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Aversive Response of Grey (*Halichoerus grypus*) and Harbour (*Phoca vitulina*) Seals Exposed to Camphor: A New Approach to Keep Seals Away from Sensitive Areas?

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Pinnipeds are semi-aquatic mammals that face a constantly changing environment as they move from land to water. Such an amphibious lifestyle has required specific physiological adaptations—for example, in their sensory systems: visual, acoustic, tactile, and chemical (olfactory and gustatory) modes. Their gustatory system has not been studied in detail, so little is known about their chemoreception abilities. Still, some information is available; in comparison to terrestrial mammals, pinnipeds have a reduced number of taste buds, suggesting a limited sense of taste (Kastelein et al., 1997; Yoshimura & Kobayashi, 1997). The ability to detect acidic and salty solutions has been demonstrated in Steller sea lions (*Eumetopias jubatus*; Kuznetsov, 1982) and California sea lions (*Zalophus californianus*; Friedl et al., 1990), and both species did not respond to sweet tastes. Indeed, the TAS1R2 and TAS1R3 genes encoding the sweet taste receptors are not functional, at least in several species of pinnipeds (Jiang et al., 2012; Wolsan & Sato, 2020), including nine species of phocids and six species of otariids (Wolsan & Sato, 2020). The umami taste receptors were also found to be pseudogenized (Jiang et al., 2012; Wolsan & Sato, 2020). Although the gustatory abilities of pinnipeds appear limited, a high sensitivity to slight differences of salt concentration has been demonstrated in harbour seals (*Phoca vitulina*). As salinity levels represent a potential source of information for orientation in marine environments, sensitivity to salt could be involved in fine-scale underwater movements (Sticken & Dehnhardt, 2000).

Pinnipeds also have a generally reduced olfactory apparatus in comparison with their terrestrial relatives (Harrison & Kooyman, 1968; Van Valkenburgh et al., 2011; Bird et al., 2020). Both peripheral (Kuzin & Sobolevsky, 1976) and central (Harrison & Kooyman, 1968) olfactory structures are present, and much more prominently so in Otariidae compared to the Phocidae and Odobenidae (Harrison & Kooyman, 1968; Reynolds & Rommel, 1999). Pinnipeds employ odours in different social interactions (Lowell & Flanigan, 1980; Insley et al., 2003), especially in mother–pup recognition (Pitcher et al., 2011) as part of a multimodal process that includes vocalizations and visual cues. However, mother Australian sea lions (*Neophoca palatina*) are able to recognize their pups based solely on scent (Pitcher et al., 2011). Several studies in captivity have shown that South African fur seals (*Arctocephalus pusillus*) can differentiate artificial odours (Laska et al., 2008, 2010). Captive California sea lions were also able to discriminate between different odours (social and non-social odours), both in the air and underwater (Brochon et al., 2021). In phocids, behavioural experiments conducted on harbour seals demonstrated that they were able to respond to familiar and unfamiliar odour (fish and eucalyptus, respectively) and were highly sensitive to dimethyl sulphide, a chemical compound released in productive marine areas (Kowalewsky et al., 2006). Furthermore, genetic evidence indicated that pinnipeds still retain large numbers of functional olfactory receptor genes, although the number is lower than in their related terrestrial mammals (Liu et al., 2019).

Despite the scant available literature on chemoreception in pinnipeds, it appears that all studies so far have focused on few species (mainly otariids) among the 34 extant species. This is probably explained by the availability of the studied species in human care and/or their better accessibility in the wild. In phocids, data on chemosensory perception are only available for harbour seals (Sticken & Dehnhardt, 2000; Kowalewsky et al., 2006).

A preliminary study on phocid abilities to perceive and behaviourally react to chemicals was started, focusing on species with very little data available such as grey seals (*Halichoerus grypus*). Similar trials were conducted in similar environments and at the same time with harbour seals. Comparing these two sympatric species that share similar diets (Brown et al., 2012) but display different patterns of social interactions should provide clues about whether their chemical sensory perception(s) are the same or not.

During the development phase of this project, several chemical compounds were tested as well as different methods of presentation. Observations were made on one adult male grey seal at the University of Southern Denmark's Marine Biological Research Center and one adult female harbour seal at Fjord&Bælt (Kerteminde, Denmark). All seals were born in human care. All individuals had a long history in training for various research projects but had never before experienced olfactory trials. In one trial, one drop of organic, pure camphor essential oil (Thibène, France) was directly deposited onto a sterile cotton gauze (Mercurochrome, Paris, France) and presented to the male grey seal (see Supplemental Video 1; the supplemental video for this paper is available in the "Supplemental Material" section of the *Aquatic Mammals* website: https://www.aquaticmammalsjournal.org/index.php?option=com_content&view=article&id=10&Itemid=147) and the female harbour seal (data not shown). Similar experiments were carried out, but the cotton gauze with camphor was inserted into an iron tea ball infuser or in a plastic box with holes to avoid any direct contact with the odour source. All trials were conducted during daily training sessions in which the individuals were asked to touch their nose to a target stick.

Camphor is a naturally occurring compound extracted from the wood of a camphor laurel tree (*Cinnamomum camphora*). It is widely used in human health as a nasal decongestant and cough suppressant (Burrow et al., 1983), and also as a topical analgesic (Burkhart & Burkhart, 2003).

When camphor was presented directly on a cotton gauze, the grey seal started to behaviourally react as soon as the bottle of camphor essential oil was opened by shaking his head with an open mouth while the experimenter was standing a few meters away. Head shaking could be regarded as a response to aversive or disturbing stimuli as observed in birds after being exposed to deterrent food (Skelhorn & Rowe, 2009) or noxious odours (Burne & Rogers, 1996). An even stronger aversive behaviour was monitored when the seal was closer to the chemical source (Supplemental Video 1). The animal moved back, chewed, and refused the primary reinforcement (i.e., fish). The vacuum "chewing" behaviour (i.e., chewing with nothing in the mouth) has been described in horses as a possible displacement activity performed in stressful situations (Scopa et al., 2018). The trial was then immediately ended to avoid stressing the animal further. The same experiment was carried out with a female harbour seal (data not shown). When the camphor was presented for the first time, her spontaneous behaviour was slightly different compared with the male grey seal: the harbour seal chewed several times but did not move away or shake her head.

After these initial responses, new testing was done two days later, with the cotton pad soaked with camphor inserted into an iron tea ball diffuser to avoid seal whiskers or the nose from touching the compound directly. In this set-up, the male grey seal still displayed aversive behaviour when exposed to the camphor stimulus, but it was less intense and only repeated mouth openings were recorded (data not shown). However, a new behaviour was documented as the male started to vocalize just after the removal of the diffuser. Camphor was also presented to the harbour seal, and her chewing behaviour was again observed; however, after seven close approaches to the camphor, this seal moved back and spontaneously dove into the pool. Trials using camphor were then stopped in agreement with the trainers to not stress these seals nor impact their usual training, which is based on positive reinforcement. Interestingly, no aversive behaviour was observed when grey and harbour seals were exposed to another unfamiliar chemical (lavender essential oil) suggesting that the observed responses to camphor were probably not a neophobic reaction. Camphor appears then to be a possible repellent compound for these two phocids, or at least for these two individual animals. In a recent study on odour discrimination in captive California sea lions, camphor was included in the different chemical stimuli following our suggestion (Brochon et al., 2021). In this otariid, camphor was not a powerful repellent by itself, but it had a negative effect when paired with an attractive food odour. Indeed, the animals displayed a reduced response to a fish odour when a camphor odour was added (Brochon et al., 2021).

To our knowledge, camphor has been shown to act as a repellent in at least two species of mammal: (1) snowshoe hares (*Lepus americanus*; Sinclair et al., 1988) and (2) common voles (*Microtus arvalis*; Schlötelburg et al., 2019). Camphor is also a known repellent in many insects such as anopheles (Asadollahi et al., 2019). Although the scent of camphor is mediated by odorant receptors (Sicard, 1985), camphor also has other, less understood, sensory properties; for example, camphor was able to potentiate the perceived intensity of both hot and cold stimuli when applied on hairy skin (Green, 1990). Interestingly, camphor has been shown to interact with several transient receptor potential (TRP) ion channels in mammals (Moqrich et al., 2005) such as TRPV3. Mammalian TRP genes are involved in trigeminal nociception and in an animal's ability to detect their environment through thermosensation, mechanosensation, and gustation (Clapham, 2003; Montell, 2005). Several members of the TRPV subfamily (V1 to V4), as well as TRPM8 and ankyrin-repeat TRP 1 (TRPA1), are important in temperature detection (thermoTRPs) (Patapoutian et al., 2003). All thermos-TRP channels are apparently also chemosensitive, potentially enabling these channels to detect multiple sensory modalities. For example, TRPV1 is stimulated by capsaicin, TRPM8 is sensitive to menthol, and TRPA1 can be activated by mustard and cinnamon oil (Patapoutian et al., 2003). The slight "burning" sensation of camphor application to the skin (Green, 1990) is, therefore, consistent with its activation of TRPV3 (Moqrich et al., 2005).

The observed strong aversive behaviour by these two seals in a direct presentation of camphor could be related to a repellent feature of the compound itself or via activation of some TRP channels, possibly through contact with the whiskers and nose skin. Indeed, several TRP channels have been shown to be present in the whisker pad skin of the rodent TRPV1 channel in trigeminal ganglions (Shinoda et al., 2011; Ando et al., 2020). Since pinnipeds have 10 times more nerve endings around their vibrissal follicles than terrestrial mammals (Marshall et al., 2006; Hyvärinen et al., 2009), it cannot be excluded that contact with pure camphor essential oil could have triggered a strong and noxious trigeminal excitation. When the camphor was presented to seals using a plastic box with holes or an iron tea ball diffuser, the grey seal behaviour was more moderate; this could be explained by the container diffusing the odour, by potential habituation to the camphor, or by a less effective stimulation of trigeminal neurons.

Overall, the female harbour seal's reaction to camphor appeared less pronounced compared to the grey seal, but it cannot be ruled out, given a sample size of two animals, whether sensitivity to camphor might have been related to sex. Also, a species effect is not possible to rule out. Unfortunately, it was not possible to further investigate the reaction to camphor by replicating the trials and involving more individuals due to potential stress on the seals.

Considering the previous findings, the use of camphor as a natural chemical deterrent of seals in sensitive areas seems both achievable and reasonably adaptable given the easy production of camphor. Moreover, camphor is highly volatile and has been detected up to 800 m from its source (Müller et al., 2004). Ballard Locks in Salmon Bay (Seattle, USA) is, for example, a sensitive area as the locks create a migration bottleneck for salmon returning to their spawning grounds, enhancing predation by seals in this area. Acoustic deterrent devices have been widely used to prevent pinniped predation (reviewed in Götz & Janik, 2013), but several concerns have been raised, including lack of long-term efficiency and possible hearing damage to animals (Findlay et al., 2021). The combined use of acoustic and chemical stimuli may offer a solution by decreasing sound exposure and potentially limiting the habituation of seals.

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