Olfaction: From pre-receptorial to behavioural aspects, 1996

Olfactiòn and anosmia in animals

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ABSTRACT

Olfactory chemoreception governs many functions critical for the survival and environmental adaptation of animal species. The striking peculiarities of the mammalian olfactory system, as well as its influence on individual homeostasis and endocrine equilibrium, are still difficult for biomedical scientists to understand. The sense of smell is amplified by four olfactory accessory systems perceiving scents of non-volatile odorous molecules.
Anosmia is a condition which produces remarkable changes in individual behaviour, revealing its extensive influence on vital functions. Most of our knowledge about olfaction comes from studies comparing the behaviour of normosmic and anosmic animals.
Such studies, along with electrophysiological techniques, should be encouraged, since they represent an open field still rich in promising discoveries. There is also a need for neuroanatomical, as well as biochemical and genetic research.

INTRODUCTION

Senses allow organisms to perceive the surrounding world. Perception is a state of being of all living forms, achieved by means of different systems, that have evolved from elementary organisms. Elementary precellular and unicellular life forms are provided with chemical sensitivity, whereas more complex organisms have developed sensorial systems, based on specialized receptor cells called chemoreceptors. These are specifically located at moist surfaces such as mucous membranes, the tongue and blood vessels.
Olfaction is a chemical sense playing a crucial role in all living organisms. Insects, fish, amphibians, reptiles, birds and mammals rely on their sense of smell for vital functions such as food search, reproduction, orientation and communication (1). Indeed even vegetables can perceive, as is demonstrated by sensitive and carnivorous plants.

MAIN OLFACTORY SYSTEM

The fundamental role of olfaction is revealed in vertebrates by the anatomic arrangement of the olfactory system, which is remarkably constant in all species (1).
The olfactory system has a prominent position in the head and brain, it is forward projected and constitutes the first pair of cranial nerves. The olfactory brain, rhinencephalon, included in the telencephalon, is made up of an olfactory portion (*paleopallio*) and of a non olfactory portion (*archipallio* or limbic system). The *paleopallio* represents the special visceral afferent olfactory system that allows the conscious perception of odours, while the *archipallio* is concerned with the emotional response to afferent stimuli, one of which is the olfactory receptions. The limbic system is highly developed in mammals also involving the nuclei and brain stem tract. (2).
According to the anatomic prominence of the olfactory system, animals are classified as

macrosmatics or microsmatics (4); among the latter are man, other primates, birds and cetaceans.
The anatomic organization of the mammalian olfactory mucosa is quite variable. The receptors are located in a postero-dorsal pocket of the nose, alongside the respiratory pathway. In some mammals, supplementary olfactory folds enlarge the olfactory area. These macrosmatic species show a more complex organization of the nasal walls and their olfactory mucosa extends as far as the ethmoturbinates, frontal and sphenoidal sinuses (4).
The specialized olfactory chemoreceptors are peripheral receptors, consisting of bipolar neurons lying in the olfactory mucosa and embedded in mucous. Their axons run deep in the mucosa, joining other axons to form the olfactory nerves. The fibres of the olfactory nerves are the smallest axons of the mammalian nervous system (3); they are unmyelinated, and continuously regenerate throughout life. These fibres run separately, crossing the cribriform plate of the ethmoid bone, to reach single glomeruli in the olfactory bulbs, in a switchboard fashion (5).
Odorous molecules are volatile substances conveyed to the olfactory system by respiratory flows and other active inhalatory patterns, such as smelling and sniffing. There is evidence that physical contact between the odorous stimulus and its receptor (located on the olfactory cilia) is necessary for pure chemoreception to take place. The olfactory receptor neurons are the only ones in the nervous system which are exposed directly to the external environment (6). Furthermore, the olfactory system is thought to mediate an even deeper chemical contact between stimuli and elaborating centres; it represents the sole example of extraneous substances entering the brain, in a way similar to food and air entering other parts of the body. The transport of non volatile metabolites into the olfactory bulbs of the mouse brain has been documented after inhalation of radioactive solvents (7). Why is there a need for a direct contact of the odorant with the brain? One plausible hypothesis, supported by the work of Axel (5), suggests that an odour activates a combination of glomeruli in the olfactory bulbs. From the bulbs the information is then sent to the limbic system, hypothalamus, pituitary, olfactory cortex, thalamus and neocortex, where complex innate and associative reactions are unleashed.
An even deeper link must exist between olfactory information and the physiological and psychological status, as shown by the influence of olfactory stimulation produced by odorant inhalation on pentobarbital sleeping time in mice (8, 9).
The ability to perceive smell without any contact with the signal source (telereception) makes olfaction a specialized "distance" chemical communication system, while sight and hearing represent specialized "distance" physical communication systems. Touch, taste and vomeronasal sense, instead, are specialized communication systems working through physical and/or chemical contact.
Sniffing is a striking behaviour shown by terrestrial mammals. By increasing the flow rate and resistance it is possible to divert variable quantities of air toward the olfactory receptor area. Sniffing is quite a complex activity requiring several components such as attention, orientation, and special ventilatory patterns. Sight, hearing, esteroceptors and proprioceptors are all projected toward the smell source. Attention, discrimination, memory, association, innate or learned behaviour are further elements of the perception of olfactory cues.
Several excellent reviews on olfactory transduction mechanisms and pathways are available (5, 6, 10-15), supporting different hypotheses which attempt to explain how over 10.000 different odours can be identified, memorized, compared and recognized. The olfactory image of a new stimulus is constructed each time a particular smell is connected to a new experience. This makes the olfactory memory virtually infinite.

ACCESSORY OLFACTORY SYSTEMS

Olfaction, strictly speaking, is carried out by the olfactory mucosa lying in the nose, and represents the main olfactory system (MOS). The discovery of other smell sensitive systems, also situated in the nose, has led to the concept of multiple chemical sensitivity (16). According to this concept, smell extends also to the perception of non volatile molecules and macromolecules perceived by secondary olfactory systems. The gustatory system also relies to some extent on olfaction and its accessory systems.

The chemoreceptor systems linked to olfaction in vertebrates are: the organ of Jacobson, the organ of Rudolfo-Masera, the trigeminal system and the terminal nerve.

The vomeronasal or Jacobson organs (VNO), vestigial in man, birds and adult primates, play a relevant role in most vertebrates' reproduction. These are paired structures lying on the floor of the nasal cavity on each side of the septum, and are lined with a sensorial epithelium. In many species these organs open into the mouth, giving rise also to a theory of food sampling function (1, 17). In the herbivore, the vomeronasal organs open into the nasopalatine channel of Stenson, and are associated with the behaviour known as "flehmen". Such a behaviour, which is abolished by VNO section, is generally shown by intact males during courtship (18, 19). After a lip contact with female urine or genitalia, the upper lip is lifted, partially obstructing the nostrils, while the incisive ducts are exposed and widened (18). An "active pump" mechanism, controlled by the nasopalatine nerves, favours the migration of non-volatile substances to VNO during flehmen (19). The animal inhales through its mouth, extends head and neck, and shows a sense of well-being and sexual arousal. It appears evident that other senses are involved in this kind of perception.

The structure and arrangement of the vomeronasal organs are quite similar to those of the main olfactory system: their receptors lack cilia, and send their axons to the accessory olfactory bulbs (20). The VNO pathway is separate from olfaction in all species. This organ appears to be a striking chemical communication sense, acting on contact. Many of the available studies on VNO ascribe to it a sexual function, but this assumption can be compared to a sense of hearing which only perceives music, just because of its obvious pleasant reaction.

The organ of Rudolfo-Masera, or "septal organ", is a patch of smell-sensitive tissue resembling the olfactory epithelium, and is located on the nasal septum above the ethmoturbinates of the cornet. The pathway of the neurons of the septal organ is not known. It shows no connections with olfactory or vomeronasal fibres (17).

The main olfactory system, the organ of Jacobson and the organ of Rudolfo-Masera seem to be separate chemoreceptive systems, individually reaching the brain through their own pathways.

Two further chemoreceptive systems have been shown to react to odorous molecules; their pathways are linked to the first and fifth pairs of cranial nerves. One of these is the *nervus terminalis* (terminalis system), present in all vertebrates, and consisting of a group of free, thin nerve terminations which reach the olfactory bulbs from the septum without joining other formations (21). This system may be relevant to reproduction. The distal part of the trigeminal nerve (trigeminal system), located in the anterior part of the nasal cavity, also shows smell sensitive termination. This nerve is involved in adapting the ventilatory system to expulsive functions, stimulating the areas devoted to respiratory control in the medulla (22). It responds to irritating odours, acting as a guard against toxic substances, by sneezing and odour avoidance behaviours.

The secondary olfactory system epithelia, like the main one, display a continuous turnover of the sensory neurons and their axonal connections throughout life (11).

PHEROMONES AND COMMUNICATION AMONG ANIMALS

The olfactory communication system works in two directions, requiring both reception and production of chemosignals. A new-born will have little, if any, capacity or knowledge of odours, except that of its mother: it will only react to irritating odours stimulating the trigeminal nerve. As it grows up, it will learn and memorize scents as they occur. This will enable it to recognize several thousands of different odours, and to build up an enormous smell memory throughout its life. With its experience of the surrounding world, each individual will develop its characteristic scent containing manifold chemical information to transmit to other animals. The individual scent is made up of a multitude of chemosignals released by several excretory glands and specialized odorous glands. Ethologists distinguish an individual baseline scent, *odore sui generis,* from other temporary scents (an array of mutable, seasonal, and environmental odours) (23). Such a chemical "identification card" is modified by the release of odorous substances called pheromones, which will signal to other individuals most physiological and social circumstances, such as anger, fear, sexual status, submission, domination, well being, and so on. Pheromones are single or multiple molecules secreted by an animal which provoke a specific reaction in individuals of the same species (23). They are produced by skin glands, micro-organisms and excretions, and reveal diet, hormone levels and other information about the individual releasing them. The specialized glands, the genito-anal area, urine and faeces, represent the different chemical "languages" an individual can speak. Pheromones allow the expression of complex concepts (e.g. individual social status), but only target individuals respond to them. An animal in its own environment marks the path to find its way back and around; stimulated by the perception of other scents, it will respond to those cues by voluntarily marking the environment. Territorial marking, self marking and marking of congeners are necessary for complex intraspecies communication (23). Striking examples of specialized odorous glands and different kinds of marking in several species are the dominion of ethology.

The extent to which odours participate in animal communication depends on the social life of a given species and individual experience. Generally speaking, sexual, parental and social behaviours rely on specialized signals, producing mainly innate responses aimed at the survival of the species. Higher mammals also experience moods, as shown by pets and primates. Fear, anger, hostility, despair, wonder, impatience, cheerfulness, dignity, pleasure, affection, love, victory, sympathy and other feelings belong to intraspecies communication and are probably expressed through odours as well. Under these circumstances, neuroendocrine changes in target individuals can either produce or follow pheromone release. An example of fast (30 min.) hormonal response is given in both sexes by the release of Luteinizing hormone (LH), following olfactory stimulation (21). releasing hormone (LHRH) has been shown to be deeply linked to the main and accessory olfactory systems (21). The role of olfaction in reproduction is shown in man by the Kallmann syndrome (hypogonadotropic hypogonadism) (24), and in some animals by the depression or suppression of sexual activity following lesions to the primary and/or accessory olfactory systems. Central anosmia totally suppresses courtship and copulation in male Golden hamsters (25) and in Syrian hamsters (26), while peripheral anosmia does not. In several mammals, individual identification, mate selection (28), puberty acceleration and inhibition, oestrus synchronization, maternal behaviour and parental care are also impaired by anosmia (29). Oestrus synchronisation is a well known phenomenon occurring in social females; by determining the contemporary birth of the herd offspring, it represents a strategy for the conservation of the species. This phenomenon has been observed in women living in communities such as convents, jails, or colleges. Mc Clintok (1971) observed that the menstrual cycles of women living in a dormitory became synchronized as the school year progressed (30).

Parental care is also strongly linked to olfactory cues in most mammals. The specific literature is rich in behavioural examples simplifying the source of the strong parental bond and the unleashing of aggressive behaviour in lactating mothers (29, 31). A target role of the olfactory bulbs toward oxytocin has recently been shown to be related to maternal behaviour.(30)

The influence of olfaction on the social behaviour of animals is shown by the "Christian

effect" and the "Ropatz effect": male mice placed in overcrowded accommodation become aggressive and their adrenal cortex increases, while their testicles decrease in size. If rendered anosmic, these animals cease to show aggressive behaviour and their adrenal cortex does not increase (31).
Olfaction also influences animal orientation in the environment. Much of this influence can be understood by observing the grazing behaviour of herbivores and the hunting behaviour of predators. Pigeons (33, 34), migratory (35) and rapacious birds, rely on olfaction to orient their flight. Such behaviours disavow the theory of a vestigial olfactory system in birds.
Most of the olfactory behaviour, thus, involves hormonal changes brought about by olfactory chemoreception. On the other hand, evidence of hormonal influence on the olfactory system exists. Steroid dependent anosmia has been reported (36, 37), while $ZnSO_4$ damaged olfactory mucosa has been shown to promptly regenerate after topical beta-metasone application (38). Recovery after olfactory bulb lesions likewise follows chronic administration of an ACTH analogue (39).

ANOSMIA

Anosmia means loss of the sense of smell.
From a clinical point of view, anosmia is classified as "essential" or "true" when it follows lesions of the olfactory mucosa, nerve, tract or bulb; "mechanical" or "respiratory" if due to nasal obstruction (polyps, tumours, aspergillosys...); "reflex" when due to disease in other parts or organs (neurosyphilis, meningioma, zinc deficiency); and "functional" if it occurs without any apparent causal lesion. (40). All these pathological conditions, not very frequent in man, are seldom observed in animals because of the difficulty of detecting them, as well as the complex expensive tests necessary for diagnosis. Few cases of central anosmia following spontaneous or experimental canine parainfluenza and distemper are reported in the veterinary literature (41, 42). Spontaneous peripheral anosmia has been reported in dogs (43), where it represents a frequent lesion in dolichocephalic breeds, more easily affected by turbinate pathology. A kind of anosmia more often reported in man is represented by the genetic disorder known as Kallman's syndrome (24).
From an experimental point of view anosmia can be classified as "peripheral" or "central", "irreversible" or "reversible".
Comparing the behaviour of intact animals with that of anosmic congeners is one of the oldest methods used to study the role of olfaction in animals. Through this comparison, when the experiment is properly carried out, the meaning of a specific signal may become obvious to man. Individual identification and recognition, social status, territoriality, migration paths, grazing behaviour, maternal imprinting, mate selection, oestrus detection and many other associative functions have been studied through experimental anosmia. Quite often these studies rely on central irreversible lesions, associated with neuroanatomical observations carried out on laboratory animal models. These models are mostly represented by small rodents and amphibians. The effects of anosmia produced by physical lesions are often evaluated by behavioural olfactometry and, less frequently, by objective electrophysiologic recordings. At the end of a lesion study, anatomical, histological, and cytochemical observations provide further evidence of correct lesions and methods. Studies on food preferences, flavour validation, food aversion are often carried on higher mammals as pets and ruminants rendered anosmic. Ethical reasons discourage the use of lesion studies in higher mammals, where less invasive techniques are required in order to produce and evaluate of anosmia.
Central experimental anosmia is produced by bilateral massive lesions of olfactory bulbs, peduncles and olfactory cortex. Single lesions to non sensory areas of the brain- such as the olfactory tubercle, lateral olfactory tract, anterior commissure and anterior olfactory nucleus produce little effect on odour detection, as shown by behavioural olfactometry. Combined lesions of these structures, however, produce severe deficits in odour discrimination and smell associative functions (14).
Since olfactory bulbectomy also results in

accessory olfactory bulb destruction (21), affecting also other chemoreceptor systems, the behavioural disruptions produced by bulbectomy cannot be ascribed merely to the impairment of the main olfactory system.
Irreversible lesions follow any technique producing central experimental anosmia.
Peripheral experimental anosmia (olfactory deafferentiation) is produced either by preventing contact of the odorous molecule with the receptors, or by impairing olfactory epithelium depolarization.
An easy method of preventing the olfactory mucosa activity, has been reported in rats, and consists of naris closure (44). This leads to a shrinkage of omolateral olfactory bulb due to the loss of granule cells (45). A similar effect is achieved by a temporary tracheostomy, diverting the air flow from the nasal cavity through a modified tracheotomy tube (46). Both methods, when correctly carried out, prevent the contact of odorous molecule with the olfactory mucosa thus producing complete anosmia. These relatively invasive techniques seem more humane than physical destruction of the olfactory mucosa or central olfactory structures. Being reversible, they also allow to use the subject as its own control.
Local anaesthesia of the olfactory mucosa represents an attractive method of producing reversible peripheral anosmia. The rationale for such an approach is given by 1) the ability of local anaesthetics to block sensory nerve endings; 2) the extreme thinness of olfactory nerve fibres with the greatest surface per unit of volume, thereby promptly affected; 3) the absence of the relatively impermeable myelin sheath, favouring contact of the cell membrane with the local agent. Further arguments for the use of this technique are provided by the non invasiveness and reversibility of the method, as well as by the easy anatomical approach to the olfactory mucosa.
Sodium and potassium appear to be the main ions underlying olfactory cell depolarization (12). Local anaesthetics prevent nervous cell depolarization by blocking the cation channels through which Na^+ and Ca^{++} enter the positively charged cell membrane, thus altering the sodium pump. As a result, less sodium enters the cell during the action potential, the threshold potential is not reached and no action potential is produced (47). Unfortunately, the unpopularity of this method indicates that something behind the rationale prevents its validity. Personal experience with topical application of 2% lidocaine, 2% procaine and 4% tetracaine on the olfactory mucosa of sheep and goats had no effect on olfactory acuity. The topical application of 10% cocaine has been reported to produce anosmia in goats, as shown by behavioural olfactometry (48). On the basis of this report the author treated 4 adult sheep and 4 goats, after an 18 hr fasting, by intranasal application of 5 ml of a 10% cocaine solution. Behavioural olfactometry - by means of concentrate-based food pellets scented with pig's faeces - showed an obvious degree of anosmia in sheep, but not in goats (49).
Irreversible experimental peripheral anosmia is not an easy task to perform. As they course separately, the single nerve fibres are quite difficult to cut peripherally, because of the large area occupied and the ethmoid bone protection. Furthermore, thanks to the regenerative power of the olfactory nerve, the irreversible effect of neurotoxic substances like phenol, alcohol, methyl bromide and zinc sulphate, causes only temporary anosmia.
The most popular system adopted till now in order to produce experimental peripheral anosmia has been the irrigation of both nostrils with a $ZnSO_4$ solution (50, 51). With this method, the superficial layer of the nasal mucosa is destroyed by the precipitation of its proteins (46). As it prevents receptor depolarization, this method produces complete temporary anosmia, without affecting the trigeminal system completely (52). Analogous effects, with a more moderate morbidity and 80% positive results, have been achieved in rats by spraying small volumes of $ZnSO_4$ into both nostrils (53).
Methyl bromide (54) and Triton X-100 (55) inhalation have also been imposed to produce peripheral temporary anosmia.
The time necessary for smell resumption after such methods is quite variable, as it is affected by the neurotoxic substance used, its concentration, exposure time, depth of the

lesion (), animal species, and age of the individual. In rats, olfactory mucosa regrowth has been reported within 2-4 weeks after $ZnSO_4$ irrigation (56), and within 1-8 weeks after methyl bromide gas exposure (53). Olfactory mucosa smell response can also be blocked by lecitins or pre-treatment with sulfidryl reagents (12).

Also the vomeronasal system has been frequently impaired by different techniques. Central lesions to the accessory olfactory bulbs can be quite selective and are irreversible. Peripheral lesions can be either reversible or not. The VNO can be removed (21), or cauterized (56), producing irreversible vomeronasal deafferentiation. Reversible methods for suppressing the VNO function in rams include $ZnSO_4$ lesions. An alternative simple method has been reported by Blissit (1990). This technique involves occlusion of the nasopalatine channels by inserting vinyl polyosanone bungs (57).

Extracranial lesions to the fifth pair of cranial nerve can suppress the trigeminal system mysterious function.

Selective terminalis system lesions are impossible to produce (21).

Experimental peripheral anosmia is a very useful tool in the hands of physiologists and ethologists. The advent of new, non invasive, humane techniques to impose experimental olfactory deafferentiation is mandatory for an higher diffusion of the studies on anosmic animals.

DIFFICULTIES IN OLFACTORY BEHAVIOUR INTERPRETATION

It would probably be easier for man to understand the role of olfaction in animals if he, too, were a macrosmatic species. Ethology clearly shows differences in the sense of smell in various species, some of which rely on scent (hedgehog, skunk, muscus deer, goat) much more than others (birds, primates and man), and whose communication relies mainly on visual and acoustic stimuli and spoken or written language.

It may be very well possible for man to analyze the chemical composition of many, or even all, animal pheromones, but it is impossible for him to understand their influence on individual experience. The interpretation of any given message is subjective and, obviously, it will not have the same meaning for all those perceiving it. Concepts such as threshold or fatigue express situations that are variable from one individual to another, and from time to time in the same individual. Furthermore, some physiological and pathological conditions modify individual olfactory sensibility, causing quantitative and qualitative changes known as hyperosmia, hyposmia, and parosmia. Respiratory flow changes may induce hyperosmia or hyposmia by diverting air flow toward or away from the olfactory mucosa. Hyposmia follows olfactory mucosa congestion or dehydration. A central parosmia, often leading to nausea, occurs in women during the first weeks of pregnancy. The same has been reported in hysteria, and during epilectoid aura (58). Olfactory hallucinations, i.e. perceiving an odour that is not present, occur in man in certain mental illnesses (58).

Specific anosmia occurs when the perception of only one class of odorants is impaired (12). All these changes must take place in other animals without man's awareness.

A major problem connected with anosmia in higher mammals is represented by the difficulty of assessing the degree of olfactory impairment. The ideal method must be non invasive and objective. Behavioural olfactometry is a non invasive method, but its results are based on a subjective interpretation of the behaviour of the anosmic individual. In the case of irreversible lesions, such behaviour is compared to that of intact congeners, observed under the same experimental circumstances.

Electrophysiologic techniques are based on the recording of electrical phenomena involved in a physiological process. In the case of chemoreception, a chemical stimulus of known intensity and duration must be applied to measure the receptor response. Due to the extreme sensitivity of the olfactory system, and to the characteristic volatility of odorous molecules, a quite sophisticated apparatus (olfactometer) is required, to deliver a controlled, time locked odoriferous stimulus to the olfactory mucosa. Once the stimulus is applied, different electrical phenomena (odour-evoked electrical responses) can be recorded at different levels. The techniques so far used to measure olfactory electrophysiologic activities are electroolfactography (EOG), electroencephalographic olfactometry (EEGO), and olfactory evoked potentials (OEP). Electroolfactography (EOG) has been known for 40 years (59). A modified, less invasive technique has more recently been developed and applied to animals (60). EOG measures the overall electrical response of the olfactory mucosa to a stimulus and has been extensively used in experimental and clinical anosmic animals (61, 62).

Electroencephalographic olfactometry is the combined recording of the standard EEG and EMG of the neck muscles in response to a controlled olfactory stimulus. In fact the EMG of the neck and head musculature increases in amplitude with the increasing concentration of odoriferous substances. This technique has also been used in olfaction research, too(63).

The recording of evoked potentials is a relatively modern technique based on time-gated recording of the brain cortical electrical activity, following a repetitive stimulus. The OEP, recorded from the olfactory cortex (olfactory cortical response) have been extensively used in man, but no reports exist in veterinary literature of the clinical or experimental application of this method to animals.

CONCLUSIONS

Olfaction rules many basic physiologic functions that are imperative for the survival of individuals and, even more so, for a group. Very little is known about the way olfaction influences reproduction, aggression, social status, territoriality, parental care, and other complex life functions. However it is evident that olfaction works through complex nervous pathways modifying the subject's endocrine equilibrium. Electrophysiologic techniques and behavioural olfactometry can reliably reveal olfactory influences. Most of the known pheromones are involved in reproduction and social status, but it is feasible that many more languages are spoken through the sense of smell. These languages are probably used by animals to transmit individual experience to congeners.

Studies on the influence of olfaction on mammalian alimentary behaviour should be encouraged.

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