.1242/JEB.174.1.139>.
30. Ridgway, S.H., Moore, P.W., Carder, D.A., and Romano, T.A. (2014). Forward shift of feeding buzz components of dolphins and belugas during associative learning reveals a likely connection to reward expectation, pleasure and brain dopamine activation. *J. Exp. Biol.* 217, 2910–2919. <https://doi.org/10.1242/JEB.100511>.
31. Osiecka, A.N., Jones, O., and Wahlberg, M. (2020). The diel pattern in harbour porpoise clicking behaviour is not a response to prey activity. *Sci. Rep.* 10, 14876. <https://doi.org/10.1038/s41598-020-71957-0>.
32. Schusterman, R.J. (1980). Behavioral methodology in echolocation by marine mammals. *Anim. Sonar Syst.* 11–41. https://doi.org/10.1007/978-1-4684-7254-7_2.
33. Wisniewska, D.M., Johnson, M., Beedholm, K., Wahlberg, M., and Madsen, P.T. (2012). Acoustic gaze adjustments during active target selection in echolocating porpoises. *J. Exp. Biol.* 215, 4358–4373. <https://doi.org/10.1242/JEB.074013>.
34. Johnson, M.P., and Tyack, P.L. (2003). A digital acoustic recording tag for measuring the response of wild marine mammals to sound. *IEEE J. Oceanic Eng.* 28, 3–12. <https://doi.org/10.1109/JOE.2002.808212>.

35. Kyhn, L.A., Tougaard, J., Beedholm, K., Jensen, F.H., Ashe, E., Williams, R., and Madsen, P.T. (2013). Clicking in a killer whale habitat: narrow-band, high-frequency biosonar clicks of harbour porpoise (*phocoena phocoena*) and dall's porpoise (*Phocoenoides dalli*). *PLoS One* 8, e63763. <https://doi.org/10.1371/JOURNAL.PONE.0063763>.
36. Kastelein, R.A., Hoek, L., de Jong, C.A.F., and Wensveen, P.J. (2010). The effect of signal duration on the underwater detection thresholds of a harbor porpoise (*Phocoena phocoena*) for single frequency-modulated tonal signals between 0.25 and 160 kHz. *J. Acoust. Soc. Am.* 128, 3211–3222. <https://doi.org/10.1121/1.3493435>.
37. Ladegaard, M., and Madsen, P.T. (2019). Context-dependent biosonar adjustments during active target approaches in echolocating harbour porpoises. *J. Exp. Biol.* 222, jeb206169. <https://doi.org/10.1242/JEB.206169>.
38. Wenz, G.M. (1962). Acoustic ambient noise in the ocean: spectra and sources. *J. Acoust. Soc. Am.* 34, 1936–1956. <https://doi.org/10.1121/1.1909155>.
39. Mellen, R.H. (1952). The thermal-noise limit in the detection of underwater acoustic signals. *J. Acoust. Soc. Am.* 24, 478–480. <https://doi.org/10.1121/1.1906924>.
40. Kastelein, R.A., Janssen, M., Verboom, W.C., and de Haan, D. (2005). Receiving beam patterns in the horizontal plane of a harbor porpoise (*Phocoena phocoena*). *J. Acoust. Soc. Am.* 118, 1172–1179. <https://doi.org/10.1121/1.1945565>.
41. Simon, M., Johnson, M., and Madsen, P.T. (2012). Keeping momentum with a mouthful of water: behavior and kinematics of humpback whale lunge feeding. *J. Exp. Biol.* 215, 3786–3798. <https://doi.org/10.1242/JEB.071092>.

STAR★METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|--|---------------------|--|
| Deposited data | | |
| Raw data and MATLAB code | This paper | Mendeley Data https://doi.org/10.17632/8nb2fzwcns.1 |
| Experimental models: Organisms/strains | | |
| <i>Phocoena phocoena</i> | Fjord&Bælt, Denmark | N/A |
| Software and algorithms | | |
| MATLAB | MathWorks v. R2021b | The MathWorks, Inc., Natick, MA, USA |

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Peter Teglberg Madsen (peter.madsen@bio.au.dk).

Materials availability

This study did not generate new materials.

Data and code availability

All original data and code have been deposited with Mendeley Data and are publicly available as of the date of publication. DOIs are listed in the [key resources table](#). Any additional information required to re-analyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Research facility and animal

Experiments were conducted at Fjord&Bælt in Kerteminde, Denmark, from February to April 2022. During the experiments, a trained adult harbor porpoise (*Phocoena phocoena*), Freja (27-year-old female, 70 kg) was housed in a 20 × 11 m semi-natural research pool ([Figure 1](#)). Freja is a long-term captive animal experienced in performing trained research experiments with no known health issues to influence the cardiac and respiratory responses to external stimuli. Freja was trained using operant conditioning and positive reinforcement: Desired behavioral responses were marked by a whistle (conditioned reinforcer), and a subsequent reward of freshly thawed herring, capelin, or sprat. Animal diets were not constrained for experimental purposes. The data collection at Fjord&Bælt was conducted under approval by the IACUC of Aarhus University #3412.

METHOD DETAILS

Experimental setup and equipment

Freja was presented with a two-alternative forced-choice task,³² where she was rewarded for selecting a standard target (aluminum sphere, target strength of −39 dB) against an alternative target, either a more challenging stainless-steel target (target strength −37 dB), or a slightly easier brass target (target strength −36 dB), in both cases placed 1 m from the standard target.³³ Both targets were 50.8 mm (diameter) solid spheres attached via lines and hooks to a metal frame that was lowered into the water to allow for easy target repositioning. Two calibrated custom-built cylindrical hydrophones (sensitivity −212 dB re 1V/1 μPa) were placed 4 cm above the target centers, continuously recorded sound during trials (sampling rate 500 kHz, 16 bits, flat frequency response of ± 2 dB between 100 and 160 kHz). High frequency noise was played from a B&K 8105 hydrophone lowered 1 m into the water and placed 1 m behind the targets. Freja was free-swimming and carried a multisensor tag attached dorsally behind the blowhole with suction cups ([Figure 1B](#)). The tag, either an ECG-DTAG3 or ECG-DTAG4,^{8,34} recorded ECG data (sampled at 500 Hz),

and sound (sampled at 500 kHz for DTAG3 or 576 kHz for DTAG4), with 16-bit resolution, a clip level of 189 dB re 1 μ Pa (DTAG3) and 178 dB re 1 μ Pa (DTAG4) and a flat (± 3 dB) frequency response from 80 to 150 kHz. The tag also recorded depth, magnetic field, and acceleration, which were commonly down-sampled to 25 Hz.

Experimental conditions

Six different possible combinations of conditions were measured to determine the effects of auditory masking or visual deprivation on the dive response. The trials combined three alternative conditions for visual deprivation: 1) no eye cups (control), 2) eye cups 10 cm behind the eyes (to control for the effect of having cups attached to the body causing drag), and 3) opaque silicone eye cups on the eyes; and two different conditions for acoustic masking: 1) no noise (control), and 2) high-frequency noise (one-third-octave band filtered noise centered at 125 kHz with maximum received levels of 125 dB re 1 μ Pa). The 125 kHz noise band was used to offer maximum masking of echolocation, as it fully overlaps with the frequencies and bandwidth of porpoise biosonar clicks,^{24,35} and the received masking noise exposure levels were between 40 and 80 dB above porpoise hearing thresholds.³⁶ These conditions are summarized, and the setup demonstrated in Figure 1.

Trials began with a stainless-steel alternate target and high frequency noise with received levels of 125 dB re 1 μ Pa (rms) at the targets. After 15 trials, in an attempt to increase the masking of the porpoise clicks, the noise level was increased to 135 dB re 1 μ Pa. However Freja became non-cooperative, at which point the high-frequency noise level was returned to 125 dB re 1 μ Pa, and the steel target was replaced with a brass target, a slighter easier echolocating task.³³ These settings were used for the rest of the data collection, and none of the 135 dB noise trials were included in the final analysis.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data processing

All data were processed in MATLAB v. R2021b (The MathWorks, Inc., Natick, MA, USA) using custom-written scripts and the animal tags toolbox (www.animaltags.org). ECG data were filtered and downsampled to 250 Hz to reduce noise. A supervised heartbeat peak detector was used to process ECG data. Automatic peak detections were manually checked to identify potential false and missed beat detections. Instantaneous heart rate (f_H) was calculated as 60 s divided by the time difference between two neighboring heart beats. The f_H was assigned to the time of the second beat. Less than 1% of ECG data had low signal-to-noise ratio, and were removed from further analysis. The median diving f_H per send was established by taking the median f_H across the 10 s before Freja touched a target. The median f_H was used rather than the mean as the median removes any outliers in the ECG data either due to incorrect peak detection, low signal-to-noise ratio or sudden increases in heart rate.

To determine the effects of both noise and the presence of eye cups on the difficulty in solving the task, and on the effort that Freja put into completing the task acoustically and behaviorally, we measured 1) number of on-axis scans of the biosonar beam across the targets, used as a proxy to determine the number of times Freja acoustically examined each target before making her final choice, 2) click source level, 3) trial duration, and 4) target range at buzz start. Porpoise clicks were detected in the sound recordings of both the DTAG and the target hydrophones using a supervised click detector. Sound recordings from both devices were synchronized using an estimated offset and matching the same click from both the DTAG and target recording using the inter-click interval (ICI) as described in Ladegaard & Madsen, 2019.³⁷ On-axis scans were determined by selecting the click with the highest target received level (RL) within a click sequence of increasing and decreasing RL (sensu Ladegaard & Madsen, 2019).³⁷ 1) The number of scans was taken as the sum of on-axis scans in the aluminum and alternate (steel/brass) sphere recording. 2) Click source level was back-calculated by adding $20\log_{10}(\text{range})$ to the received levels of the on-axis clicks, quantified as peak-to-peak (pp), on the aluminum target. 3) Trial duration was estimated as the time that Freja dove below the water until the time that she touched the target. 4) Buzz range was determined by selecting the beginning of the buzz (i.e., series of rapid clicks with ICI < 10 ms, sensu Ladegaard & Madsen, 2019)³⁷ and determining the corresponding animal-to-target range. The animal-to-target range was measured using the time delay between the same click recorded on the DTAG and the hydrophone, assuming a sound speed of 1500 m/s (see Ladegaard & Madsen, 2019³⁷ for details).

To address the level of biosonar masking and assess task difficulty, the echo-to-noise ratio (ENR) was estimated relative to the aluminum target. First, the recordings were band-pass filtered (sixth order filter) at the one-third octave band centered at 125 kHz. The echo level (EL) received at the porpoise was then estimated from the target recordings by subtracting a transmission loss estimate of $20\log_{10}(\text{range})$ from the RMS RL at the aluminum target and adding a TS of -39 dB. The RMS RL was computed within the click duration defined by the -10 dB points on either side of the peak of the amplitude envelope. The masking noise level (NL) was estimated from the on-animal recordings by dividing the 10 ms window prior to each click detection into ten 1 ms bins, then computing the RMS level for each bin, and then reporting the median value of those. For approach clicks, where ICI exceeds 10 ms, this NL estimation approach served to estimate only continuous noise while excluding occasional transients such as surface and bottom reflections and clicks from conspecifics. In trials without masking noise playback, the recorders were limited by self-noise in the 125 kHz band. We therefore opted for estimating ambient NL within the 125 kHz one-third octave band based on thermal noise,^{38,39} which amounted to an estimated isotropic NL of 72 dB re μPa . From this NL estimate we then subtracted 11.7 dB to correct for the high-frequency hearing directivity of the porpoise hearing system.⁴⁰ In trials with playbacks of masking noise, we did not correct NL estimates for hearing directivity as the masking noise was directly in the sonar beam of the approaching porpoise.

A post-hoc analysis revealed that f_H after Freja touched the target slightly increase during a short period of time while still diving (Figure 2). To analyze the percent change in f_H after the buzz, we binned data into 2 s from 4 s before to 10 s after the start of the buzz, took the difference from the median diving f_H between subsequent bins and converted to percent increase. The median of each bin was taken across sends for each condition. To assess the association of increase in f_H following the buzz to exercise, minimum specific acceleration (MSA, m s^{-2})⁴¹; a proxy for swimming effort) was determined from the three axis acceleration data recorded by the DTAG. Median MSA was taken for each point across all sends for each condition for 4 s before and 10 s after the start of the buzz.

Statistical analysis

The effects of visual deprivation or masking of the echolocation on the dive response were assessed by examining the association between median f_H and deprivation condition. Data was collected over 12 days with 4 - 6 sends per day. We used Generalized Linear Mixed-Models (*fitlme* or *fitglme* functions) to account for the dependent nature of data coming from the same day; all models included f_H as an independent variable, date as a random intercept and deprivation condition (i.e., visual deprivation and acoustic masking) as a fixed effect. The interaction between the two conditions was examined; however, no significance was found and hence the interaction was excluded from the models. In addition, we used the same model structure to investigate the effect of the conditions on the send duration, number of scans on both targets, buzz range, and click source level. The response variable was modeled using a Gaussian function for all variables except number of scans on both targets, where a Poisson (link = log) was used, as the response variable represented a count. The assumptions of normality and homoscedasticity of residuals were checked and satisfied for all models. The threshold for statistical significance was set to 0.05. Results are reported by an estimate (alpha, in the unit of each parameter), its 95% confidence interval (CI) and a p value (p).