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BRIEF REPORT

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Report on the brain of the monk seal (*Monachus monachus*, Hermann, 1779)

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Abstract

The Mediterranean monk seal (*Monachus monachus*, Hermann, 1779) is an endangered species of pinniped endemic to few areas of the Mediterranean Sea. Extensive hunting and poaching over the last two centuries have rendered it a rare sight, scattered mainly in the Aegean Sea and the western coast of North Africa. In a rare event, a female monk seal calf stranded and died in southern Italy (Brindisi, Puglia). During due necropsy, the brain was extracted and fixed. The present report is the first of a monk seal brain. The features reported are remarkably typical of a true seal brain, with some specific characteristics. The brain cortical circonvolutions, main fissures and the external parts are described, and an EQ was calculated. Overall, this carnivore adapted to aquatic life shares some aspects of its neuroanatomy and physiology with other seemingly distant aquatic mammals.

KEYWORDS

anatomy, brain, californianus, histology, *Monachus monachus*, neuroanatomy, *Zalophus californianus*

1 | INTRODUCTION

The Mediterranean monk seal (*Monachus monachus*, Hermann, 1779) is a phocid belonging to genus *Monachus*. Before 2014 the genus *Monachus* also included two additional species, now classified into the germane genus *Neomonachus* (Jemison, 2014; Scheel et al., 2014), that is, the Hawaiian monk seal *Neomomachus schauinslandi* (Matschie, 1905) and the Caribbean monk seal *N. tropicalis* (Grey, 1850), the latter presumed extinct.

The Mediterranean monk seal is an endangered species included in the IUCN Red list (https://www.iucnredlist.org/species/13653/117647375#population; Karamenlidis & Dendrinos, 2015) counting fewer than 700 surviving individuals in sparse sub-populations that inhabit the Aegean and Ionian Sea coasts (the most consistent group), the coasts of Mauritania and the Portuguese island of Madeira. Once abundant in the Thyrrenian and Adriatic Seas, its presence in these waters is now limited to a few individuals along the Croatian coastline. Recent rare sightings indicate a possible sporadic return to the coasts of Southern and insular Italy, where the species was formerly common (Fioravanti et al., 2020; Nicolaou et al., 2021).

Studies on the anatomy and physiology of this rare species are mostly limited to skeletal remains, museum specimens and remote data collection (Dendrinos et al., 2007; Karamanlidis et al., 2021; Salman et al., 2001; Samaranch & Gonzalez, 2000). It has, however, been described centuries ago by various naturalists and authors from the Mediterranean basin, including Aristotle (Johnson, 2004).

In January 2020, a female monk seal calf stranded alive on the shoreline close to Brindisi in Puglia, Italy (Mazzariol et al., 2021). The calf subsequently died despite emergency veterinary medical support. A necropsy was performed within 12 h *post-mortem*. The body was in good conservation status. This note reports some key facts about the morphology of the brain. The brain of a California sea lion (*Zalophus californianus* Lesson, 1828) was also considered for comparison and reference. The Californian sea lion is one of the best studied pinniped to date, especially regarding the brain (Cook et al., 2018, 2021; Fish, 1898, 1899; Howell, 1928).

2 | MATERIALS AND METHODS

2.1 | Animals and brains

On 26 January 2020, a very young female monk seal pup was spotted swimming close to the coast not far from Brindisi, Puglia, Italy. The animal later stranded and subsequently died notwithstanding veterinary medical aid. Measures indicated a young individual of 22.5 kg, with a total length of 118 cm. The body weight is compatible with a newborn specimen (Jefferson et al., 2015).

A necropsy was performed and revealed a co-infection by Cetacean morbillivirus and *Toxoplasma gondii* that affected several internal organs (Mazzariol et al., 2021; Petrella et al., 2021).

The brain was extracted from the skull using an oscillating surgical saw and its fresh weight recorded. The brain was then immersed in 4% buffered formalin for preservation. Photographs were taken immediately after sampling and then again 2 months after formalin fixation.

The brain showed no apparent macroscopic damage or lesion at first. However, slicing for fixation and subsequent microscopic pathological examination revealed multifocal brain haemorrhages and non-suppurative meningoencephalitis.

A serial sampling of the cortex was performed for various pathological and anatomical motives and immersed immediately after necropsy in formalin or fresh frozen, depending on the end purpose (bacteriological, viral, contaminant or DNA later investigations). Anatomical blocks were fixed in formalin and processed for paraffin embedding 4 weeks after fixation, embedded in paraffin and microtome-cut at 8 μm thickness and mounted on glass slides. Mounted sections were coloured for Nissl bodies, using 2% cresyl violet. Briefly, sections were dewaxed in xylene baths, then hydrated in decreasing alcohol series, then in distilled water. Sections were stained in a 2% cresyl violet bath for 3 minutes, then quenched in tap water. Then, sections were passed in an ascending series of alcohols for dehydration, and subsequently in xylene baths. Coverslip glass was mounted using mounting medium.

This article refers mainly to the monk seal, the brain of an adult male California sea lion (*Zalophus californianus*) is described only as a reference. The pinniped died in a marine theme park of causes unrelated to the nervous system. Its body was refrigerated and then transported to the facilities of the Department of Comparative Biomedicine and Food Science of the University of Padova for *post-mortem* examination. Death was attributed to pulmonary lesions, and the brain was not affected. The interval between death and brain removal was approximately 12 h. After removal from the skull the brain was photographed and fixed by immersion in buffered formalin.

2.2 | Encephalization quotient

The weight of the brain was related to body weight to obtain the encephalization quotient (EQ), calculated with the formula EQ = Ei/0.12P2/3, where Ei and P are the mean weights of the brain and body, respectively (Jerison, 1973). We maintained the value of the exponent (2/3 or 0.67) originally indicated by Jerison (1973), although we are aware of alternative values for the slope (for review see Boddy et al., 2012; for details see also Cozzi et al., 2014; Minervini et al., 2016; Graïc et al., 2017).

3 | RESULTS

The brains of the monk seal (Figures 1 and 2) was well preserved and externally showed no apparent macroscopic sign of traumatic lesion. The brain haemorrhages and meningoencephalitis that affected the animal were evident upon dissection, but did not alter the outer surface of the encephalon or distorted its outline and shape (see Mazzariol et al., 2021 Figure 1).

The fresh weight of the brain was 342.4 g. The calculated monk seal brain volume, based on weight, was 327.4 cm^3, applying the density of 1.046 g/cm^3 (standard conversion factor for human brain tissue). The overall shape of the brain was round, somewhat shorter rostro-caudally (approx. 105 mm) than that of the sea lion and wider laterally (approx. 100 mm; Figures 1–3).

The external aspects of the brain showed no sign of immaturity. On the dorsal side, the anterior frontal pole of the isocortex formed a strong curvature in the monk seal. The external surface of the telencephalon revealed intense gyrification in the anterior part. The rostral position of the cruciate sulcus observed in the monk seal is present also in the California sea lion, although on a more flat frontal pole (Figure 3). This feature is typical of carnivores and even more so in pinnipeds as reported in the literature (Fish, 1896; Hoeksema et al., 2021; Howell, 1928; Langworthy et al., 1938; Rioch, 1937). The longitudinal direction of the sulci was particularly evident in the temporal and occipital lobes of the monk seal. These were markedly different in the brain of the California sea lion, whose gyrification was more intense and not as markedly longitudinal. The pseudosylvian fissure was marked and deep, relatively anterior, vertical and reached the ventral surface of the brain in both species. The suprasylvian sulci on both sides were convoluted. In particular, the endolateral and the lateral sulci of the monk seal were quite straight and extensive.

The pineal gland, placed just rostral to the two large anterior colliculi, was globous and related anteriorly to the well evident *striae*

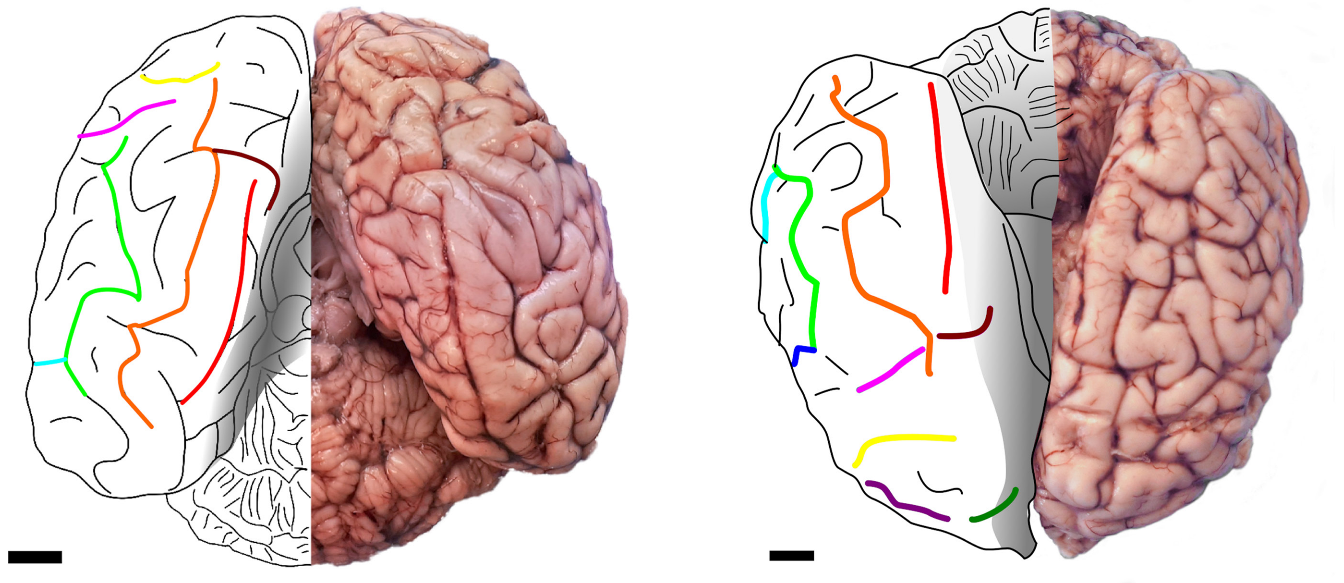

FIGURE 1 Dorso-caudal and dorso-rostral view of the monk seal brain (*Monachus monachus*). The gyrification is redrawn on the left side to illustrate the sulci patterns. In green: the suprasylvian sulcus; orange: lateral sulcus; red: entolateral sulcus; brown: splenial sulcus; fuchsia: ansate sulcus; yellow: coronal sulcus; light blue: post-sylvian sulcus; dark blue: pre-sylvian sulcus; dark green: cruciate sulcus; dark purple: cruciate sulcus. Bar: 1 cm.

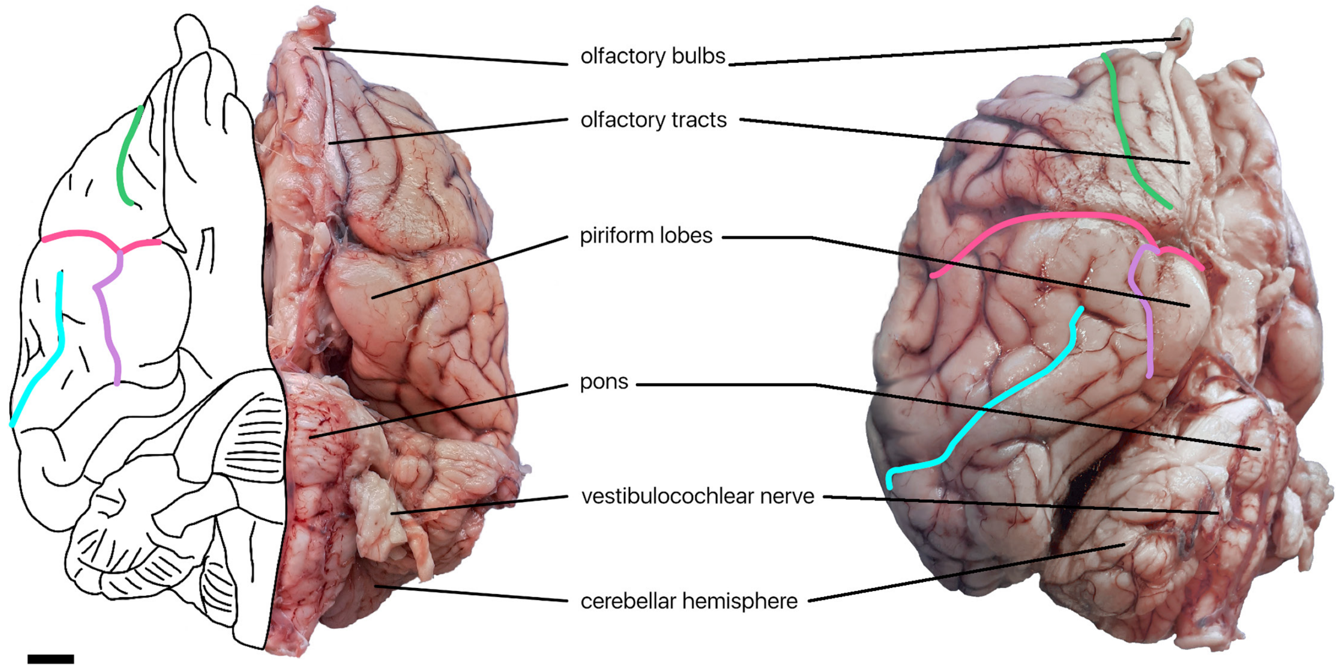

FIGURE 2 Ventral and lateral view of the monk seal brain (*Monachus monachus*). The left side is redrawn to illustrate the main features of the brain. In water green: the orbital sulcus; in pink: the pseudosylvian sulcus; in purple: the rhinal sulcus; in light blue: post-sylvian sulcus. Bar: 1 cm.

medullaris (=*habenularis*) thalami. The *corpus callosum* was without particularity.

The ventral side of the brain showed relatively small olfactory bulbs and tracts for a carnivore (Figure 2) and a well-developed piriform lobe. The rhinal fissure was relatively short. The orbital sulcus was lateral to the olfactory tract, running diagonally. The wide cerebellum, partially covered by the occipital lobes of the cerebrum, showed clear subdivisions of the folia and prominence of the vermis over the lateral lobes. These latter were nonetheless relatively large and almost bent in posterior direction.

The pons was developed and convex, with the basilar artery clearly visible along the midsagittal plane. The pyramids and the inferior olivary nuclei were clearly recognizable on the ventral surface of the brainstem. The origin of the left trigeminal nerve and part

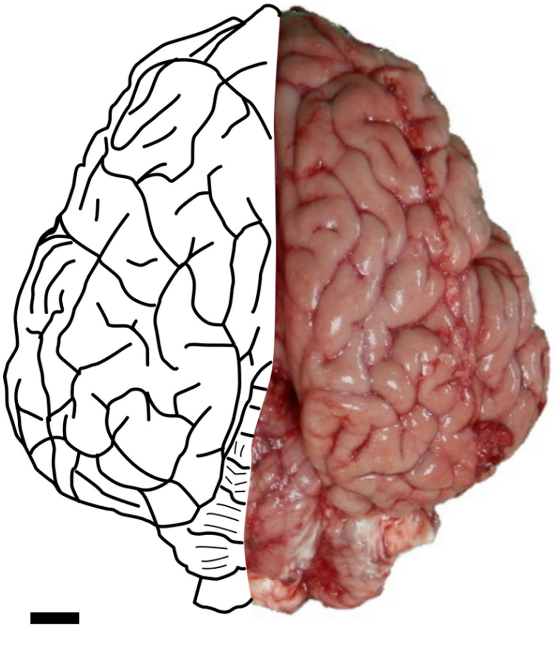

FIGURE 3 Dorsal and ventral view of the brain of the California sea lion (*Zalophus californianus*). The left side is redrawn. Bar: 1 cm.

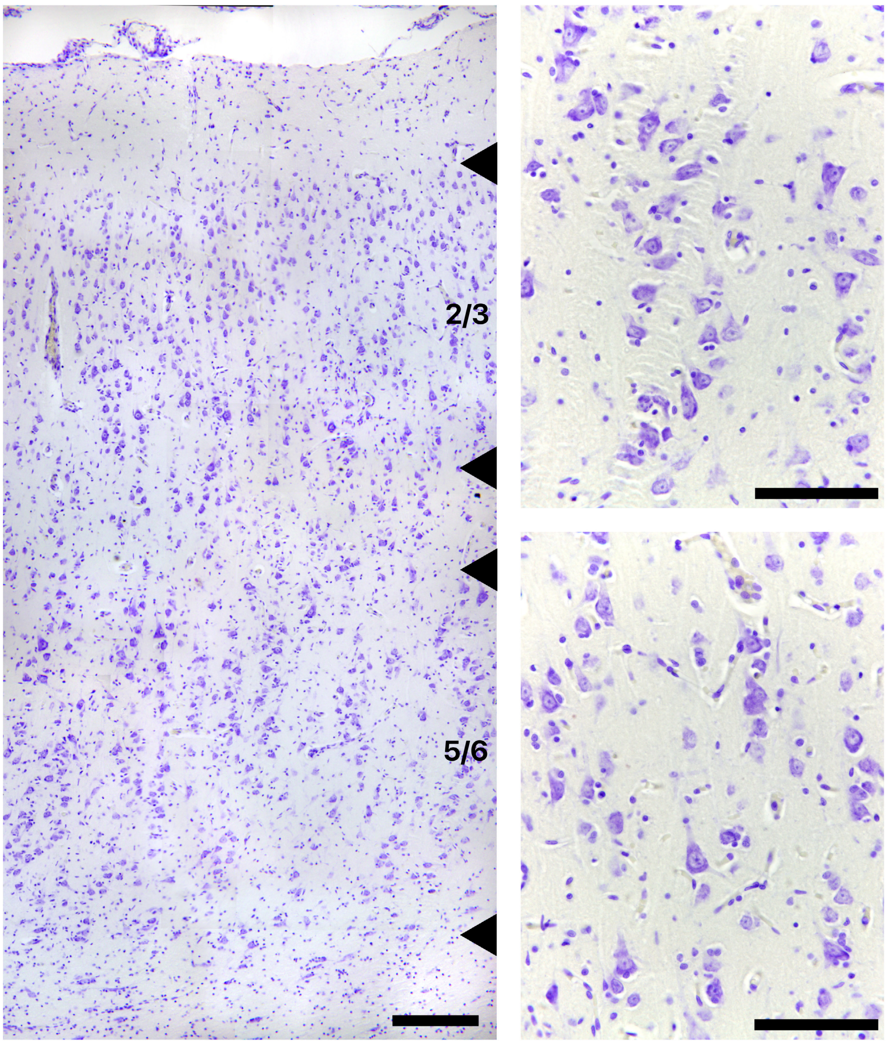

FIGURE 4 Microphotograph of the cerebral cortex of *Monachus monachus*. On the left, the whole cortical column. Top right, Layer 3. Bottom right, Layer 5. Nissl staining; the bar on the left is 200 µm, the bars on the right are 100 µm.

of the relative ganglion were well preserved, and their size resulted conspicuous in respect to adjacent structures. The *crus cerebri* remained hidden under what seemed to be a slight ventral flexure cranial to the pons.

The EQ of this very young individual specimen was 3.46.

Histologically, the lamination (Figure 4) form the parietal cortex amounted to approximatively 2.5 mm. Below the rather thin molecular layer, the second layer (external granular) is hardly discernible from the external pyramidal layer. The absence of large pyramidal neurons in the upper third of the layer 2/3 suggests that the second layer is relatively thick compared to the rest of the cortical column. The lower two-thirds of layer 2/3 are populated by smaller and medium to large neurons. While the smaller neurons are similarly arranged to the second layer, the medium to large neurons are quite conspicuously lined in columns interspersed by neuropil space. These arrays are slightly irregular and some collide partially or completely (see upper right Figure 4). Between layer 2/3 and the inner pyramidal layer seems to appear a space populated only by smaller neurons. This strip, corresponding to layer 4 is thin and irregular in its borders with layer 2/3 and layer 5/6. Below still lies a large layer, at least as thick as layer 2/3, comprising medium to large neurons, seemingly partially lined in columns by pial space. In the lower third, the neurons are sparser and tend to gradually get sparser towards the white matter, making the limit between the two difficult to pinpoint.

4 | DISCUSSION

To the best of our knowledge, the brain of the monk seal has never been described before, and the only existing documentation concerns the extinct species *Monachus tropicalis* (Fish, 1898, 1899).

The mass of the brain is apparently close to the average of what previously calculated based on the volume of the brain cavity of a relatively large cohort of young and adults of the same species and Mediterranean habitat (Mo, 2005; Mo et al., 2009). In these latter studies, the volume of the cranial cavity measured in several museum monk seal specimens (Mo, 2005; Mo et al., 2009) was comprised between 225 and 540 cm^3. Consequently, the brain mass and volume that we recorded here is consistent with previous data based on skull measurements. However, the body mass of this young (newborn) specimen (22.5 kg) is much smaller of that of adults of the same species, that may reach 240–300 kg in females and up to 350–400 kg in males (Boitani et al., 2003; Jefferson et al., 2015). The EQ of 3.46 reported here theoretically indicates that this monk seal possessed a brain larger than expected (>1) for an animal of its size (body mass). However, as our specimen is a newborn in thin body condition, the EQ that we obtained has little general value for the species, but is only indicative of the early development of the brain of the species, as in many other mammals, relative to their body at birth. This is possibly related to the need for a relatively immediate motor coordination and orientation to be present at birth, although the Mediterranean monk seals is known to breed inland in remote caves (Gilmartin and Forcada, 2009).

Macroscopic observations indicate that the shape of the brain is remarkably similar to that of other Pinnipeds with some differences from *Carnivora*, for the general pattern of the gyri and marked piriform lobe (Hoeksema et al., 2021; Montie et al., 2009; Walløe et al., 2010). There are although, remarkable differences in the shape of the cortex between the California sea lion and the Mediterranean monk seal (Figures 1–3). We emphasize that we were able to analyse only one juvenile specimen of Monk seal, and that the comparison with the California sea lion reported here is meant only for general orientation and reference to a more described pinniped neuroanatomy. The large temporal development, or rather, the narrow frontal pole of the cortex in the seal may show a differential development of the parietal lobes, or may be the product of a different skull architecture. Unlike in the California sea lion, where the frontal part of the brain resembled more that of other carnivores, with an elongated frontal pole (Figures 1–3), the frontal pole was rather round and with comparatively reduced olfactory bulbs. We note that, on the whole, the presence of the olfactory bulbs and related archicortical structures suggests a role of the olfaction in general orientation, foraging and social behaviour. The notable development of the rostral colliculi also suggests a certain importance of the visual system, which is coherent with previous data (Schusterman & Balliet, 1970). The presence of a well-developed pineal gland in a newborn individual supports previous studies that describes this condition as typical of fetal life and the early afterbirth period Aarseth and Stokkan (2003). The noteworthy size of the trigeminal nerve is consistent with a key role of the vibrissae in environmental perception and hunting (for review see Gant & Goss, 2021). The importance of the somatosensory and, within it, of the cortex dedicated to the vibrissae, is likely considerable, given their importance in other pinnipeds. This is stark contrast to some other aquatic mammals (Gerussi et al., 2021). The macroscopic aspect of the brain does not allow for a precise localization, but it will likely be next to the primary motor cortex (Figure 1).

In the cerebellum of the monk seal, the vermis is prominent and convoluted over the lateral lobes, as it is the case in several terrestrial mammals including carnivores. Its relative size is also consistent with that of other carnivores. We also note that most terrestrial mammals display the same cerebellar anatomy, except for primates and elephants (Larsell & Jansen, 1970; Voogd, 1998; see also below). It is now well known that the lateral lobes of the cerebellum are involved in a number of neural processes, including cognitive functions (Bernard et al., 2015; Bernard & Seidler, 2013). We therefore cannot infer any exceptional development in the monk seal compared to other carnivores.

Intense gyrification is related to the development of the cerebral cortex and the pattern observed in other aquatic mammals such as delphinids and whales compared to terrestrial artiodactyls, is not unlike that of the monk seal compared to some terrestrial carnivores of similar brain size. A pronounced curve of the frontal pole is also notable (Cozzi et al., 2017; Huggenberger et al., 2019). There stops the parallel, living in the same medium might have driven the brain development in seemingly convergent ways; however, the genetic background of the seal places it quite clearly among carnivores. On the other hand, in the cerebellum of cetaceans, and especially in that of members of the family *Delphinidae*, the relatively straight vermis is surrounded by two lateral lobes whose volume is only comparable to that of humans and elephants, comparatively much larger than in the monk seal (Cozzi et al., 2001; Cozzi et al., 2017; Flanigan, 1972; Huggenberger et al., 2019; Jansen & Jansen, 1969; Morgane & Jacobs, 1972; Ridgway et al., 2019).

At the microscopic level, the cortex of the monk seal showed a typical mammalian lamination, although a precise delimitation between the layer 2 and 3 and then 5 and 6 was far from obvious. This feature is not unprecedented and may vary in function of the cortical area (Brodmann, 1909). In most cases along the cortex, a suggestion of a fourth layer was found, which is coherent with their phylogeny, as compared to cetaceans, closer to terrestrial artiodactyls which also generally lack or hide a proper layer 4 (Graïc et al., 2021, 2022). This has direct implications on the circuitry underlying the cognitive performance potential of the animal, rather typical of carnivore predators (Ratcliffe et al., 2006; Shultz & Finlayson, 2010).

The physiology of the monk seal, including the changes related to diving, are unknown. Recent evidences from other pinnipeds suggest peripheral vasoconstriction and specific perfusion during breath-holding dives (Hindle et al., 2019), and—in general—adaptive biological mechanisms to accommodate for prolonged and potential deep diving. Our data does not allow any speculation in this sense. However, we emphasize that some of the macroscopic features of the brain of the monk seal suggest an intermediate adaptation to life in the water, with well-developed temporal lobes (as in cetaceans where the acoustic sense prevails) and well-built vibrissae for orientation in the liquid medium, maintaining a persistent olfactory system (as in many terrestrial mammals and pinnipeds).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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