Chem. Senses 28: 361–370, 2003

Olfactory Discrimination of Short Chain Fatty Acids in Rats with Large Bilateral Lesions of the Olfactory Bulbs

Stephanie Bisulco and Burton Slotnick

Department of Psychology, American University, Washington, DC 20016, USA

Correspondence to be sent to: Burton Slotnick, Department of Psychology, American University, Washington, DC 20016, USA. e-mail: slotnic@american.edu

Abstract

Rats trained preoperatively to discriminate between acetic acid and caproic acid and between acetic acid and propionic acid were tested for their memory of these tasks and ability to discriminate between these odorants and between the enantiomers of carvone after receiving large bilateral bulbar lesions that included most of the fatty acid responsive areas identified in prior physiological studies. Concentrations of acid odorants were varied to insure that discrimination was based on the qualitative difference between acids. Experimental rats performed somewhat poorer than controls on the memory test but had no significant deficits in performing the acid discrimination tasks or discriminating between the enantiomers of carvone. These results demonstrate that removal of most bulbar sites identified as responsive to fatty acids and the consequent disruption of patterned input to the bulb is largely without effect on discriminating odor qualities of structurally similar acids.

Key words: aliphatic acids, odor coding, odor discrimination, odor memory

Introduction

Because most or all olfactory sensory receptor neurons respond to a number of different odorants and one odorant molecule may be recognized by multiple receptors, each odor activates combinations of receptors (Malnic *et al.*, 1999; Duchamp-Viret *et al.*, 2000). Thus, it is expected that an odor would activate multiple glomeruli, i.e. those that receive input from all sensory neurons for which that odorant serves as a ligand (Ressler *et al.*, 1994). Precisely this outcome has been obtained by mapping bulbar responses to odorants in optical imaging and 2-deoxyglucose (2-DG) studies (Johnson *et al.*, 1999; Rubin and Katz, 1999; Johnson and Leon, 2000a,b; Uchida *et al.*, 2000; Meister and Bonhoeffer, 2001). Moreover, structurally related odorants (e.g. members of a homologous series) activate similar, even overlapping, but not identical groups of glomeruli (Johnson *et al.*, 1999; Johnson and Leon, 2000a,b). For example, Johnson and colleagues showed that odorants with different functional groups (e.g. pentanoic acid and pentanol) produced activation in different groups of bulbar glomeruli, while those with the same functional group (e.g. 2-methylbutyric acid and isovaleric acid) produced activity within a common group of glomeruli. Even when two odorants from a homologous series differed only by one carbon, they produced similar but discriminable patterns of activation.

Of particular interest to this study is a series of electrophysiological, optical imaging, c-fos and 2-DG studies demonstrating that restricted areas of the olfactory bulb are responsive to stimulation by short-chain fatty acids. Mitral cells in the dorsomedial rabbit olfactory bulb were activated by fatty acids, and individual cells responded best to a particular acid (Imamura *et al.*, 1992). Uchida *et al.* (Uchida *et al.*, 2000), using intrinsic imaging, identified the same region in the rat olfactory bulb as being responsive to fatty acids. These data confirm earlier 2-DG and c-fos studies that identified this bulbar area as being responsive to propionic acid (Bell *et al.*, 1987; Slotnick *et al.*, 1989; Onoda, 1992). In more recent mapping studies using high resolution 2-DG, Johnson *et al.* (Johnson *et al.*, 1999) identified additional glomerular areas in the anterior dorsolateral, caudal midlateral and caudal ventromedial portions of the bulb that were activated by short-chain fatty acids. These areas or domains were viewed as occurring in pairs of related sites. Thus, the dorsolateral and dorsomedial sites had similar degrees of activation to an odor, as did the caudal midlateral and caudal ventromedial sites. In particular, acetic, propionic, butyric, valeric, caproic and octanoic acid odors activated specific but not necessarily the same glomeruli in each of the four identified bulbar areas. These outcomes provide specific examples for the notion that, at the molecular level, odor space is mapped into neural space such that different odorants produce distinct 'odotope maps' at the level of the olfactory bulb (Xu *et al.*, 2000). One implication of this odotope map or 'odotopic' view is that

© Oxford University Press 2003. All rights reserved.

Downloaded from http://chemse.oxfordjournals.org/ by guest on October 3, 2012

the pattern of activation across the olfactory bulb produced by exposure to an odorant may represent or be responsible for the sensory quality of that odor and the small differences in patterns produced by similar odorants (e.g. structurally similar aliphatic acids) may underlie an animal's ability to discriminate between such odorants (Johnson *et al.*, 1999; Xu *et al.*, 2000).

Thus, it is reasonable to expect that disruption of these patterns of activity by bulbar lesions would alter the perceived quality of odors and degrade the ability to detect them and discriminate among them. This prediction has not been supported in behavioral studies. Slotnick and colleagues reported that rats with relatively large lesions of the dorsomedial bulb had no deficits in detecting propionic acid even at relatively low concentrations (Slotnick *et al.*, 1987) and could discriminate the acid from structurally unrelated odorants (Lu and Slotnick, 1998) and quite similar odorants (Lu and Slotnick, 1994). More recently, Slotnick and Bodyak (Slotnick and Bodyak, 2002) found that rats with lesions of the two most anterior bulbar fatty acid sites and those with more extensive bulbar deafferentation produced by the olfactory epithelial toxin 3-methyl indole had few or no deficits in a test of odor recognition or in discriminating among several fatty acids and aldehydes. However, there were several shortcomings in these various lesion studies. In the earlier studies only one of the four bulbar areas or domains identified by Johnson *et al.* (Johnson *et al.*, 1999) as responsive to fatty acids was removed. Further, in the Slotnick and Bodyak study, only single concentrations of each of the C3, C5, C7 and C9 acids were used. Because rats can discriminate between small differences in odor concentrations (Slotnick and Ptak, 1977), discrimination could have been based on intensity rather than odor quality cues. The purpose of this study was to reexamine the effects of bulbar lesions on discrimination between structurally closely related (C2 versus C3) and less closely related (C2 versus C6) fatty acids. To insure against or minimize the possibility that intensity could be used as a discriminative cue, stimulus concentration was varied in tests designed to assess odor memory and odor discrimination. Finally, relatively large bulbar lesions were made in an effort to destroy or disrupt the majority of the bulbar sites known to respond to these fatty acid vapors.

Materials and methods

Subjects

Twelve adult male Sprague–Dawley rats weighing 224–345 g at the beginning of the study were housed individually in plastic cages on sawdust bedding in a temperature- and humidity-controlled room that was maintained on a 12:12 h light dark cycle (lights off at 8 p.m.). They received Harlan brand rat chow *ad libitum* and were allowed 8–10 cc of water per day, except for the day before and the week following surgery when supplemental water was given. Under this water deprivation schedule, body weights were maintained at ~85% of baseline. All procedures were performed according to a protocol approved by the American University Institutional Animal Care and Use Committee.

Olfactometers

The two multiple-channel liquid dilution olfactometers used were similar to those described in detail by Slotnick and Schellinck (Slotnick and Schellinck, 2002). Briefly, each unit had eight independent odor channels and the input and output lines (C-flex tubing) of each channel were controlled by normally closed pinch valves. Operating the upstream and downstream valves for a selected channel produced a 50 cc/min stream of air over the surface of 20 ml of odorant in the saturator tube. This stream was manifolded to a 1950 cc/min stream of clean air that was connected to the animal sampling port.

The operant chamber was a 15 cm wide, 25 cm long, 23 cm high Plexiglas box with a stainless steel floor. A 20 mm diameter glass odor sampling tube was mounted vertically on one outside wall of the chamber. A flexible hose connected the top of the tube to a 5 cubic feet per minute exhaust fan. The bottom of the tube connected to the normally open port of the three-way valve. A 20 mm hole cut through the Plexiglas chamber wall and the odor sampling tube served as a port through which the rat could insert its snout. Snout insertions were detected by an infrared photocell unit. A 14-gauge stainless steel drinking tube terminating in a three mm stainless steel ball extended 10 mm into the odor sampling tube such that the animal could lick at the stainless steel tube when its snout was inserted into the odor sampling tube. Lick responses were detected using a lickometer circuit connected between the chamber floor and the water delivery tube. Water was delivered to the tube by gravity flow from a reservoir whose output was controlled by a normally closed two-way solenoid. Prior to training and when different odorants were used, pinch valve tubing and saturator tubes were replaced with clean (previously unused) tubes and the system was washed with 95% ethanol and air-dried.

PC computers and digital interfaces controlled all training and test procedures. The olfactometer, operant chamber, and software were provided by Knosys Olfactometers (www.chemsenses.com).

Odorants and odorant concentrations

The odorants used in this study [ethyl acetate, acetic acid, propionic acid, caproic acid, (+)-carvone, and (–)-carvone] were obtained from Sigma or Fisher Scientific/Acros Organics (carvones) and were the highest purities available. Ethyl acetate was dissolved in deionized water, and all other odorants were dissolved in odorless mineral oil. Odorant concentrations given below were those of the liquid odorant in the saturator bottle. Because, as described above, the odorant vapor in the saturator tube was manifolded with

Downloaded from http://chemse.oxfordjournals.org/ by guest on October 3, 2012

clean air before being introduced to the sampling port, the odor concentration experienced by the rat at the sampling port was ~2.5% of the concentration of the headspace above the liquid odorant. Gas chromatographic analyses indicate that the headspace concentrations of several hydrocarbons from mineral oil dilutions are proportional to their liquid dilution (Cometto-Muniz *et al.*, 1999, 2001).

To determine concentrations of acetic, propionic and caproic acid that were reasonably similar in perceived intensity, we had four or five observers select from a series of concentrations of propionic acid and caproic acid those that best matched the perceived intensity of 0.1% acetic acid. On average, these observers judged that a 0.2% propionic acid and a 0.5% caproic acid concentrations best matched the perceived intensity of 0.1% acetic acid. We also generated multiple and overlapping concentrations of these acids to use in the multiple concentrations tasks described below. These tasks were used to reduce or eliminate the possibility that perceived strength of the stimulus could provide a cue for discriminating between them. For this purpose, three distinctly different concentrations of acetic acid (0.04, 0.2 and 1%) were used and three concentrations each of propionic (0.08, 0.4 and 2%) and caproic acid (0.2, 1 and 5%) were selected that best matched the perceived intensity of the low, medium, and high concentrations of the acetic acids. Subjects judged the highest concentrations of propionic and caproic acids as more intense than the lowest concentration of acetic acid and judged the highest concentration of acetic acid as more intense than the lowest concentrations of caproic and propionic acid.

Sequence of behavioral tests

The primary purpose of this study was to determine whether extensive bulbar lesions would significantly alter the ability of rats to discriminate between fatty acid odors on the basis of the qualitative difference between such odors. To accomplish this, rats were initially trained to detect a non-fatty acid odor (ethyl acetate), then trained to detect acetic acid, to discriminate between acetic acid and caproic acid and to discriminate between acetic acid and propionic acid. Then, additional training was given on these acid discrimination tasks but, as described below, the concentration of the odorants was varied within the training session (multiple concentration tasks). In the last few multiple concentration tasks a partial reinforcement schedule was used. This was done to increase resistance to extinction in preparation for the planned extinction-based postoperative memory task.

Postoperatively, rats were first tested on the ethyl acetate detection task to insure that they were under olfactory stimulus control and then tested under extinction for their memory of the multiple concentration acid discrimination tasks. They were then retrained on the acetic acid detection task and the acid discrimination tasks and, finally, tested for their ability to discriminate between two other odors (enantiomers of carvone) whose bulbar representation overlapped those of fatty acids (Linster *et al.*, 2001).

Preoperative training procedures

Beginning 2 weeks after the initiation of their water deprivation schedule, rats were trained using standard operant conditioning procedures to respond by licking the reinforcement tube in the presence of the vapor from 5% ethyl acetate. Details of the training procedure are given by Slotnick and Bodyak (Slotnick and Bodyak, 2002). Briefly, snout insertion into the odor sampling tube produced a 2.5 s presentation of the odor stimulus. A criterion response (at least one lick on the reinforcement tube in each 0.5 s of the last 2 s of stimulus presentation) was reinforced with 0.04 ml of water. Trials were separated by a 5 s intertrial interval (ITI). Ethyl acetate has proven to be an effective training odor, has been widely used in a variety of rodent studies [e.g. (Apfelbach *et al.*, 1998; Doty *et al.*, 1998; Dhong *et al.*, 1999)], and its primary foci of activity in the olfactory bulb lies largely outside the intended lesion sites for experimental rats (http://leonlab.bio.uci.edu).

After completion of this initial training, rats were trained on an ethyl acetate detection task using the go, no-go discrete trials procedure described in detail by Slotnick and Schellinck (Slotnick and Schellinck, 2001). The procedures used in initial training were continued except that both positive (ethyl acetate) and negative (no odor) stimuli were used. On each trial, the vapor generated from either 5% ethyl acetate (the S+ stimulus) or deionized water (the S– stimulus) was presented. S+ and S– trials were presented in a modified random sequence with the restriction that there was an equal number of each in every block of 20 trials. Making a criterion response on an S+ trial was scored as a hit and was reinforced with 0.04 ml of water, while failing to make a criterion response to an S+ trial was scored as a miss. Failing to make a criterion response on an S– trial was scored as a correct rejection, while making a criterion response on an S– trial was scored as a false alarm and was punished by a 15 s increase in the ITI. Hits and correct rejections were totaled to yield a percent accuracy score for each block of 20 trials. Criterion performance for this and all subsequent tasks was set at achieving at least 85% accuracy on two consecutive blocks of trials.

Rats were given 600 trials on this task in 200-trial daily sessions. In the next 200-trial session, a 1% solution of ethyl acetate served as the S+ stimulus. Rats were then trained to detect acetic acid (S+, 0.1% acetic acid; S–, mineral oil), to discriminate between 0.1% acetic acid (S+) and 0.5% caproic acid (S–), and to discriminate between 0.1% acetic acid (S+) and 0.2% propionic acid (S–). Each rat was given a minimum of 200 trials on each task. For a few rats that did not reach criterion performance, training was continued in a second session on that task.

Next, rats were given additional training on each acid discrimination task but using multiple concentrations of

Downloaded from http://chemse.oxfordjournals.org/ by guest on October 3, 2012

each stimulus. This was done to insure that discrimination was based on the qualitative difference between the acids rather than perceived differences in intensity. In the first multiple concentration task the S+ stimuli were 0.04, 0.2 and 1% concentrations of acetic acid and the S– stimuli were 0.2, 1 and 5% concentrations of caproic acid. Each stimulus was maintained in a separate channel of the olfactometer, and all six stimuli were presented in a modified random order such that each was given five times in each block of 30 trials for a total of 240 trials. To increase resistance to extinction for the postoperative memory test described below, rats were given three or four additional sessions on this task in which reinforcement probability on S+ trials was reduced to 0.5.

Next, similar procedures were used to train rats to discriminate between the three acetic acid stimuli (S+ stimuli) and 0.08, 0.4, and 2% concentrations of propionic acid (the S– stimuli). Rats were then given three or four additional sessions on this task in which reinforcement probability on S+ trials was reduced to 0.5.

Postoperative tasks

In the first postoperative task, given 3 weeks after surgery, 1% ethyl acetate was the S+ stimulus, and water was the S– stimulus. In preparation for their memory tests, all rats completed an additional 200 trials of ethyl acetate detection training in which reinforcement probability was reduced to 0.5.

Memory for the S+ and S– assignments of the acetic and caproic acid stimuli was then assessed by testing rats under extinction on each of the multiple concentration tasks. These tests were identical to the preoperative multiple concentration tasks except that only 60 trials were given, responses to the S+ stimuli were not reinforced, and responses to the S– stimuli were not punished by an extension of the ITI. Thus, rats had no feedback for correct or incorrect responses.

Following these memory tests, all rats were retrained to criterion on the 0.1% acetic acid detection task, the 0.1% acetic versus 0.5% caproic acid discrimination task, the 0.1% acetic versus 0.2% propionic acid discrimination task, the multiple concentration acetic versus caproic acid task, and the multiple concentration acetic versus propionic acid task. Then the later two tasks were repeated except that all stimulus concentrations were reduced by a factor of 10. Finally, all rats were trained to criterion to discriminate between 1% (+)-carvone (S+) and 1% (–)-carvone (S–). This later task was given because it was anticipated that bulbar lesions in experimental rats would remove the dorsal bulbar sites that are activated by the enantiomers of carvone (Linster *et al.*, 2001; Rubin and Katz, 2001).

Surgical procedures

Rats were anesthetized with Ketamine and Xylazine, secured in a stereotaxic apparatus, and operated on ~1 week after completing all pretreatment tasks. Seven experimental rats received bilateral olfactory bulb lesions. Both olfactory bulbs were exposed, and the dorsal surface of the left olfactory bulb was removed by gentle aspiration through a fine glass pipette under 10× magnification. Larger lesions were made in the right olfactory bulb. The remaining five rats served as controls. In these rats the left olfactory bulb was not damaged, but large lesions were made in the right olfactory bulb. Lesion sites were covered with gelfoam and scalps were closed with metal wound clips. The rats were kept on a heating pad until they regained their righting reflex and were then returned to their home cages. They were given soft food the day after surgery and supplemental water for the next 6 days and then returned to their standard water deprivation schedule.

Assessing and mapping bulb lesions

After completing the postoperative tasks, each rat was deeply anesthetized with chloral hydrate and killed by cardiac perfusion with saline and phosphate-buffered 10% paraformaldehyde. The brains were removed and stored in a solution of 10% formalin and 25% sucrose for several weeks. Following an hour-long rinse in tap water, the brains were photographed and then embedded in 12% gelatin and stored in 10% formalin for 24 h.

Because the brains were embedded in gelatin and there was extensive damage to the dorsal surface of the olfactory bulbs in experimental rats, we defined the angle of section as one parallel to a frontal transection of the forebrain at the level of the optic chiasm. This transection was made at a right angle to the dorsal surface of the cortex. The resulting plane of section through the olfactory bulbs closely approximated that described in the Slotnick and Hersch (Slotnick and Hersch, 1980) stereotaxic atlas. The olfactory bulbs were sectioned on a freezing microtome at 50 μm. Every fourth section was saved, mounted on glass slides and stained with thionin.

Sections were inspected microscopically and drawn using a projection microscope. The locus and extent of lesions in the right (lesioned) bulb in control animals and the bulb with the smallest lesion (left bulb) in experimental animals were plotted on a metric chart of the olfactory bulb based on the Slotnick and Hersch (Slotnick and Hersch, 1980) atlas of the rat olfactory system. This chart was constructed by having a vertical line represent the anterior–posterior (AP) coordinate and using horizontal lines extending to the right and left of this vertical line to represent the extent of the glomerular layer on the medial and lateral walls of the bulb at each AP level illustrated in the atlas. The horizontal extent of these lines was based on the length of the glomerular layer from the dorsal surface to the ventral surface of the bulb. Thus, the most dorsal glomeruli were represented at the center of the AP line, while the ends of the right and left horizontal lines represented the most ventral glomeruli. For purposes of illustration, the peripheral points of this chart

Downloaded from http://chemse.oxfordjournals.org/ by guest on October 3, 2012

were connected and the connecting lines smoothed. Next, we plotted on this chart the approximate positions of the fatty acid fields described by Johnson *et al.* (Johnson *et al.*, 1999). The two-dimensional chart Johnson *et al.* used by for illustrating these 2-DG defined glomerular fields was based on a rolled out map representation of the bulb, but points in that chart are not defined metrically. On the basis of information provided by Johnson *et al.* (Johnson *et al.*, 1999), it was not possible to locate precisely on the Slotnick and Hersch atlas the position of the glomerular fields that were activated by short-chain fatty acids. However, by comparing the relative positions of these fields in their charts [i.e. figures 4, 5 and 6 in Johnson *et al.* (Johnson *et al.*, 1999)] and illustrations on their web site (http://leonlab.bio.uci.edu), it was possible to represent the approximate position of each of their fields on the frontal sections of the Slotnick and Hersch atlas. The position and approximate extent of these fields were then transposed to the metric chart. This chart and the approximate positions of the four fatty acid 2-DG foci as described by Johnson *et al.* (Johnson *et al.*, 1999) is shown in Figure 1. Because the most anterior aspect of olfactory bulbs that received lesions had been removed, frontal levels of sections through the bulbs were defined relative to their position rostral to anterior pole of the accessory olfactory bulb.

Statistical analysis

Mann–Whitney *U* tests were used to compare groups. Errors to criterion served as the measure of performance on the detection and discrimination tasks and percent accuracy was used to score performance on the two memory tests. A total of 11 Mann–Whitney tests were performed and, thus, the adopted alpha level of 0.05 was adjusted to 0.004 using the Bonferroni correction.

Results

Location and extent of bulbar lesions

In each control rat the left olfactory bulb was intact and the rostral one-half to two-thirds of the right olfactory bulb was removed. In each experimental rat, the first two or more mm of the left olfactory bulb was removed and the bulb lesions extended posteriorly to destroy the dorsal, dorsolateral and dorsomedial aspects of the left bulb anterior to the accessory olfactory bulb. In all cases, lesions in the right olfactory bulb were larger than those in the left bulb.

Photomicrographs illustrating sections of the left olfactory bulb of two experimental rats with the largest lesions (rats A4 and A7) and of the rat with the smallest lesions (rat A1) are shown in Figure 2. The charts in Figure 3 show regions of the olfactory bulb glomerular layer that were destroyed in the left olfactory bulb in the rat with the

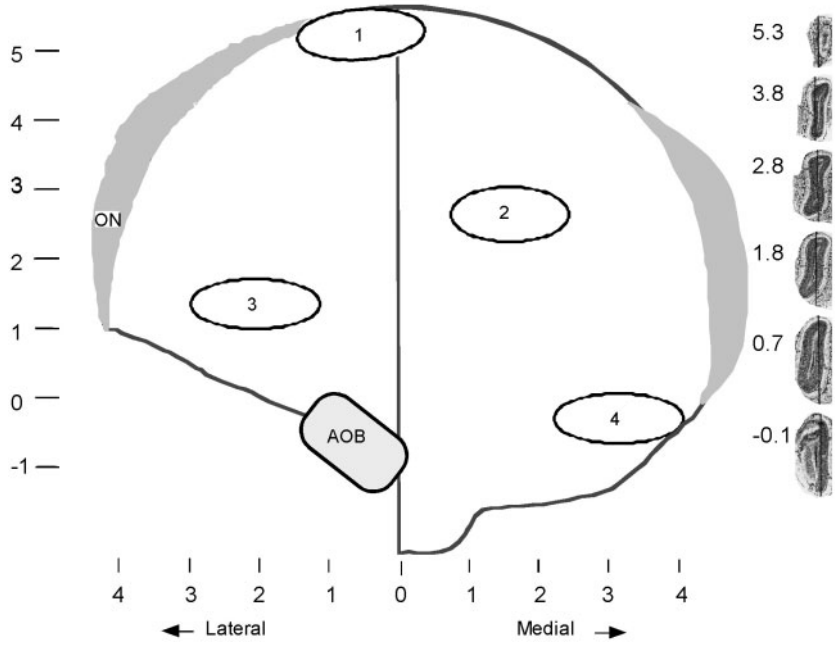

Figure 1 A metric two-dimensional representation of the glomerular layer of the olfactory bulb illustrating the anterior, medial and lateral position of the bulb glomerular layer. As described in the text, the vertical line represents the anterior–posterior (AP) extent of the bulb midline (anterior towards the top) and the medial and lateral extent of the glomerular layer are represented to the right and left, respectively, of the midline. The frontal zero coordinate is defined by the first section in which the granule cell layer of the accessory olfactory bulb could be clearly identified. The figures to the right of the chart show selected frontal levels through the olfactory bulb and the vertical line through those figures represents the stereotaxic midline of the bulb (Slotnick and Hersch, 1980). Glomerular fields 1–4 show the approximate position of the four fields identified by Johnson *et al.* (Johnson *et al.*, 1999) as responsive to short-chain fatty acids. AOB = accessory olfactory bulb; ON = olfactory nerve layer.

Downloaded from http://chemse.oxfordjournals.org/ by guest on October 3, 2012

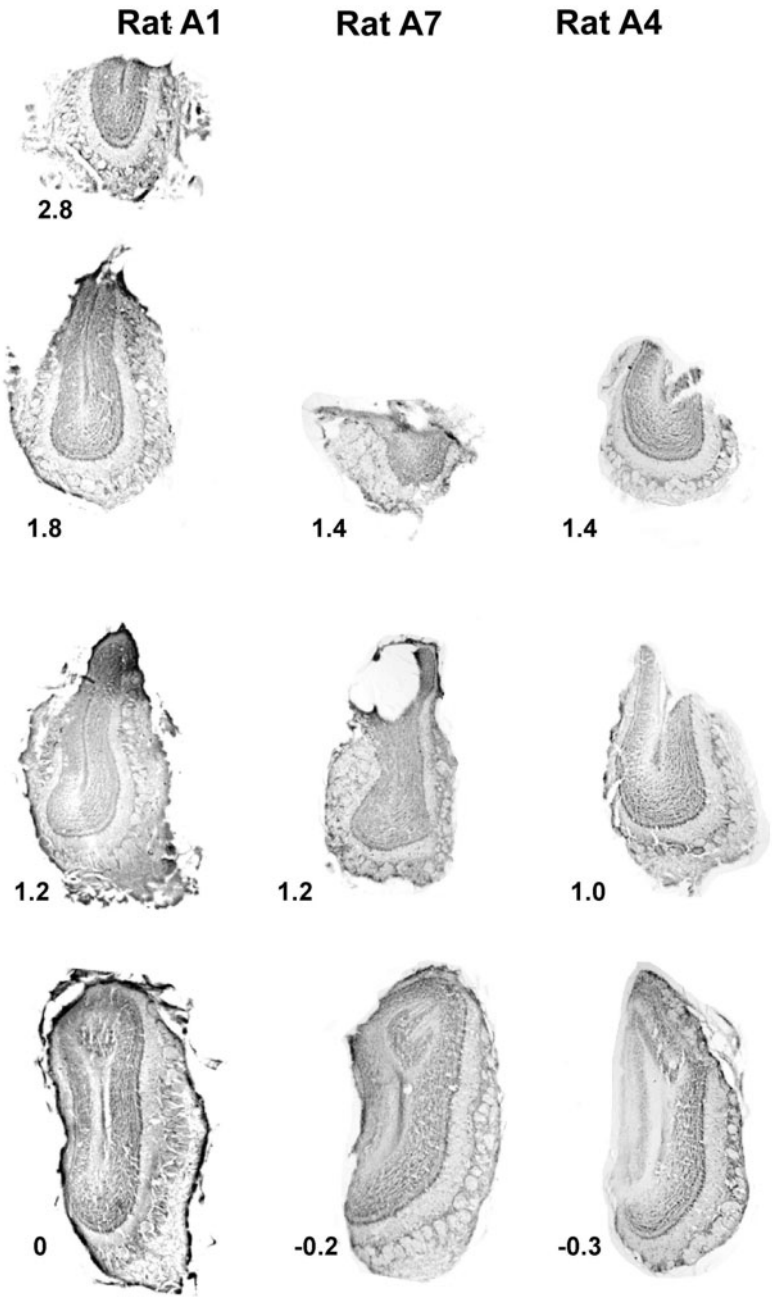

Figure 2 Photomicrographs of selected frontal sections of the rat with the smallest lesion (A1) and the two rats with the largest lesions (A4 and A7). Numbers at the bottom of each photomicrograph give the approximate distance in mm from the rostral aspect of the accessory olfactory bulb. Dorsal is to the top and medial is to the right in each section.

smallest lesion (Rat A1) and one of the rats with large lesions (Rat A7). Within the experimental group, the anterior field (Field 1) identified by Johnson *et al.* (Johnson *et al.*, 1999) as being activated by exposure to fatty acids was completely removed in each rat. Field 2 was completely removed and Field 3 was largely removed in three rats. In the remaining four rats, Field 2 was completely removed (one rat) or largely removed and Field 3 was at least partially removed. Field 4 was intact in all cases.

Preoperative Behavioral results

All rats achieved criterion performance on each task within one or two 200-trial sessions, with a median of 10 errors to criterion across all problems. Rats maintained high levels of performance (85–100% correct responding) when trained with multiple concentrations of the S+ and S– acid odorants and when they were trained on partial reinforcement. The performance of the designated control and experimental

Downloaded from http://chemse.oxfordjournals.org/ by guest on October 3, 2012

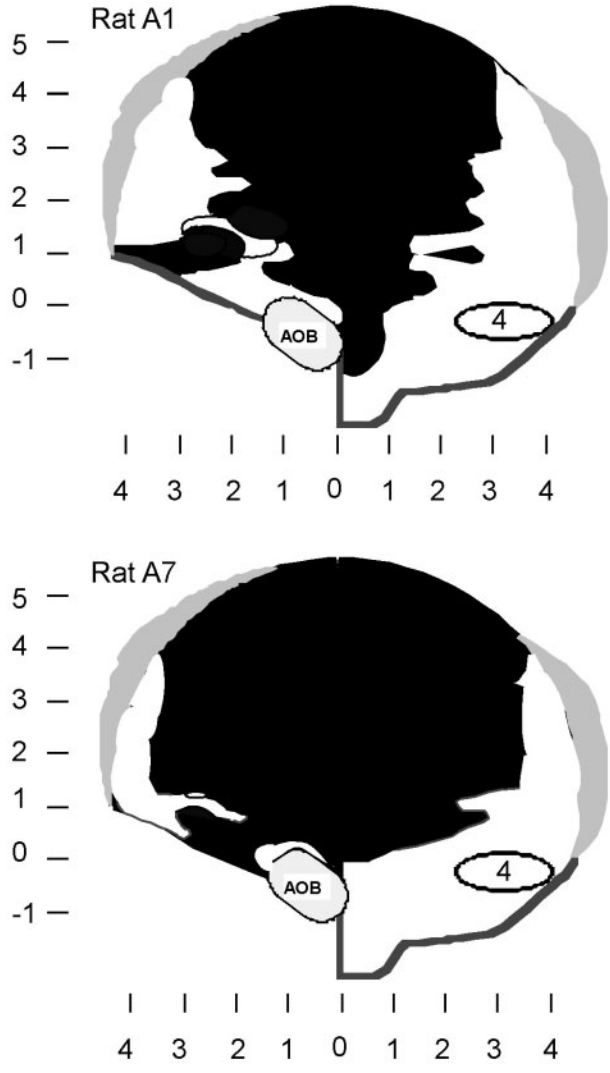

Figure 3 Representation of the extent of glomerular destruction in a rat with one of the smaller lesions (A1) and in a rat with one of the larger lesions (A7).

groups was similar on these tasks and the groups did not differ on median errors across all tasks ($U = 14.5$, $P < 0.62$).

Postoperative behavioral results

Ethyl acetate detection

Experimental rats made somewhat but not significantly more errors than controls in achieving criterion performance on the ethyl acetate detection task (Table 1).

Memory tests

The memory tests were the first postoperative exposure to acid stimuli and were given after retraining on the ethyl acetate detection task. Median accuracy scores for control and experimental groups and outcomes of statistical tests are given in Table 2. In general, most rats had relatively low accuracy scores on the acetic versus caproic acid memory test, and the median score for control rats (83%) was somewhat but not significantly greater than that for experimental rats (70%). Five of the seven experimental rats and one of the five controls rats had accuracy scores that were less than 75% (Figure 4A).

Most rats had even lower accuracy scores on the acetic versus propionic acid memory test (Figure 4A), and the median accuracy score for control rats (76%) was somewhat but not significantly greater than that for experimental rats (60%). Each experimental rat but only two of the five controls had accuracy scores that were <75% (Figure 4A).

Acid detection and discrimination tasks

Median errors to criterion for control and experimental groups and outcomes of statistical tests for the postoperative detection and discrimination tasks are given in Table 1 and illustrated in Figure 4B. As shown in Table 1, on average, experimental rats had somewhat higher error scores than controls on several of these tasks but none of these differences was significant and the groups did not differ with regard to median errors over all tasks.

Table 1 Median errors to criterion for the control and experimental groups and results of Mann–Whitney tests for each of the postoperative detection and discrimination tasks

Task[a]	Control group	Experimental group	*U*	*P* <
EA detection	3	22	7	0.09
C2 detection	3	10	9	0.16
C2 versus C6 discrimination	3	3	16	0.81
C2 versus C3 discrimination	3	7	11.5	0.32
C2 versus C6 multiple concentrations	2	4	10.5	0.25
C2 versus C3 multiple concentrations	0	3	6	0.05
C2 versus C6 multiple low concentrations	7	4	13.5	0.52
C2 versus C3 multiple low concentrations	9	8	6	0.81
Carvone discrimination	11	18	12	0.37
Overall median score[b]	3	8	10.5	0.25

[a]EA, ethyl acetate; C2, acetic acid; C3, propionic acid; C6, caproic acid.

[b]Median of all errors to criterion scores across all subjects of each group for the detection and discrimination tasks.

Downloaded from http://chemse.oxfordjournals.org/ by guest on October 3, 2012

Table 2 Median percent correct responding on the 60-trial memory test for control and experimental groups

Memory test	Control group	Experimental group	*U*	*P* <
C2 versus C6	83	70	8	0.12
C2 versus C3	76	60	3	0.018

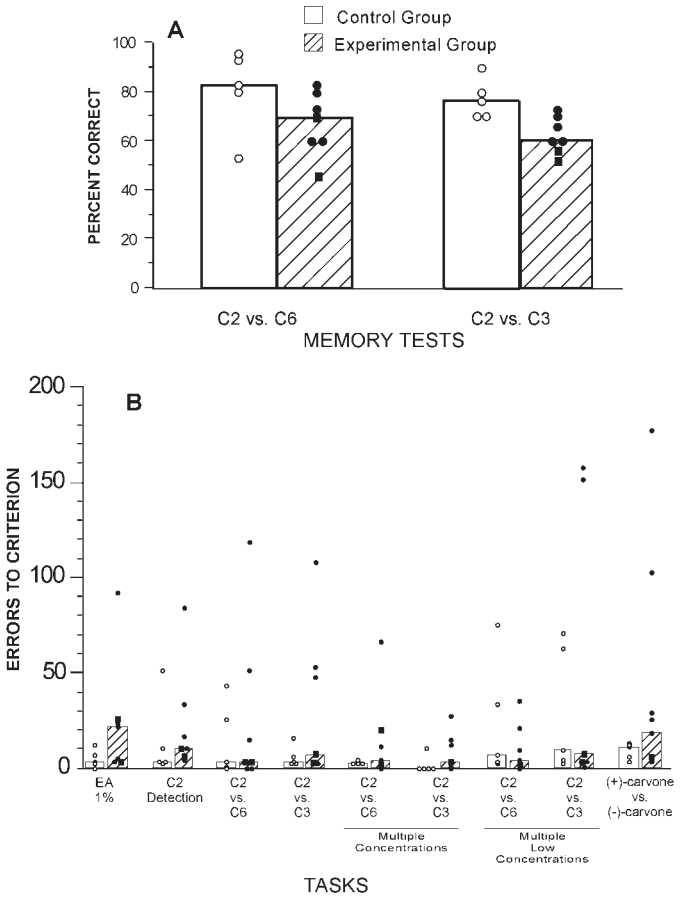

Figure 4 **(A)** Vertical bars show median percent correct responding in the acetic versus caproic acid (C2 versus C6) and acetic acid versus propionic acid (C2 versus C3) memory test for control (open bars) and experimental rats (striped bars). **(B)** Vertical bars show median errors made on the ethyl acetate (EA) and acetic acid (C2) detection tasks and on single concentration and multiple concentration acid discrimination tasks and the carvone discrimination tasks. In both graphs, open circles represent the scores of individual control rats and closed symbols the scores of individual experimental rats. The scores of the two experimental rats with the largest lesions are represented as solid squares.

Of particular interest is the performance on the initial block of trials on the multiple concentration tasks. If rats had been using intensity difference cues in the prior two-odor acid discrimination tasks then one might expect a marked drop in performance when they were first retrained on the multiple concentration tasks. However, this did not occur: the median accuracy on the first block of trials on the acetic acid versus caproic acid multiple concentration task was 93% for controls and 86% for experimental rats. For the acetic acid versus propionic acid multiple concentration task first block accuracy was 95% for controls and 90% for experimental rats.

Relationship between performance and size of bulbar lesion. There was no obvious relationship between size of lesion and performance in any of the odor detection or discrimination tasks. The two rats with the largest lesions (A4 and A7) had the lowest accuracy scores on the acetic versus propionic acid memory test (Figure 4A) but their error scores on the subsequent detection and discrimination tasks were within the range of rats with smaller lesions. Further, one or both of these two rats had scores similar to or below the median score of controls on each of the detection and discrimination tasks.

Discussion

The present results demonstrate that rats with relatively large lesions of the dorsal one-third of the olfactory bulbs had some deficits in remembering the positive and negative assignments of fatty acid odors but performed nearly as well as controls in discriminating among them. Because rats continued to perform at high levels of accuracy when concentrations of odors were varied over a wide range during the discrimination tasks, it is unlikely that performance was based on perceived differences in intensity. The present results extend those of Slotnick and Bodyak (Slotnick and Bodyak, 2002) by controlling for odor intensity cues and by showing that rats with quite large bulbar lesions may readily discriminate two odors that differ only by one carbon atom.

A decision as to whether the lesions in experimental rats in this study invaded or removed fatty acid responsive areas defined in the Johnson *et al.* study (Johnson *et al.*, 1999) depends on two factors: the accuracy with which we could identify the location of these fields on frontal sections of the olfactory bulb from the maps provided by Johnson *et al.* (Johnson *et al.*, 1999) and the concentrations of odorants used. The later consideration is relevant because, for at least some odorants, the number of glomeruli activated increases with higher odor concentration (Rubin and Katz, 1999; Johnson and Leon, 2000a). Rubin and Katz (Rubin and Katz, 1999) and Uchida *et al.* (Uchida *et al.*, 2000) used headspace vapors from 10% (or higher) mineral oil dilutions of fatty acids while Johnson *et al.* (Johnson *et al.*, 1999) used an air dilution olfactometer to generate odors at 7.2 ppm. The odors used in the present study were generated using a combination of liquid and air dilution steps. The highest concentrations used, calculated using Henry's law, were 8.8 ppm for acetic acid, 1.3 ppm for caproic acid, and 4.4 ppm for propionic acid. Thus, the highest acid concentrations used in this experiment were similar to those used in

Downloaded from http://chemse.oxfordjournals.org/ by guest on October 3, 2012

by Johnson *et al.* and appreciably below those used by Rubin and Katz and Uchida *et al.*

It should also be noted that it is highly unlikely that these relatively low concentrations of acids would stimulate trigeminal receptors. Cometto-Muniz *et al.* (Cometto-Muniz *et al.*, 1998) have determined in humans the 'pungency' thresholds for carboxylic acids as a function of carbon chain-length. For C2–C7 acids the pungency threshold is 4 or more orders of magnitude above odor detection threshold and the lowest pungency threshold for this series (C2) is ~100 ppm, i.e. more than 10 times the highest C2 concentration used in this study.

Insuring that the glomerular fields for fatty acids identified by Johnson *et al.* are accurately mapped on standard frontal sections was somewhat troublesome because, as described in Methods, the precise positions of these fields were not defined metrically. Nevertheless, the bulbar lesions in experimental rats were quite large and they undoubtedly removed all of Fields 1 and 2 in most cases and largely removed or at least invaded Field 3 in all cases. In addition, these lesions destroyed other bulbar regions not identified as significantly responsive to fatty acids in the Johnson *et al.* (Johnson *et al.*, 1999) study.

Response accuracy on the memory test used in this study provides a measure of the extent to which bulbar lesions altered the perceptual quality of the odor. Thus, responding appropriately under conditions where no feedback was given is possible only to the extent that the perceived odor matched or was sufficiently similar to a stored image of that odor. In both the acetic acid versus caproic acid and acetic acid versus propionic acid memory tests, experimental animals performed more poorly than controls. However, these results provide, at best, only weak evidence for the notion that disruption of the pattern of inputs produced by exposure to these odors alters significantly their identification or perceptual quality. This is because memory scores for experimental rats were only marginally poorer than those of controls and because lesions in experimental rats were large and extended into areas of the bulb well outside of those identified as responsive to fatty acids. Nevertheless, the memory test outcomes were in the direction predicted by an odotopic view of odor coding and these are perhaps the first such results from a brain lesion study to provide support for this view.

In contrast, the outcomes of the discrimination tasks offer little or no support for the notion that differences in the pattern of bulbar inputs for two closely related odors (as defined by optical imaging and 2-DG studies) are essential for discriminating between such odors. The extensive lesions in experimental rats would certainly have disrupted the normal patterns of bulbar inputs for both fatty acids and the enantiomers of carvone. Nevertheless, both experimental and control rats made few errors when retrained on each of the multiple acid concentration problems and experimental rats had no deficits in discriminating between the enantiomers of carvone despite the fact that they had not been pre-trained on this task. The excellent performance of these rats when shifted from the single concentration two-odor acid discrimination tasks to the multiple concentration tasks indicates that discrimination was based on the qualitative difference between odors and that this performance was not compromised by removal of the majority of 2-DG-identified fatty acid inputs to the bulb.

It is possible, as Johnson *et al.* suggest, that there is some functional redundancy among the multiple glomerular areas that are activated by an odor. Further, this redundancy may support discrimination but not preservation of odor quality. While this notion of redundancy is consonant with the present outcomes, it may prove difficult to reconcile that explanation for behavioral savings with a model of odor coding in which odor quality is represented as a spatially distributed pattern of activation across the olfactory bulb. As Slotnick and Bodyak (Slotnick and Bodyak, 2002) observed, redundancy implies a certain amount of equipotentiality among identified glomerular fields while, according to an odotopic model, differences in the patterns of stimulation across the olfactory bulb play a significant role in odor discrimination. In any case, the present results, together with those of Slotnick and Bodyak (Slotnick and Bodyak, 2002) provide strong evidence that the 2-DG pattern of activation produced by exposure to an odor is not essential for discriminating that odor from odors that produce quite similar patterns of activation.

References

Apfelbach, R., Weiler, E., Asselbergs, W.F., Polak, E.H. and **Slotnick, B.** (1998) *Selective and reversible reduction of odor sensitivity in the rat by concanavalin A.* Physiol. Behav. 65, 513–516.

Bell, G.A., Laing, D.G. and **Panhuber, H.** (1987) *Odour mixture suppression: evidence for a peripheral mechanism in human and rats.* Brain Res., 426, 8–18.

Cometto-Muniz, J.E., Cain, W.S. and **Abraham, M.H.** (1998) *Nasal pungency and odor of homologous aldehydes and carboxylic acids.* Exp. Brain Res., 118, 180–188.

Cometto-Muniz, J.E., Cain, W.S., Abraham, M.H. and **Gola, J.M.** (1999) *Chemosensory detectability of 1-butanol and 2-heptanone singly and in binary mixtures.* Physiol. Behav., 67, 269–276.

Cometto-Muniz, J.E., Cain, W.S., Abraham, M.H. and **Gola, J.M.** (2001) *Ocular and nasal trigeminal detection of butyl acetate and toluene presented singly and in mixtures.* Toxicol. Sci., 63, 233–244.

Dhong, H.J., Chung, S.K. and **Doty, R.L.** (1999) *Estrogen protects against 3-methylindole-induced olfactory loss.* Brain Res., 824, 312–315.

Doty, R.L., Li, C., Bagla, R., Huang, W., Pfeiffer, C., Brosvic, G.M. and **Risser, J.M.** (1998) *SKF 38393 enhances odor detection performance.* Psychopharmacology (Berl)., 136, 75–82.

Duchamp-Viret, P., Duchamp, A. and **Chaput, M.A.** (2000) *Peripheral odor coding in the rat and frog: quality and intensity specification.* J. Neurosci., 20, 2383–2390.

Imamura, K., Mataga, N. and **Mori, K.** (1992) *Coding of odor molecules*

Downloaded from http://chemse.oxfordjournals.org/ by guest on October 3, 2012

by mitral/tufted cells in rabbit olfactory bulb. I. aliphatic compounds. J. Neurophysiol., 68, 1986–2002.

Johnson, B.A. and **Leon, M.** (2000a) *Modular representations of odorants in the glomerular layer of the rat olfactory bulb and the effects of stimulus concentration.* J. Comp. Neurol., 422, 496–509.

Johnson, B.A. and **Leon, M.** (2000b) *Odorant molecular length: one aspect of the olfactory code.* J. Comp. Neurol., 426, 330–338.

Johnson, B.A., Woo, C.C., Hingco, E.E., Pham, K.L. and **Leon, M.** (1999) *Multidimensional chemotopic responses to n-aliphatic acid odorants in the rat olfactory bulb.* J. Comp. Neurol., 409, 529–548.

Linster, C., Johnson, B.A., Yue, E., Morse, A., Xu, Z., Hingco, E.E., Choi, Y., Choi, M., Messiha, A. and **Leon, M.** (2001) *Perceptual correlates of neural representations evoked by odorant enantiomers.* J. Neurosci., 21, 9837–9843.

Lu, X.-C.M. and **Slotnick, B.M.** (1994) *Recognition of propionic acid vapor after removal of the olfactory bulb area associated with high 2-DG uptake.* Brain Res., 639, 26–32.

Lu, X.-C.M. and **Slotnick, B.M.** (1998) *Olfaction in rats with extensive lesions of the olfactory bulbs: implications for odor coding.* Neuroscience, 84, 849–866.

Malnic, B., Hirono, J., Sato, T. and **Buck, L.B.** (1999) *Combinatorial receptor codes for odors.* Cell, 96, 713–723.

Meister, M. and **Bonhoeffer, T.** (2001) *Tuning and topography in an odor map on the rat olfactory bulb.* J. Neurosci., 21, 1351–1360.

Onoda, N. (1992) *Odor induced fos like immunoreactivity in the rat olfactory bulb.* Neurosci. Lett., 137, 157–160.

Ressler, K.J., Sullivan, S.L. and **Buck, L.B.** (1994) *Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb.* Cell, 79, 1245–1255.

Rubin, B.D. and **Katz, L.C.** (1999) *Optical imaging of odorant representations in the mammalian olfactory bulb.* Neuron, 23, 499–511.

Rubin, B.D. and **Katz, L.C.** (2001) *Spatial coding of enantiomers in the rat olfactory bulb.* Nat. Neurosci., 4, 355–356.

Slotnick, B. and **Bodyak, N.** (2002) *Odor discrimination and odor quality perception in rats with disruption of connections between the olfactory epithelium and olfactory bulbs.* J. Neurosci., 22, 4205–4216.

Slotnick, B. and **Schellinck, H.** (2001) *Behavioral methods in olfactory research with rodents.* In Simon, S.A. and Nicolelis, M.A.L. (eds), Methods in Chemosensory Research. CRC Press, Washington, DC, pp. 21–61.

Slotnick, B.M. and **Hersch, S.** (1980) *A stereotaxic atlas of the rat olfactory system.* Brain Res. Bull., suppl. 5, 1–55.

Slotnick, B.M. and **Ptak, J.E.** (1977) *Olfactory intensity-difference thresholds in rats and humans.* Physiol. Behav., 19,795–802.

Slotnick, B.M., Graham, S., Laing, D.G. and **Bell, G.A.** (1987) *Detection of propionic acid vapor by rats with lesions of olfactory bulb areas associated with high 2-DG uptake.* Brain Res., 417, 343–346.

Slotnick, B.M., Panhuber, H., Bell, G.A. and **Laing, D.G.** (1989) *Odor-induced metabolic activity in the olfactory bulb of rats trained to detect propionic acid vapor.* Brain Res., 500, 161–168.

Uchida, N., Takahashi, Y.K., Tanifuji, M. and **Mori, K.** (2000) *Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features.* Nat. Neurosci., 3, 1035–1043.

Xu, F., Greer, C.A. and **Shepherd, G.M.** (2000) *Odor maps in the olfactory bulb.* J. Comp. Neurol., 422, 489–495.

Accepted April 4, 2003

Downloaded from http://chemse.oxfordjournals.org/ by guest on October 3, 2012