THEORETICAL REVIEW

Producing and Interpreting Experimental Olfactory Deficits

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PRODUCING and measuring olfactory deficits is a significant problem in experimental studies of olfaction and behavior. On the basis of anatomical considerations a multicomponent olfactory organ system is described, consisting of an interrelated group of chemosensitive receptor systems: the olfactory (I) nerve, the vomeronasal apparatus, portions of the trigeminus, and the nervus terminalis, plus a set of efferent projections to olfactory structures. Within this framework, nine olfactory system manipulations and a variety of experimentally and clinically recognized olfactory abnormalities are reviewed. The various manipulations of the olfactory organ system can differentially affect component organ subsystems, produce different olfactory deficits and, in some instances, introduce secondary, nonsensory effects which can confound interpretation of behavioral and physiological measures. Suggestions are made for conceptual, procedural, and terminological standardization in studies of olfaction and behavior.

Elimination of the sense of smell is one relatively simple, direct experimental approach to the study of olfactory control of behavior. Over the years, a surprising variety of surgical, nonsurgical, chronic, and reversible techniques have been used in such investigations. In theory, the effects of these manipulations should be the same if the observed behavioral changes are the result of total olfactory loss. In practice, however, this is not the case. A number of recent studies provide good evidence that various manipulations of the olfactory system do not produce equivalent effects [2, 36, 49, 131].

There are two kinds of explanation for this discrepancy. The sensory explanation is that the olfactory deficit produced by the various techniques may be different. The nonsensory explanation involves the possibility that some methods may produce secondary effects unrelated to olfactory deficits which alter the behavior or physiology of the organism studied. Both kinds of explanation indicate the necessity for consideration of the techniques themselves, the sensory system involved, as well as conceptual and procedural standards used in the investigations.

The present review is primarily addressed to problems involved in making and interpreting olfactory system manipulations in animal studies. On the basis of anatomical considerations a multicomponent olfactory organ system is described; it consists of a number of discrete afferent chemosensitive receptor systems and receives central efferent projections. The anatomical arrangements of these subsystems are examined in terms of their vulnerability to the various olfactory manipulations employed in behavioral studies. The nature and extent of olfactory deficits are discussed as a framework for establishing conceptual and terminological standards for defining the effects of olfactory manipulations. Finally, the various manipulations are reviewed and evaluated regarding their efficacy, applicability, adequate control procedures, and possible characteristics of the preparations which should be taken into account in interpreting results derived from such manipulations.

It is hoped that this review will contribute to standardization of experimental procedures for workers already in the field of olfaction and behavior, as well as aid others,
contemplating the use of experimental olfactory manipulations, in the choice and proper application of appropriate methods in their research and theoretical considerations.

THE OLFACTORY ORGAN SYSTEM

The I Nerve and its Central Connections

The vertebrate olfactory receptor is a bipolar neuron whose central process forms the olfactory nerve fiber (Ist cranial nerve). Allison [7] has estimated $10^7$ olfactory receptors in the rabbit. The receptors are densely packed and embedded in a complex of turbinates in the posterior nasal cavity. Incoming olfactory fibers ensheath the olfactory bulbs, mostly on the rostral and ventral aspects, and terminate in a glomerular layer. There is an ipsilateral projection of fibers from each half of the nasal cavity to each olfactory bulb.

The olfactory bulb is an ovoid mass of neatly layered cortical tissue organized around an evagination of the lateral ventricle, situated at the rostral end of the telencephalon. The olfactory stalk, or peduncle, is an area of paleocortex lying caudal to and continuous with the main olfactory bulb; it extends from the base of the bulb to the olfactory tubercle. The peduncular region consisting of the retrobulbar area to the zone of junction with the telencephalon proper is termed anterior olfactory nucleus [146]. The arrangement of cells and fiber-architectonics led Herrick [59] and others [7, 142, 146] to further subdivide the anterior olfactory nucleus into four to seven areas. The cell bodies of pars bulbaris, one of these subdivisions, actually lies within the olfactory bulb. Also residing in the olfactory peduncle is the anterior limb of the anterior commissure.

The olfactory bulb projects to the anterior olfactory nucleus, olfactory tubercle, prepyriform cortex, and amygdala [76, 79, 106, 120, 146]. All sensory efferents from the bulb are believed to travel within the lateral olfactory tract, which forms circumferentially around the anterior end of the olfactory peduncle. The fibers collect into a discrete bundle on the ventrolateral surface of the posterior peduncle as they move caudally along the lateral edge of the olfactory tubercle, apparently ending at the level of the lateral olfactory nucleus in the amygdaloid complex. Remaining fibers are distributed over the posterior portion of the prepyriform lobe [146]. Additional olfactory projection areas include the anterior, preoptic and lateral areas of the hypothalamus, anterior and dorsomedial thalamus, and lateral habenula [10, 40, 106, 124]. Although some of the latter projection sites may not receive fibers emanating from the olfactory bulb proper, degeneration is frequently observed in these structures after olfactory bulb removal, which typically encroaches on olfactory cortex in addition to the main olfactory bulbs.

The anatomy and physiology of the peripheral olfactory system has been reviewed by Moulton and Beidler [91]. There are numerous excellent review of central olfactory connections [7, 46, 76, 79, 97, 120] to which the interested reader is directed for detailed discussions.

The Vomeronal System

The vomeronal apparatus consists of (a) the peripheral receptor, the vomeronal (Jacobson’s) organ, (b) the vomeronal nerves, and (c) the target organ in the central nervous system, the tuberculum vomeronasal or accessory olfactory bulb [1, 96] where the nerves spread out to form a superficial fiber layer.

The vomeronal system is extremely well developed in reptiles and most mammals [58, 79, 81, 96, 113, 138]. In the rat the vomeronal organ is a paired tubular structure situated slightly above the floor of the nasal cavity within the nasal septum. It extends in the rostrocaudal direction, beginning roughly behind the buccal margin of the upper incisors and reaching about three-fourths the length of the diastema of the upper jaw [1].

The opening of the vomeronal organ is variable, depending on the species. In the rat, for example, the organ is open only to the nasal cavity by a small duct in the anteroventral septal wall [66]. In other forms it is open to both the nasal cavity and the mouth via the nasopalatine canal, which penetrates the hard palate via the incisive foramen. Jacobson’s organ in adult lizards has no connection with the nasal cavity but opens directly into the mouth [138]. There is a discrete vomeronal epithelium in turtles but no outpocketing in the nose and it is thus freely open to the nasal cavity [138].

In all species the vomeronal organ directs to the CNS a distinct fiber: the vomeronal nerve. In the rat the nerve takes an oblique course in the septum as it leaves the neuroepithelium and passes through openings in the dorsal cribiform plate to synapse in the accessory olfactory bulb, which is embedded in the dorsal part of the (main) olfactory bulb. The laminae of the accessory olfactory bulb are distinct from the cells of the main olfactory bulb.

Thus, the vomeronal system is separable from the main olfactory system (the I nerve and its central connections) in terms of its receptor apparatus and the projections from the neuroepithelium to its target organ in the central nervous system [1, 79, 80, 86]. Furthermore, the second order projections of the vomeronal organ system are distinct from those of the main olfactory bulb. Herrick [58], using a Golgi preparation, described a direct fiber projection from the accessory olfactory bulb to the “nucleus amygdalae” in frog. In the tegu lizard Heimer [52] finds a similar projection to the nucleus sphericus. Winans and Scala [149] studied the central projections of the mammalian accessory olfactory bulb. With selective lesions and Fink-Heimer staining they observed discrete terminal degeneration in the medio cortical complex of the rabbit amygdala, which is not found after lesions restricted to the main olfactory bulb tract, sparing accessory bulb neurons. These results are in complete agreement with the earlier observations of Mann [80] who described specific myelinated accessory olfactory bulb projections to the same portions of the amygdala in four genera of phyllostomatoid bats (Phyllostomus, Glossophaga, Artibeus, and Desmodus). The main and accessory olfactory bulbs can be further dissociated in terms of embryological origins [63], growth rates [128], and histochemical characteristics [150].

McCotter [86] has reviewed a number of exciting hypotheses of vomeronal system function but there is a lack of positive evidence concerning the contribution of this system in behavioral control [98, 148]. The prevalence of vomeronal apparatus across many species — mammalian and submammalian — invites fruitful investigation.

The Trigeminal Nerve System

The trigeminal, or V cranial nerve, is a large segmented
bundle of fibers having three divisions: mandibular, maxillary and ophthalmic. In a variety of species, including rat and man, some of these divisions distribute free nerve endings into the nasal mucosa which have been shown to be chemosensitive [137]. Therefore, these aspects of the trigeminal nerve system are here defined as another functional component of the olfactory organ system.

Most trigeminal innervation of the interior nasal cavity appears to be situated in the airway region although terminations are found in olfactory mucosal areas as well. The entire nasal mucosa can be viewed as a chemoreceptive organ in which the respiratory and olfactory components are separable but overlapping in both topography and response characteristics [56, 137, 138].

The maxillary division of the trigeminal nerve delivers terminals to the nasal cavity via its posterior nasal rami as well as the nasopalatine nerve. The ophthalmic contribution to the nasal mucosa, the ethmoid nerve, passes through the orbit as a branch of the nasociliary nerve, coursing over the optic nerve and then penetrates the cranium via the ethmoidal foramen. The fiber then proceeds over the olfactory bulb, embedded in the dura mater, and enters the nasal cavity through foramina in the cribiform plate. Tucker [138] judges the ophthalmic division of the system to be its most surgically accessible component.

The precise functional contribution of the chemosensitive portions of the trigeminal nerve system is unclear but nonetheless intriguing. Traditionally regarded as mediating the "common chemical sense" [90] the trigeminus has been assumed to be sensitive only to chemical irritants. More recent empirical evidence, however, seriously questions the classical view. Tucker [137] recorded from "twigs" of the ethmoid nerve in the gopher tortoise and found the nerve sensitive to virtually all odors which stimulate its olfactory nerve. Indeed, concentrations necessary to excite trigeminal fibers were sometimes lower than the minimum required to evoke responses from the olfactory nerve. Other authors have emphasized the need to consider the trigeminal contribution to odorant perception and recognition [55, 56, 121]. Might this nerve, which synapses directly into the brainstem, be involved in behavioral arousal to specific odorants or pheromones? Whatever its function proves to be, the evidence available at this time indicates that the trigeminal is chemosensitive and can be easily included in the olfactory organ system. For this reason olfactory manipulations which affect or spare the trigeminal system are liable to have important differential effects on the organism's responsiveness to chemical cues from the environment.

The Nervus Terminalis System

For at least 70 years the nervus terminalis has been familiar to comparative anatomists but has been all but ignored in behavioral and physiological investigations; thus its functional significance is solely speculative. The potential importance of the terminal nerve is suggested by its anatomical associations and its persistent representation across virtually all classes in vertebrate phylogeny [69].

The nervus terminalis is first apparent as it emerges in several small bundles from the ventromedial aspect of the forebrain. It courses anteriorly along the ventromedial surface of the olfactory peduncle and bulb, forming a loose plexus, to the region where the vomeronasal nerves join into a main strand above the cribiform area. Nervus terminalis migrates through vomeronasal and trigeminal fibers [61, 62, 67, 87, 101] to its area of termination. Free endings of the nervus terminalis have been distinguished from vomeronasal and trigeminal receptors by Huber and Guild [67] who described their anterior course through the nasal cavity and distribution in the vomeronasal and anterior part of the nasal septum of the rabbit. Larsell [67] describes a similar distribution in dog, squirrel, human, pig and sheep. McCotter [87] could not identify nervus terminalis in the rat.

The central projections of the n. terminalis are described as being direct to the septal area, preoptic zone, hypothalamus and/or preoptic area [61, 67, 101]. Larsell [68] has suggested that the nerve may function as a chemosensitive afferent. Riss, Halpern and Scalia [115] also discuss some exciting possibilities as to its functional role. Tucker [138] has recently emphasized the need for (and possibly difficulty of) electrophysiological studies to assess its chemosensitive characteristics.

Centrifugal Fiber Projections to the Olfactory Organ System

In the last 10 years there have been a number of anatomical studies identifying specific efferent fiber projections to the olfactory bulbs, peduncle, and tubercle, arising from a variety of olfactory and telencephalic nuclei, and possibly from cell bodies in the brain stem. Anatomically, there is little justification for considering these centrifugal fibers to constitute a unitary efferent system since the origins and organization of the projections are varied. It is only for purposes of this presentation that they are grouped together for discussion.

The lateral olfactory tract carries centrifugal fibers [51, 108, 109], corresponding to those originally described by Cajal [26]. The anterior limbs of the anterior commissure also contribute efferent fibers, with projections from the contralateral anterior olfactory nucleus, pars medialis and ipsilateral olfactory tubercle [77]. Dennis and Kerr [30] sectioned both the lateral olfactory tract and anterior limbs of the anterior commissure and were nonetheless able to record evoked potentials in the olfactory bulbs after stimulation in the posterior olfactory cortex. The pathway mediating this response, however, remains to be observed histologically.

The origin of these centrifugal fibers has been a matter of some controversy. There is general agreement that contributions are made from ipsilateral olfactory brain and contralateral anterior olfactory nucleus. In a detailed and thorough study Price and Powell [110] identified the cells of the horizontal limb of diagonal band, the substantia innominata, as the nucleus of the origin of centrifugal olfactory fibers. The afferent connections of this nucleus, as described by Price and Powell, emphasize the widespread integrative complexity of the efferent and afferent connections between the olfactory bulbs and telencephalon.

Ungerstedt observed ascending noradrenergic fibers extending to the olfactory bulbs from brainstem nuclei [139]. These tracts run near but not quite within the lateral olfactory tract. Efferent fibers projecting to the olfactory bulbs and containing acetylcholinesterase have also been described [125].

The accessory olfactory bulbs receive efferent projections [111] originating in ipsilateral cortical and medial amygdaloid nuclei which pass through the stria terminalis.
The close anatomical association of the cortical and medial nuclei of the amygdala and ventromedial and preoptic areas of the hypothalamus [74] have been discussed by Raisman [111] in terms of possible olfactory--endocrine relations involving the vomeronasal system.

Stone and his co-workers have reported in a series of papers [132, 133, 134] electrophysiological results which they interpret as evidence for an efferent component in the olfactory-trigeminal system. According to their studies trigeminal efferents may normally interact with olfactory bulb so as to modulate its electrical activity as well as mediate autonomic responses accompanying behavioral arousal; neural structures and pathways long considered to be substrated for transmission of sensory afferents also contain or consist of a complex of efferent fibers from a variety of sites in the CNS. The functional role of these centrifugal pathways has been the subject of several discussions [111,115] as well as the basis of some pioneering experimental work [99].

The Concept of an Olfactory Organ System

These anatomical and physiological considerations indicate that chemosensitivity and/or the sense of smell of most animals is normally subserved by a complex of neural systems, composed of afferent and efferent projections which has been described here as the olfactory organ system. The inclusion of both peripheral and central processes extends the notion of nasal chemoreception, recognized by some previous workers, and emphasizes the unity and integration of the neural apparatus for smell and its functional relationship with other portions of the CNS.

The lesser-known subsystems are not merely "olfactory esoterica" [138]; the concept of a multicomponent olfactory organ system emphasizes the importance of giving careful consideration to the effects of different olfactory system manipulations, a major point of the present review. Although much remains unknown about the interactions of the subsystems it is apparent that the individual components do not function independently. Disruption of any one component is likely to alter the operations of remaining components. Likewise, lesions restricted to the organ system can affect other, non-olfactory portions of the brain, i.e. telencephalic and diencephalic nuclei often associated with extra-olfactory functions, as well as endocrine [147] and metabolic pathways [102]. While the use of selective lesions is an invaluable tool for recognizing functional neural relations it cannot be assumed that removal of any main or ancillary portion of the olfactory organ system leaves the remainder of the system or the remaining olfactory sense otherwise functionally normal.

THE NATURE AND EXTENT OF OLFACTORY DEFICITS

Anosmia means literally, without smell. This condition is simple enough conceptually, but there are several important practical problems encountered in adequately defining and demonstrating the nature and extent of olfactory deficits. To be totally without smell implies that all chemosensitive components of the olfactory organ system are inoperable or unresponsive in the presence of odorant stimuli. The term anosmia can justifiably be applied to any condition of complete loss of olfactory capability, permanent or temporary, regardless of etiology. Inaccurate conclusions and confounded theoretical implications can arise, however, if anosmia, per se, is not distinguished from other less complete olfactory deficits or conditions of olfactory impairment accompanied by secondary, nonsensory effects. There are a variety of olfactory disabilities which have been recognized in the clinical and experimental literature. These are worthy of consideration, for they shed light on some empirical and conceptual problems encountered in producing and understanding olfactory deficits.

The Anosmias

According to some definitions a totally inoperative first nerve results in "general anosmia" [9]. Functionally, the I nerve is a primary component of the olfactory organ system. However, in the absence of the olfactory nerve or its central projections the vomeronasal, trigeminal and nervus terminalis systems can continue to operate. Allen [5] found olfactory conditioning to persist after complete lateral olfactory tract lesions in dogs. Patients were able to detect, and in one case recognize the odor of geraniol, after "transection of all olfactory nerve fibers" and total surgical excision of the olfactory epithelium, a treatment for invasive carcinoma of the nasal sinuses [56]. On the basis of their observations and measurements, Henkin and Hoye [56] define "primary" and "accessory" areas of olfaction. Primary olfactory areas receive I nerve innervation. Accessory olfactory areas, on the other hand, contain cranial nerve innervation (and are thus not necessarily related to the vomeronasal-accessory olfactory bulb system).

Clinically, the most common olfactory abnormality is a lowered sensitivity toward particular odors, while general olfactory perception remains unchanged. In 1918 Blakeslee described this condition in respect to particular flower scents [18] and from this concept of "odor blindness" arose. Amoore [9] prefers the term "specific anosmia" and has studied the phenomenon in human populations [8]. Careful measurements and the use of a wider range of odorant stimuli in olfactory testing would probably reveal that many experimentally anosmic animals can, in fact, perceive and respond to at least some olfactory cues.

Hyposmia

This term usually refers to a relatively generalized and mild olfactory deficit. Hyposmia may be experienced during the common head cold and frequently develops after severe cases of influenza. The latter form of hyposmia or " uncomplicated anosmia" can reportedly be relieved in human patients with massive injections of Vitamin A [35].

Henkin and Hoye describe patients without the I nerve as hyposmic, although the olfactory deficit in such persons is considerable; they reserve "complete anosmia" for cases in which the entire range of chemoreceptors are inoperable [56].

Dysomia and Parosmia

These terms describe a syndrome of perverted sense of smell. Dysomia is a pathological state in which common odors are experienced as "foul", "obnoxious" and often uncontrollably nauseating [57]. In some cases, autosmia [88], the continuous experience of odors in the absence of actual stimuli is also reported. Parosmia [88] refers to a condition in which certain scents evoke sensations that differ qualitatively from the odor perceived by a normal
EXPERIMENTAL OLFACTORY DEFICITS

person; cacosmia [88] and heterosmia are generally synonymous with parosmia.

Cryptosmia

This refers to all conditions in which odor stimuli are blocked from reaching odor-sensitive tissues [88]. Peripheral and central structures remain intact but are somehow obstructed.

MANIPULATIONS OF THE OLFACTORY ORGAN SYSTEM

Olfactory Bulb Removal

Bilateral surgical destruction of the olfactory bulbs has probably been the most widely used method of producing anosmia experimentally. The general routine for this nonstereotaxic lesion involves making a scalp incision to bare the skull, removing a bone flap overlying the olfactory bulbs and, under visual guidance, aspirating brain tissue comprising the olfactory bulbs, taking care to scrape the cribiform plate. Excessive bleeding is sometimes experienced after aspiration and foreign materials such as Gel-foam or bone wax are frequently packed into the operated space. The neural damage involved in this lesion is, of course, enormous and even with care tends to be quite variable in extent. Relatively few studies report the precise extent of damage beyond gross inspection of the brain, although some experiments have included histological descriptions of damage revealed by degeneration stains of prepared sections. Following olfactory bulbectomy widespread degeneration is found throughout the telencephalon [106, 120, 146].

As was noted earlier, the olfactory bulb is not a discrete structure. Lying within the olfactory bulbs are the accessory olfactory bulbs, as well as at least one subdivision of the anterior olfactory nucleus. Thus, it is difficult, if not impossible, to reliably perform a lesion by aspiration limited solely to the olfactory bulbs. Additional structures often destroyed during bullectomy include anterior olfactory nucleus, anterior limbs of the anterior commissure, lateral olfactory tract, projection fibers of the accessory bulbs and even olfactory tubercle. Furthermore, efferent systems projecting to the bulbs will also be involved. Scraping of the cribiform plate, in many species, can produce variable amounts of damage to trigeminal branches, the vomeronasal nerve, and possibly the nervus terminals. Olfactory bulb removal also alters unoperated brain areas, as evidenced by widespread terminal denervation observed after ablation [146]. Pohorecky [105] and others [38] have measured significantly lowered levels of telencephalic norepinephrine after both bullectomy and transection of the olfactory peduncle.

The olfactory deficit produced by bullectomy should be permanent and is usually assumed to be complete. However, it is sometimes found that the resultant olfactory deficit is not complete [78] in all animals. Whether the retained olfactory abilities are trigeminal, vomeronasal, or can be attributed to some remaining first nerve fibers has not been determined.

The most serious problem with olfactory bulb removal as a technique for inducing anosmia is that the lesion can produce significant behavioral and physiological effects which are not directly related to the sensory deficit. As early as 1907, J. B. Watson and his contemporaries noted atypical behavior and performance in bullectomized rats [114, 143]; there are now several lines of evidence which indicate that olfactory bulb damage does indeed affect more than the sense of smell. The behavior and performance of bullectomized animals in nonolfactory learning tasks can change dramatically [103, 144]. Sensitivity to some, but not all exteroceptive stimuli increases [23]. Rats often become irritable and hyperemotional after the lesion [2, 20, 34, 38, 80]. It is conceivable that such changes could be due to the “psychological stress” associated with loss of important information from the environment [34]. However, rats rendered anosmic by certain other, non-surgical olfactory manipulations, did not display the general behavioral changes [2] and learning deficits [49] observed in bullectomized subjects.

Table I summarizes a wide range of alterations, behavioral and physiological, which have been observed following bilateral olfactory bulb removal. Many, but clearly not all of these changes are attributable to olfactory debilitation. Recently, various alternative olfactory system manipulations have been tested and in some instances, as can be seen in the second and third columns of Table I, conflicting results have been obtained. These data provide evidence that olfactory bulb removal can produce more than a sensory deficit. Interruption of pathways within the olfactory organ system and between the olfactory system and other portions of the nervous and endocrine system seem to be implicated in the induction of many of the changes observed in bullectomized animals.

Furthermore, if bilateral olfactory bulb removal is achieved with two unilateral lesions separated by a recovery period, then some behavioral alterations observed after ordinary bullectomy are absent [41, 119]. Female hamsters, sustaining unilateral olfactory bulb removal during infancy do not lose olfactory abilities yet display deficits in maternal behavior later in life [73].

These considerations, taken together, make olfactory bulb removal a generally undesirable procedure for producing a sensory deficit. The potential complications resulting from secondary or nonsensory effects greatly limit interpretation and conclusions about changes related to sensory loss, per se, after bullectomy. In most cases other methods should be considered, taking into account the characteristics of the particular alternatives.

Lateral Olfactory Tract Lesions

Anatomical evidence suggests that the lateral olfactory tract is the sole sensory projection from the olfactory bulbs to the forebrain [146]. Therefore, destruction of this fiber bundle should eliminate all sensory information from the olfactory bulbs to the rest of the brain. This procedure would seem to offer several advantages over aspiration of the olfactory bulbs. First, the actual extent of surgical insult is far less than that involved with traditional bullectomy; less neural tissue is destroyed and problems with excess bleeding may be avoided. The lesion can be made with stereotaxic guidance, which affords some degree of uniformity. Structures which are normally included in bullectomy, such as the accessory olfactory bulbs, can be spared.

Long and Tapp [78] produced radio frequency lesions of the lateral olfactory tract in rats and tested them in a variety of olfactory detection tasks using food odors (lab chow). They found that when the lesion included 100% of the tract, animals performed as though anosmic. Hist-
TABLE 1

BEHAVIORAL AND PHYSIOLOGICAL CHANGES OBSERVED AFTER BILATERAL OLFACTOR Y BULB REMOVAL. SIMILAR EXPERIMENTS UTILIZING ALTERNATIVE EXPERIMENTAL MANIPULATIONS ARE INDICATED IN SECOND COLUMN.†‡

<table>
<thead>
<tr>
<th>Effect of Bilateral Olfactory Bulb Removal</th>
<th>Corroborating Results with Other Manipulations</th>
<th>Alternate Manipulation*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Species-Typical Behaviors</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disruption of maternal behavior (mouse [44], rat [41])</td>
<td>No</td>
<td>ZS (rat [42])</td>
</tr>
<tr>
<td>Elimination or disruption of male sexual behavior (rat [19,53], mouse [117], hamster [95])</td>
<td>Yes</td>
<td>ZS (mouse [117], hamster [32, 75]); B+Occl. (hamster [32]); Anes. (sheep [12]); Anes. (hamster [107])**</td>
</tr>
<tr>
<td>Altered or decreased &quot;scent-marking&quot; (hamster [94], gerbil [13], dog [43])</td>
<td>Yes</td>
<td>ZS (hamster [32]); B+Occl. (hamster [32]); Occl. (dog [50])</td>
</tr>
<tr>
<td>Reduction or elimination of intermale aggression (rat [11], mouse [116], gerbil [28], hamster [93])</td>
<td>Yes</td>
<td>ZS (wild rat [4], mouse [116], gerbil [28], hamster [32]); B+Occl. (hamster [32])</td>
</tr>
<tr>
<td>Elimination of cold-induced huddling (mouse [135])</td>
<td>No</td>
<td>ZS (mouse [135])</td>
</tr>
<tr>
<td>Altered hoarding behavior (rat [136], hamster [47])</td>
<td>No</td>
<td>ZS (rat [42])</td>
</tr>
</tbody>
</table>

| Feeding and Drinking                     |                                               |                         |
| Potentiated hyperphagia with ventromedial hypothalamic lesions [70] | – |                         |
| Altered meal patterns [71] | – |                         |
| Increased taste finickiness [21] | No | ZS [21] |
| Decreased taste aversion thresholds [45,64] | – |                         |
| Increased saline intake [27] | – |                         |

| Neurological and Physiological Measures   |                                               |                         |
| Decreased ovary weights (mouse [147]) | – |                         |
| Decreased hormonal sensitivity (mouse [135]) | – |                         |
| Disrupted estrous cycles (mouse [140]) | Yes | ZS (mouse [140]) |
| Increased adrenal weights [38] | – |                         |
| Decreased telencephalic norepinephrine levels [105,38] | – |                         |
| Altered electrophysiological activity of intact brain regions [14] | Yes | XNE (rabbit [17]) |
| Increased heart rate changes [104] | – |                         |
| Increased glucose tolerance and insulin sensitivity [102] | – |                         |

| Learning Tasks                           |                                               |                         |
| Deficits in passive avoidance [49, 83, 126] | – |                         |
| Alterations in active avoidance [83,126] | – |                         |
| Deficits in visual discrimination problems (pigeon [144], rat [103]) | Yes | X.O.N. (pigeon [144]) |
| Inability to acquire taste-aversions [49] | No | ZS [49] |

| General Performance and Behavior         |                                               |                         |
| Increased "emotionality" or "irritability" (rat [2, 11, 20, 34, 38], hamster [47]) | No | ZS (rat [2]) |
| Altered locomotor activity (rat [83], gerbil [28]) | Yes | ZS (gerbil [28]) |
Increased reactivity to some exteroceptive stimuli [23]

Persistent behaviors competing with performance in learning situations [114, 143]

Altered operant (bar-pressing) performance (rat [83, 127], gerbil [28], hamster [47])

Lesions of Other Central Olfactory Structures

There is a fairly extensive literature on the effects of lesioning a variety of neural structures which, anatomically, are considered to be olfactory-related components of the central nervous system. None of these lesions totally abolish odor detection, although some have been interpreted to impair recognition or interfere with some aspects of encoding. Such structures include: the anterior limbs of the anterior commissure [15], olfactory tubercle [78], pyriform cortex and amygdaloid nuclei [6], temporal lobes [24], lateral hypothalamus [84], and habenula [112]. In some of these experiments interpretation is difficult since the lesions may produce changes in behavioral responsiveness which may mask or alter the effects of the lesions on sensory processing.

Destruction of the Ist Nerve

The anatomical arrangement of the first nerve and olfactory bulbs allows surgical access to first nerve fibers rostral to the bulbs in a variety of species. Wenzel and her co-workers destroyed the olfactory nerve in pigeons with a dental burr at a level between the orbits just anterior to the olfactory bulbs [144, 146]. Boycott and Guillery [22], working with turtles, lesioned the first nerve fibers as they entered the bulbs, using a heated platinum wire.

Wenzel [144] has noted that degeneration is apparent in the olfactory bulbs of olfactory nerve-sectioned birds, several months after the operation. Alterations of electrophysiological activity in a variety of brain regions was found in rabbits following surgical destruction of the olfactory membrane [17]. Thus, this method may directly interfere with normal bulbar functions beyond interruption of sensory input. Wenzel has in fact, examined this possibility in some detail, and observed a number of behavioral changes in nerve-sectioned pigeons such as differential heart rate changes to auditory stimuli and disrupted orientation toward relevant visual signals [144, 145]. In these respects nerve-sectioned pigeons resemble bulb-ablated subjects. Likewise, Boycott and Guillery [22] noted feeding disruptions in turtles (Pseudemys scripta) after olfactory nerve destruction.

Unfortunately, the olfactory apparatus of many species does not present the experimenter with a morphologically discrete and isolated bundle of first nerve fibers for transection. In some forms, most rodents for example, the first nerve does not collect neatly anterior to the cribiform plate and the bulbs lie adjacent to the posterior surface of the bone. Thus, if destruction of the Ist nerve is attempted at its junction with central olfactory structures, trauma to the bulbs is likely.

Depending on the anatomy of the particular species and the destructive mode employed, olfactory afferents other than the first nerve and centrifugal fibers may be left intact. This method then, while sparing the massive tissue damage accompanying bulbectomy nonetheless may induce behavioral changes, not related to sensory loss which could confound experimentation.

Surgical Removal of the Neuroepithelium

Another surgical approach to producing anosmia without direct invasion of the central nervous system has been applied experimentally to rats, gerbils and therapeutically in humans. This technique involves exposure of the nasal cavity, either by drilling trephine holes or elevating a bone flap and systematically destroying, by mechanical aspiration, the total olfactory epithelium.

The procedure is arduous and is complicated by the convoluted turbinates of the nasal cavity which must be freed of receptor cells. In small animals the turbinates themselves may have to be destroyed; aspiration is sometimes necessary to prevent choking [131]. The surgical procedure for humans, employed by Henkin, is performed in two stages [56].

Since the division between the olfactory and airway epithelium is only approximate, and the damage sustained by sensory cells other than I nerve receptors will be vari-
able, the effects on the vomeronasal organ and nerves will also depend on the species and surgical conservatism.

The olfactory deficit resulting from this procedure remains uncertain. Observations made on human patients, discussed earlier, indicate that in some cases sensitivity to some odorants may prevail, perhaps due to intact cranial nerve components. Olfactory tests in animal preparations have been quite limited and rather uncontrolled to date. The reactions of operated rats and gerbils have only been observed to volatile odorants such as ether and Hik Karate soaked cotton swabs.

The performance of the resultant olfactory deficit is also uncertain. Smith surgically removed discrete portions of frog olfactory epithelium and observed histological recovery after as little as 15 days; within 70 days regeneration appeared complete [130]. Smith's observations were limited to cellular recovery and no olfactory tests were made. Regeneration of olfactory cells has also been observed after mechanical, chemical, and inflammatory destruction in frogs [130], rats [129], rabbits [92], and monkeys [122]. The reversibility of this procedure thus requires investigation.

**Intranasal Zinc Sulfate Treatment**

Since the 1930's physiologists interested in regenerative processes of sensory cells have utilized zinc sulfate as a necrotic agent. At about that time nasal sprays, containing 1% zinc sulfate were being used as a prophylactic for poliomyelitis and some human patients complained of loss of the sense of smell [129].

Recently, Alberts and Galef [3] described a technique for intranasal administration of zinc sulfate in rats and demonstrated, with a foodseeking task, that a single treatment rendered trained, food deprived rats temporarily unable to locate buried scented food in an open field. Before zinc sulfate treatment and after intranasal saline treatment these food deprived animals located and ate the hidden food within 30 seconds. The duration of this olfactory deficit is usually about 5–7 days.

Since their original report, this method has been replicated in several laboratories using a variety of odorants, as test stimuli. The procedure has been found to be effective in rats [2, 42, 49], mice [36, 37, 118], hamsters [32, 107], gerbils [28], and sea turtles *Chelonia mydas* for an underwater discrimination task [81]. The uniformity of the olfactory epithelium across most vertebrate species suggests that this technique may be generally applicable to most forms.

Histological reports indicate widespread and general cellular degeneration in the nasal epithelium, and subsequent regeneration after zinc sulfate application, for the rat [129], frog [130], monkey [122] and rabbit [92], suggesting that the olfactory deficit is due to coagulation necrosis of the sensory neuroepithelium. Mulvaney and Heist [92] found evidence of regeneration of sensory cells in the rabbit epithelium corresponding to the time course of behavioral recovery [3, 42, 123].

Intranasal zinc sulfate treatments are advantageous for several reasons. First, CNS intervention is avoided. The odors of amyl acetate and citroen evoke reliable slow-wave patterns in bulbar EEG and stimulate a large percentage of olfactory bulb mitral cells during unit recording. Two days after zinc sulfate treatment both electrophysiological responses were abolished in hamsters, although 3 days later amyl acetate again evoked characteristic slow-wave patterns (Macrides, personal communication). Since the sensory deficit is not permanent, behavioral recovery can be correlated to the reappearance of olfactory abilities. The procedure can also be repeated and it has been found possible to thus maintain the olfactory deficit [2]. Effective intranasal treatments can be performed rapidly, usually in less than one minute.

The nature of recovery period and the exact extent of olfactory abilities after recuperation remains unclear, however. During post-treatment recovery of olfaction the ability of rats to accurately locate scented food seems to develop gradually, as measured by latency to grasp the buried pellets. Whether this reflects increasing olfactory acuity or learning to use an impaired olfactory sense (perhaps trigeminal cues?) is not yet known. However, Scott [123] has reported an experiment in which rats were trained in a conditioned suppression procedure to olfactory stimuli delivered via a dilution olfactometer. Detection thresholds, measured behaviorally, were near the lowest values for these odors in electrophysiological tests. Responses returned to normal in 5–8 days, after being abolished by zinc sulfate. Specific responses to biological odorants that are believed to be highly specialized also reappear [4, 32, 36, 75].

As with any technique, there are potential hazards using zinc sulfate to induce an olfactory deficit. In humans, zinc sulfate is an emetic if ingested in quantity [85]. Nevertheless, hungry rats, mice and turtles perform foodseeking tasks and eat readily after treatment [2, 81, 118]. The intranasal irrigation should be performed carefully and excess solution remaining in the mouth should be aspirated. Control procedures to account for possible effects of ingestion can be run.

It is usually assumed that CNS structures are not altered by intranasal zinc sulfate [3]. However, Margolis (personal communication) has found that after intranasal zinc sulfate irrigation in mice, the olfactory bulb weight decreases significantly. Furthermore, a protein unique to mouse olfactory bulbs is absent after zinc sulfate administration. It has been shown that this protein is synthesized in the olfactory receptors and transported, by axoplasmic flow, into the CNS [82]; thus the disappearance of mouse olfactory bulb protein and the corresponding change in olfactory bulb weights may be directly related to the deproteinizing action of zinc sulfate. These results and their implications for olfactory processing are being studied.

Applied carefully and appropriately intranasal zinc sulfate appears to be a useful and valuable method for producing an olfactory deficit. Behavioral and physiological evidence suggests that the apparent anosmia seen in treated animals is quite general and probably involves, initially at least, all chemosensitive receptors in the nasal cavity and pharynx. The events during and after olfactory recovery promise to be useful for our understanding of the nature of olfactory control of behavior and possibly for investigating peripheral mechanisms of olfaction in general.

**Anesthesia of the Nasal Mucosa**

The application of topical anesthetics to the nasal mucosa, to temporarily abolish local neural (receptor) activity is an appealing and promising approach for producing a broadspectrum, reversible olfactory deficit. This method has been utilized in several experiments.
Perhaps the earliest published report of topical olfactory anesthesia is that of Banks, Bishop and Norton [12], who temporarily abolished the courtship behavior of rams after applying 3 cc of xylocaine hydrochloride 2% to the nasal epithelia and sinuses. To accomplish this rams were restrained on their side and a polyethylene tube inserted into each naris. The tube was sealed at one end and attached to a 5 cc syringe at the other. Several small pinholes were made in the wall of the tube, effecting the delivery of a fine spray. For control purposes physiological saline was similarly applied. Behavioral tests were run 30 min after the nasal spray.

The efficacy of this procedure was investigated with a behavioral test. Normal rams, blindfolded and standing in a crate violently withdrew their heads when an ether soaked cotton ball was held 6 in. from their nose; 30 min after treatment this procedure did not evoke a noticeable behavioral response and the condition appeared to prevail for up to 2 hr post-treatment (Banks, personal communication).

Michael and Kerowrne [89] demonstrated olfactory involvement in the sexual arousal of male rhesus monkeys with a related treatment. Their preparation involved the insertion of a nasal plug, soaked in bismuth-iodoform paste. With reflected light, direct visual guidance and a child's nasal speculum, the plugs were inserted above the superior turbinate adjacent to the nasal olfactory area. In monkeys the nasal airway is sufficiently large that normal breathing is not disturbed. The vomeronasal nerve was cut immediately above the organ of Jacobson.

Confirmation of olfactory impairment was tested with crushed banana pellets, an attractive odor to monkeys. No additional or volatile odors were tested since these workers were not concerned with trigeminal responses which may have still persisted (Michael, personal communication).

There is a report of application of viscuous xylocaine to rat nasal mucosa, which retarded a withdrawal response to NH4 OH [72]. No nonirritating odorants were tested in this study and it is unclear if a viscous solution could penetrate the full extent of the turbinate system without disrupting the normal nasal respiration of rats.

More recently, however, Doty and Anisko [3] administered 2% procaine hydrochloride solution intranasally to hamsters using the choanal approach of Alberts and Galef [3]. Intranasal procaine HCl eliminated normal behavioral sex odor preferences and temporarily abolished mating behavior in male hamsters [33]. Control subjects, receiving similar intranasal treatments with water alone, or intranasal water treatments with intraperitoneal injections of procaine HCl performed normally in mating tests and demonstrated behavioral preferences for sex odors. Thus, in the concentration and dose used by Doty and Anisko, procaine HCl appears to be a "relatively non-toxic" [48] topical anesthetic capable of temporarily blocking activity of olfactory receptors without exerting more general debilitating effects on the animal.

The use of topical anesthetics, particularly with small animals, should be examined carefully for possible anesthetic effects secondary to its intended action on receptors. This is especially important when the results of the treatment are disruptive or involve the elimination of a behavior. It is possible that the anesthetic itself may penetrate the cribiform plate or directly affect the CNS via the vascular system of the nasal cavity. Thus far, the range and type of odorants tested in these cases has been quite limited and requires systematic study. Similarly, the technique's action on trigeminal and vomeronasal receptors remains unknown and may be variable.

These procedures are promising, however, and offer several advantages for experimental purposes. The olfactory deficit is reversible and it is possible that no permanent damage to the peripheral nervous system will be sustained. Thus, repeated within-subject testing is possible. The effects, to the extent that they have been measured, are shortlived. For some experiments this can be very useful. Finally, the technique, ideally at least, exerts its effects on the receptors in the nasal cavity and does not disrupt bulbar or other CNS functions beyond interruption of normal olfactory input.

Nasal Blockade with Tracheostomy

Hart and Haugen [50] employed an elegant system for reversible olfactory blockade, which functionally bypasses all olfactory receptors (cryptosmia). After tracheostomy male dogs were fitted with custom constructed plastic tubes which could be opened and closed. When the tubes were open, dogs breathed through their exteriorized trachea; sealing the tube permitted normal breathing and nasal sniffing. Olfactory blockade was achieved by opening the tracheal tube and plugging the dogs' nostrils with cotton. The efficacy of this preparation was tested by presenting bacon strips or canned dog food one inch from the animals' noses when blindfolded. This elicited no response until the food was touched to the dogs' lips. It is necessary to apply xylocaine to the outer portion of the dogs' nasal cavity to "reduce sneezing-like, head shaking" which occurred without local anesthesia. Such minimal precautions are probably necessary for sniffing animals in general; the problem of peripheral blockade of normal sniffing in terms of general behavioral disruption has not been examined.

It is unfortunate that mere blockade is an untenable approach to this problem, but bilateral occlusion of the nostrils in the rat, for example, produces either suffocation or such severe behavioral disruption associated with abnormal mouthbreathing that experimentation is impossible. Such an olfactory block can, in theory at least, be maintained indefinitely (although Hart and Haugen removed and cleaned the tubular inserts daily), yet can be reversed rapidly, permitting pre- and post- within subject tests. The olfactory loss should be complete; even receptors located in the oro-pharynx can be bypassed.

The procedure used by these authors did not include controls for inserts in the dogs' nostrils or the local anesthesia, but as no major effects on scent marking or male sexual behavior were discovered, these were probably unnecessary for their experiment. Whether the small size, communal living habits and mutual grooming behaviors of other animals, such as the rat, will obviate widespread applicability of this approach will require further technical investigation.

Unilateral Nasal Occlusion with Unilateral Bullectomy

This method of reversible olfactory blockade was developed and employed independently in two laboratories, one studying pigeon navigation (100) and the other hamster social behavior [32]; it combines a central and peripheral manipulation.

The technique is based on the anatomical division of the nasal cavities and ipsilateral projections of olfactory recep-
tors onto the olfactory bulbs. The animal undergoes unilateral olfactory bulb removal by standard technique. If the naris contralateral to the ablated bulb is mechanically occluded, inspired air passes over receptors which are no longer functional, producing the desired olfactory deficit. Switching the nasal block in the same animal, however, allows inspired air to pass over receptors which still have intact synapses with the intact olfactory bulb. In pigeons the nasal occluder was a cotton plug, held in place with tape and for hamsters, a surgical wound clip dipped in local anesthetic was used. The procedure of switching the nasal occluder thus incorporates a necessary control condition — for any discomfort or the effects of unilateral air flow through the sinuses. Workers using this technique must be aware of general effects of unilateral olfactory bulb removal (e.g. [73]). In some species the nasal septum does not extend completely in the anteroposterior plane and inspired air could cross the incomplete septum, bypassing the blockade. However, Bennett [16] has devised a solution to this problem. There is, as yet, little information on possible retronasal diffusion of odors with this preparation.

Unilateral olfactory blockade should produce a complete anosmia, blocking all nasal receptors, permit repeated within-subject procedures, and offer the experimenter complete control over the duration of the deficit. One apparent advantage of this reversible procedure is that when reversed, the animals' olfactory abilities are immediately unimpaired, beyond the general effects of the unilateral bullectomy, mucus blockade or degenerative changes in the epithelium (uninvestigated). Reversible preparations using chemical treatments of the mucosa, on the other hand, require variable periods for recovery during which olfactory acuity and sensitivity have not been defined.

DISCUSSION

The concept of an olfactory organ system emphasizes the functional unity of an integrated complex of neural structures and pathways which, in the normal intact animal, mediates sensitivity to both general and specific chemical cues from the external environment. Also germane to this problem, but not included in this paper, are specialized structural adaptations of the external receptor apparatus.

In the past, little attention has been given to the anatomical characteristics of the olfactory system, particularly in investigations utilizing direct manipulations designed to interfere with olfactory processing. There are perhaps two notions responsible for this. The first is that there is a single function served by tissue termed olfactory cortex. Clearly the olfactory bulbs which receive direct, first order synapses from the I nerve is a primary analyzer of chemical stimuli from the external environment. But is that all? Herrick [60], as long ago as 1933, suggested other “nonspecific” functions for this paleocortical mass, including integration of information from both the external and internal milieu. Second, the traditional division between main and ancillary olfactory pathways supported the tacit assumptions that the systems were naturally divisible, and that individual components can be removed without altering remaining portions of the system. Apparently, these assumptions are erroneous.

The various subsystems of the olfactory organ system are not functionally autonomous. Although much is yet to be learned about their function, it is clear that they are closely interrelated. The fact that olfactory stimuli exert powerful influences on feeding, reproductive, parental, and other behaviors clearly indicates the olfactory organ system is further integrated with other important neural and endocrine systems of the organism.

Thus, it is always critical to recognize behavioral effects or alterations in performance after olfactory manipulations which are not due solely to the sensory deficit. This hazard is particularly likely with manipulations that involve intervention into the central nervous system. In general, techniques which involve CNS damage are the least desirable studies of the effects of anosmia on behavior. There is now a wealth of evidence on apparent nonsensory effects of olfactory bulb removal, likely to confound interpretation of specific behavioral problems (see Table 1). Therefore it is suggested that: (1) Olfactory bulb removal should no longer be considered a normally acceptable means of inducing anosmia, per se. (2) Animals sustaining olfactory bulb destruction should not merely be referred to as anosmic. It should be emphasized that they are bullectomized-anosmic animals, likely to possess peculiar behavioral and physiological characteristics in addition to their olfactory deficit.

Measurement and evaluation of olfactory deficits remains a major problem in studies of anosmic animals. Total, complete anosmia — the absence of sensitivity to all odors at any concentration — can never be demonstrated directly. The extent of an olfactory deficit can only be defined operationally, in terms of the number and range of odorant stimuli tested. Nevertheless, the concept of anosmia is critical in experimental analyses, particularly in situations where olfactory involvement in behavioral control is being assessed by a method which eliminates or disrupts the sense of smell. Most of the clinically recognized olfactory abnormalities found in human subjects, e.g., dysosmia and parosmia, have not yet been demonstrated in other species. It is possible, however, that analogous perceptual pathologies may be produced by some experimental manipulations, or develop during the recovery stages of the various reversible preparations.

Thus, it is further suggested that: (3) Olfactory deficits or anosmia be defined with respect to the olfactory stimuli tested. (4) In tests of olfactory impairment the odorant selected should be the same as or related to the olfactory cues believed to be involved in the behavior in question. (5) Olfactory stimuli should be presented in appropriate concentrations; that is, at intensities no lower than those found in the natural situation. (6) More generalized olfactory deficits can be substantiated by testing with broad-spectrum chemical odors such as amyl acetate, in addition to specific odorant stimuli.

For example, if the effects of anosmia on sexual behavior are being assessed the most relevant olfactory deficit to measure is with respect to the sex odors of the species. If an animal is found to be without smell in such tests, then anosmia can be defined in respect to sex odors — at least for the concentrations tested. Additional considerations using pure chemical odors can be used to broaden the demonstrated olfactory deficit. Irritant odorants, such as ether, ammonia or other volatile substances are poor choices, because they are unrelated to olfactory cues involved in sexual behavior, and because they are likely to yield data on the sensitivity of cranial components of the olfactory organ system other than the Ist nerve.

Prospectus

Available evidence indicates that bullectomized animals

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present a complex syndrome of altered behavioral and physiological function. Starting with gross ablation of the olfactory bulb, this syndrome may be further studied and defined. Experiments utilizing more selective lesions have begun [31, 126, 127], and it appears that the syndrome of olfactory bulb organization (e.g., [29]) may provide the basis for application of specific chemical stimulation, blockade, and lesion techniques to this problem. The study of bulbectomized subjects, while of limited usefulness in sensory investigations, has opened a new and exciting area of research into the possibility of nonsensory functions of central structures traditionally viewed as olfactory.

REFERENCES


