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The role of two anatomically separate olfactory bulbs in shark food odor tracking

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THE ROLE OF TWO ANATOMICALLY SEPARATE OLFACTORY BULBS IN SHARK FOOD ODOR TRACKING

by

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SHARK FOOD ODOR TRACKING

ADRIENNE LOHE

ABSTRACT

Most sharks have well-developed olfactory systems and depend to a large degree on odor information to locate food, home and navigate, and possibly detect predators and mates. The aim of this investigation is to determine the behavioral function of two paired bilateral olfactory bulbs in the smooth dogfish shark, *Mustelus canis*. The paired olfactory bulbs are a rare and unique feature among elasmobranchs and are absent in bony fishes. Given that the olfactory system of bony fishes contains lateral and medial nerve bundles with behavioral functions in feeding and social behavior respectively, we hypothesize that sharks have an elaborate functional division in which the medial bulb is processing social odors and the lateral bulb food odors. This functional division would parallel the division into an olfactory and an accessory olfactory system, also known as the vomeronasal organ or Jacobson’s organ, which evolved in tetrapods. Our study is based on the behavioral effects of selective transection of the two olfactory tracts to reveal how the brain is processing input from two anatomically distinct olfactory systems. The results show that animals with lateral tract transections showed impaired ability to track a food odor plume while those with medial transections showed no change. Attempts to identify a reliable social odor (pheromone) were not successful, preventing us from determining the deficits expected from medial tract lesions.
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List of Abbreviations

BU........................................................................Boston University
MRC..................................................Marine Biological Laboratory Marine Resource Center
MOS.....................................................................Main Olfactory System
OB........................................................................Olfactory Bulb
OE........................................................................Olfactory Epithelium
OR........................................................................Olfactory Receptor
ORN.....................................................................Olfactory Receptor Neuron
TL........................................................................Total Length
V1R/V2R..............................................................Vomeronasal Receptors 1 and 2
VNS......................................................................Vomeronasal System
WHOI..............................................................Woods Hole Oceanographic Institution
Introduction

Often referred to as “swimming noses,” sharks have impressive olfactory abilities reflected in the surface area of the olfactory epithelium as well as the size of the olfactory bulbs (Meredith and Kajiura, 2010). Odor information serves to identify prey items and motivate food search behavior. In this capacity it is a critical information channel for all sharks and indispensible for some species (Gardiner and Atema, 2014). Because odor itself is non-directional and its dispersal unpredictable in space and time, other senses such as flow detection by the lateral line combine with chemoreception to guide food search behavior along odor plumes (Gardiner and Atema, 2007; Gardiner and Atema, 2014). To make order of chaotic intermittent plumes when tracking, sharks use bilateral odor arrival time differences to turn toward the side stimulated first rather than the side with a higher concentration of odor. In this way it decreases its chances of steering out of the plume and enhances connection with the plume (Gardiner and Atema, 2010). The degree to which a shark relies on olfaction to locate food or mates varies by ecological niche, and functional pressures drive differences in olfactory morphology and capabilities (Schussel et al., 2008; Yopak et al., 2014). Here we isolate the shark’s use of chemoreception to locate a food source in order to examine a unique olfactory anatomy in the context of the separate vomeronasal and olfactory systems seen in higher vertebrates.

*M. canis* and several other elasmobranch species, including lemon sharks (*Negaprion brevirostris*), Atlantic sharp-nose sharks (*Rhizoprionodon terraenovae*), broadnose
sevengill sharks (*Notorynchus cepedianus*) and blue sharks (*Prionace glauca*) have two distinct, anatomically separated olfactory bulbs on either side of the head (Meredith et al., 2013; Northcutt, 1978). These two olfactory bulbs innervate two distinct sides of the large nasal epithelium. Medial and lateral olfactory bulbs are completely segregated and have separate olfactory tracts fusing into a single tract leading to the forebrain (Figure 1). This feature is not universal in sharks and is not known to be present in skates or rays (Northcutt, 1978).

Studies have confirmed a somatotopic organization of olfactory bulbs in elasmobranchs, where olfactory receptor neurons (ORNs) in the medial half of the olfactory epithelium (OE) project immediately to the medial half of the olfactory bulb (OB) and ORNs in the lateral OE project to the lateral half of the OB (Meredith et al., 2013). It is still unknown, however, what role the two anatomically separate bulbs play in elasmobranchs such as *M. canis*. In 2013, Meredith et al. put forth two not mutually exclusive hypotheses to explain this unique morphology: somatotopic versus chemotopic organization. In a somatotopic organization each bulb would receive input from the adjacent portion of a functionally uniform olfactory epithelium. In a chemotopic organization the two bulbs would be processing information from distinct classes of ORNs that might be spread throughout the epithelium (Meredith et al., 2013). A third possibility is that the OE is also functionally segregated, leading to two anatomically and functionally segregated neural pathways. In teleost fishes, functionally different ORNs are scattered through the OE and project each to their own glomerulus in an anatomically single OB (Hamdani and Døving, 2007).
Higher vertebrate classes including most amphibians, reptiles and mammals have evolved separate olfactory and vomeronasal systems, the latter documented to detect intraspecific pheromones (Grus and Zhang, 2006). Though it is often assumed that the vomeronasal system processes pheromones exclusively, it can also respond to some non-pheromone stimuli such as prey odor and environmental odors, while the main olfactory system can respond to pheromones (Baxi et al., 2006). The overlap between the two odor detecting systems is not yet completely understood.

The two chemosensory systems seen in tetrapods have sensory epithelia in differentially located organs and are characterized by receptors of different gene families. Receptor neurons in the olfactory epithelium express receptors of the olfactory receptor (OR) gene family while receptor neurons in the vomeronasal epithelium express receptors of the gene family vomeronasal receptors 1 and 2 (V1R and V2R) (Ferrando et al., 2009). Different types of G-protein alpha subunits are associated with each receptor gene family. ORs are coupled to subunit $G_{\text{olf}}$, V1Rs to $G_{\text{i}}$, and V2Rs to $G_{\text{o}}$ (or $G_{\text{aq}}$ in fish) (Ferrando and Gallus, 2013; Gonzalez et al., 2010). These receptor types have been further linked to receptor neuron cell type; vomeronasal receptors are generally microvillar cells while olfactory receptor cells are commonly ciliated (Gonzalez et al., 2010). Based on these connections, it is likely that in the olfactory and vomeronasal systems of tetrapods, the location, morphology, receptor gene family and G-protein alpha subunit of a receptor can all be linked and each can indicate to which olfactory system it belongs (Ferrando et al., 2009).
Teleosts have a single olfactory bulb on each side of the head adjacent to olfactory epithelium and connected to the forebrain by an olfactory tract. Despite this single bulb anatomy, there is evidence that the lateral portion of the olfactory tract serves a feeding behavior function while the medial portion of the olfactory tract is involved in social behaviors (Eisthen and Polese, 2006). In fact, each olfactory tract was found to have 3 distinct nerve bundles that activated different responses: stimulation of the medial part of the medial nerve bundle caused an alarm response, the lateral part of the medial nerve bundle induced reproductive behavior, and the lateral nerve bundle mediated feeding behaviors (Hamdani and Døving, 2007). Though lacking the morphological components of a true vomeronasal system including a vomeronasal organ, vomeronasal epithelium and accessory olfactory bulb, teleosts show VNS-specific genes and a separation of nerve bundles for VNS- and MOS-type signals (Grus and Zhang, 2006). This suggests that a VNS-specific transduction pathway existed in the common ancestor of tetrapods and teleosts.

VNS-specific genes have also been identified in agnatha and chondrichthyes, both of which arose before the split between sarcopterygians (which gave rise to tetrapods) and actinopterygians, including teleosts (Grus and Zhang, 2009; Hamdani and Døving, 2007). The genome of the jawless sea lamprey contains V1Rs as well as Trpc2, a calcium channel protein functioning in vomeronasal signal transduction (Grus and Zhang, 2009).
The genome of the elephant shark *Callorhinichus milii* contains V1Rs, Trpc2, and V2Rs (Grus and Zhang, 2009).

Sharks with dual olfactory bulb anatomy have physically separated organs of yet unknown function, which could serve as a VNS and MOS, though not developed to the degree seen in tetrapods. Furthermore, lungfishes, the closest living relative to tetrapods, possess all components of an accessory olfactory system fully comparable to the vomeronasal system in higher vertebrates (Gonzalez et al., 2010). These data suggest that the VNS may have evolutionary origins in the most primitive jawless fish, with a more developed system in close relatives of tetrapods such as lungfish. Though vomeronasal-specific genes have been identified in sharks, their expression in medial versus lateral bulbs has yet to be explored.

In this research we study for the first time if the dual olfactory bulb system of certain sharks may represent a functionally segregated chemical sensing pathway similar to the olfactory and vomeronasal system of tetrapods. We examined *M. canis* behavior before and after a lesion of either a lateral or medial olfactory tract to determine the role of each in food odor tracking. *M. canis* is an excellent model for testing the function of this dual olfactory system based on some detailed knowledge of its odor tracking behavior and its demonstrated adaptability to testing in captivity.
Methods

Experimental Design

Sharks were behaviorally tested for tracking response to food odor in a series of four treatments: control, unilateral nose plug, post-plug control, and post-surgical lesion. The experimental cycle spanned 5 days and allowed for maintained feeding motivation as well as adequate recovery time from handling and surgery.

On day one, sharks were control tested for response to food odor in the flume. If sharks were judged to behave normally in this initial control test, they continued in the trial. The few that did not were kept another week to acclimate to lab conditions and control tested the following week. Normal behavior was defined by successful tracking of a food odor to its source, locating and eating the squid piece located at the source, and repeating this reliably for the majority of trials. This indicated that the shark was motivated to eat, comfortable in the flume setting, and providing reliable data.

After initial controls, sharks were tested on day two for response to food odor in a unilateral nose-plug treatment. Nose plug trials, first used by Sheldon and Parker in 1913, form a complete physical block at the entrance of the nose and prevent any chemoreception from taking place. Animals with a unilateral nose plug receive chemosensory information from only one side of the head, and we hypothesized that this
effect would not be significantly different from the effect of a lateral olfactory tract lesion. The plug was removed after the last test of the day.

On day three of the cycle, sharks were tested again in a normal control trial for response to food odor in the same manner as day one. This control ensured that there were no lingering effects from the nose plug being inserted into the nostril, for instance irritation or infection. The shark’s normal tracking and feeding behavior was critical to continue on in the experiment.

After controls were complete, a surgical lesion of one of the four olfactory tracts (right lateral, right medial, left lateral or left medial) was completed under anesthesia on day four. Animals were allowed to recover for 24 hours prior to behavioral testing.

Following surgery, sharks were behaviorally tested on the fifth day for response to food odor. Any changes in behavior, tracking ability, and feeding success were noted. After tests were completed, animals were euthanized using MS-222 overdose and decapitated; heads were cut into left and right halves to facilitate fixation and both halves were placed in formalin for later fine dissection.

*Animal Collection & Care*
Smooth dogfish sharks (*Mustelus canis*) were purchased from the Marine Biological Laboratory’s Marine Resource Center (MRC) in Woods Hole, Massachusetts. Sharks had been housed at the MRC for up to 3 months after being caught by trawler in Nantucket Sound. While at MRC they were fed a diet of frozen squid (*Loligo pealeii*) and capelin (*Mallotus villosus*). In batches of 5 or fewer individuals per week, sharks were transported to the Shark Flume Lab at Woods Hole Oceanographic Institution (WHOI).

Experiments were conducted under animal care protocol “Tracking food and pheromones: function of olfactory bulbs in sharks, *Mustelus canis*” valid from 5/1/2014 to 4/30/2017 with veterinary oversight by Roxanna Smolowitz DVM and approved as an off-site project by Boston University.

Animals at WHOI were housed in 3-meter diameter soft kiddie pools with up to 5 animals (mixed sex) per pool. Each pool had a thin layer of natural beach-sand lining the bottom so that sharks could rub their skin to remove ectoparasites. The pools had a constant flow of filtered seawater maintained at temperatures between 15° and 20° C. Each pool had two large air stones to constantly oxygenate the water. Temperature, flow, and physical condition of animals (skin condition and breathing rate) were checked daily in each pool. Sharks were fed on alternate days with squid (*L. pealeii*) if they were not being behaviorally tested. During testing cycles, sharks were not fed outside of behavioral experiments in order to maintain feeding motivation. Upon arrival, sharks were sexed, measured (TL) and occasionally tagged for individual identification with
colored T-bar anchor tags through the dorsal fin. Sharks with unique markings, size, or other feature were left untagged.

For behavioral testing, we moved individual animals to the 10-meter flume by net. The flume had a flow rate of approximately 4cm/second at a water height of 40cm. Water temperatures were maintained between 15° and 20° C, equal between pools and flume. Cameras suspended above the flume allowed for video capture and tracking of shark behavior. The shark lab containing both the pools and flume is covered by transparent plastic, allowing for natural light cues.

**Experimental Procedure**

Food odor was created by blending 50g of thawed squid with 1L seawater and then straining solids from the extract. The flume supported two separate parallel plumes introduced by a peristaltic pump at a rate of 25 ml/minute: one with undiluted food odor and the other unflavored seawater (Figure 3). Each plume emerged from a nozzle at the end of clear plastic tubing suspended half a centimeter from the bottom of the flume. The placement of the food odor and the control seawater introduction nozzles alternated from right to left side every other trial to control for possible learning or acclimation to feeding on one side of the flume. Though the food odor was presented in a predictable pattern (right-left-right-left) there was no evidence that the sharks learned this pattern or expected to receive food in a certain spot or flume side.
Sharks were tested individually in 8 trials per daily treatment. An individual was netted and introduced to the flume, where it was given 10 minutes to acclimate to the new surroundings and establish a normal behavior. If an animal was visibly stressed after 10 minutes, additional time was allowed for the animal to start behaving normally as evidenced by slow swimming, slow breathing and/or resting.

The shark was then gently corralled behind a flow-through start gate while the peristaltic pumps were turned on delivering odor and seawater in separate parallel plumes (Figure 3). A small piece of squid was placed beneath the food odor introduction nozzle as a reward for the shark. The uniform flow of the flume, at roughly 4cm/s, carried the food odor plume and seawater plume down to the start gate within 3 minutes. After waiting the 3-minute period, the gate was lifted and the shark became free to move about the flume. Cameras began recording and the trial lasted for 2 minutes. A successful individual tracked the food odor plume to its source and fed, typically within one minute, ignoring the seawater plume altogether. Whether the shark was successful or not, the recording continued for 2 minutes and this procedure was repeated for a total of 4 trials per individual. After four trials were complete, the individual was taken out of the flume and put back in its home pool until its afternoon trials. The next individual animal was then placed into the flume and allowed to acclimate. In all, four 2-minute trials were run for each individual in the morning and repeated in the afternoon, resulting in eight 2-minute
trials per individual per treatment. Trials were run according to this procedure in each treatment.

To create a unilateral nose-plug, two small (1cm x 0.5 cm) balls of cotton were soaked in petroleum jelly. The shark was removed from the water, wrapped in a seawater soaked towel, and held upside-down in tonic immobility. Forceps were used to gently insert one plug in the inflow and the other in the outflow of one nostril of the shark. The shark was then immediately released into the flume for a 10-minute acclimation period before testing. This was sufficient to recover normal swimming and food search behavior. Nose plugs were removed after testing was completed for the day.

Surgeries were completed on the fourth day of experimentation. MS-222 (Table 1) was used to sedate the individual first in induction and then in maintenance concentrations while the short surgery was performed. Two liters of induction anesthesia were placed in a heavy-duty black trash bag. A netted shark was placed head-down into this bag and held in the solution until movement ceased, usually within a minute. The bag allowed a soft and flexible environment for the shark to avoid injury from initial thrashing. The sedated animal was then transferred to a surgical table with maintenance anesthesia delivered orally and moving over the right and left gills. The animal was wrapped in a seawater soaked towel, strapped in place, and monitored for gill movement and pupil size to indicate level of sedation. When needed, seawater or induction MS-222 solution could be added to regulate the sedation level.
Using a scalpel, a small rectangle of skin directly over the olfactory tracts was cut, followed by a deeper cut through the cartilaginous skull. These layers were removed to allow access to the brain. Eye spears were used to drain the wound and to visualize the tracts. An olfactory tract was crushed and severed with two sets of fine forceps under a surgical microscope. When the surgeon was satisfied with the surgery, the wound was filled with a small amount of Neosporin and petroleum jelly, and covered over with a plastic (~7x12mm) skull plate. The animal was returned to the flume for recovery and if needed, given “artificial respiration” by moving it through the water with open mouth to facilitate water flow over the gills. Fish recovered to breathe on their own within 0-3 minutes, and to fully normal behavior within 40 minutes.

Analysis

Surgery: To validate the success of the lesion, formalin-fixed heads were carefully dissected to expose the olfactory tracts and bulbs. Lesion damage was qualitatively described after a visual inspection and lesions were deemed successful if damage was localized to the tract of interest and if the tract was less than ~5% intact (Figure 2).

Tracking behavior: Videos were captured through a lab-specific MatLab code and saved as .AVI files. Each video was randomly assigned a different three-digit code to ensure there would be no bias for the viewer. Blind video analysis was done by hand, and
numbers of right and left turns were scored for each trial. A ‘turn’ was defined as a sudden movement of the animal’s head resulting in a change in the sharks heading. It should be noted that these sharks are constantly undulating their heads right and left as part of normal swimming, but because the shark keeps moving forward in one direction, these movements are not counted as ‘turns’ in this experiment. By overlaying images from video of normal swimming in ImageJ the turn angle of these normal undulating motions was estimated to be within $10^\circ$; sudden turns with an angle greater than $10^\circ$ were scored. A second observer watched a random 10% subset of the videos and counted turns independently. This dataset was compared to the data for the same videos counted by the first observer using a paired t-test. The two datasets were found to be virtually identical, establishing “counting turns” as a reliable method of data analysis ($t=-0.0155$, $p=0.9966$).

Statistics

Left and right turn counts were used to calculate turn bias for each trial. In control conditions one expects an average of 50% left and right turns, varying slightly by individual. Animals with left side treatments (nose plugs as well as surgical lateral tract lesions) were expected to make more right turns, while animals with right side treatments were expected to make more left turns. Bias was calculated as \((\text{turns towards expected side})/\text{(total turns)} \times 100\%\).
An ANOVA was performed on turn bias data using several independent variables including lesion type (lateral or medial), plug and lesion side (left or right), treatment (control, nose plug, post-plug control, lesion), and individual animal. A Student’s t test was used to test for significant differences between each treatment.

Results

Of the 23 individual sharks tested, 4 were eliminated due to incomplete or incorrect surgical lesions. The 19 individuals used in this analysis ranged from 55 to 93cm in total length and were evenly distributed between sexes; 10 of the animals were male and 9 were female (Table 2). Ten were given lateral lesions while 9 were given medial lesions. These were further split into left and right side lesions; 3 left lateral, 7 right lateral, 6 left medial, 3 right medial. Though it would have been preferable to have an even number of each, it was impossible to predict which animals would be eliminated during experimentation for abnormal behavior, lack of feeding motivation, or incomplete surgeries. A one-way ANOVA of turn bias by side showed no significant difference in effect between left and right side lesions (F-ratio=0.38, p=0.54), allowing us to combine the two groups: 10 lateral and 9 medial lesions.

A one-way ANOVA of mean turn bias evaluated the effect of treatment by lesion type. Animals that received a lateral lesion showed significant variance across the four
treatments (F-ratio=17.61, p<0.0001) and a Student’s t test revealed that both the nose plug and lesion treatments had a significant effect on mean turn bias compared to the control and post-plug control. Nose plug and lesion treatments were also significantly different from each other, with the nose plug (57.2%) showing a greater effect on mean turn bias than a lesion (53.2%). Comparatively, animals that received a medial lesion also showed significant variance across the four conditions (F-ratio=8.81, p<0.0001) but in this case, a Student’s t test revealed that only the plug condition was significantly different from the other three treatments; the effect of the plug was strong, resulting in a mean turn bias of 55.0%. Finally and critical for our hypothesis, the medial lesion treatment showed no difference from controls (p=0.5648) (Figure 4).

A separate one-way ANOVA evaluated the treatment effects of lateral versus medial groups. Only in the lesion treatment did animals show a significant difference in mean turn bias between lateral and medial lesions (F-ratio=5.96, p=0.016). Those with lateral lesions had a mean turn bias of 53.24% in the lesion condition, meaning that they turned more towards the (intact) expected side instead of turning towards each side equally. Those with medial lesions had a mean turn bias of 49.95%, meaning that the number of times they turned left and right in the lesion condition was approximately the same (Figure 5). In the other 3 treatments, animals in the lateral and medial groups showed no significant difference in turn bias: initial control (F-ratio=0.64, p=0.42), plug (F-ratio=2.17, p=0.14), and post-plug treatments (F-ratio=0.21, p=0.64).
From an observational point of view, it was very easy to discern when sharks detected the presence of food odor and began tracking behavior. In control treatments sharks swam in large circles around the flume at the bottom of the tank, typically in a slow and controlled manner. Upon detecting the food odor, sharks made a fast and dramatic turn towards the odor. Picking up speed, sharks became excited and made frequent, quick turns in and out of the odor plume to track it upstream to its source. Once the food was consumed, sharks typically circled the odor source and struck at it multiple times depending on hunger level. In plug treatments sharks detected the odor in the same manner, but made multiple turns towards the incorrect side while tracking, each time taking the shark out of the odor plume. Though sharks could successfully locate the food in nose plug treatments, it often took longer. They sometimes travelled upstream by swimming in circles (towards the unplugged side), which successively moved them closer to the source. After a lateral tract lesion the same behavior was observed, though less dramatically than in the plug treatment. Medial lesions did not cause any observable change in food odor tracking behavior.

**Discussion**

Surgical lesioning of a left or right lateral olfactory tract had a significant effect on the food odor tracking behavior of the smooth dogfish shark *M. canis* resulting in an increased mean turn bias towards the intact side. Lesioning of a medial tract had no such effect and turn bias in this treatment was not significantly different from control tests. The lateral lesion and the nose plug caused similar tracking errors; both were
significantly different from the control and post plug treatments, neither of which caused side bias. However, the nose plug had a stronger effect than the lateral tract lesion. This may suggest that the medial olfactory system contributes partially to food search behavior and that lateral and medial olfactory systems have some overlap in function. Tests in goldfish have shown that both medial and lateral olfactory systems respond to food odors while only the medial olfactory system responds to pheromones (Sorensen et al., 1991). A similar functional overlap may exist in the elasmobranch olfactory system studied here. A less likely explanation for this result could be that not all lesions completely severed the lateral tract allowing for some food odor processing. Regardless, these results support the hypothesis that the dual olfactory bulbs of *M. canis* do in fact serve different behavioral functions, with the lateral being influential in the efficient tracking of food odors. Unfortunately we did not have a social odor or a social behavior available to test the reciprocal hypothesis that the medial system specializes in social odor recognition.

Feeding and reproduction are among the most critical tasks for any living organism. Across both vertebrate and invertebrate classes, olfactory systems are often functionally split between sensing food/habitat and social odors. In teleosts, lateral and medial nerve bundles of the olfactory tract serve feeding and social functions respectively. Tetrapods have evolved a completely separate vomeronasal system to detect pheromones or social odors, leaving the main olfactory system to handle environmental cues including food odors, though there is some functional overlap between the two systems. Though we can not consider the dual olfactory bulb system seen here as a direct precursor to the split
vomeronasal and main olfactory systems of tetrapods, it is possible that certain sharks have developed a parallel system of functionally segregated olfaction.

It seems likely and has been speculated that during precopulatory behavior female elasmobranchs exude sex pheromones that trigger a reproductive response in males (Pratt and Carrier, 2001). Based on frequent observations that males closely follow females prior to mating, this theory is widely believed, but has no supporting experimental data (Forlano and Bass, 2010). Prior to the olfactory experiments described here, we tested cloacal fluid from both male and female *M. canis* sharks for any sort of behavioral response from either sex in the lab. We also introduced water from a home pool containing sharks of one sex to a home pool containing sharks of the opposite sex. No evidence of social stimulation was observed with either method (Lohe, unpublished). It is quite possible that captivity and/or seasonally inappropriate conditions made these tests unsuccessful. Mating behavior in *M. canis* occurs in spring and early summer (Conrath and Musick, 2002) while we tested in mid-late summer.

Teleost fishes and most tetrapods show an olfactory organization that is chemotopic; ORNs of the same morphology and function are spread throughout the olfactory epithelium and converge to functionally distinct glomeruli in the OB. Patterns of activity across these different glomeruli within the OB are thought to distinguish between odor types and concentrations (Meredith et al., 2013; Derby and Sorensen, 2008). Elasmobranch OE has been shown to contain widely distributed microvillar ORNs (and
some rare crypt ORNs) that project only to glomeruli in the OB directly posterior to them (Meredith et al., 2013). In elasmobranchs with a dual olfactory bulb structure similar to the one described here, ORNs in the medial and lateral half of the OE project directly to the medial and lateral OB respectively (Meredith et al., 2013). Though the OE in *M. canis* appears anatomically singular it is unknown if different types of ORNs are scattered throughout the OE. In blue sharks, *P. glauca*, the OE seems to be separated into two distinct halves (based on pigmentation), perhaps suggestive of functional separation (Atema, unpublished).

To investigate the function of the medial olfactory system in sharks with dual olfactory bulb anatomy, future studies should determine both the morphology and gene family (OR or V1R/V2R) of olfactory receptor neurons in the corresponding olfactory epithelium. If ORNs projecting from the medial half of the epithelium to the medial bulb are morphologically distinct from those projecting to the lateral bulb, and are of the gene family V1R or V2R, this could provide some evidence that the medial bulb and tract not only serve a separate function from the lateral, but may have ties to the vomeronasal signal-transduction pathway that detects social odors. Final conclusive evidence might then come from behavioral studies of pheromones in courtship, something we could not accomplish due to a pervasive lack of knowledge of shark social behavior.

To suggest explanations for the emergence of dual olfactory bulbs in certain sharks, we can look for commonalities in their ecology and phylogeny. As ecological niche may be a
factor in the presence of the dual olfactory bulb system seen in *M. canis, P. glauca, N. brevirostris, N. cepedianus* and *R. terraenovae*, we must consider the reproductive strategy, feeding habits and preferred habitats of these species. Sharks use sensory information in different ways depending on their ecological niche; while certain sharks will not commence food search behavior without an odor stimulus, pelagic species may hunt after only seeing prey, allowing them to track from upstream (Gardiner et al., 2014).

*M. canis* is a benthic shark living in the northwest Atlantic that feeds mainly on crustaceans and mollusks (Gelsleichter et al., 1998). They are viviparous and very active, commonly patrolling the bottom for food (Ebert and Stehmann, 2013). *P. glauca* is a wide ranging epipelagic shark that makes trans-Atlantic migrations. There is sexual segregation shown in this species, with more females at higher latitudes than males. They are also viviparous. *N. brevirostris* is a large, inshore tropical shark living commonly among coral reefs and mangroves. They form groups of up to 20 individuals and show some sexual segregation, feed primarily on fishes, and are also viviparous. *R. terraenovae* is a temperate to tropical shark living in the western Atlantic commonly close to the surf zone of sandy beaches. They are viviparous and commonly feed on small teleosts (Ebert and Stehmann, 2013). Finally, the broadnose seven gill shark, *N. cepedianus*, is a powerful predator commonly found in coastal shallow waters worldwide. It is ovoviviparous with up to 82 young per litter, and preys on other elasmobranchs as well as bony fishes (Compagno, 1984). In sum, these five sharks exhibit widely varying preferred habitats, ecological niches and life history strategies, making it difficult to associate dual olfactory bulb anatomy with any of these factors.
The sharks known to have dual olfactory bulb anatomy are also diverse phylogenetically. *M. canis*, *N. brevirostris*, *P. glauca*, and *N. terraenovae* are all from the Order Carcharhiniformes. *N. cepedianus* belongs to the Order Hexanchiformes. These two Orders are divided between two major groups that make up sharks taxonomically: Galeomorphii and Squalimorphii. Galeomorphii (containing the Order Carcharhiniformes) make up the modern sharks which all have an anal fin, whereas Squalimorphii (containing the Order Hexanchiformes) lack an anal fin (Vélez-Zuazo and Agnarsson, 2011). We see that *N. cepedianus* is an outlier from the other four sharks because it is ovoviviparous as opposed to viviparous and belongs to the Squalimorph superorder. Here too, it is unclear what has driven the development of this anatomy, if it has evolved multiple times in sharks, or how many shark species share this feature.

Finally, we consider a functional explanation. A split between medial and lateral olfactory systems may be functionally beneficial for animals that rely heavily on their sense of olfaction. Separated olfactory systems may provide more neural tissue devoted to each system. A large and complex network of glomeruli within each olfactory bulb could allow sharks to recognize many different pheromones with high sensitivity, gain information about age and sexual maturity, and also remember and learn certain odors. Similar to the pheromone-specialized macrogglomerulus of some insects, the evolution of dual olfactory bulbs may allow sharks to narrowly tune one of their olfactory systems to
detect social odors with greater precision, acting as a filter against the “noise” of the general odor background.

This study aimed to determine the behavioral function of a unique dual olfactory bulb anatomy seen in certain sharks. More research is needed to determine the function of the medial olfactory bulb, but it is clear that the lateral bulb plays a dominant role in *M. canis* food odor tracking behavior while the medial likely serves a different function. The findings of this study have implications for several distantly related species of shark and offer clues to the evolution of this olfactory system. Multisensory integration is a fundamental aspect of the guidance of all animals including sharks (Gardiner et al., 2014) and understanding the function of this unique dual olfactory bulb anatomy leads us to better understand the neural mechanisms of behavior. This significant finding lays the groundwork for future research in shark olfactory anatomy and behavior and it broadens our understanding of odor information processing and the evolution of vertebrate olfactory systems.
Tables and Figures

Table 1.

<table>
<thead>
<tr>
<th>Anesthesia Type</th>
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<tr>
<td>Induction</td>
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<tr>
<td>Maintenance</td>
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<tr>
<td>Euthanasia</td>
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Table 2.

<table>
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<tr>
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<tr>
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<td>C</td>
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<td>Right Lateral</td>
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</table>
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Table Captions

Table 1. Concentrations of MS-222 anesthesia used in induction, maintenance and euthanasia.

Table 2. Size, sex, and surgical lesion for each of the 19 animals used in this experiment. Size is total length in centimeters.

Figure Captions

Figure 1. *M. canis* olfactory system including olfactory epithelium, lateral olfactory bulbs, medial olfactory bulbs, medial olfactory tract, lateral olfactory tract, main olfactory tract, and forebrain. Photo taken from a formalin-preserved specimen.

Figure 2. Photograph of a dissection of an animal with a successful left lateral lesion. Photo taken from a formalin-preserved specimen.

Figure 3. Photograph of two parallel rhodamine dye plumes as they slowly disperse downstream in the flume without mixing.

Figure 4. Bar graph of mean turn bias by treatment in lateral and medial groups. Error bars show standard error. Red columns represent the group of animals receiving a lateral lesion, blue columns represent the group of animals receiving a medial lesion. Letters of the same color indicate significant differences as determined by Student’s t test. Gray line indicates 50% turn bias, or equal turns towards left and right sides.

Figure 5. Bar graph of mean turn bias in lesion treatment for animals receiving medial versus lateral lesion. Error bars show standard error. Gray line indicates 50% turn bias, or equal turns towards left and right sides.
Bibliography


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EDUCATION

**Boston University** Boston, MA  
Master of Arts in Biology: Ecology, Behavior and Evolution  
**Courses:** Biostatistics, Marine GIS, Conservation Biology, Sensory Biology, Ichthyology, Scientific Diving and Underwater Research Methods **GPA: 4.0**

**Santa Clara University** Santa Clara, CA  
Sept. 2009-June 2013  
Bachelor of Science in Environmental Science, *Cum Laude* GPA: 3.6

**School for Field Studies** Turks & Caicos Islands, BWI  
Sept. 2011-Dec. 2011  
Marine Resource Management Studies GPA: 4.0

EXPERIENCE

**Woods Hole Oceanographic Institution** Woods Hole, MA  
Sept. 2014-present  
*Graduate Student Researcher, Environmental Systems Laboratory*  
- Conducted original research for thesis: “Role of two anatomically separate olfactory bulbs in shark food odor tracking”  
  - Designed and ran sensory biology experiments  
  - Led animal care and laboratory maintenance  
  - Completed olfactory tract lesion surgeries in sedated *Mustelus canis* sharks  
  - Led behavioral tracking analysis and statistical data analysis using JMP software  
  - Managed high school and undergraduate volunteer teams in the lab

**Boston University Department of Biology** Boston, MA  
Sept. 2014-present  
*Teaching Fellow*  
- Courses taught include Marine Megafaunal Ecology of Stellwagen Bank, Systems Physiology, and Ichthyology.

**Smithsonian Institution** Washington, DC  
*Research Intern, National Museum of Natural History*  
- Assisted Dr. James Mead in marine mammal division maintaining cetacean distributional database, assisting in specimen dissection/preparation, managing and analyzing collections.
- Assisted entomology department documenting parasitic wasp collection, identifying and photographing 10 drawers of wasps weekly for use by researchers worldwide.

**The Whale Camp** Grand Manan, New Brunswick, Canada June 2013-Aug. 2013  
*Environmental Science Instructor*  
Planned and facilitated daily science and adventure education programs for students on land and at sea, including lessons in marine mammal ecology, marine science, geology, forest ecology, botany, oceanography, coastal / island ecology.

**North and South Rivers Watershed Association** Norwell, MA June 2012-Sept. 2012  
*Field Research Intern*  
- Surveyed benthic marine invertebrate populations to monitor changes resulting from the introduction of a tidal gate to the area.  
- Tagged horseshoe crabs to monitor population size and coastal health.  
- Tested and compared various water quality indicators in local reservoirs.  
- Identified and surveyed wetland plant species to monitor ecosystem health

**VOLUNTEERING**

**Sea Run Brook Trout Coalition** Newburyport, MA Sept. 2016  
Participated in electrofishing and PIT tagging of sea run brook trout with MassWildlife as part of monitoring project at Red Brook.

**BIOBUGS at Boston University** Boston, MA May 2015 & 2016  
Led high school students through Urban Ecology lab activities in the field and classroom.

**SKILLS & ACHIEVEMENTS**

Certifications: ESCI CPR/AED, ECSI First Aid, SSI Open Water Diver, AAUS Scientific Diver, NAUI Rescue Diver, NAUI Nitrox Diver, DAN First Aid for Professional Divers  
Academic Awards:  
- Dana Wright Research Fellowship, Summer 2016  
- Santa Clara University Dean’s Scholarship 2009-2013  
- Santa Clara University Women’s Rowing Captain, 2012-2013, Division I  
- West Coast Conference All-Academic Award Spring 2013  
- Collegiate Rowing Coaches Association Scholar Athlete Award 2013  
- West Coast Conference All-Scholastic Honorable Mention Spring 2011, 2012