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The underestimated role of olfaction in avian reproduction ?

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Abstract

Until the second half of the 20th century, it was broadly accepted that most birds are microsmatic if not anosmic and unable to detect and use olfactory information. Exceptions were eventually conceded for species like procellariiforms, vultures or kiwis that detect their food at least in part based on olfactory signals. During the past 20–30 years, many publications have appeared indicating that this view is definitely erroneous. We briefly review here anatomical, electrophysiological and behavioral data demonstrating that birds in general possess a functional olfactory system and are able to use olfactory information in a variety of ethological contexts, including reproduction. Recent work also indicates that brain activation induced by sexual interactions with a female is significantly affected by olfactory deprivation in Japanese quail. Brain activation was measured via immunocytochemical detection of the protein product of the immediate early gene *c-fos*. Changes observed concerned two brain areas that play a key role in the control of male sexual behavior, the medial preoptic nucleus and the bed nucleus of the stria terminalis therefore suggesting a potential role of olfaction in the control of reproduction. The widespread idea that birds are anosmic or microsmatic is thus not supported by the available experimental data and presumably originates in our anthropomorphic view that leads us to think that birds do not smell because they have a rigid beak and nostrils and do not obviously sniff. Experimental analysis of this phenomenon is thus warranted and should lead to a significant change in our understanding of avian biology.

Keywords

avian olfaction; ducks; Japanese quail; medial preoptic area; copulatory behavior; immediate early gene

1. Introduction

The importance of olfaction in birds has long been questioned and the acuity of the avian olfactory system has been the subject of strong controversies. Many classical textbooks of zoology used to report that birds are anosmic with a few possible exceptions such as petrels and kiwis [33,60] and even the Handbook of the Birds of the World that is currently being published states that “generally the sense of smell is poorly developed, perhaps partly due to the regression of the upper jaw” [20]). There is, however, a substantial amount of experimental evidence generated during the last half century by a slow but continuous stream of research

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that contradicts this conclusion and demonstrates in birds the presence of exquisite olfactory discriminations.

The earliest reports concerning avian olfaction were usually anecdotal and were not part of a long-term systematic research program. However, since the 1960s thanks to the pioneering work of Bang [6] describing the anatomy of the olfactory cavity in several avian species and to the measurement by Bang and Cobb [7] of the olfactory bulbs in over 100 species, a continuously growing array of anatomical, neuroanatomical, physiological and behavioral evidence has demonstrated the complexity of avian olfactory structures and indicated the relative importance of olfaction in species as diverse as kiwi, vultures, petrels, albatrosses, ospreys, penguins, cormorants, ducks and geese, but also in species with much smaller olfactory bulbs such as starlings, garden warblers, robins, hummingbirds, pigeons, quail, turkeys and chickens.

Although it is now more broadly accepted that birds are not anosmic, it is still generally believed that their sense of smell is less developed than in other vertebrate groups such as reptiles and mammals. However, the importance of a functional sense of olfaction has now been demonstrated in a diversity of biologically relevant contexts in multiple avian species, including orientation in homing pigeons (reviewed by [106]), food location in kiwis [107], turkey vultures [34,46], and petrels [18,47,69,70], recognition of familiar odors in domestic chicks [14,58,80] and nest localisation by petrels and other procellariiforms [11,12,36] (see [40,89] for reviews). Nevertheless, when compared to other vertebrate classes, research on the production or social use of bodily odors in birds has been largely neglected. A number of studies suggest, however, that species-specific odors may play an important role in mate choice of birds [3,11,43,93]. We agree, however, with the recent conclusion of Hagelin [40] stating that it, to this date, “*premature to claim that any avian odor signal functions as a pheromone*” (a pheromone being described as a species-specific signal capable of eliciting a behavior or a physiological or developmental response).

The available work on odor perception and function in birds will be the focus of the present review that will also integrate recent data from our laboratory indicating that brain activation following copulation is significantly modified in quail that were allowed to copulate after their nostrils were plugged so that they could no longer detect olfactory stimuli [97].

2. Morphological and neuroanatomical evidence for olfactory function in birds

The avian olfactory system in birds does not differ much in gross anatomy from that of amphibians, reptiles or mammals. The avian nasal cavity is fully developed. Most birds have paired external nares situated near the base of the top mandible of their bills. Birds breathe through these nares that lead air into a series of three internal nasal cavities. The function of the first two chambers is probably to warm and moisten the incoming air before it enters the respiratory system. The second chamber has an opening into the buccal cavity allowing a part of the air to go directly to the lungs. The remaining air then passes into the third chamber, which is the only one to contain olfactory epithelium [52]. As in other vertebrates, this epithelium contains olfactory receptors supported by cartilaginous structures that may be elaborately scrolled or convoluted so as to increase their surface area. Histological studies of the avian olfactory epithelium have revealed detailed features of organization that are basically similar to those found in mammals. Olfactory receptors in the epithelium connect, via paired olfactory nerves, with olfactory bulbs located at the rostral end of the brain. The avian olfactory bulbs exhibit a high degree of structural similarity and follow a similar developmental pathway as in other vertebrates (see [31] for a recent review). Interestingly, the anatomical features (such as the complexity of the nasal cavity or the size of the olfactory bulb in relation to the

total brain size) vary widely across avian species [7,60], suggesting the existence of correlations between differences in lifestyles (environmental and biological constraints) and the degree of development of the olfactory system. This in turn indicates that the olfactory system is presumably functional and evolutionary important at least in some species. It was indeed suggested a century ago that the importance of smell in a particular species can be inferred from the size of its olfactory apparatus in relation to the size of the rest of its brain [27] and the ratio of the longest diameter of the olfactory bulb to that of the ipsilateral hemisphere has subsequently been used to rank a wide range of avian species on a microsmatic/macrosmatic continuum [52].

In 1978, Rieke and Wenzel [88] published a detailed neuroanatomical report providing clear evidence for neural projections from the olfactory bulbs to several central brain sites (piriform cortex, hyperstriatum ventrale [now mesopallium], lobus parolfactorius [now medial striatum]; see [84] for the revision of the avian brain nomenclature)[88]. The projection targets of the olfactory bulb in pigeons were subsequently investigated by Reiner and Karten by autoradiographic techniques [83]. They found that the olfactory bulb projects to the entire rostrocaudal extent of a portion of the dorsolateral telencephalon (which is here recognized as the piriform cortex in birds) and to portions of the medial telencephalic wall including the medial septal region. This connection was confirmed by retrograde tracing with wheat-germ agglutinin horseradish peroxidase (WGA-HRP) showing that the piriform cortex in homing pigeons receives a dense projection from the olfactory bulb [8]. Interestingly, this study indicated that the network of connections of the piriform cortex to the rest of the brain is similar in homing pigeons to the connections of the piriform cortex in mammals.

3. Electrophysiological evidence for olfactory perception in birds

Compelling neurobiological evidence of olfactory sensitivity has been provided by electrophysiological recording of responses to odors. Electrophysiological data were collected from both peripheral (directly on olfactory nerves) and central (within the olfactory bulbs and the brain itself) sites beginning in the 1960s. Electrodes implanted in the olfactory bulbs of rock doves (*Columba livia*) identified the classic olfactory spindles associated with detection of odor stimuli that were already well-known in mammalian olfactory bulbs and nerves [92]. Similar results were obtained later in several other species [113].

Tucker recorded action potentials from fibers in the olfactory nerves of 14 species representing a wide range of olfactory bulb sizes and therefore presumably variable olfactory abilities. The electrical activity recorded in the olfactory nerves was also proportional to the concentration of the olfactory stimuli that the animal was exposed to [99]. Single unit responses to odors were similarly recorded from the turkey and black vultures' olfactory receptor cells as well as from the pigeon's olfactory bulb [47]. Several studies also revealed that olfactory bulb neurons are spontaneously active but odor stimulation increases their firing rate [61,62,92]. Electrophysiology was further used to demonstrate that the electrical activity induced at the level of olfactory nerves by the detection of olfactory stimuli is transmitted to the various parts of the brain. These include parts of the basal ganglia and multiple locations in associative zones implicated in the decoding and integration information that the animal gathers from the outside world [57].

Taken together, these studies clearly demonstrate that a wide variety of avian species detect olfactory stimuli, some of them at very low concentration, and transfer corresponding signals to the brain. These action potentials actually reach telencephalic sites that are likely to be able to decode the information and then transmit it through mono- or poly-synaptic connections to multiple brain areas where they could play a role in the control of a variety of physiological or behavioral reactions.

4. Behavioral evidence for olfactory perception in birds

The fact that these electrical inputs reach behavior-relevant brain areas does not, however, prove that the related olfactory information can actually be used to control behavior. Detailed experiments have, however, demonstrated that a) the detection of olfactory stimuli can control physiological or behavioral responses in standardized laboratory conditions and also more importantly b) that these stimuli play a role in the control of spontaneous behaviors expressed in a variety of natural situations.

4.1. Tests of olfactory sensitivity in laboratory settings

Quantitative studies have been performed in order to assess the olfactory acuity in several avian species commonly used in the laboratory such as pigeons or chicken. Common assessments of olfactory perception in birds include conditioning/learning procedures, maze experiments and evaluation of overt reactions following odor presentation such as changes in heart beat or respiratory rates. Monitoring heart rate or respiratory changes to study olfactory perception in birds has been used in several investigations [67,109,110].

Experiments demonstrated for example that very dilute concentrations of olfactory stimuli such as amyl acetate significantly modify the respiratory or cardiac rate of greylag geese or pigeons [103,109]. In another species that was already recognized as gifted for olfactory detection, the shearwater (*Puffinus puffinus*) exposure to olfactory stimuli induces an acceleration of the respiratory rate. This acceleration is related directly to the concentration of the olfactory stimuli that are used but also to their nature. The birds reacts to a number of chemical compounds such as amyl acetate or pyridine, that they have no a priori reason to have met before, but also to the odor of fish that represents a physiological stimulus since it constitutes a significant part of their food [111]. These experiments thus demonstrated that birds are sensitive to olfactory stimuli but the responses that were used to measure the reaction of the subject are difficult to quantify. Quantitative analyses were therefore difficult to perform with this experimental approach.

Psychologists have however developed a series of conditioning techniques that make it possible to ask specific questions to animals concerning the way they perceive the outside world. These techniques have been broadly used to analyze various sensory modalities and their acuity in animals. They have also been used to study olfaction in birds. Two broad types of conditioning have been defined. One is called Pavlovian or type 1 conditioning, the second is called Skinnerian or operant or type 2 conditioning. These two conditioning techniques are based on different principles but they both allow the experimenter to ask very specific questions to an animal about its perception of the world.

In a Pavlovian experiment assessing olfactory capacities, one bird, for example, a pigeon will be immobilized in a conditioning cage that it is connected to an olfactometer. The olfactometer is an apparatus that allows exposing the animal to olfactory stimuli that have been calibrated and are therefore completely controlled. The pigeon is equipped with two electrodes that will permit the measurement of its cardiac rate and two other electrodes are also implanted in order to deliver mild electric shocks that will be used as aversive stimuli. When exposed to such an electric shock, a pigeon will normally increase its cardiac rate. During the first phase of an experiment, the exposure to a specific odor will be repeatedly associated with the delivery of a small electric shock. The shock will increase the cardiac rate but also the pigeon will progressively associate the shock with the olfactory signal. This implies of course that the olfactory signal can be perceived. In a second phase, it then becomes possible to expose the pigeon to the olfactory stimulus alone and record whether an increase in cardiac rate is observed. If that is the case the results will unambiguously demonstrate that the olfactory stimulus has been perceived by the animal. It will later be possible to decrease the concentration

of the olfactory stimulus during subsequent tests to determine when the cardiac response disappears. This will provide a measure of the sensitivity of the subject to the stimulus that is investigated.

This experimental approach demonstrated that pigeons are sensitive to extremely low concentrations of chemical compounds such as butanol, butyl acetate and amyl acetate. This sensitivity to olfactory stimuli is actually mediated by the olfactory system and the olfactory nerves because section of these nerves completely abolishes the conditional response. However a huge increase in the concentration of the olfactory stimuli can induce again a change in cardiac response because these super-stimuli are now perceived by other sensory modalities such as taste and sensitivity of the facial branch of trigeminal nerve [103]. This approach similarly demonstrated excellent olfactory capacities in five passerine species. Their olfactory thresholds, as quantified in this study, were similar to those of other avian species and even of macrosmatic mammalian species such as rats or rabbits despite the relatively poorly developed olfactory anatomy in passerines [16].

It is also possible to evaluate olfactory sensitivity by the technique called operant conditioning. In this case the subject is trained to form an association between two types of stimuli but here the animal will have to produce a deliberate behavioral response in the conditioning situation. The experimental protocols that can be used are extremely complex and varied but the principle is always the same. The animal must produce a specific action (e.g., peck at a key) when it is presented to a stimulus that's being tested. He must either not produce this behavior or produce another action if the stimulus is not present. An obvious stimulus is always presented in association with the stimulus being tested in order to tell the animal when a trial is beginning.

In a task of olfactory discrimination, a pigeon would for example be trained to peck to a key of one color if the olfactory stimulus is present and peck at another key if the stimulus is not present. The correct response is rewarded by the distribution of a small amount of food. A wrong response can be either ignored or punished for example by putting the animal in the dark for a few seconds. One can in this way obtain measures of olfactory sensitivity that are quite comparable to those obtained by the technique of Pavlovian conditioning (see [44,65, 102] for examples). This experimental approach confirmed not only that pigeons can detect subtle odors but also demonstrated that they are very sensitive to small changes in the concentration of an olfactory stimulus. It is indeed important from a functional point of view that a subject be able to identify the presence or absence of an odor but also to discriminate whether the intensity of the stimulus is increasing or decreasing. An animal that is for example trying to identify the location of food or a potential sexual partner must indeed be able to appreciate changes in concentration of an olfactory trace in order to be able to locate its target. This differential sensitivity has been shown to be in the same order of magnitude in pigeons as it is in rats [19].

In summary electrophysiological data as well as conditioning techniques clearly demonstrate that pigeons have an olfactory perception that is perfectly functional and presumably comparable to the olfaction seen in humans if not in rats. These data were mostly established for pigeons that have relatively small olfactory bulbs. *A fortiori*, other species with more developed olfactory bulbs should be capable of finer discriminations.

4.2. The use of olfaction in spontaneous behavior

Numerous ethological experiments have additionally demonstrated that many avian species actually use olfaction to determine their reaction in specific behavioral tasks. It has, for example, been demonstrated that tits are able to smell the aromatic environment of their nest and modify it presumably to decrease the parasite load in the nest [77]. Domestic chicks prefer familiar odors and can associate odors to food [52]. Many birds from the procellariiform order

(albatrosses, shearwaters, petrels, and fulmars) also use olfaction to locate their food at sea as do vultures but also kiwis (see below). Many procellariiforms also return to their nest (usually at night) located in a dense colony of similar burrows largely based on olfactory stimuli [12, 35–37].

When tested on particular tasks, most bird species thus demonstrate a sense of smell that is surprisingly developed [89]. These studies clearly indicate that birds exhibit the full spectrum of olfactory abilities that mammals possess and use them in a variety of behavioral contexts. A few particularly well studied examples will be considered here before we finally focus on the control of reproductive behavior.

4.2.1. The use of olfaction in homing pigeons—Pigeons have been credited with one of the most amazing capacities in olfactory discrimination. In 1972, Floriano Papi discovered that pigeons who had their olfactory nerves sectioned were either unable to return to their loft or returned with much longer latencies [75]. Although this experiment was not proving by itself that olfaction is involved in homing, Papi and his colleagues began thinking that olfaction was indeed a critical sense for returning to the loft. A large number of experiments were from that time carried out and actually tended to confirm this idea. Based on these data, Papi developed the olfactory navigation hypothesis that could explain why homing ability is disrupted in anosmic subjects [71]. At their loft, pigeons would build up an olfactory map by associating the environmental odors carried by the winds with the directions from which they blow to the home area [72,73]. For example, they could associate the odor of pine trees with wind blowing from the north and the odor of the sea with wind blowing from the south. At the release site, pigeons would smell the local environmental odors, determine the direction of displacement (e.g. I smell pine trees, I must be north of my loft) and orient towards the home direction by using the sun or, in overcast conditions, the magnetic compass [53,54,91].

This olfactory navigation hypothesis has been tested in diverse experiments namely by lesioning the olfactory system, exposing birds in their loft to winds whose direction had been artificially manipulated or exposing them during their displacement to the release site to odors that do not match the location where they will be released. Most of these experiments have consistently produced a disruption of pigeons' navigational abilities from unfamiliar sites (for example [29,72,74,105,106]). Many researchers thus believe that the olfactory information constitutes at least a part of the map that they use to determine their location when released at unfamiliar sites (for review see [100,104,106]). Other scientists, however, do not however agree with this interpretation and believe that the experiments supposedly supporting the olfactory hypothesis have alternative interpretations (e.g. [76,116]). It is beyond the scope of the present review to consider this controversy in detail but in our opinion, if it is likely that some experiments supposedly demonstrating a role of olfaction can have an alternative interpretation (e.g. the experimental modification of winds direction at the home loft would not only deflect the wind but also manipulate the direction of the sun and thus the sun compass [45,101]), it is unlikely that all these experiments were similarly flawed. It seems therefore probable that environmental odors are part of the signals used by pigeons for orientation and this definitely would represent the most amazing olfactory prowess of birds.

4.2.2. Nest recognition—Behavior observations in nature associated with a few experiments carried out in the laboratory demonstrate that several species of procellariiformes (e.g. Leach's storm petrel, *Oceanodroma leucorhoa* or Wilson's petrel, *Oceanites oceanicus* or greater shearwaters, *Puffinus gravis*) use olfaction to locate their nest when they return from their fishing trips out at sea (reviewed in [40]). Petrels and fulmars nest on the cliffs at the edge of the sea and some species have their nest in burrows located in vast colonies. Bird nests are sometimes very close to the each other and birds must be capable of locating their own nest in relation to the nest of the neighbors. One can imagine that the female and the

chicks present in the nest could guide the male by their calls. However it has also been shown that olfactory information contributes to this localization [36]. On the one hand, it can be observed that these birds in the field find their nest among a big colony even if the nest is not occupied and thus in the absence of acoustic stimuli that could be originating in the female or in the chicks. The petrels are also able to find their burrow even during a dark night with no moon shining or on foggy days. The bird will land on a location close to its nest and then walk to the entrance. The paths followed by the birds returning to nest are in the vast majority moving upwind indicating that they can potentially use the cone of odors originating from the nest to get orientation cues. If humans interfere experimentally with this behavior by plugging the bird's nostrils or making them anosmic by injection of zinc sulfate, they become unable to find the entrance of their nests [10].

Additionally studies carried out in the laboratory have shown that birds of these species are capable to orient in a Y maze based only on olfactory cues released by nest material that had been collected in their own nest. The birds will systematically orient in the maze towards the arm containing material that was collected in their own nest as opposed to material collected in the nest of another individual of the same species [12].

4.2.3. Food detection by kiwis, procellariiforms and new world vultures—Food searching is probably the activity of birds for which olfaction is most easily accepted as playing a significant role, at least for some species. It has long been recognized that kiwis (*Apteryx australis*) locate their food during the night based on its odor. This idea was confirmed in controlled experiments in which it was shown that these birds are able to discriminate between boxes covered with canvas that contain or do not contain food [107,108]. Accordingly olfactory bulbs in kiwis are very large by comparison with brain size.

Observations of spontaneous behavior had also suggested that several species of procellariiforms gather in areas of the sea that contain fish debris. A finer analysis revealed that these birds approach the fish material more frequently in a zig-zag movement navigating against the wind and therefore moving within the cone of odors presumably originating in this potential food source. Field experiments formally confirmed that food location was in these birds based on odor rather than visual stimuli. It could, for example, be shown that procellariiforms approach in larger number a sponge that has been soaked in cod oil as compared with sea water only [38]. In another experiment, various compounds were placed on a small raft in an apparatus that allowed the free diffusion of odor but prevented detection of any of their visual features. In these conditions, large numbers of procellariiforms approached in an upwind direction the raft when it contained smelly products related to their food such as tuna oil, tuna meat, or squids but not other compounds such as sea water, mineral oil, hexane or puffed cereals [47]. This work has been continued more recently and controlled behavioral experiments have shown for example that different species of procellariiforms are attracted by natural scented compounds associated with their food such as dimethyl sulphide, a odor produced by phytoplankton associated with high primary productivity where birds normally feed [68]. Even young chicks who are known to fledge and survive the open seas without aid or instructions of a parent show such an attraction to dimethyl sulphide suggesting that it has not to be learned from the parents. There is therefore compelling evidence that procellariiforms find their food based, at least in part, on olfactory cues (reviewed by [69]).

Various species of vultures have also been shown to be attracted by the odors of decaying animal carcasses. The vision of groups of vultures hovering over carrion is familiar to many. It can be demonstrated that vision plays no role in governing this behavior. Vultures will indeed detect these preys many kilometers away (when vision is impossible) and very generally will approach them flying upwind. Controlled experiments in the field have shown that hidden carcasses or synthetic olfactory stimuli mimicking the odor of decaying meat (e.g. ethyl

mercaptans) attract vultures from considerable distances [32,34,94,110]. Vultures thus clearly locate their food by olfaction and accordingly, their olfactory bulbs are extremely developed.

4.2.4. Predator detection in the blue tit—The use of chemical cues from predators could be important for an accurate assessment of the predation risk in many bird species. This is especially the case in species that use habitats in which visual detection of predators is constrained. Hole-nesting songbirds use cavities for breeding where they can encounter predators such as mustelids (e.g. weasels). A most efficient assessment of the predation risk will obviously be possible if the birds before entering in their nesting cavity can detect a potential predator based on olfactory cues rather than by vision. A recent study of Amo and collaborators [1] suggested that blue tits (*Cyanistes caeruleus* L.) are able to recognize the chemical cues of mustelids and use them to assess the risk of predation. Birds delayed their entry into the nest-box and refused to enter the box when the predator scent had been placed inside the nest by comparison with a control scent consisting of absorbent paper soiled by Japanese quail. In addition, birds decreased the time spent inside the predator-scented nest-box when feeding nestling. Because only two olfactory stimuli were tested and their relative concentration was not assessed, it remains possible that the nest box avoidance when the mustelid odor was present related only to the potentially more prominent nature of this stimulus as compared to quail odor. Data are nevertheless consistent with a use of odors for detection of predators and additional work would be warranted on this topic.

An older study also analyzed the responses of domestic chicken of different ages to cat odor and found that only 7-day-old chickens avoid the cat odor, whereas this avoidance response was not observed when chickens were 4 or 10 days old [28]. The reason underlying such an unexpected age difference is unknown. Interestingly, birds can also use chemical signal as a chemical defense, providing protection against predators (see [42] for a detailed review on this topic).

5. The role of olfaction in the control of reproduction

5.1. Parental behavior in ring doves

Another early experiment indicated that olfactory information could potentially be involved in the control of another phase of the reproductive cycle, namely the feeding of the young. In mammals it is clearly established that olfactory signals play a key role in the establishment of the relationship between the mother and its young. On the one hand the young mammal is able to recognize his mother based on its odor and inversely the mother is capable of recognizing its own young also based on olfactory cues only. Very few information of this type is available in birds but one experiment carried out in ring doves suggests that olfaction might be involved in birds as in mammals in the relationship between the young and its mother [17]. It was shown that if young ringdove squabs are impregnated with an artificial odor (for example an apple or citrus smell), these young will be progressively abandoned by their parents who will stop feeding them. This suggests that extraneous odors interfere with the social relationship established between the dove parents and their young. Control experiments demonstrated that toxic effects of the chemical products that were used can be excluded as a factor explaining the absence of feeding. If the same odorant compounds were injected sub-cutaneously to the young squabs, no change in feeding behavior was detected. This clearly indicates that it is the olfactory components of the manipulation that was interfering with the social relationship. One possible interpretation of this experiment could be that the parents recognize their young based on olfactory stimuli produced by the young and that artificial odors placed on the squabs would prevent this olfactory recognition. This is, however, only one of several possible interpretations and additional experimental work would be needed to confirm this notion.

5.2. Reproductive behavior in mallard ducks

During the 1970s, I became interested in the role that could be played by olfactory stimuli in the control of reproductive behavior of ducks. During the annual cycle, male mallards go through different phases. After the molt that takes place during the end of the summer and the beginning of the fall, males begin to gather in groups of 4–10 individuals that will exhibit a series of behaviors directed either to other males or to females. These displays called social displays are extremely stereotyped and include multiple behavior patterns such as the grunt whistle, head-up-tail-up, or down up. These behaviors have two main functions: they express competition between males for access to females and additionally they are directed towards the females to attract them and form stable couples that will last during the entire breeding season. These social displays are mostly observed from September to February. Later in the season, sexual behavior *sensu stricto* becomes the most prominent activity that can be observed and many copulations will take place with a maximum frequency observed during the month of March. Females will then lay eggs and spend the rest of the spring and summer incubating them and taking care of the young. We wondered whether olfactory stimuli could play a role in the complex interactions that take place between males and between males and females.

Olfactory nerves were therefore sectioned in one group of males to make them completely anosmic while another group of males were submitted to the control manipulation (i.e., sham surgery without touching the nerves). The behavior of these birds was then tested regularly throughout the winter to observe what kind of behavior deficits would be present [3]. We observed that birds with olfactory nerve sections displayed a pronounced decrease and sometimes a complete suppression of most social displays and most sexual behaviors such as grasping that neck feathers of the female, mounting and copulation itself. (See figure 1).

One possible interpretation of these observations is that this section of the olfactory nerves, because it suppressed all potential olfactory interactions between sexual partners, was the cause of the behavior inhibition. It is however well known that the olfactory nerve section induces a retrograde degeneration of the olfactory bulbs. It is also known that, besides their role in the perception of olfactory stimuli, the olfactory bulbs play a relatively complex role at the level of behavioral control. The bulbs act as a general activating factor and modify (increases or decreases depending on specific experimental conditions) the level of arousal and of activity of the animal (at least in mammals) and this independently of the perception of olfactory stimuli [55].

This is also the case in birds where the olfactory system affects in a tonic manner the general level of responsiveness and arousal through its connections to higher brain centers. In several experiments, Wenzel and collaborators were able to demonstrate, by a lesion approach, these non-olfactory functions of the olfactory pathway. After lesions of the olfactory nerves and/or bulbs, performance was significantly decreased in learning situations [48,112] and changes in tonic immobility, aggression [114] and reactivity in new situations [115] were also detected.

An alternative interpretation of these behavioral results is thus that the deficits are the result of a general inhibition of the activity of the birds and not simply of the blockade of their olfactory capacities. This second interpretation is however relatively unlikely because other behaviors that are not directly linked to sexuality such as aggressive behaviors were not affected. These data therefore suggest that olfaction could play a significant role in the control of social interactions in ducks.

This notion was reinforced by another experiment during which two groups of male ducks were continuously exposed during 6 months (during the fall and winter) to 2 females each that were impregnated with the artificial odor of amyl acetate (AA) for one group and of ethyl acrylate (EA) for the other group [3]. Females wore on their back a small capsule filled twice

a week with these chemicals. During the next spring, the sexual preferences of these two groups of males were repeatedly tested by presenting them with multiple pairs of unknown females impregnated with one or the other odor. Males in the AA group significantly preferred to direct their sexual activity to the female in the pair bearing the AA odor but the reverse preference was not observed in the EA group. One interpretation of these data is that AA males associated during the six months of training the odor of AA with the performance of sexual behavior and oriented towards the females bearing this odor during the choice tests. Why then did the EA males fail to develop a similar preference for EA females? This could relate to the acrid odor of EA as opposed to the more pleasant fruity odor of AA. Alternatively, one could postulate that AA females used during the choice tests were intrinsically more attractive than the EA females but this would then not explain why AA males choose the AA females while EA males failed to find them attractive (the experiment was run on a matched design so that in the end all males were tested with the same females). More work is obviously needed to refine these conclusions but this experiment clearly suggests that olfactory stimuli might be involved in mate choice of ducks.

The next question was then to identify the nature of the olfactory stimuli that are implicated in these social interactions. No definitive answer to this question has been obtained to this date. However in one suggestive experiment was carried out in collaboration with Dr. Jürgen Jacob of Ahrensburg, Germany [49]. Behavioral observations demonstrate that ducks spend an important part of the day taking care of their feathers. They spread on the feathers an oily secretion that is produced by a small gland located on the dorsal side of the tail, the uropygial gland. The secretion of this gland is collected with the beak, which is then used to spread it on the feathers. This oily secretion plays a critical role in maintaining the integrity of the plumage [96] and is thus very important for the bird's survival.

The chemical composition of the secretion the uropygial gland in ducks and other birds is very complex. It can however be analyzed in great detail by gas chromatography. During one experiment we collected the secretion of this gland from males and females during a period of seven months ranging from December to June. This period covered the entire duration of the reproductive activities. Secretions were collected on absorbing paper, placed in alcohol and then sent for a chemical analysis by gas chromatography. The fatty acids that were identified in these analyses fell in three broad categories: ramified ester waxes, non ramified ester waxes and diesters waxes [49]. During December, the secretion in males and females were relatively similar. All males and females had branched and unbranched ester waxes in their secretion and none of the subjects had diesters independent of their sex. This situation did not change in males during the next six months but in contrast, all females stopped producing branched and unbranched ester waxes during the breeding season from January to April and then started secreting these compounds again after the period of active copulation (i.e. in May and June). Furthermore, females produced diester waxes in their uropygial gland secretion throughout the reproductive season from February to June while these compounds were never found in the male secretions (see Fig. 2). This seasonal variation in the composition of the uropygial gland secretion was demonstrated more recently to be under the control of estrogens [9].

These data indicate that during the breeding season specifically the chemical composition of the secretion of the uropygial gland is sexually differentiated. Females produce a certain number of compounds that are not present in males and they stop secreting other compounds that remain present in males. Given that this secretion is broadly spread on the plumage, it represents a potential chemical signal that could be exploited by congeners to recognize the sex of their partners and their endocrine condition. Seasonal variation in uropygial gland secretion has now been observed in a variety of avian species but its function remains elusive (e.g., adaptation to the higher feather abrasion during incubation, signaling of mate quality, decrease in olfactory detection by predators, regulation of parasite load in the feathers) [85–

87]. The interpretation of uropygial gland secretions as sexual olfactory signals thus remains a hypothesis based only on the chemical composition of the secretion and additional behavior experiments should be carried out to test whether these chemical differences do have a true functional meaning. This work has unfortunately not been done to this date.

This early experimental work was suggesting that odors could play a role in the control of reproductive behaviors of ducks. No formal demonstration of such a role was however obtained but recent work on other species has produced more definitive data.

5.3 Auklets

To this date, the best evidence for a role of olfactory stimuli in the control of reproduction in any avian species is clearly coming from the work of Hagelin and collaborators on the Crested auklet (see [40] for a recent review). The Crested auklet (*Aethia cristatella*) is a seabird of the family Alcidae (auks, guillemots, murres, ...) which nests in huge colonies (>1 million individuals) in the western arctic (Bering Sea and Sea of Okhotsk). Interestingly auklets produce an unusual, tangerine- or citrus-like odor specifically during the breeding season. This odor is present on the plumage and associated with a frequently repeated display that was called "Ruffsniff" during which birds place their bills within the nape feathers of the display partner, a region that appears to be strongly scented.

This odor has intrigued researchers because it displays several features suggesting that it may represent a chemo-signal with potential significance in the field of reproduction [39,40]. First of all, the scent is produced seasonally by both sexes. Second, the scent is associated with the prominent "Ruffsniff" display that could obviously represent a mean of odor assessment. Third and finally, experimental manipulations in T-maze in the laboratory and on taxidermic models in nature revealed that individuals are able to distinguish and respond preferentially to the odor of the natural plumage as well as to a mixture of artificial compounds (mostly octanal and cis-4-decenal) reproducing this odor.

In laboratory, auklets were attracted to the arm of a T maze that contained either natural plumage scent or the synthetic plumage odor but they were repelled by the odor of mammalian musk [43]. Novel odor with no biological relevance such as amyl acetate induced no preference for one arm of the T maze versus another, indicating that the preference related to the plumage scent was not due to novelty but to an intrinsic property of the plumage. Experiments performed in the field with taxidermic models scented with a synthetic odor mixture demonstrated that both males and females approach more closely a scented male model than a control one [50]. No effect was, however, observed in both sexes when the scent was added to a female taxidermic model. The presence of the scent also did not induce the expression of ruff-sniff displays by itself [50]. These data therefore suggest that the tangerine odor of auklets may serve a general social function but cannot be considered as a pheromone *sensu stricto* that would elicit behavioral reactions by itself. An interaction with visual, acoustic or behavioral stimuli might be necessary for releasing behavioral displays [42]. Additional work is thus needed to clarify the specific role of these odors in male-male competitions or in male-female interactions.

It must also be mentioned that an alternative, completely independent, function for this olfactory signal has been proposed and investigated in a few experiments. It has been suggested that the auklet scent could provide protection against predators, ectoparasites, or microbes. Some of the compounds present in the auklets scent are indeed similar to invertebrate repellants found in nature [25]. They could therefore play a significant role as repellent of a tick (*Ixodes uriae*) that is commonly found in auklet colonies and transmits a number of infectious diseases. Experimental analysis of this possibility has provided only partly supporting results.

Initial tests with synthetic compounds were found to repel or invalidate a number of parasites [26] but comparison of the test conditions with concentrations of putative repellants actually present in natural plumage indicated that experiments had been performed with concentrations higher than the ecologically relevant concentrations [40]. Furthermore, fresh auklet plumage did not repel or increase morbidity of feather lice [24] and auklet ticks experimentally exposed to fresh piles of scented or unscented feathers were not repelled by scented plumage [40]. The potential role of the auklet scent thus remains questionable and would require additional experimental test (see [23,41] for a recent discussion about this putative function of the auklet scent).

5.4. Japanese quail

Little appears to be known about olfaction in Japanese quail (*Coturnix japonica*). This species has comparatively rather small olfactory bulbs but quail do possess an olfactory epithelium and several studies have demonstrated that they are capable, like domestic chicks [52] of discriminating between various chemical compounds and diets based on smell only (see [66] for review). Tentative evidence has also been presented that smell plays a role in the rejection of aposomatic insects during avian foraging behavior [59]. These findings show that quail do have a sense of smell even though this aspect has rarely been taken into account in studies of their behavior.

It is widely accepted that visual and acoustic cues represent the primary signals that are implicated in the control of socio-sexual interactions in quail and that these signals seem to be sufficient to elicit copulatory responses. In male quail, as is true of other Galliform species, visual cues alone appear to be sufficient to elicit sexual behaviors. It was for example shown that male quail will learn to spend time in front of a window providing a view of a female if they are allowed subsequently to copulate with her, a behavior called the learned social proximity response [21,22]. The behavior could be induced by a taxidermically prepared female model and was primarily elicited by visual cues from the head and neck region that are sexually dimorphic in quail.

However, based on the fact that quail have a functional olfactory system and that olfaction seems to play a role in the organization of reproductive behavior in some avian species, we wondered whether olfactory stimuli could also play any role in the control of sexual behavior in Japanese quail. We therefore studied males tested for sexual behavior after their nares were occluded so that they were unable to detect olfactory stimuli and compare them with birds tested in a control situation.

Castrated male quail that had been treated with exogenous testosterone were lightly anaesthetized and one group of birds was made anosmic by a complete blockade of the 2 external nares with a layer of rapid-drying dental cement (see Fig. 3A). Control males were also treated with cement that was applied at the base of the beak, avoiding contact with the nares. Two days later, we assessed the efficacy of these treatments on odor perception by measuring the behavioral reactions of the subjects following exposure to a strong olfactory stimulus.

The subject was held loosely by hand and when it became immobile, a soft plastic squeeze pipette containing a few drops of acetic acid was held with its open tip 3–4 mm from the subject's nares and was gently squeezed 3 times next to each nostril alternatively. The subject's response during odor exposure was scored on a nominal scale ranging from 0 to 2 with 0 = no observable response, 1 = slight movement of the head, beak clapping, and 2 = abrupt shaking or jerking of the head, beak clapping and pecking toward the pipette. Most control subjects reacted during these tests by showing the most vigorous of these responses (score 2) while none of the males with their nares occluded obtained this maximal score; most of them were

assigned a zero score (no reaction at all) and only a few subjects (3 out of 13) showed the intermediate reaction, possibly mediated by trigeminal sensitivity. A few days later, these birds were tested again for sexual behavior during one 10 min presentation to a sexually mature female. Most subjects in both experimental groups showed at least once the complete copulatory sequence. The frequency of these sexual behaviors was not obviously affected by the occlusion of the nares that was still present at that time and as illustrated in Figure 3B, the frequency of mount attempts and of cloacal contact movements were similar in control subjects and subjects with the nares occluded [97].

Ninety minutes after the end of this behavioral test, the brain of all subjects was collected, sectioned and stained by immunohistochemistry for the protein product of the immediate early gene *c-fos*. The expression of this immediate early gene in the brain is enhanced in neurons experiencing an increase in metabolic activity. This induction is relatively non-specific and can thus be the result of the detection of sensory inputs, of neural activity associated with integration of multiple inputs or of pre-motor and motor outputs. The interpretation of such an increase in *c-fos* expression has thus to rely on the anatomical localization of the induction and on the specificity of the experimental situation that induced it [78].

As expected based on our previous work [15,63,98] a dense expression of Fos was observed in the caudal part of the medial preoptic area (mPOA) and throughout the bed nucleus of the medial part of the stria terminalis (BSTM) of male quail that had been exposed to a female and allowed to copulate with her (Fig. 3C). Quite surprisingly, the density of Fos-ir cells in these two nuclei was markedly reduced in birds that had copulated with their nostrils plugged (Fig. 3D).

This qualitative observation was then confirmed by a standardized counting of the numbers of Fos-immunoreactive (Fos-ir) cells in several brain regions that were known to be implicated in the expression of sexual behavior. Counts were obtained in three groups of birds: one group that had copulated with the female in control conditions, one group that had copulated after the nares had been occluded and one final group that had been kept in their home cage without being exposed to the female. In the caudal part of the mPOA and in the two subregions of the BSTM (rostral and caudal subdivisions), the numbers of Fos-ir cells were significantly larger in males that had copulated with the female than in control birds that had remained in their home cage and had not been exposed to the female. This brain activation was, on average, decreased in all three brain regions in birds that had their nostrils plugged and this decrease was statistically significant caudal mPOA. In the rostral BSTM, the number of Fos-ir cells was not significantly smaller in the SEX/PLUGGED than in the SEX group but the former group was no longer significantly higher than the controls [97].

For largely unidentified reasons, Fos immunostaining in the olfactory bulb was highly variable in these subjects: only a small number of subjects showed any Fos immunoreactivity and in all cases immunostaining was very weak. However another immediate early gene called *zenk* (also called *egr-1* or *zif-268* in mammals [64]) provided a more consistent pattern of activation in this structure. Zenk had been shown before to be induced by sexual stimulations in male quail [2,15].

As shown in Figure 4A (left panel), in many subjects that had copulated with a female, a large number of Zenk-immunoreactive (-ir) cells was observed in the central part of the olfactory bulb that should correspond to the granular cell layer in other amniotes. These cells were not present or present in smaller densities in control birds that had not copulated with the females and in birds that had copulated but with a nasal plug (Fig. 4A, right panel). These Zenk-ir cells were counted in a standardized manner in these three groups of birds which clearly indicated the presence of a larger number of positive cells in the group allowed to copulate freely (SEX)

as compared to the controls who stayed in their home cage or with birds who copulated with their nares occluded. This confirmed the qualitative trends that had been observed but the differences between groups were not statistically significant, even after removing from each group a significant number of subjects that showed no Zenk expression at all, presumably for technical reasons (Fig. 4B). This study, however, contained also other groups of birds with plugged or unplugged nostrils that had in parallel been submitted to an anesthesia of the cloacal gland region to assess the effect of this manipulation on behavior expression and brain activation (see [97] for detail). When these birds were included in the analysis, a significant difference of Zenk expression in the olfactory bulbs was then detected between birds copulating with plugged nostrils and birds that could smell freely.

It is therefore interesting to note that deprivation of olfactory inputs affected brain activation induced by copulation without affecting in any detectable manner the pattern of copulatory behavior itself. Several interpretations of these seemingly conflicting results can be offered. It could first be hypothesized that the blockade of the nares with dental cement did not fully deprive birds from their sense of smell. This is however contradicted by the facts that this method has been validated in previous studies in birds [51,81,82] and that our behavioral tests with acetic acid confirmed the complete lack of reaction to this very strong olfactory stimulus.

The fact that birds used in the present experiment were not sexually naïve (they had been pre-tested several times for the presence of active copulatory behavior) could also be a confounding variable. The previous sexual experience of the subjects might indeed mitigate the disruptive effects of olfactory deprivation on male sexual behavior as is clearly the case in male hamster [79,117]. The present data therefore do not rule out completely a possible effect of olfactory deprivation on male sexual behavior that would be only observed in sexually naïve birds. Further experiments are needed to address this possibility.

It must also be noted that in Galliform species, including quail, visual cues may be sufficient to elicit copulatory responses [22]. Additional stimuli, especially vocalizations but also possibly olfactory stimuli might enhance these responses and probably play a role under natural conditions but may be masked in the experimental conditions used for this experiment (see [40,42] for additional discussion). Male were indeed kept continuously in physical isolation between the tests and their sexual motivation was consequently extremely high when they were introduced to a female. This could explain why they reacted by showing sexual behavior despite the fact that they were not experiencing the full range of stimuli that normally activate this behavior. Thus the present experiment does not completely exclude a potential role of olfactory on the expression of copulatory behavior.

Although the lack of such a behavioral effect was at first sight surprising, it facilitates the interpretation of the deficits in brain activation that were detected. This experiment indeed demonstrated that blockade of the nostrils decreases the number of Fos-ir cells in brain areas that are classically activated following expression of male copulatory behavior [5,98]. Given that no decrease in motor output was detected in these birds deprived of their sense of smell, it is thus likely that the decrease in brain activation was due to the lack of olfactory inputs to the mPOA and BSTM. This suggests therefore very strongly that these inputs reach brain areas controlling sexual behavior, an observation also supported by the increased expression of Zenk in the olfactory bulbs and its inhibition following blockade of the nostrils. To our knowledge, this is the first report of immediate early gene activation in the avian olfactory bulb and further experiments should be carried out to confirm this pattern of activation.

In mammals, the olfactory information that plays a role in the activation of sexual behavior in males and females reaches the POA through a pathway that includes the cortico-medial amygdala and the bed nucleus of the stria terminalis (BSTM) [13,56,90]. *In vivo* tract-tracing

has revealed the presence in quail of an important projection from the arcopallium (homologous to parts of the mammalian amygdala) and in particular the nucleus taeniae of the amygdala to the medial preoptic nucleus [4], suggesting that olfactory inputs could reach the medial preoptic nucleus through a similar route in quail. However, we did not find in this experiment any effect of olfactory deprivation on Fos expression induced by male sexual behavior in the nucleus taeniae. Furthermore, no effect of the olfactory deprivation implemented in this study was detected on Fos expression in brain nuclei such as the medial and lateral striatum, the medial septum or the piriform cortex that are presumably part of the olfactory pathway [13,30,83, 88]. Thus either olfactory information is transmitted to the mPOA and BSTM of quail by a route that does not involve these nuclei or olfactory cues originating from the female during copulation are not intense enough to induce Fos expression in the first relays of olfactory pathway or may activate other aspects of the functioning of these pathways that could only be detected by other markers of neuronal activation (e.g. other immediate early genes).

6. Conclusion

Despite the presence of claims suggesting the absence of a functional sense of olfaction in birds in general, there is now a substantial amount of research demonstrating that a wide variety of avian species are clearly able to detect odors based on electrophysiological and behavioral evidence (e.g. sensitivity thresholds measured by conditioning procedures). Furthermore, ethological investigations have also demonstrated that many birds use olfactory stimuli in various contexts such as searching for food, avoiding predators, orienting toward their nest. The presence of a well-developed sense of olfaction in birds is also supported by recent biochemical studies demonstrating that nine avian species from seven different orders possess olfactory receptor genes homologous to those of other vertebrates and that the sequence of these genes apparently codes for potentially functional proteins that should then be expressed in the sensory olfactory neurons [95]. A few experiments have also suggested that birds might use olfactory stimuli for the control of their reproductive behavior. The origin of these stimuli has in general not been identified except in the case of the Crested auklets in which a tangerine odor produced by males and females was clearly demonstrated to be able to control sexual and/or aggressive reactions of the males. Our recent work in quail also suggests the presence of a significant brain activation (as identified by the quantification of the induction of immediate early genes expression) by olfactory stimuli in relation to the expression of male sexual behavior. The amount of available information thus clearly demonstrates that the commonly held view of birds being anosmic or even microsmatic is obviously wrong. Research on the sense of olfaction in birds, the neural pathways mediating the detection of olfactory stimuli and their use in the control of various aspects of the behavior is therefore warranted and should lead to a substantial re-evaluation of many aspects of avian biology.

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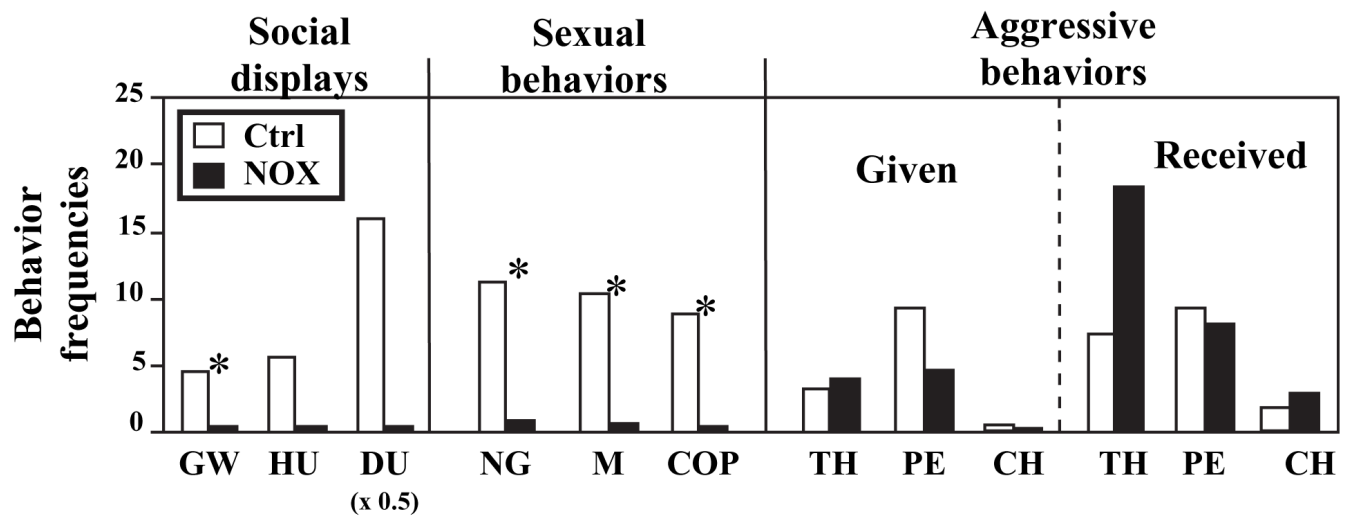


Figure 1.

Behavior frequencies recorded during four hours of observations (16×15 min in January) in male ducks that had their olfactory nerves sectioned bilaterally (black bars) and in control subjects (open bars). Frequencies were statistically compared in the two groups and asterisks above a pair of bars indicate a significant difference ($p < 0.05$). A marked decrease in the expression of social displays and sexual behaviors was detected after the nerves section but aggressive behaviors (given or received) were not affected. Abbreviations: JGW: grunt-whistle; HU: head-up tail-up; DU: down-up; NG: neck grab; M: mount; COP: copulation; TH: threat; PE: peck; CH: chase. Modified from data in [3].

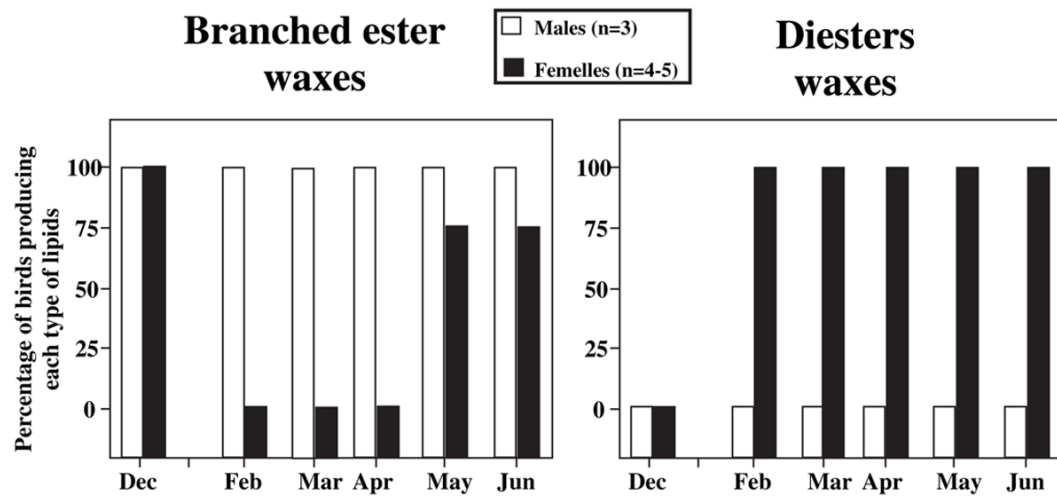
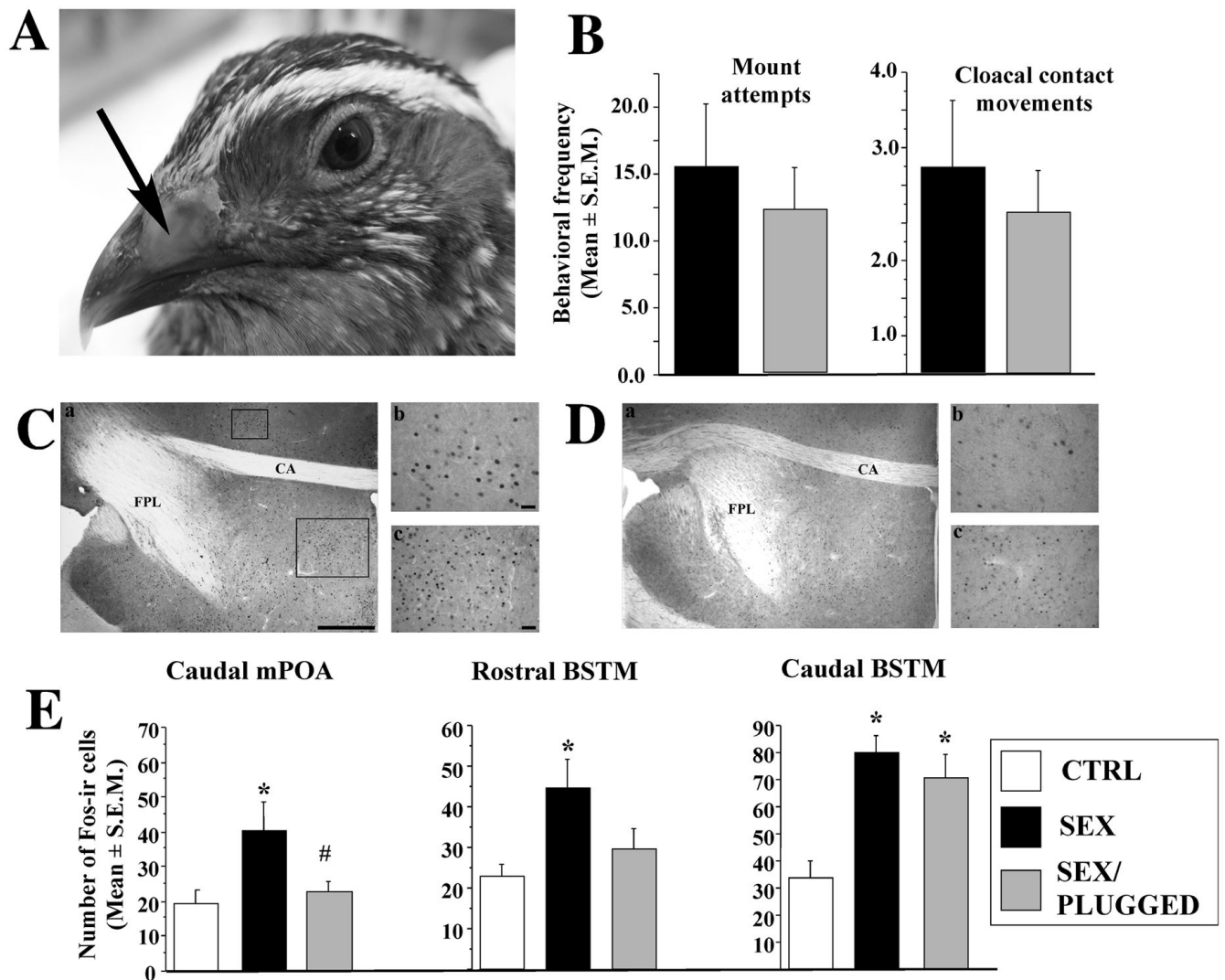


Figure 2.

Seasonal changes from December to June of the percentage of male or female domestic ducks (Rouen breed) synthesizing two types of lipids in their uropygial (preen) gland: branched ester waxes and diester axes. The pattern of changes for unbranched ester waxes was similar to the pattern observed with branched esters. Drawn from data in [49].

**Figure 3.**

Occlusion of the nostrils in Japanese quail does not lead to any significant change in sexual behavior but alters the expression of immediate early gene Fos in their brain. **A.** Photographs of the head of a male Japanese quail in which the nostrils were blocked by dental cement (arrow). **B.** Frequency of two sexual behaviors (mount attempts and cloacal contact movements) recorded in males that were either used as control (black columns) or had their nose blocked by dental cement (gray columns). **C–D.** Photomicrographs of sections through the preoptic area stained by immunohistochemistry for the immediate early gene Fos in a male that had been allowed to copulate in control condition (C) or a male which had copulated after his nose was blocked by dental cement (D). In both panels, Fos expression is illustrated in: (a) the caudal mPOA and the rostral BSTM at low magnification (4x objective); (b) the rostral BSTM at high magnification (40x objective); (c) the caudal mPOA at medium magnification (20x objective). The boxes in panel C indicate the position of the fields shown at higher magnification. Scale bars = 500 μ m in a, 50 μ m in b and 100 μ m in c. CA, commissura anterior; FPL, fasciculus prosencephali lateralis. **E.** Numbers of Fos-immunoreactive (Fos-ir) cells observed in three brain areas of male quail that had been allowed to copulate with a female either in the normal condition (SEX) or after their nose was blocked with dental cement (SEX/

PLUGGED). Cells were also counted in control birds that stayed in their home cage and were not exposed to a female (CTRL). $\ast = p < 0.05$ compared to CTRL, $\# = p < 0.05$ compared to SEX. mPOA: medial preoptic area; BSTM: medial part of the bed nucleus striae terminalis. Redrawn from data in [97].

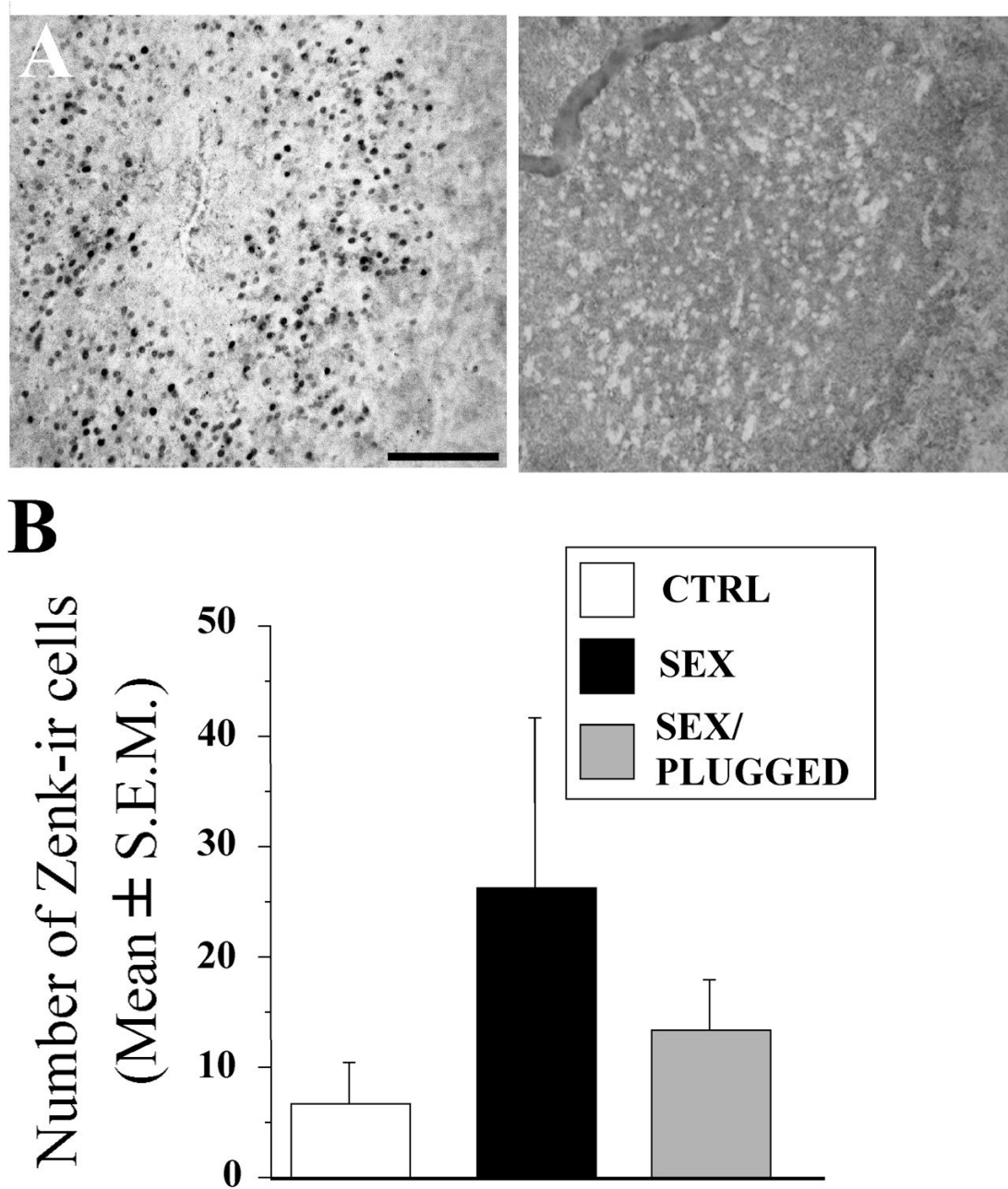


Figure 4.

A. Photomicrographs illustrating *zenk* expression in the olfactory bulb of male quail that copulated with a female in the absence (left) or the presence (right) of a nasal plug. Scale bar = 100 μ m. **B.** Numbers of zenk-immunoreactive cells observed in the olfactory bulb of male quail that had been allowed to copulate with a female (SEX; n=4) or copulated with that female after their nose was blocked with dental cement (SEX/PLUGGED; n=5). Control birds (CTRL; n=2) stayed in their home cage and were not exposed to a female. Birds that showed, for unexplained technical reasons, no Zenk expression at all in the olfactory bulbs were removed from this analysis resulting in the reduced numbers of subjects mentioned here. Although the average difference illustrated here was not statistically significant, it became significant when results of the present subjects were pooled with results of other birds that had additionally their

cloacal gland anesthetized before the behavioral tests (see [97] for additional description of these results). Redrawn from data in [97].