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Research report

Olfaction and peripheral olfactory connections in methimazole-treated rats

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Abstract

Methimazole has been reported to produce extensive degenerative changes in olfactory epithelium and a severe deficit in odor detection [Genter BM, Owens DM, Carlone HB, Crofton KM. Fundam. Appl. Toxicol. 1996;29:71–77; Genter BM, Owens DM, Deamer NJ, Blake BL, Wesley DS, Levi PE. Toxicol. Pathol. 1995;23:477–486.]. To examine this further, rats were tested on olfactory detection and discrimination problems before and after intraperitoneal injection of 300 mg/kg methimazole. In the first 2 days after treatment, experimental rats had nasal congestion and a modest decrement on odor detection and odor mixture discrimination tasks. They performed almost as well as control rats on the third post injection day. In a separate group of rats, anterograde transport of horseradish peroxidase from olfactory epithelium to the bulb was examined 1, 2, 3, and 5 days after administration of methimazole. The treatment produced a modest but progressive disruption of bulbar input: 2 days after administration only approximately 10% of bulbar glomeruli had reduced levels of reaction product while 30–40% of glomeruli had little or no reaction product in 3–5 day survival rats. These results indicate that methimazole is not a particularly effective olfactotoxin and does not produce anosmia or even a severe hyposmia.

Keywords: Methimazole; Olfactory epithelium; Odor detection; Odor discrimination

1. Introduction

A promising method for examining the relation between sensory input to the olfactory bulb and the sense of smell is the use of 'olfactotoxins' to disrupt discrete areas of the olfactory epithelium [1,4–8,21]. These agents produce degeneration of olfactory epithelium and, in many cases, there is a gradual recovery of structural integrity. For example, 3 methylindole (3-MI) acts, in part, by bioactivation of at least some P450 enzymes, resulting in selective degeneration within the olfactory mucosa [11,21]. Setzer and Slotnick [15] reported that 3-MI produced an initial severe disruption of axonal transport of HRP from the epithelium to the bulb followed by marked recovery over a 3-month posttreatment period, particularly for lower doses of the agent (150–350 mg/kg). However, their attempts to assess behavior when the effect of the toxin on olfactory epithelium was maximal were compromised because rats were ill for some days after treatment [16]. The illness was probably due to the widespread effects 3-MI has on P450 enzymes, particularly in the lungs and liver [11].

A potentially more promising agent, methimazole, a thioureylene antithyroid drug used to treat hyperthyroidism, has recently been studied by Genter et al. [5,6]. They reported a nearly complete sloughing off of the neuronal and sustentacular layer of the olfactory mucosa within 32 h after a single 300 mg/kg dose of methimazole. The toxicity is probably due to reactive intermediates produced in the metabolism of the drug [6]. Rats trained to find a food pellet buried in bedding failed in this task on the first 3–4 days after treatment although they appeared healthy and motivated and, in fact, performed normally on a control task (Morris Water Maze). With additional training, performance on

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the olfactory task improved and, within 10 days, returned to control levels [5].

The purpose of the present study was to provide a quantitative measure of the methimazole-induced disruption of input to the olfactory bulb and to assess the olfactory capacity of rats treated with the toxicant. If methimazole produced site-specific disruption of the olfactory epithelium and specific deficits in odor detection or discrimination, it would be a useful agent for studies of regeneration and odor coding. To this end, olfactometric methods were used to test methimazole-treated rats on a variety of odor detection and discrimination problems. Disruption of epithelium was assessed by examining anterograde transport of horseradish peroxidase conjugated with wheat germ agglutinin (WGA*HRP) from the epithelium to the glomerular layer of the olfactory bulbs.

2. Methods

2.1. Subjects

Twenty-seven adult male Long–Evans strain rats were individually housed in plastic cages with woodchip bedding. The rats were approximately 90 days old and weighed 300–400 g at the beginning of the study. Purina lab chow was provided ad libitum. The vivarium was maintained on a 12:12 light/dark cycle (lights on at 08:00 h). Except for 2 days prior to experimental treatment when supplementary water was given, each subject was maintained on a 10 ml/day water deprivation schedule.

2.2. Apparatus

Rats were tested using three identical eight channel olfactometers similar in design to that described by Slotnick [18]. These systems have been used in numerous studies in our laboratory and a variety of tests have validated their use for odor detection and odor discrimination studies [9,18–20]. Odors were generated by passing a stream of air over the surface of a liquid odorant contained in 38-cm-long, 2-cm-diameter glass saturator tubes. Airflow through each tube was controlled by separate solenoid valves on the upstream and downstream ends of the tube. Operating the solenoid valves manifolded odorized air from the selected channel into a clean air stream connected to the animal's chamber.

Odor stimuli (isoamyl acetate, ethyl acetate, and pyridine (Sigma)) were diluted with deionized water and concentrations given below are those of the liquid odorant. The odor vapor, generated by passing 50 cc/min over the surface of the odorant material in the saturator tubes, was manifolded with a 1950 cc/min stream of clean air before being introduced to the sampling port. Thus, the concentration of odor stimuli experienced by rat at the sampling port was approximately 2.5% of the concentration of the headspace above the liquid odorant.

The test chamber was a $24 \times 24 \times 16.5$ cm Plexiglas box. A 2.3-cm-diameter vertical glass tube, attached to an outside wall of the chamber was used to present odor stimuli. The animal could insert its snout into a 2-cm-diameter hole cut through the chamber and stimulus tube. Insertion of the snout was detected by a photobeam. Water reinforcement was delivered via a 13-gauge stainless steel tube connected to a water reservoir via a 2-way normally closed solenoid. Operation of the solenoid valve delivered 0.04 ml of water and produced an audible click. Responses on the reinforcement tube were detected by a touch-sensitive circuit between the chamber floor and the tube [3].

2.3. Initial training

Rats were trained using the go, no-go discrete trials discrimination procedure previously described [18]. Standard operant conditioning methods were used to train rats to insert their snout into the odor sampling tube (nose poke) and then respond on the reinforcement tube. In initial sessions, only the S+ odor stimulus (0.1% isoamyl acetate) was presented in each trial. Rats were then trained on an isoamyl acetate detection task in which 0.1% amyl acetate served as S+ and clean air served as S−. After a 4-s intertrial interval, a nose poke into the sampling port initiated an S+ or S− trial. On S+ trials the odor stimulus was presented for 2 s. A response made on the reinforcement tube within the next 3 s produced 0.04 ml of water. Identical procedures were used for S− trials except that the stimulus was clean air and responding on the reinforcement tube was not reinforced. Responding on S+ trials (hits) and not responding on S− trials (correct rejections) were scored as correct. Not responding on S+ trials (misses) and responding on S− trials (false alarms) were scored as errors. All subjects were given three 200-trial sessions on this task and performance was scored for each block of 20 trials (ten S+ and ten S− trials presented in a modified random order). Performance accuracy on the last 200 trials for each rat was between 85 and 100%.

2.4. Standard task set

Next, each rat was trained on a standard set of detection and discrimination problems as described below. The set of problems was presented in a single session and a new problem was introduced when the

rat achieved or exceeded criterion performance of 85% correct responding in a block of 20 trials. If the rat failed to achieve criterion performance within 100 trials on any one of these tasks the session was terminated and the problem set was given again on the next day. The following sequence of problems constituted the Standard Task Set:

Task 1. Isoamyl acetate detection A. The S+ stimulus was 0.1% isoamyl acetate and the S− stimulus was clean air.

Task 2. Isoamyl acetate detection B. The S+ stimulus was 0.01% isoamyl acetate and the S− stimulus was clean air.

Task 3. Two-odor discrimination A. The S+ stimulus was 0.1% isoamyl acetate and the S− stimulus was 1% pyridine. The intensity of these two odors were judged to be approximately equal by a human observer.

Task 4. Two-odor discrimination B. The S+ stimulus was 0.1% isoamyl acetate and the S− stimulus was 0.5% ethyl acetate. The intensity of these two odors were judged to be approximately equal by a human observer and the two odors were judged to be more similar to one another than those in Task 3.

Task 5–7. Odor mixture. In these problems rats were required to discriminate isoamyl acetate from a mixture of isoamyl acetate and pyridine. The concentration of pyridine in the mixture was gradually decreased as follows: In the first step of the mixture task the S+ stimulus was 0.1% isoamyl acetate and the S− stimulus was 0.1% isoamyl acetate plus 1% pyridine (Task 5). Upon achieving criterion performance, pyridine concentration in the S- mixture was reduced to 0.1% (Task 6), and then 0.01% (Task 7). The odor mixtures for these tasks were produced in the vapor phase by operating the S+ (isoamyl acetate) and S− (pyridine) control valves simultaneously. On S+ trials both the S+ control valve and the clean air control valve were operated simultaneously to insure that total air flow was the same on S+ and S− trials. Rats were given three to six sessions on the Standard Task Set. By the end of training all rats performed at criterion on all but the last odor mixture task.

2.5. Drug posttreatment test procedure

Approximately 24, 48, and 72 h after methimazole or control injections, subjects were tested on the same Standard Task Set using the same performance criterion described above. If, as occurred in some cases, a rat failed to reach criterion on a problem in the 100 trials allowed, it was not tested on the subsequent tasks on that day. In such cases the rat was assigned an error score of 50 on each of the remaining problems in the set.

2.6. Methimazole administration

After the last pretreatment training session, each rat was given 20 ml of water for each of 2 days and then injected with methimazole or vehicle. Methimazole (Sigma M-8506, Lot Number: 113H3668 kindly provided by Dr K. Crofton) was dissolved in 200 μl of dimethyl sulfoxide. Each of six rats was given an intraperitoneal injection of 300 mg/kg of methimazole and six rats received only 200 μl of the solvent.

2.7. Anatomical procedures

A separate group of 15 rats, maintained on the same water deprivation schedule, was used for an anatomical study. Eleven rats were given 300 mg/kg methimazole (as described above) and the olfactory epithelium of each rat was treated with horseradish peroxidase conjugated with wheat germ agglutinin (WGA*HRP) 1 day (Group M1, $n=3$), 2 days (Group M2, $n=2$), 3 days (Group M3, $n=3$), or 5 days (Group M5, $n=3$) later. Four rats (anatomical controls) were injected with dimethyl sulfoxide (200 μl) and treated with WGA*HRP 2–4 days later. A modification of the method described by Shipley [17] was used to administer WGA*HRP: The rat was anesthetized with 350 mg/kg of chloral hydrate (7% aqueous solution) and its head was clamped in a stereotaxic holder. The dorsal surface of the olfactory sacs were exposed through the nasal bones and each sac was injected with 15 μl of 0.5% WGA*HRP in saline made using 0.5% Fast Green. Twenty-four hours later, rats were deeply anesthetized and perfused through the heart following the procedures of Mesulam [12]. The brain was removed, stored at 4°C in sucrose phosphate buffer and either sectioned the same day or 24 h later. Fifty-micron-thick frontal sections through the olfactory bulbs were cut on a freezing microtome and every third section was saved, reacted with tetramethylbenzadine and mounted on gelatin-coated glass slides. The sections were lightly counterstained with thionin, quickly dehydrated through cold 70, 95, and 100% ethanol, cleared in xylene and covered using Permount.

2.8. Histological analyses

Anterograde transport of WGA*HRP to glomeruli was assessed in eight sections from each brain. The section containing the most anterior aspect of the accessory olfactory bulb was designated as level zero. Six sections anterior to this level and one section posterior to this level were then selected. The distance between sections was 0.4–0.7 mm. Each section was inspected and photocopied at 46× using a Bell and Howell model ABR 2000 microfiche reader/printer.

For microscopic analysis, one bulb of each of two subjects from each group (control, M1, M2, M3 and M5) were used. The photocopies of the selected sections from each bulb were divided into four quadrants (dorsolateral, dorsomedial, ventrolateral, and ventromedial) by bisecting the long and short axes of the section. Each quadrant was further divided into small segments that were about the size of one or two large glomeruli or three to four small glomeruli. The sections were inspected using brightfield and polarized light at 100 × and each segment on the corresponding photocopy of the section was scored from 0 to 2. A score of zero was assigned if most glomeruli in the segment contain little or no reaction product. A score of 1 was assigned if most glomeruli in the segment contain only light reaction product and a score of 2 was assigned if glomeruli in the segment contain predominantly dense reaction product. Because virtually all such segments in control sections had a score of 2, scores of 0 or 1 in experimental rats represented glomerular areas that had distinctly less transport than those of controls. The number of glomerular sectors that had a score of 2 in experimental rats, relative to that in controls, was used to determine the percent of the glomerular area in each quadrant in each experimental rat that contained control levels of reaction product.

Because rats were killed 24 h after application of WGA*HRP, axonal transport of the tracer from epithelium to the bulbs could have occurred anytime between (approximately) 4 h after WGA*HRP treatment and the time of sacrifice. Thus, the reaction product in glomeruli in groups M1-M5 represents axonal transport that occurred 28–48 h (Group M1), 52–72 h (Group M2), 76–96 h (Group M3) or 118–144 h (Group M5) after methimazole injection.

2.9. Analysis of behavioral results

Error scores served as the dependent variable for the behavioral tasks. Mann–Whitney *U*-tests were used to compare scores of the two groups on each of the seven Standard Task Set problems on each of the three posttreatment test days. Wilcoxon Signed-Ranks tests were used to compare pretreatment terminal performance with the first posttreatment test.

3. Results

3.1. Histological results

Virtually all glomeruli in the olfactory bulbs of control rats were filled with moderately dense or dense reaction product. The glomeruli of experimental rats in Group M1 were also filled with reaction product and this group was not distinguishable from controls on the basis of the number of filled glomeruli or density of the reaction product.

Fewer glomeruli in experimental rats of Groups M2, M3 and M5 had dense reaction product and, in each case, some glomeruli had little or no reaction product. In general, the extent of this loss was graded relative to survival time with the M5 group having the greatest loss. Across all quadrants and frontal levels, the M2, M3 and M5 groups had average score of 89.5 (93–86%), 80 (85–75%), and 68% (73–63%), respectively. Almost all glomeruli with little or no reaction product were found within 2 mm anterior to the level of the accessory olfactory bulb (AOB); virtually all glomeruli in frontal level 8 (posterior to the AOB) were filled with dense reaction product in each case (Fig. 1). As shown in Fig. 1, the loss in reaction product was most marked in the dorsomedial quadrant (quadrant 3) of the bulb for groups M3 and M5. Fig. 3Fig. 4 show photomicrographs of bulbar sections from control and experimental rats.

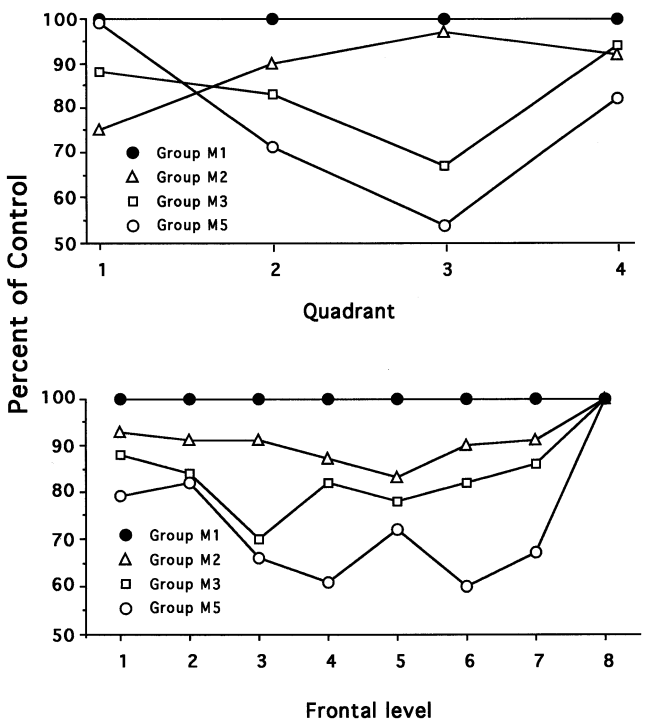

Fig. 1. Top graph shows the number of glomeruli expressed as a percent of control that were filled with dense reaction product in the ventrolateral (Q1), dorsolateral (Q2), dorsomedial (Q3) and ventromedial (Q4) quadrants of the olfactory bulb for rats sacrificed 2 (Group M1), 3 (Group M2) or 6 days (Group M5) after treatment with 300 mg/kg methimazole. Bottom graph shows the number of glomeruli expressed as a percent of control that were filled with dense reaction product at eight approximately evenly spaced frontal levels through the bulb. Level 1 is the most anterior and level 7 is at the rostral extent of the accessory olfactory bulb. Each data point is the mean of measures made on two rats.

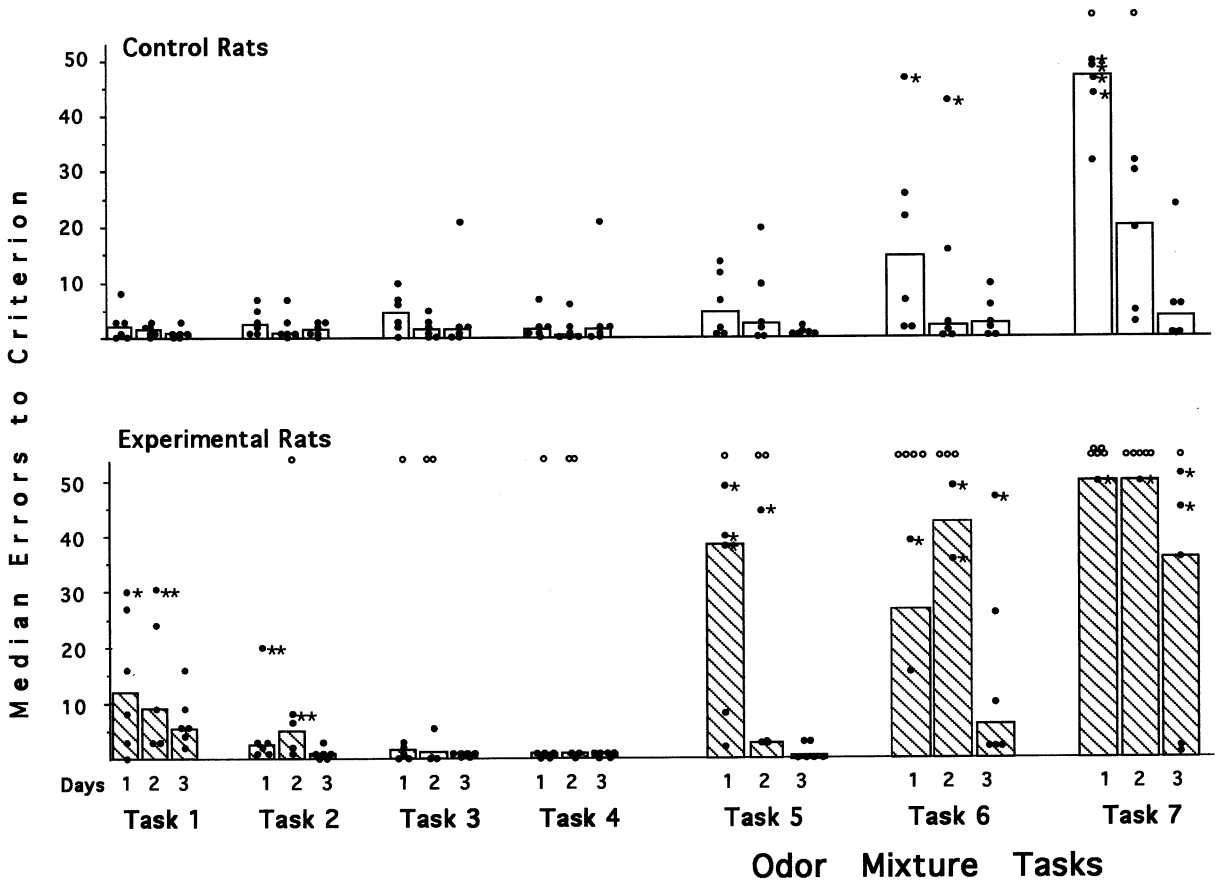

Fig. 2. Vertical bars show median error scores for control and experimental rats for each problem in the Standard Task Set on posttreatment Days 1, 2 and 3. Solid circles represent the scores of individual rats that completed a task. Solid circles with asterisks represent scores of rats that were tested on a problem but did not reach criterion performance within the 100-trial test. Open circles represent assigned scores of rats that failed to reach criterion on a prior test and were not tested on the next task.

3.2. Pretreatment behavioral performance

On the last pretreatment training session rats made few errors on all but the last odor mixture task (problem 7) and, clearly, problem 7 was the most difficult of the series. Experimental and control groups did not differ on any of these pretreatment tests.

3.3. Posttreatment behavioral performance

Day 1 posttreatment error scores of control rats did not differ from their pretreatment terminal performance on any of the tests. Performance of controls on Day 2 and Day 3 tests was equivalent to or better than that on Day 1. As shown in Fig. 2, control rats made few errors on Tasks 1–6 (median total errors, 4, 1, 2 for posttreatment days 1, 2, and 3, respectively) but performed more poorly on the last mixture problem (Task 7) on Day 1 and Day 2 (median errors, 46 and 20, respectively). However, performance on this mixture task improved on day 3 (median errors, 4).

On average, experimental rats performed more poorly than controls on most tasks and, more poorly on their first posttreatment day than on their last pretreatment test on Tasks 1, 5, 6, and 7 ($P < 0.05$ each case). On posttreatment Day 1 one rat (R56) stopped working on Task 2 and, on posttreatment Day 2, R56 stopped working on Task 1 and R57 stopped working on Task 2. Of the remaining four experimental rats, three failed to reach criterion on Task 5 or 6 on posttreatment day 1 and only one was tested on Task 7 (Fig. 2). As a result, fewer experimental rats than controls were tested on problems 2–6. The median scores illustrated by the bars in Fig. 2 are based on the rats which were tested on a problem and the individual scores of these rats are illustrated by closed circles. Performance of most experimental rats improved on Days 2 and 3 and, on Day 3, all but one of the experimental rats completed the Standard Task Set.

Two analyses of the data were performed to compare error scores of the two groups. The first analysis assigned error scores of 50 for rats that were not tested on a particular task. Based on all scores (actual and assigned), experimental rats performed more poorly than did controls on problems 1 ($P < 0.07$), 5 ($P < 0.05$) and 6 ($P < 0.025$) on posttreatment Day 1. The difference

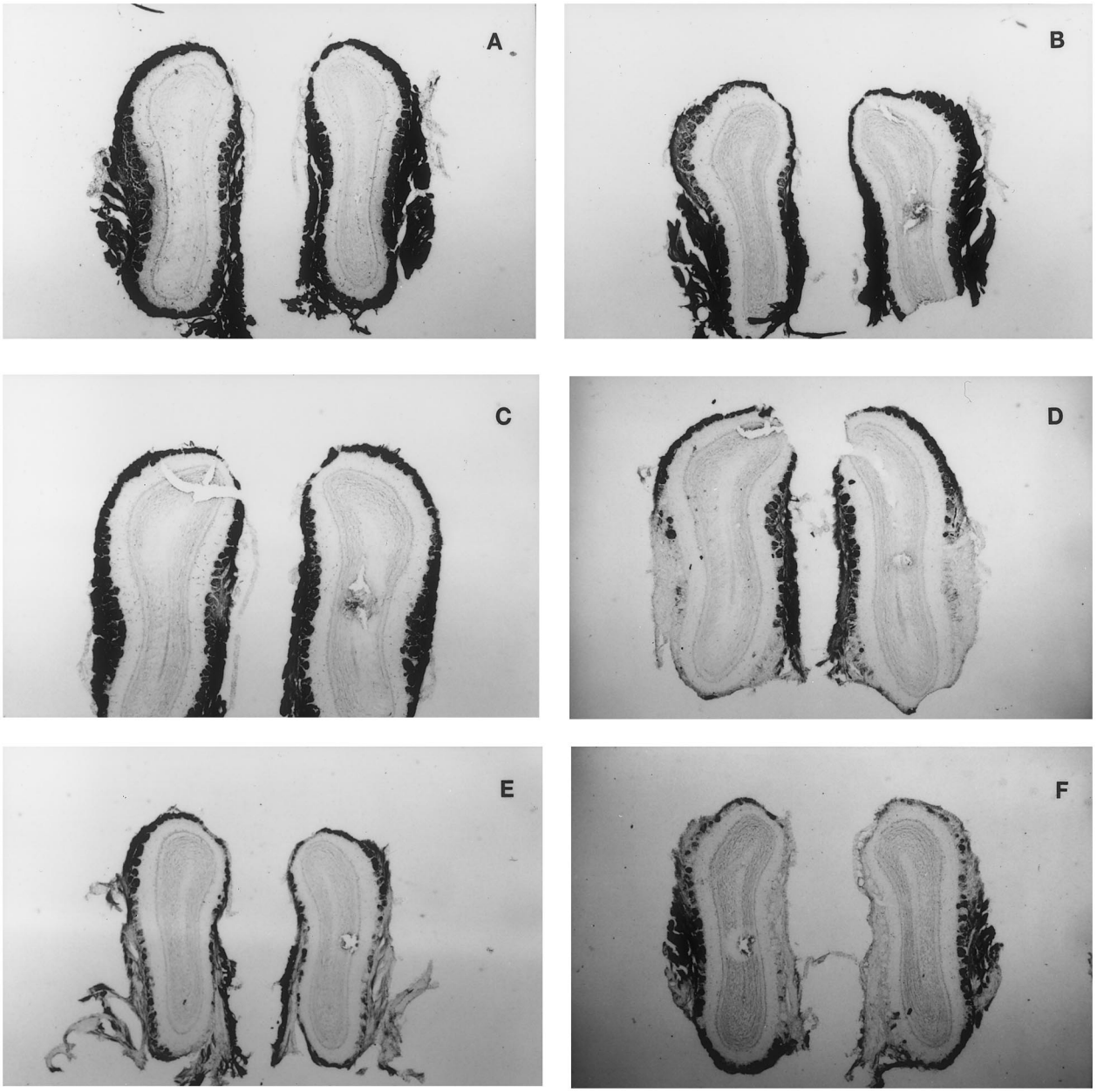

Fig. 3. Frontal sections through the olfactory bulbs of control and experimental rats. (A) Control rat showing dense reaction product in the olfactory nerve layer and in all glomeruli. (B and C) Two sections of a rat from Group M1 showing dense reaction product in all glomeruli. (D) Rat from Group M2 showing a decrement in reaction product in the lateral, ventral and ventromedial areas of the bulb. Most of these glomeruli had light reaction product that was clearly visible using polarized light. (E) Rat from Group M3 showing loss of reaction product in the lateral and ventromedial areas of the bulb. (F) Rat from Group M5 showing loss of reaction product in the dorsolateral and medial areas of the bulb.

between groups on the most difficult odor mixture discrimination (Task 7) on Day 1 was not significant because most control rats did not reach criterion on the task. Experimental rats also performed more poorly than controls on Day 2 on problems 1, 6 and 7 ($P < 0.01$, each case). On Day 3, experimental rats made more errors than controls on Tasks 1 and 7 but only the difference between groups on Task 1 achieved statistical significance ($P < 0.05$).

The second analysis was based only on error scores of rats that were tested on the task. These experimental rats performed more poorly than controls only on Task 1 on Day 2 ($P < 0.01$).

4. Discussion

The present results demonstrate that a 300 mg/kg intraperitoneal injection of methimazole produced a moderate and time-dependent decrease in axonal trans-

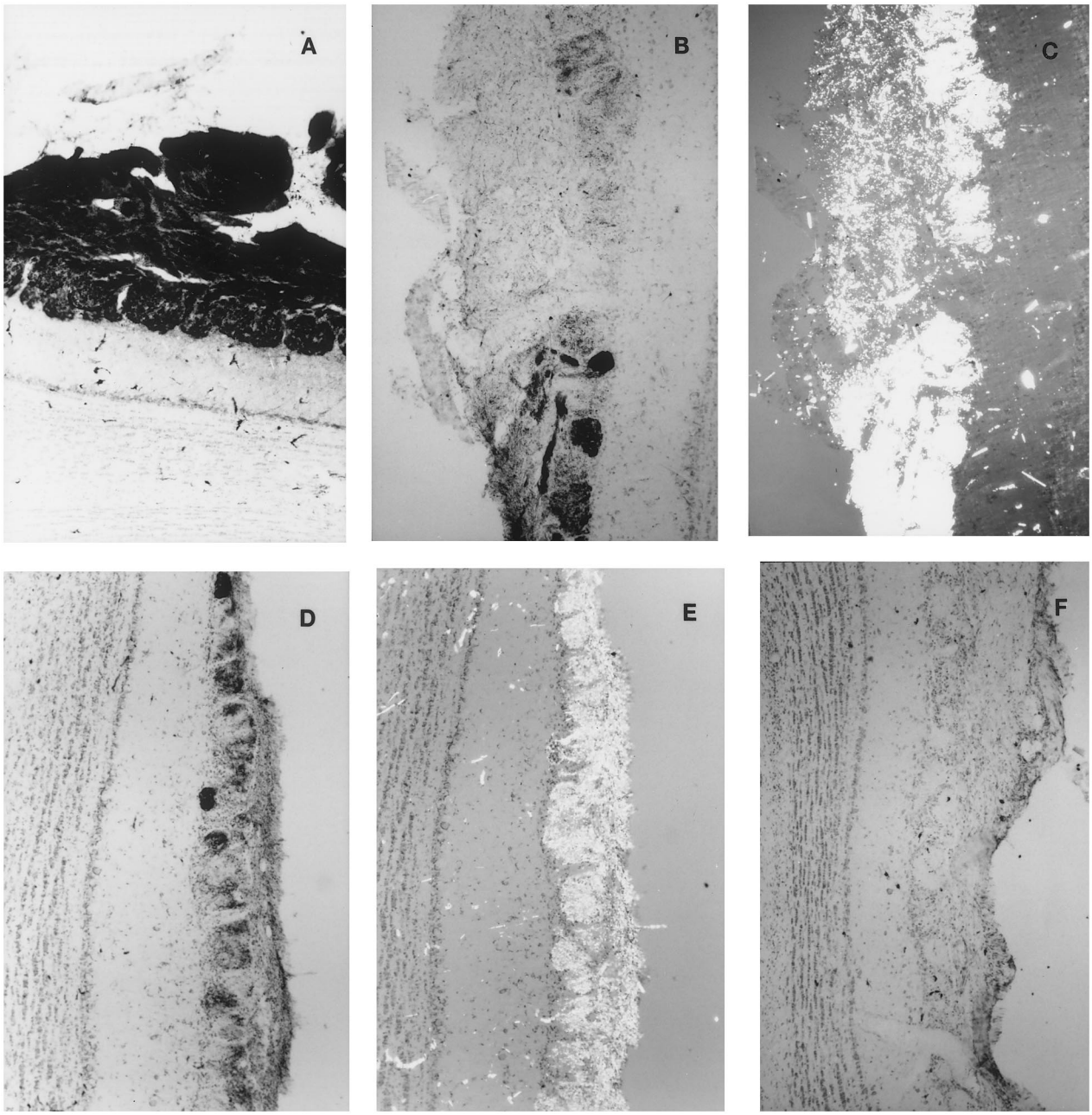

Fig. 4. Photomicrographs showing detail of anterograde transport in bulbar glomeruli of control and experimental rats. (A) Control rat. (B) Rat from group M2 showing a few glomeruli with dense reaction product in a glomerular zone with light reaction product. (C) The same section as in B but photographed using polarized light. Note that the light reaction product is clearly visible. (D) Rat from group M3 showing a few glomeruli with dense reaction product in a zone of glomeruli with light reaction product. (E) The same section as in D but photographed using polarized light. Note that all glomeruli contain reaction product. (F) Rat from group M5. None of the glomeruli in this zone had detectable reaction product. Orientation: (A) dorsal is to the left and medial toward the top; (B–F) dorsal is to the top and medial is to the right.

port from the olfactory epithelium to glomeruli of the olfactory bulb and, at least in the first 2 days after treatment, a modest decrement in odor detection and discrimination performance. In general, the anatomical and behavioral effects were much less marked than those obtained with 3-MI, an agent that produces profound but subtotal damage to the olfactory epithelium [14–16,21].

The current results stand in contrast to those of Genter et al. [5] who reported that the same dose of methimazole produced a nearly complete destruction of the olfactory mucosa 32 h after administration and, in behavioral tests, anosmia or severe hyposmia for the first 5–6 days after treatment.

The differences in anatomical outcomes between these studies are probably not due to the dose and

mode of administration of the toxicant: methimazole dose, solvent and mode of administration followed closely those described by Genter et al. Further, to insure that differences in outcomes were not due to differences in product, we used both methimazole purchased from Sigma (Sigma M-8506, lot 64H7704) and methimazole from the same batch used in the Genter et al. report (Sigma M-8506, lot 113H3668; kindly provided by Dr K. Crofton). Both batches yielded similar histological outcomes in preliminary studies but only the material supplied by Dr Crofton was used in the present experiment.

The differences in anatomical results are probably due to differences in histological methods. Genter et al. [5], using paraffin embedded sections of the olfactory epithelium, found a dose–response relationship: 25 mg/kg produced damage only to mucosa lining the dorsal medial meatus but 300 mg/kg was reported to result in nearly complete sloughing off of the neuronal and sustentacular layers. However, this finding was based on sections at level 3 of the nasal cavity (the ethmoid recess near the anterior end of the pharyngeal duct as described by Young [22]). No documentation of damage in other regions of the epithelium was reported.

In contrast, the HRP transport method used in the current study allowed us to sample the extent of bulbar input from the entire olfactory epithelium. This is because the HRP application brings the enzyme in contact with most or all areas of the epithelium (as evidenced by transport of HRP to all glomeruli in control cases). Thus, it seems possible that whatever degeneration was observed in level 3 nasal sections in the Genter et al. report may not have been representative of other mucosal areas.

Another possible reason for the marked difference in histological outcomes is that the methimazole caused a sloughing off of receptor cells but left axons intact and these axons transported HRP to olfactory bulb glomeruli. In fact, Genter et al. reported that the toxicant did not produce lesions in the subepithelial nerve bundles. The cut ends of axons do transport HRP [12] and, at least for the short survival cases, possible uptake and transport of WGA*HRP by axons severed from their connections to the receptor cells cannot be excluded. This may account for the control levels of axonal transport observed in the M1 group (those rats given HRP 24 h after treatment with methimazole). It is not known whether axons of olfactory sensory neurons could still transport HRP 2 or more days after death of their cell bodies. Olfactory axons are quite thin (0.1–0.3 μm in diameter [13]) and would be expected to degenerate rapidly after separation from their sensory neurons. However, Matsumoto and Scalia [10] found that thin axons in the frog optic nerve were viable and transported HRP for several weeks after transection of the nerve. But, several lines of evidence support the contention that severed olfactory axons in the mammal may not exhibit the same properties and that our measure of anterograde transport reflects functional connections between the epithelium and the olfactory bulb. First, if severed axons anterogradely transported HRP then we would have observed no little or decrement in the amount of reaction product in olfactory bulbs of the longer survival groups. However, there was a progressive decrease in the number of glomeruli with reaction product in these groups.

Second, Setzer and Slotnick [15] found that, in 3MI-treated rats, the decrement in anterograde transport of HRP to the olfactory bulb varied monotonically with dose of the toxin. At the highest dose used (400 mg/kg) only a few glomeruli contained reaction product 5–10 days after treatment. Also, the olfactory performance of 3MI-treated rats was closely related to the number of glomeruli that contained reaction product [16]. Finally, we have found that nasal lavage with zinc sulfate produces anosmia in mice (Bodyak and Slotnick, unpublished data). In these preliminary studies there was no transport of HRP from epithelium to the olfactory bulbs in anosmic mice. Mice were able to detect and even discriminate odors 19–22 days after $ZnSO_4$ treatment. In those animals anterograde transport of HRP could be demonstrated in small clusters of glomeruli. This evidence suggests that anterograde transport of HRP provides a sensitive index of functional connections between the olfactory epithelium and the olfactory bulb. However, it does not rule out the possibility that some of the reaction product observed in glomeruli in this study may represent a false positive, i.e. reaction product in axons separated from their cell bodies. This issue will require further study and, perhaps, an examination of both anterograde and retrograde transport in animals treated with olfactotoxins.

The differences in behavioral outcomes between this study and that of Genter et al. are probably due to differences in how behavior was assessed. Genter et al. used latency to find a buried food pellet to measure olfaction. This is a commonly used measure of olfactory ability but the method has marked shortcomings: it provides little control of the olfactory stimulus, presents the subject with a complex odor environment with uncontrolled air movement and, in addition, may make demands on the subjects' spatial memory. Also, the dependent measure, latency, does not measure accuracy. Finally, because there is no control of stimulus concentration, it is not possible to specify the stimulus parameters and, thus, the method provides only a single but physically undefined level of difficulty. Rats in the Genter et al. study required 12 or more days of training before stable short-latency responses were obtained. Because they allowed only one trial each day, it is unclear whether the food-finding task was a particularly difficult one or whether insufficient trials were given for optimal performance.

These various limitations were largely eliminated in the present behavioral study by the use of operant conditioning and olfactometric control of odor stimuli. The method allows reasonably precise definition of the stimulus, insures that discriminative behavior is based only on odor cues and provides a measure of odor detection and discrimination accuracy over a series of hundreds of trials within a daily session. Experimental rats trained using our methods performed well on most of the posttreatment tests, certainly far better than would have been predicted from the results of Genter et al.

The pattern of errors across problems suggest that, at least in some cases, poor performance of experimental rats may not reflect a sensory loss consequent to reduced afferent input. Thus, experimental rats made more errors than controls on the first task in the series (simple odor detection) but they performed about as well as controls on the intermediate tasks (2-odor discrimination and the easier steps of the odor mixture discriminations) within the same session. The poor performance in the initial detection task (0.1% isoamyl acetate) is probably not due to a sensory loss because, after achieving criterion performance, most rats were more accurate in the next test, one that used a lower concentration (0.01%) of the same odor. The deficit in the initial task probably represents a 'warm-up' effect resulting, perhaps, from a reduced salience of the stimulus or relearning to attend to the stimulus. Interestingly, this 'warm-up' effect may largely account for the behavioral deficit reported by Genter et al. Because they allowed only one trial each day, it is possible that the poor performance of their experimental rats reflected an analogous 'warm-up' and not, as assumed, anosmia.

The odor mixture tasks used in the current study were graded in difficulty and these provided a better test for sensory deficits. This gradation in difficulty was reflected in performance of rats prior to treatment and, in controls, after treatment (Fig. 2). Experimental rats made many more errors than controls on the more difficult tasks in the first two posttreatment days but performed at or near control levels on the last day. The poorer performance of experimental rats these demonstrably difficult sensory discriminations probably reflects reduced olfactory sensitivity.

If the deficits in odor sensitivity were related to the loss of sensory input then our anatomical results would predict that performance of experimental rats would progressively deteriorate over the first three posttreatment test days. But, the opposite result was obtained: behavioral performance on most tasks improved during the period of progressive loss in sensory input. The initial poor performance and subsequent improvement observed in the face of progressive loss in sensory input could be due to other effects of the toxicant. Genter et al. pointed out that debris from sloughing off of the epithelial tissue may have impaired nasal airflow and contributed to the increased latency to find buried food pellets. This suggestion is supported by observations made in the current study that, after methimazole injection, experimental rats had congested breathing and frequently 'sneezed'. Two experimental rats that were closely observed sneezed 17.5, 10, and 8 times per minute, on average, on the first, second, and third posttreatment days, respectively. Congested breathing and sneezing was not quantified in the other experimental rats but it occurred in each case and was much less apparent on the third than on the first posttreatment test day. Thus, debris in the nasal cavities of experimental rats may have resulted in both difficulty in attending to odor stimuli and reduced olfactory sensitivity. Performance may have improved as this debris was cleared. The possibility that 'olfactotoxins' may disturb olfaction because of such effects in the nasal vault seems not to be widely appreciated. Nevertheless, inflammation, sloughing off of tissue, edema and other non-neuronal effects of a toxin could disrupt olfaction by reducing access of remaining epithelial tissue to inhaled vapors (e.g. [2]).

In summary, the present results do not support the claim that transient anosmia and complete or nearly complete destruction of olfactory epithelium is produced by a single dose of 300 mg/kg methimazole. Methimazole did produce a progressive, although moderate, disruption of axonal transport of WGA*HRP from epithelium to the bulb over the first three posttreatment days but, in behavioral tests, experimental rats were able to detect and discriminate odors and recovered to or near control levels of performance during this period.

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