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Olfactory Performance of Rats after Selective Deafferentation of the Olfactory Bulb by 3-Methyl Indole

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Abstract

Rats trained to detect propyl acetate and valeric acid and to discriminate between propyl acetate and amyl acetate and between valeric acid and butyric acid were injected with a low dose of 3-methyl indole, a treatment that produces well-defined and selective deafferentation of the olfactory bulbs. Treatment completely deafferented most but not all bulbar loci for aliphatic acids and at least disrupted those for propyl and amyl acetate. In posttreatment tests, experimental rats performed somewhat but not significantly more poorly than controls and about as well on the acid detection and discrimination tasks as on the corresponding acetate tests.

Key words: axonal transport, glomerular deafferentation, 3-methyl indole, odor detection, olfactory bulb, odor discrimination

Introduction

A combinatorial mechanism for odor coding of the type revealed by 2-deoxyglucose (2-DG) and intrinsic imaging of the olfactory bulb has been challenged by several lines of evidence from behavioral studies. Thus, reports that rodents are able to detect and identify odors after a sample of only a few hundred milliseconds (Uchida and Mainen 2003; Abraham et al. 2004; Rinberg et al. 2006) raise the issue of whether maps based on averaging bulbar activity over many seconds or minutes of odor exposure reflect neural activity at the time of odor sampling.

A quite different line of evidence comes from reports in which identified bulbar loci or patterns of activity are surgically destroyed. These studies have largely failed to support predictions based on mapping studies: rats with discrete or even extensive lesions of the olfactory bulbs have not been found to have a specific anosmia or hyposmia for targeted odors, and extensive disruption of mapped patterns of inputs have little effect on a variety of psychophysical measures of odor discrimination or measures of odor quality perception (e.g., Hudson and Distel 1987; Slotnick et al. 1997; Lu and Slotnick 1998; Slotnick and Bodyak 2002; McBride and Slotnick 2006). However, limitations in the lesion method seriously constrain its usefulness and compromise the interpretation of outcomes. Thus, some bulbar sites are not surgically accessible, bulbar lesions often have an irregular geometry, potentially, undercutting projections from apparently intact regions of the mitral cell layer, and posterior lesions often extend into the lateral olfactory tract and the anterior olfactory nucleus.

To circumvent such problems, I used a nonsurgical technique to reexamine the issue of specific anosmia or hyposmia resulting from selective deafferentation of the olfactory bulb. To this end, rats, trained to discriminate between acetates and between aliphatic acids, were retested after intraperitoneal injection of a low dose of the olfactory epithelial toxin 3-methyl indole (3-MI), a treatment known to disrupt most of the bulbar areas activated by aliphatic acids (Setzer and Slotnick 1998; Slotnick and Bodyak 2002).

Materials and Methods

Thirteen 90- to120-day old Wistar strain male rats were used. Rats were housed in groups of 3–4 on sawdust bedding in plastic cages and maintained in a temperature- and humidity-controlled vivarium kept on a 12:12 h light–dark cycle (lights on at 7 AM). Rats were maintained on a water restriction schedule of 8–10 ml/day beginning 10 days prior to behavior training and throughout the experiment. All methods and procedures were approved by the University of South Florida Institutional Animal Care and Use Committee.

Apparatus

Four identical Knosys 8-channel liquid dilution olfactometers similar to those described in detail by Bisulco and

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Slotnick (2003) were used. Briefly, solenoid pinch valves controlled air streams and odors were generated by passing a 50-cc/min stream of air over the surface of odorants diluted in mineral oil and contained in disposable 240-ml PVC plastic bottles. This odorized air was diluted in 1950 cc/min of clean air before being introduced to an odor sampling tube in the rat operant chamber.

Prior to use and when a new odorant was employed, the olfactometer was washed with 95% ethanol and air-dried. Previously, unused C-flex tubing and saturator tubes used in detection and discrimination tasks described below were washed in 95% ethanol and maintained until use in a 50 °C clean stainless steel-lined convection oven dedicated to alcohol-cleaned glass and plastic ware. Each odorant and odorant concentration was maintained in its own saturator tube and the liquid odorant was refreshed daily. Previously used tubing and containers were discarded. Odorant solutions were kept refrigerated until shortly before use. As previously noted (Slotnick and Restrepo 2005), the use of pinch valves, disposable plastic tubing and bottles, and clean glass manifolds for each task eliminated any potential contamination in the system and insured that discriminative responding was based only on the odorant cues provided.

Odorants

Odorants ethyl acetate, propyl acetate, *n*-amyl acetate, isovaleric acid, and butyric acid were purchased from Sigma (St Louis, MO). The purity levels were the highest available from this supplier. All odorants were diluted v/v with mineral oil to the desired concentration, and 10 ml of solution was used as the odorant source in the odor saturation tubes. Odorant concentrations are given as the liquid dilution of the odorant in the saturator tubes, and positive and negative stimuli used in training are named with regard to the odorant and its liquid dilution. Because, as described above, the 50-cc/min odorant vapor from the saturator tube was manifolded with 1950 cc/min of clean air before being introduced to the rat sampling port, the odor concentration delivered to the animal sampling port was approximately 2.5% of the concentration of the headspace above the liquid odorant. The odorant concentration of the headspace above the liquid solution is not known precisely, but gas chromatographic analyses indicate that headspace concentrations of a wide variety of hydrocarbons from mineral oil dilutions are proportional to their liquid dilution (Cometto-Muniz et al. 2003).

Preoperative behavior tests

Initial training

All rats were first trained on a simple odor detection task using the go, no-go discrimination procedures previously described in detail (Slotnick and Schellinck 2002). Five percent ethyl acetate served as the odorant. Standard operant conditioning methods were used to train rats to insert their snouts into the odor sampling port and respond by licking at the water delivery tube (located within the odor sampling port) in the presence of the ethyl acetate vapor. The first snout insertion after a 5-s intertrial interval (ITI) initiated a trial. At the beginning of the trial, the stimulus control valves and a valve that directed the air stream away from the rat sampling tube was activated. This resulted in combining the odorant vapor with the main air stream and the diversion of that stream to an exhaust path. The diversion valve relaxed 1 s later, and the odor stimulus was presented to the odor sampling port. The stimulus valves relaxed 2.5 s later, thus terminating delivery of the odor. Delivery of reinforcement was contingent on the rat keeping its snout in the odor sampling port and licking on the water delivery tube in 7 or more of the 10 last 0.2-s periods of the 2.5-s odor presentation period. Trials in which the rat did not keep its snout in the odor sampling port for at least 0.1 s after odor onset were aborted and counted as short-sample trials. Completing the criterion response requirement produced a 0.04-ml water reward. All rats were given 2–3 such sessions before being trained on the odor detection and discrimination tasks described below.

Next, rats were trained on a detection task in which 5% ethyl acetate served as the S+ stimulus and the mineral oil solvent served as the S− stimulus. On each trial, either the S+ or S− stimulus was presented. Making a criterion response on S+ trials was reinforced with 0.04 ml of water and scored as a hit, whereas failing to make a criterion response was scored as a miss. Making a criterion response on S− trials was scored as a false alarm, and failing to make a criterion response was scored as a correct rejection. S+ and S− trials were presented in a modified random order such that there were an equal number of each in each block of 20 trials and that one type of trial was not presented more than 3 times consecutively. The trial procedures were identical to those in initial training: the first snout insertion after a 5-s ITI initiated the trial. The rat was required to keep its snout in the odor sampling port through the final valve period and for at least 0.1 s after presentation of the stimulus. If the rat did not complete this requirement, the trial was aborted, scored as a short-sample trial, and that trial type was presented on the next trial. Percent correct responding was determined for each block of 20 trials ([hits + correct rejections/20] × 100). All rats were trained to a criterion of 80% correct responding in a block of 20 trials and then given 40 additional over-training trials on this ethyl acetate detection task.

Next, rats were trained in separate sessions to detect 1% isovaleric acid, 1% propyl acetate, 0.1% isovaleric acid, 0.1% propyl acetate, to discriminate between 0.1% isovaleric acid (S+) from 0.1% butyric acid (S−), and between 0.1% propyl acetate (S+) from 0.1% *n*-amyl acetate (S−). Finally, these rats were trained on a series of odor mixture tasks in which they were required to discriminate odorant A (S+) from a liquid mixture of odorant A and B (S−). For the acetate tasks, odorant A was 0.1% propyl acetate and odorant B was a mixture of 0.1% propyl acetate and 0.1% *n*-amyl

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acetate. For the acid tasks, odorant A was 0.1% isovaleric acid and odorant B was a mixture of 0.1% isovaleric acid and 0.1% butyric acid. For the first mixture task, the S− stimulus was an equal parts mixture of the 2 odorants (i.e., 5 ml of each odorant). In successive sessions, the A component of the S- stimulus constituted 90%, 99%, and 99.1% of the mixture. The acetate and acid detection and discrimination tasks were presented alternately beginning with the 1% propyl acetate detection task for 6 of the 13 rats and with the 1% isovaleric acid detection task for the remaining 7 rats. Rats were given a minimum of 60 trials on each task. For rats that did not achieve criterion accuracy of at least 80% correct responding in a block of 20 trials, training was continued until criterion was met or for a maximum of 100 trials.

3-MI treatment

Following completion of the odor mixture tasks, 7 rats were given an intraparitoneal injection of 75 mg/kg (n = 1), 125 mg/kg (n = 2), or 150 mg/kg (n = 4) of 3-MI (99% purity, Sigma) dissolved in corn oil (Mazola) to a concentration of 7.5, 12.5, or 15 mg/ml. As shown by Setzer and Slotnick, treatment with 150 mg/kg of 3-MI results in essentially complete deafferentation of glomeruli in the dorsal one-fourth of the olfactory bulb and all but the more posterior medial and lateral walls of the bulb. The affected areas should include virtually all bulbar sites activated by butyric acid, most but not all bulbar sites activated by isovaleric but should spare input to most glomerular regions activated by amyl acetate and propyl acetate (as documented in studies using 2-DG (Johnson et al. 1998, 1999). The remaining 6 rats were injected with the corn oil vehicle.

Because rats treated with 3-MI emit a strong stench of 3-MI for 2–3 days, each rat was placed in a clean cage lined with paper towels and kept in a vented hood for 3 days prior to being housed in the vivarium.

Post-treatment tests

Beginning the 3rd day after treatment, rats were retested on the 5% ethyl acetate, 1% isovaleric acid, 1% propyl acetate, 0.1% isovaleric acid, and 0.1% propyl acetate detection tasks (in that order) and, as described above, on the discrimination tasks. In these posttreatment tests, all rats were tested using the same sequence of tasks. Because significant recovery of input to the olfactory bulb occurs within the first 12 days after treatment with 3-MI (Setzer and Slotnick 1998), it was important to complete posttreatment tests quickly. To this end, a session was terminated when the criterion of 80% correct responding was achieved or exceeded and multiple (2–6) sessions were given each day. All rats completed their posttreatment tests within 6 test days (by the 9th day following treatment).

Anatomical control

Immediately following completion of behavioral tests, each experimental rat and 2 of the controls were lightly anesthetized with Ketamine and Xylazine, and each nasal vault was injected with 60 μl of an equal parts mixture of 1% wheat germ agglutinin–horseradish peroxidase (WGA-HRP) and 1% dimethyl sulfoxide. On the next day, rats were deeply anesthetized and euthanatized by perfusion with saline and mixed aldehydes according to the procedures of Mesalum (1982). The olfactory bulbs were frozen and sectioned at 60 microns. Every third section was processed for visualization of WGA-HRP reaction product (Mesalum 1982), mounted on gelatin-coated glass slides, dried, counter stained with thionin, quickly dehydrated through cold alcohols, cleared in xylene, and covered using Permount. To assess anterograde transport from olfactory sensory neurons to olfactory bulb glomeruli, sections were inspected microscopically using bright field and dark-field optics, and selected sections were photographed using a Nikon digital camera. Density of reaction product within glomeruli was judged using the rating scale described by Setzer and Slotnick (1998).

Anatomical analysis

Glomerular regions activated by amyl acetate, propyl acetate, isovaleric acid, and butyric acid are illustrated in 2 dimensional rolled out maps of the olfactory bulb published online at http://leonserver.bio.uci.edu/. Selected frontal sections through the olfactory bulb showing loci of activity for amyl acetate, isovaleric acid, and butyric acid have been published (Johnson et al. 1998, 1999). On the pseudo-colored, dorsal-centered rolled-out maps from the leonserver Web site, glomerular regions having the strongest response (as defined by activity levels that are 2.5–3.0 standard deviations [SDs] above background) are identified with red. These regions have a surround of glomeruli, pseudocolored in yellow, that are less responsive (activity levels that are 2–2.5 SDs above background (Johnson et al. 1999). For each odorant tested, the distribution or pattern of these strong regions of activity with their weaker surrounds are characteristic of and serve to distinguish that odorant from other odorants. To identify these regions for each odor, the approximate positions of the red regions and the surrounding yellow regions were plotted on outlines of the rolled out map (Figure 3). The glomerular regions that contained identified WGA-HRP reaction product in frontal sections of experimental rats were then plotted on a separate rolled out map outline. Because points in the rolled out map are not defined metrically, it was not possible to locate precisely the regions of remaining bulbar input on the rolled out map. However, as discussed below, it is unlikely that small errors in locating these regions on the rolled out map would alter conclusions based on outcomes in the present study.

Odor concentration

At least in some cases, larger focal areas are associated with higher odorant concentrations (Johnson and Leon 2000). Thus, it would be misleading to relate histological outcomes

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to published maps if the odorant concentrations used in this study were appreciably higher than those used in creating these maps. Glomerular maps for amyl acetate, propyl acetate, isovaleric acid, and butyric acid illustrated in the leon-server Web site were made in response to stimulation at 75 ppm for propyl acetate and amyl acetate, 9.8 ppm for isovaleric acid, and 6.7 ppm for butyric acid. The headspace concentration of the mineral oil–diluted odorants used in this study were not measured but can be estimated from plots in Figure 2 of Cometto-Muniz et al. (2003). These show the measured physical concentration in the headspace above different mineral oil dilutions of various hydrocarbons. Based on these data, the estimated concentration delivered to the rat (estimated headspace concentration divided by a factor of 40 for the air dilution stage of the olfactometer) for amyl acetate, propyl acetate, and butyric acid was, approximately, 2, 50, and 0.2 ppm. Headspace concentrations for valeric acid are not given in the report of Cometto-Muniz et al. (2003), but based on the data provided for C4 and C6 acids, it is assumed that the concentration of isovaleric acid delivered to the rat in this study was less than 1 ppm. In any case, it is clear that the concentrations delivered to subjects in this study were lower than those used to create the maps illustrating major sites of glomerular activation for odorants used in this study.

Results

Anatomical results

As expected, essentially all glomeruli of control rats were filled with dense reaction product (Figure 1). In sharp contrast, most glomeruli in experimental rats contained no detectable reaction product (Figures 1 and 2A–C). Residual input to glomeruli in experimental rats were confined to 2 clusters of glomeruli: one in the posterior mid lateral to ventral lateral region and, more posteriorly, beginning at the level of the rostral accessory olfactory bulb, a second cluster in the ventromedial aspect of the bulb. Rat R4, which was treated with lowest 3-MI dose (75 mg/kg), was an exception and had more extensive and dense input on the posterior lateral wall and some input to dorsal glomeruli at this level (Figure 1). The pattern of residual bulbar inputs for the remaining experimental rats was quite similar to those reported by Setzer and Slotnick (1998) and Slotnick and Bodyak (2002) for short-survival rats treated with 150 mg of 3-MI. Glomeruli in these clusters contained, in different rats, very light reaction product (seen clearly with dark-field optics) or more dense reaction product. The transition from glomerular areas with reaction product to those containing no reaction product was fairly well defined (Figure 2A,B). Figure 3 shows maps, generated as described above, of glomerular regions that were most responsive amyl acetate, propyl acetate, butyric acid, and isovaleric acid and the glomerular regions that contained detectable reaction product in experimental rats treated with 125 or 150 mg/kg 3-MI. As shown in this figure, deafferentation produced by 3-MI disrupted the pattern of inputs for each odorant. Virtually, all glomeruli responsive to butyric acid and all dorsal glomeruli responsive to isovaleric acid had no input. As shown in Figure 3, there was probably bulbar input to posterior lateral and posterior ventromedial loci responsive to isovaleric acid. Both amyl acetate and propyl acetate have fairly extensive glomerular representation in the posterior lateral and posterior ventromedial glomeruli and, among experimental rats, most or at least approximately one-third of these glomerular regions contained reaction product. In brief, deafferentation of glomerular areas responsive to butyric and isovaleric acid was clearly more extensive than that for amyl acetate and propyl acetate.

Behavioral results

Figure 4 shows mean errors to criterion on all tasks for all rats prior to treatment and the posttreatment scores of control and experimental rats. On all tasks, experimental rats made, on average, more errors than controls, but none of the posttreatment differences between groups were significant (multiple between group *t*-tests) and the lowest P value was 0.058 for these comparisons. On most problems, rats made more errors on the acid tasks, but within group differences between the acetate and the corresponding acid tests were not significant for either control or experimental groups ($P > 0.1$ each comparison, paired *t*-tests).

The marginal posttreatment difference between groups is also reflected in total error scores averaged across problems (Figure 4, inset right). Experimental rats made, on average, more total errors than controls for both the acetate and acid tasks, but differences between groups were not significant (between group *t*-tests). Total error scores (Figure 4, inset right) on acid tasks were not significantly greater than those on acetate tasks for controls ($P > 0.1$), but this difference was marginally significant ($P < 0.06$) for experimental rats (within group *t*-tests).

Although no significant between group or within group differences were found, inspection of Figure 4 reveals 2 clear patterns in outcomes: In most problems, mean error scores for both control and experimental rats were lower when retested on these tasks and, in all cases, mean error scores of experimental rats were somewhat (but not significantly) higher than those of controls. As indexed by error scores, the last acetate and acid odor mixture tasks were the most difficult and should be the most sensitive indicator of a differential effect of deafferentation by 3-MI. Experimental rats did make more errors on the last acid mixture task, but this task was also more difficult for controls and, as a percentage of control group errors, the deficit of experimental rats on the last acetate mixture task was, in fact, greater than that on the last acid mixture task (Figure 4).

No relationship was found between density or extent of olfactory input and performance across tasks. All rats reached criterion performance on all acetate tasks and the

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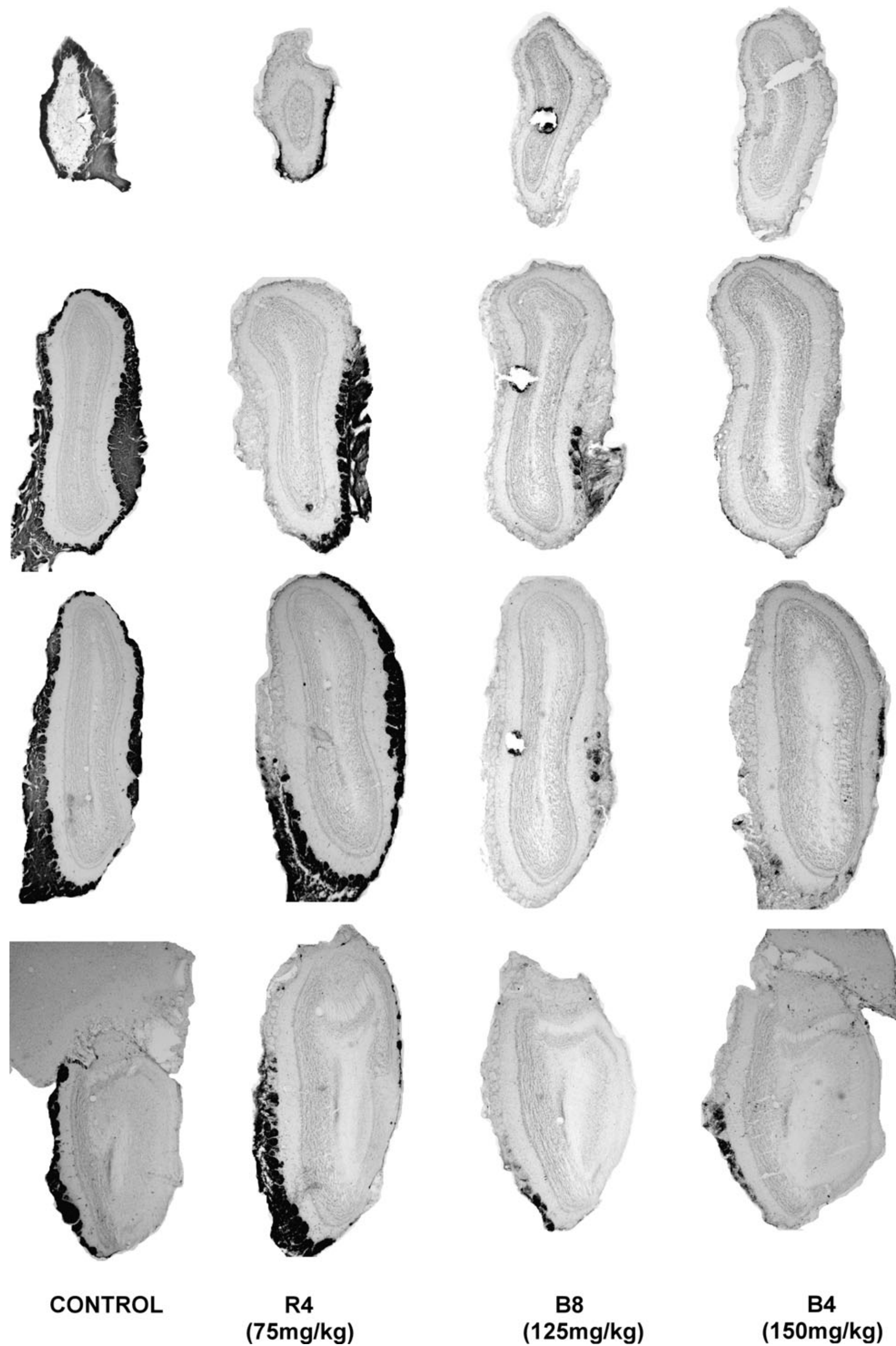

Figure 1 Photomicrographs of representative frontal sections through one olfactory bulb from a control rat and from each of 3 experimental rats. Rats are identified by their number and 3-MI dose. Among all experimental rats, R4 had the most extensive inputs and B4 had the least input. In each section, medial is to the left.

2 experimental rats with the least amount of input did not perform more poorly than others. Two control and 2 experimental rats failed to reach criterion on the last acid mixture task, but the experimental rats that performed worse on this task had more dense bulbar input than several rats that performed better. The one the rat (R4) that had the most extensive input and the only rat with input to posterior dorsal and dorsal lateral glomeruli (Figure 1) performed no better on the various acetate and acid tasks than those with less dense and more restricted inputs. However, this attempt to relate

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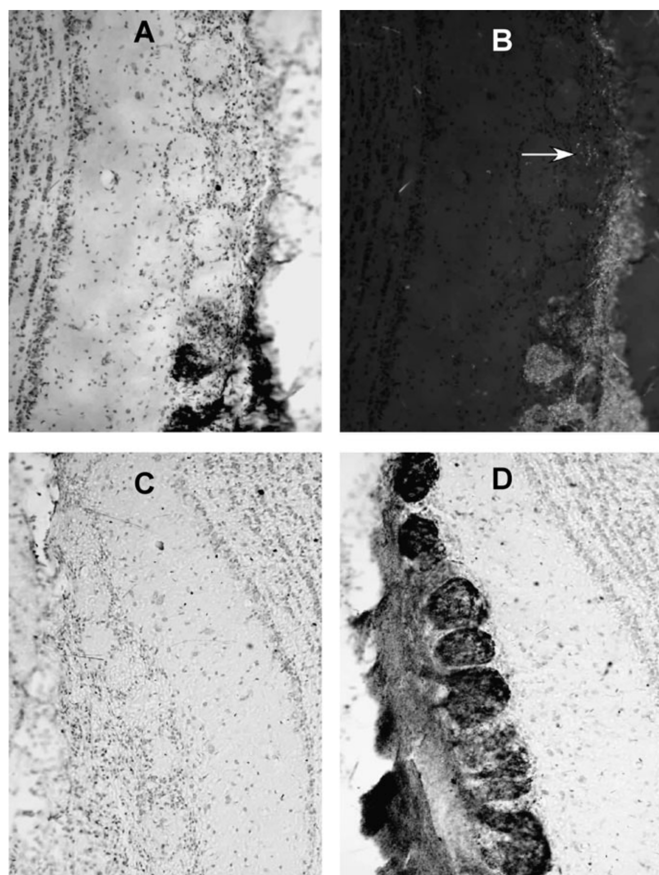

Figure 2 **(A, B)**, photomicrographs showing the transition from glomeruli with dense input to those with no input on the lateral wall of the olfactory bulb in bright field optics (A) and in polarized light (B) for rat R4 (the rat with the most extensive input). Arrow in (B) points to a glomerulus with a small sprinkling of reaction product (not clearly visible in bright field optics). **(C, D)**, photomicrographs showing glomeruli on the dorsomedial wall of the rostral olfactory bulb for a control rat (D) and an experimental rat (C).

behavioral results with anatomical outcomes is complicated by the fact that there is gradual recovery of inputs to the olfactory bulb after treatment with 3-MI (Setzer and Slotnick 1998) and because some experimental rats completed tests in fewer days than did others. Thus, the anatomical analysis defined the maximal olfactory inputs available at the end of testing and it is likely that less input was present on initial tests.

Discussion

The present results demonstrate that extensive deafferentation of the majority of olfactory bulb glomeruli had, at best, only a marginal effect on the ability of rats to detect acetate and acid odorants and to discriminate between pairs of acetates and pairs of acids. Performance on acid detection and discrimination tasks was comparable to that on the acetate tasks despite the fact that bulbar deafferentation included more of the acid than the acetate responsive sites. These outcomes are consistent with a marginal sensory loss resulting from disruption of olfactory input but do not support the notion that a major disruption or, in the case of butyric acid, virtually complete deafferentation of identified bulbar sites, will produce a specific hyposmia or anosmia. Nor do these outcomes support the notion that a greater disruption of identified sites for one class of odorants would produce a greater sensory loss for those odorants.

The results obtained with butyric acid are unique because virtually all the 2-DG–identified sites for this odorant were deafferented. Odorants used in prior lesion behavioral studies have a far more extensive bulbar representation than has butyric acid and, hence, it was not possible to surgically remove each of the identified sites without producing extensive damage to the olfactory bulbs. In those studies, discrete but subtotal damage to identified sites had little or no effect on odor detection and discrimination (Slotnick et al. 1997; Slotnick and Bodyak 2002). The significance of those outcomes as a test of combinatorial odor coding (as revealed by 2-DG and intrinsic optical imaging studies) were criticized on the assumption that undamaged identified sites could support the behaviors tested (Johnson et al. 1999; Xu et al. 2000; Johnson and Leon 2001; Johnson et al. 2004). Although this criticism would not appear to apply to lesion studies that failed to support the contention that the pattern of inputs is responsible for the perceptual quality of the odor (Lu and Slotnick 1994; Slotnick and Bodyak 2002), no prior behavior study has removed essentially all identified foci for identified odors. The present results with butyric acid are not completely satisfactory because the odor served as the S− stimulus and, hence, do not provide a direct measure of detection. However, evidence that experimental rats were able to detect butyric acid is that they discriminated between isovaleric acid and mixtures of isovaleric and butyric acids. The only cue that could support this performance, other than detection of the butyric acid component in the mixture, would be the difference in concentration of isovaleric acid in the S+ and S− stimuli. This appears to be most unlikely because acquisition of intensity discriminations generally requires extensive training, and because the intensity difference for the isovaleric acids in the last acid mixture was only 0.1%, a value more than 10 times lower than the intensity difference threshold for rats even after extensive training on intensity difference discriminations (Slotnick and Ptak 1977; Slotnick et al. 1997). Thus, our evidence favors strongly the contention that even virtually complete elimination of the major bulbar foci for an odorant does not produce a specific anosmia or even a marked hyposmia for that odorant.

The present results, using chemical deafferentation of the olfactory bulb are, in general, in good agreement with a variety of olfactory bulb lesion studies that have failed to find support for the notion that a mechanism for odor coding is revealed by the outcomes of 2-DG and intrinsic optical imaging mapping studies (e.g., Lu and Slotnick 1994; Slotnick et al. 1997, 1998; Slotnick and Bodyak 2002; Bisulco and

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Figure 3 Representations of major bulbar inputs plotted on the dorsal centered rolled out map adapted from the leonserver Web site for amyl acetate (AA), propyl acetate (PA), isovaleric acid (VA), and butyric acid (BA). The open regions in the map-labeled WGA-HRP show the approximate position of olfactory bulb areas that contained detectable WGA-HRP reaction product for all but the lowest 3-MI dose rat (rat R4). Open regions in the AA, PA, VA, and BA maps show the approximate locations of glomerular areas activated by each odorant (regions shown in yellow in the leonserver Web site). Solid areas within these open regions represent the approximate location of the strongest odorant response within this area (regions shown in red in the leonserver Web site). Horizontal and vertical lines through these maps are provided as an aid for comparisons among figures.

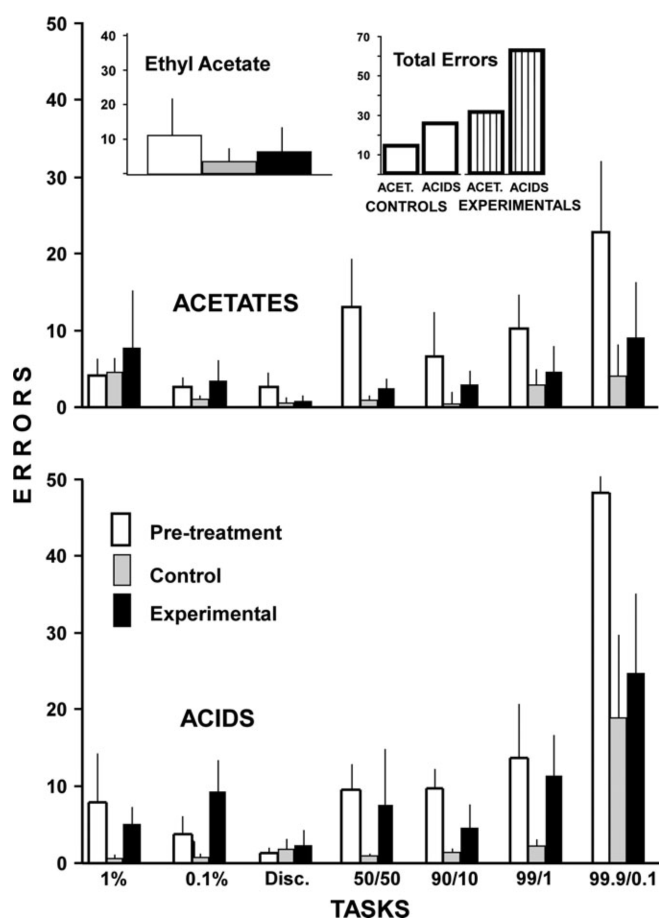

Figure 4 Large plots: mean error scores for all acetate and acid tasks prior to treatment (open bars) and for control (shaded bars) and experimental (black bars) after treatment. Vertical lines show 0.5 SDs. Odor mixture tasks are identified with regard to the proportions of the A and B odorants in the S− stimulus (see text for details). Insets show mean errors on the initial 5% ethyl acetate task (inset left) and the total errors on all acetate and acid tasks for controls (open bars) and experimental rats (striped bars) after treatment (inset right).

Slotnick 2003; Slotnick and Bisulco 2003; McBride and Slotnick 2006). Although the issue is not completely resolved by conditioning studies using surgical or chemical deafferentation of the olfactory bulb, there appears to be little support at the level of behavior that identified loci are essential for odor detection or that differences in identified patterns of input provide the basis for odor discrimination. One exception to this line of negative evidence are the studies of Linster et al. (2001, 2002) showing that rats more readily discriminate between the enantiomers of carvone, odorants that produce somewhat different patterns of bulbar inputs, than between the enantiomers of limonene or terpinen-4-ol, odorants that produce very similar patterns. However, in a recent study, we (McBride and Slotnick 2006) failed to replicate those outcomes: normal rats discriminated equally well between the enantiomers of carvone and those of terpinen-4-ol, and surgically disrupting patterns of inputs for both sets of enantiomers had little effect on discriminative performance.

It is unclear why these various behavioral studies of rats with olfactory bulb lesions have failed to find some functional correlate for olfactory bulb maps produced using 2-DG and intrinsic imaging. The maps themselves, particularly those of the Johnson and Leon group, display considerable order relative to physical properties of the stimulus. Thus, for example, overall map patterns are predictive of odorant class and, within a homologous series of odorants, bulbar patterns vary in an orderly manner with some physical parameter of the odorant such as carbon chain length or functional group. The maps undoubtedly reflect some average of responses from populations of olfactory sensory neurons for which the target odor is a more or less adequate ligand plus the convergence of inputs to specific bulb glomeruli. But despite these orderly relationships, identified loci do not appear essential for odor detection or sensitivity and even massive disruption of odor-specific bulbar patterns appears to have little effect on a variety of odor discrimination tasks. These

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outcomes may be because our tests are not sufficiently sensitive to uncover potential lesion-induced changes in odor perception, because the odotopic maps mediate some olfactory capacity that is not tapped in simple odor detection and discrimination tasks or, as discussed by McBride and Slotnick (2006), that rats can readily use features of the odorants that do not contribute to the 2-DG odotopic maps. Another possibility is that olfactory bulb lesions alter the perceived quality of the odor but not the ability to detect or discriminate between odors. Although Lu and Slotnick (1994) and Slotnick and Bodyak (2002) found no gross changes in their measures of odor quality perception in rats with bulbar lesions, it is, admittedly, difficult to assess this aspect of perception in animals and different or more sensitive measures of odor quality need to be developed. However, it is also true that a spatial representation of the stimulus in neural space does not, ipso facto, reflect the mechanism by which a sensory stimulus is coded. In the auditory system, for example, the representation of tonotopic organization of the basilar membrane on auditory cortex plus the progressive sharpening in receptive fields of auditory neurons at each ascending component of the system was assumed to provide strong evidence that the cortex was critically involved in making fine frequency discriminations. However, initial behavior studies failed to find deficits in frequency discrimination or even in frequency stimulus generalization gradients in animals with substantial or complete lesions of auditory cortex (e.g., Cranford et al 1976; Heffner 1978; Ono 2006). The most striking deficits in animals with auditory cortical lesions were in auditory localization and in discriminating patterns of sound. Those behavioral deficits could not have been predicted from the then current physiological and anatomical studies, but such outcomes provided the impetus for more sophisticated physiology (e.g., Doan and Saunders 1999; Heffner 1978; 1997).

The obverse may also be true: spatially ordered information does not necessarily require topographic organization. Thus, pyramidal "place cells" in the CA1 region of the hippocampus are not topographically organized with regard to the physical arrangement of their place receptive fields. In this case, positional or place cues appear to be mediated by spike frequency of activated neurons and not by the actual position of these neurons within CA1 (Wilson and McNaughton 1993; Muller et al. 1996).

As shown in a number of studies (Bodyak and Slotnick 1999; Slotnick and Schellinck 2002; Uchida and Mainen 2003; Abraham et al. 2004; Rinberg et al. 2006), rats and mice require only a few hundred millisecond sample to correctly identify an odor. The use of a fast bulbar imaging method provides some insight into the neural activity during the first second or so of odor stimulation (Spors et al. 2006). It is evident from this and related reports (Cinelli and Kauer 1995; Cinelli et al. 1995; Kent et al. 1996; Scott-Johnson et al. 2000; Wachowiak and Cohen 2001; Spors and Grinvald 2002; Lehmkuhle et al. 2003) that odor stimulation produces temporally dynamic changes or waves of activity that vary with sniff rate and other parameters of stimulation. Although such studies do not identify the neural code underlying odor identification, they do highlight the fact that, within the first few hundred milliseconds of stimulation, a variety of parameters such as sniff rate, the temporal sequence of input patterns, activation latency, the instantaneous pattern within a sequence, or the phase of mitral cell firing may all contribute to coding (Spors et al. 2006). These temporally dynamic changes provide potential cues for the behaving animal but are not captured in time-averaged and static representations of input patterns, and this may largely account for the fact that disruption of such patterns has little effect on odor detection and discrimination.

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