

Canine Olfaction:
An Overview of the Anatomy, Physiology and Genetics

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A dog's sensory experience of the world is guided largely by its nose. Unlike upright humans who have evolved to depend on high acuity vision, the macrosmatic dog relies heavily on a highly developed olfactory system to detect environmental information relevant for survival. A dog's sense of smell is many orders of magnitude more sensitive than a human's. This is largely due to anatomical, physiological and genetic differences that allows dogs to more effectively collect, sample and cognitively process chemosensory information from their environment. The dog's partnership with humans evolved in part because man wanted to take advantage of the dog's keen sense of smell to aid his own needs for tracking and hunting. The modern working dog continues to provide odor detection for human directed tasks at a level of sensitivity that has yet to be replicated or surpassed by man-made instruments.

Anatomy:

Air enters the dog's nasal cavities through two external nostrils or "nares." The dog has bilateral, symmetrical nasal cavities separated by the nasal septum. Inside the nasal cavity lie the turbinate bones and the paranasal sinuses. The turbinate bones (or conchae) are convoluted folds of bone covered in a mucous membrane containing the olfactory epithelium. The turbinates in macrosmatic animals (animals with higher olfactory acuity compared to low acuity microsmatic animals, such as humans) dramatically increase the surface area of the olfactory epithelium. The surface area containing olfactory cells in a human is roughly 5 cm² compared to 150-170 cm² in a dog. Inhaled air is first exposed to the maxilloturbinates where it is warmed, moistened and cleaned by specialized ciliated epithelium and support cells. The inspired air then continues to flow into the more posterior ethmoturbinates and the frontal, maxillary and sphenoid sinuses. The majority of odor processing is accomplished by the main olfactory epithelium (MOE) lining the ethmoturbinates and frontal sinuses; the mucosa of the maxilloturbinates contains only a small number of olfactory sensory neurons. (See Figure 1)

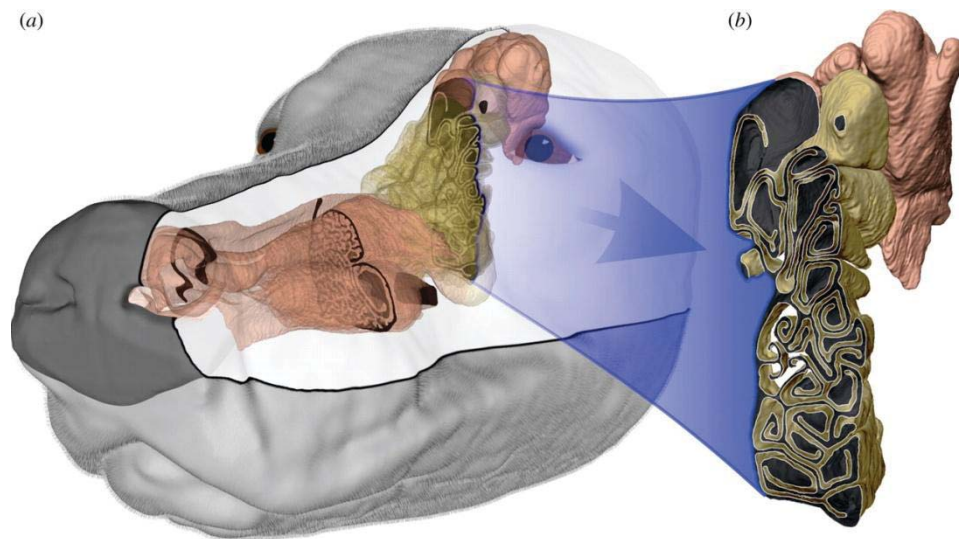


Figure 1. Canine Olfactory Structure (from Craven et al., 2007)

In addition to the MOE, the dog has an additional olfactory epithelium, the vomeronasal sensory epithelium (VNSE), found in the vomeronasal organ (VNO). This tissue is present bilaterally in the bottom of the nasal cavity above the roof of the mouth just behind the canine teeth. The VNO is open to the nasal cavity and connected to the mouth by small channels. Licking brings chemicals into the mouth that are perceived by the specialized receptors of the VNSE which detect pheromones important for species-specific communication, as well as other volatile molecules. Substances in water licked up by dogs can be detected through this system. The information from these sensory cells are transmitted through a separate neuronal path to the brain. Two other olfactory systems, the septal organ (SO) and Gruneberg's ganglion (GG), have been identified in some macrosmatic animals (such as the mouse, marsupials, rabbits), but a recent morphological study of dog olfactory epithelium by Barrios et al. (2014) failed to find these subsystems in adult, neonate, or fetal dogs. They posited that these other systems may not be necessary because the large nasal cavity of the dog allows for a greater physical separation to mediate discrimination of odors.

Environmental odorants, the molecules that selectively bind to olfactory receptors to initiate the perception of odor, are transported to the olfactory epithelium via the unique air flow patterns that are generated during sniffing. The highly evolved and complex structures of the external nares and the recessed olfactory cavities both create aerodynamic flow that optimizes capture of odorants. Sniffing behavior is a sinusoidal pattern of inspirations and expirations through the nose with a frequency of up to 20 sniffs per minute or 4-7 Hz. Sniffing rate has been observed to vary with the difficulty of the task. Although these rapid bursts of sniffing appear to be the norm when a dog is hunting odor, single long sniffs are also observed, e.g. in dogs that are air-scenting. The flow of air through the nostrils takes separate pathways on inspiration versus expiration mediated by nostril flexure (controlled by the muscles and ligaments) and the external design of the nose (Settles et al., 2003). Expired air exits the nostril in a ventral-lateral vortex. This pattern minimizes re-breathing of expired air and promotes inspiration of fresh air samples. Secondly, the lateral vortex stirs up ambient odorants and particulates that may have otherwise been inaccessible and the warm, moist expired air may promote volatilization of latent odorants. (Craven et al., 2007)

Inspiratory air flow during sniffing is distinctly different than respiratory air flow. When a dog is breathing or panting, not sniffing, the majority of inspired air travels to the lungs via the nasopharynx, with only 12-13% of the inspired air reaching areas with olfactory epithelium. During sniffing, however, high velocity air flow is generated that transports larger volumes of air to the most dorsal nasal cavities where it turns 180° and flows back over the ethmoturbinates. During the expiration phase of sniffing, no air enters or exits the olfactory recess, resulting in prolonged exposure of the inspired air to the chemoreceptors of the olfactory epithelium. Acquisition of critical olfactory information on inspiration during sniffing is also optimized by the aerodynamic patterns created by the external morphology of the nares. Craven et al. (2007) observed that the nostril "reach," the inspired air that is in the immediate vicinity of each naris, is approximately 1 cm. This distance is "smaller than internostril separation, indicating that each nostril samples air from spatially separate regions" (Craven et al., 2007). This feature of spatial-temporal sampling, along with separate bilateral neuronal pathways to the olfactory lobe, and preserved spatial topography from epithelium to olfactory cortex, allows the dog to localize odor source. These features are believed to contribute to a dog's ability to determine direction when tracking/ trailing. (See Figure 2)

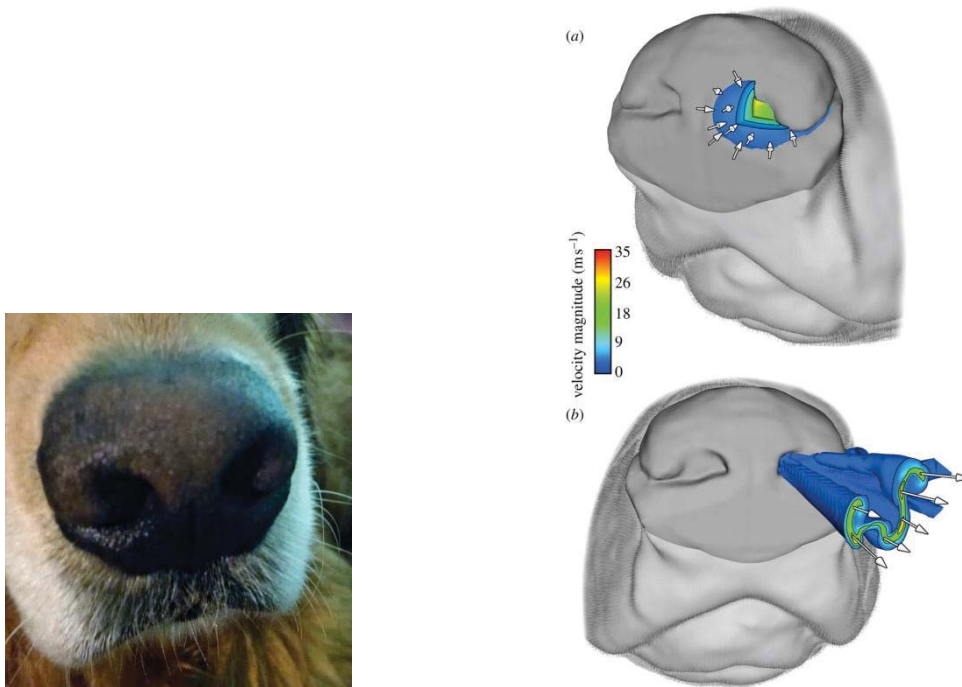


Figure 2. External Nares & Air Flow (from Cravens, et al., 2007)

Physiology:

Once the vapor or particulate carrying the odorant enters the nasal cavities, the olfactory receptor cells (ORC) in the nasal epithelium initiate the transduction process that results in odorants being perceived as odors by the brain. These unique neurons are embedded in the nasal epithelium surrounded by other cells types that support the olfactory mucosa. The number and density of these ORC vary between species. It is estimated that humans have about 5 million and some dog breeds have 200-300 million. The density of olfactory cells in a dog's nasal epithelium is 100 times greater than in humans. It has been suggested that greater numbers of ORCs may not be responsible for olfactory acuity, but may contribute to enhanced odor discrimination. Within species, the number of ORC change during developmental. Kavoi et al. (2010) found that ORC density in dogs increased 22.5% from birth to adulthood, whereas this value decreased by 26% in sheep. These inter-species differences may reflect the relative importance of olfaction in survival. These findings also suggest that a postnatal developmental window exists during which exposure to certain odorants may shape the animal's olfactory sensitivity.

The ORC is a bipolar neuron with a dendrite and axon that connect directly to the cell body. The dendrite extends out toward the epithelial surface and terminates in a rounded olfactory knob covered with radiating cilia that are embedded in the fluid of the mucous membrane. Species differences in the number of cilia per ORC have been observed; humans and sheep may have 6-8 per cell, while the dog may have 20-100 cilia per cell. The olfactory receptors (OR) are located on the cilia. Odorant molecules must dissolve in the watery mucous surrounding the cilia in order to attach to the receptor proteins. Many odorants are hydrophobic molecules and require an interaction with olfactory binding proteins found in the nasal mucosa to transport the odorant to

the olfactory receptors. It is believed that all the cilia on a single ORC have the same type of olfactory receptor. Each olfactory receptor binds to one specific physio-chemical property (e.g., carbon chain length or attached functional groups) of an odorant. An odorant molecule will have multiple properties and therefore bind to and activate several different ORC. Although the encoding algorithm is not fully understood, this combination of multiple, different OR being activated is translated in the brain as the perception of both odor quality and intensity.

A very unique property of the ORC is that they are constantly regenerating. No other mammalian sensory cell has this capacity. Olfactory neurons live 30-60 days and then are replaced by new cells. One of the support cells types in the nasal epithelium, the basal cell, appears to be the source of new cells. Replacement neurons do not automatically contain the same OR of the neuron that died. This system demonstrates remarkable plasticity in the fact that "the type of receptor that is generated on the new replacement neuron is (partly) triggered by the odors the animal most often smells" (Gerritsen & Haak, 2015). Several lines of research have demonstrated that animals develop more receptors for odors on which they are regularly trained (Wang et al., 1993; Youngentob & Kent, 1995). This may explain the findings in a study by Walker et al. (2006). These researchers examined the olfactory acuity, i.e. the lowest concentration of chemical that can be sensed, in dogs trained to detect a single target odor (*n*-amyl acetate or nAA). They systematically lowered the concentration of nAA over a period of several months and used regression analysis to determine the threshold for detection. Previous studies (Krestel et al., 1984) found beagles could detect nAA at concentrations around 10 parts per billion (ppb), whereas the dogs in the Walker et al. (2006) study had thresholds of detection in the 1-2 parts per trillion (ppt) range. In the latter study, the exposure to the target odor over a period of several months may have increased the number of OR that bind to nAA, thus increasing the acuity to this odorant. Another variable that could have influenced the differences in these two studies is the different dog breeds used (Beagles vs. Rottweiler & Schnauzer). It has been demonstrated that there are genetically determined breed differences in OR types and affinities mediated by polymorphism in genes coding for olfactory receptors (Tacher et al., 2005).

The axons of all the ORC form the olfactory nerve bundles that projects directly into the olfactory bulb that sits behind the cribriform plate, a structure of the ethmoid bone that separates the nasal cavity from the brain cavity. The ORC axon covers a short, but direct distance to the brain without any intermediate relays, a feature that is unique to the olfactory system. This allows for a very rapid transmission of information which may have evolutionary value if it promotes survival. These axon terminals make synaptic contact with clusters of second order neurons in the olfactory bulb called glomeruli. When an odorant molecule binds to the receptor protein, a chemical cascade is triggered by the release of cAMP. This chain of events causes the depolarization of the olfactory neuron resulting in the propagation of an electrical signal along the axon of the ORC that stimulates the neurons of the glomeruli. A striking structural feature of each glomerulus is that all olfactory neurons that terminate on it have the same type of olfactory receptor. This spatial mapping of information is preserved when it is projected to the cortex, a feature that is relevant to the higher level encoding of odorant information. The very precise structural layout of the glomerular layer and the correlation between the number of glomeruli and number of odorant characteristics that can be detected suggest glomeruli are the functional units of olfaction (Goldblatt et al., 2009). The glomeruli converge with the dendrites of mitral cells,

the largest cells in the olfactory bulb, and tufted cells. The axons of the mitral and tufted cells form the lateral olfactory tract that conducts the olfactory signal to the piriform cortex. There are also additional projections to the limbic system of the brain from both the olfactory bulb and the vomeronasal organ (VNO). The VNO, with its unique set of receptor types, projects solely to the accessory olfactory bulb which only has connections with the limbic system.

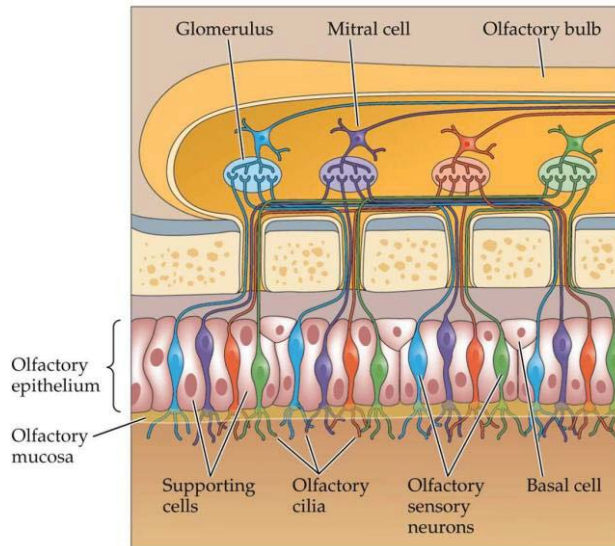


Figure 3. Olfactory System (from web image)

The mitral cells of the olfactory bulb are interconnected laterally and therefore receive information from numerous glomeruli. This puts them in a unique position to "refine" and modify the olfactory signal before it is sent to the cortex. It has been suggested the processing that occurs at these cells allows for enhanced discrimination of odors. Wei et al. (2008) examined the histological structure of the olfactory bulb in dogs across age and sex. They observed that the olfactory bulb grew in size from juvenile to adult, but the density of the mitral cells decreased. However, the size of the mitral cells increased, which may be related to an expansion of dendritic connections. The most interesting finding was that the quantity of mitral cells was significantly greater in adult females than adult males. Studies across species have found that females possess greater olfactory sensitivity than males. This anatomical difference may contribute to these sexual variations.

The primary perception of odor appears to occur in the piriform cortex. The piriform neurons possess a plasticity that may play a role in learning novel odors. Unlike the encoding that occurs in the olfactory bulb, which is more analytical and feature-oriented, the neurons in the piriform cortex do not respond in an analytical fashion. Also, the neural response of these cells has been shown to change after initial exposure to mixtures of odorants, followed by exposure to individual components (Wilson, 2003; Wilson and Stevenson, 2003). All the studies that have observed changes in cortical cell response following extended experience with a complex odor are consistent in demonstrating that "experience with an odor (or odor mixture) in a variety of conditions allows that odor-specific activity pattern to be synthesized as a unique perceptual whole through cortical synaptic plasticity" (Wilson & Stevenson, 2003). This theory appears to hold when the complex odor is a mix of three or more odorants. A similar cortical process may also explain why differences in quantity of an odor are perceived as qualitative differences.

Understanding how the brain processes individual odorants versus complex mixtures of odorants is especially important for detection dogs that are trained to locate complex odors, such as explosives or decomposing human remains. This topic has received particular attention in the training of explosive detection dogs (EDD). The salient question for training is whether to train on the naturally occurring mix of odors or a particular individual odorant that is likely to be present in the complex mix. Goldblatt et al. (2009) noted that the reports published for EDD training were consistent for their *inconsistency* in results. This may be due to significant differences in variables such as the experience time/exposure a dog is given with either the complex odor and/or the individual odorant before they are asked to detect the other. Clearly, more research is needed in this area. Based on current research, Gerritsen and Haak (2015) suggest dogs will learn complex odors more quickly if they have been trained first on individual components of this odor. They also point out that dogs that are sensitive to small amounts of an individual odorant may not recognize it in a complex mix. Ideally, training protocols should use single and complex odors, as well as small and larger quantities to optimize olfactory perceptual learning.

The limbic system of the brain, which coordinates basic physiological states (e.g., hunger and thirst), sexual behaviors, emotional states (e.g., fear, aggression), and memory, also processes olfactory information based on separate projections from the VNO. The olfactory cells of the VNO possess a unique subset of receptors found only in the VNO epithelium that respond primarily to pheromones. These chemical compounds are associated with primitive functions such as determination of reproductive status and sexual behaviors. Other hormonal molecules trigger VNO receptors that convey species-specific communications such as recognition of pack members and offspring, states of dominance, or territory. This information from the VNO to the limbic system is rapid and unconscious. The odor information from the VNO with its separate pathway to the limbic system is processed rapidly and outside the influence of the higher cortical processing of odors from the MOE. Odor information and subsequent behaviors from VNO receptors have the potential to interfere or override behaviors resulting from cortical processing. A male dog's detection of a female in estrus while searching for a target odor is a good example. In a separate nerve bundle, the limbic system also receives information from the MOE. Therefore, it is possible for the perception of all odorants to be influenced by emotional components. For example, when perception of an odor is associated with a positive reward, this contributes to the motivation of the dog to find the odor. Similarly, this mechanism is probably at play in "proofing" dogs off certain odors (e.g., rattlesnakes) using aversive conditioning (e.g., electric shock).

Genetics:

The study of olfaction has advanced at a more rapid pace since the discovery of the genes encoding odorant receptors and the structural characteristics of these receptors (Buck & Axel, 1991). The sequencing of mammalian genomes "have made it possible to infer how smell has evolved to suit the needs of a given species and how variation within a species may affect individual olfactory perception" (Keller & Vosshall, 2008). Genetic research has determined that olfactory receptors are encoded by the largest gene family in the mammalian genome. A larger number of different olfactory receptors translates to an olfactory system that can recognize and discriminate across an expansive number of odorant molecules. Comparative genetic studies

have found that macrosmatic animals, like rodents and dogs, have much larger olfactory gene repertoires than microsmatic animals. The number of functional olfactory genes in humans has been estimated to be around 450, compared to 800+ in the dog and 1500+ in the rat and mouse (Quignon et al., 2003; Quignon et al., 2005). There is some inter-species overlap in olfactory repertoires suggesting evolution from a common mammalian repertoire.

The difference in the olfactory receptor gene repertoires across species may, in part, account for the findings that the threshold of detection for a given odorant differs among species. Through the course of evolution, the olfactory gene repertoire of a species will be shaped by natural selection, allowing the species to acquire the ability to detect biologically relevant odors at thresholds that are functionally relevant. Within any given species, different odors will have different thresholds of detection based on the physical properties of the odors and the importance of the odors for the species (Goldblatt et al., 2009). However, it would not be parsimonious for every biologically relevant odor to have a unique receptor. Rather, the animal would be better served if it possessed the capability to learn new and complex odors that are relevant. The large array of ORs expressed in macrosmatic animals allows for these needed expansive combinatorial computations. The neuronal encoding for the learning of novel complex odors occurs in the higher brain levels (as discussed above) and is also influenced by evolutionary pressures.

Genetic variation within individuals of a species is well documented and these differences partially account for the differences in olfactory ability that have been reported. Studies in humans and mice have linked large genetic variability in ORs to inter-individual sensitivity to odors. For example, Keller et al. (2007) demonstrated that polymorphisms in specific OR genes accounted for sensitivity to specific odorant molecules in humans. Large genetic differences also exist within dogs, as witnessed by the 350+ breeds. It has been suggested that variations in olfactory gene repertoires between breeds and polymorphisms within a breed account for the range of ability in olfactory acuity and discrimination observed in dogs. Tacher et al. (2005) investigated the level of polymorphism in 16 OR genes in 95 dogs representing 20 breeds. They found a high level of polymorphism (multiple allelic variants per gene site) at all 16 genes. Some of the allelic variants were specific to one breed. They also found different breeds had different subsets and frequencies of pseudogenes (genes that are functionally inactive due to mutations). Some studies have linked high frequencies of pseudogenes to poor odor sensitivity; for example, quantification of the human olfactory subgenome found 51% of the genes were pseudogenes.

Lesniak et al. (2008) reasoned that the performance of odor detection dogs might be linked to specific polymorphisms, i.e. that particular alleles at an OR locus would enhance odor recognition accuracy. They examined 5 OR genes, all from subsets of the olfactory subgenome that are thought to be crucial in odor discrimination, in 35 dogs that were trained in various detection disciplines including human identification, ED, drug detection, and cancer marker detection. The dogs were predominantly males GSDs. The dogs were field tested and then ranked relative to their overall performance. Their performance was correlated to the genotypes at the specific 5 ORs that were examined. The study found that specific alleles at two gene loci appeared to be linked to odor recognition efficiency, regardless of the dog's specialty. The researchers discuss the methodological weaknesses and small sample size regarding their interpretations. However, they are encouraged that larger scale studies of this kind may provide

genetic information that will be useful in the breeding and selection of dogs suitable for work in various canine detection disciplines.

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