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A comparison of the discriminatory ability and sensitivity of the trigeminal and olfactory systems to chemical stimuli in the tiger salamander

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Summary. Trigeminal receptors can respond to a wide variety of chemical stimuli, but it is unknown whether these receptors mediate discrimination between chemical stimuli matched for equal perceptual intensity. The present electrophysiological and behavioral experiments address this issue using tiger salamanders, *Ambystoma tigrinum*, and four compounds (amyl acetate, cyclohexanone, butanol, and d-limonene). In addition, the relative sensitivities of the trigeminal and olfactory systems to these compounds are compared. In electrophysiological cross-adaptation experiments (amyl acetate vs cyclohexanone; butanol vs d-limonene), there was complete cross adaptation such that only concentrations above the background (cross-adapting) stimulus concentration elicited responses, suggesting that chemical stimuli may stimulate trigeminal receptors nonspecifically. In behavioral experiments (amyl acetate vs cyclohexanone; butanol vs d-limonene), only animals with intact olfactory nerves could discriminate between perceptually equivalent concentrations, that is concentrations that elicited the same level of responding. Both electrophysiologically and behaviorally, the trigeminal system exhibited higher thresholds than the olfactory system. We conclude that trigeminal chemoreceptors, at least in salamanders, are unable to discriminate between these two pairs of compounds when matched for equal perceptual intensity, and that trigeminal chemoreceptors are less sensitive than olfactory receptors.

Abbreviations: *AA* amyl acetate; *CH* cyclohexanone; *LI* d-limonene; *BU* butanol; *EOG* electro-olfactogram; *ISI* interstimulus interval; *ONX* olfactory nerve cut; *ppm* parts per million (1 µl of compound in vapor phase/1 l of air = 1 ppm)

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Introduction

Chemical stimuli entering the nasal cavity of most terrestrial vertebrates may elicit responses from the olfactory, vomeronasal, and/or trigeminal systems (Tucker 1971). Olfactory and vomeronasal receptor cells are located in discrete areas of sensory epithelium, while trigeminal cell bodies, located in the trigeminal (Gasserian) ganglion send their axons, via the ethmoid and nasopalatine nerves, throughout the respiratory epithelium (Bojsen-Møller 1975). Trigeminal receptors usually are considered primary mediators of the sensations of pain, touch, temperature, and proprioception. In addition, trigeminal receptors which respond to chemical stimuli constitute part of the common chemical sense, whose major function is often purported to be the protection of the body from noxious chemicals (Parker 1912; Keele 1962). Indeed, stimulation of trigeminal chemoreceptors by irritating vapors elicits a wide variety of protective physiologic reflexes (Silver 1987).

Besides its apparent protective function, the trigeminal system may have a role in the perception of chemical stimuli. Stimulation of the trigeminal nerve with a chemical stimulus appears to contribute to the overall perceived intensity of that stimulus (Cain 1974). More recently, others have demonstrated that birds can be trained to respond to apparently non-irritating compounds on the basis of trigeminally mediated information (Mason and Silver 1983; Walker et al. 1979, 1986).

While it has been established that many chemical stimuli can stimulate trigeminal receptors (Ito 1968; Doty et al. 1978; Silver and Moulton 1982) there are at least two major questions concerning the role of trigeminal chemoreception in the perception of odors. The first question is whether the

trigeminal system can discriminate between chemical stimuli matched for equal perceptual intensity. One attempt to address this question behaviorally in salamanders suggested that the trigeminal system could discriminate between chemical stimuli, although concentrations which elicited equal responding were not compared (Mason et al. 1981). Another attempt (Walker et al. 1979), which compared a range of stimulus concentrations in pigeons, concluded that discrimination could not take place.

The second question involves the relative sensitivities of the olfactory and trigeminal systems. Few quantitative data are available which compare trigeminal chemoreception and olfaction in the same animal. Tucker (1971) reported that electrophysiological trigeminal thresholds for amyl acetate were about 4 log units higher than olfactory thresholds (approximately 400 ppm compared with 0.04 ppm) in the tortoise and rabbit. Walker et al. (1979) demonstrated that behavioral thresholds for amyl acetate in pigeons with their olfactory nerves cut were at least 2.6 log units higher than thresholds in intact birds. Using a cardiac acceleration paradigm, Walker et al. (1986) also reported a 2 to 4 log unit difference in thresholds for amyl acetate, butanol, butyl acetate, and benzaldehyde thresholds in olfactory nerve-sectioned as compared to intact birds.

The present electrophysiological and behavioral experiments were designed to address both of these issues: to examine whether trigeminal chemoreceptors can distinguish between chemical stimuli and to compare trigeminal chemoreception and olfaction. Tiger salamanders (*Ambystoma tigrinum*) were chosen as experimental subjects both because they are widely used in olfactory research and because their olfactory and vomeronasal systems (and nervus terminalis) are easily accessible to surgical manipulation (i.e., it is possible to eliminate the olfactory and vomeronasal contribution to odor detection and discrimination for behavioral assays; Mason and Stevens 1981).

Material and methods

Electrophysiology. Adult terrestrial-phase tiger salamanders were purchased from Amphibians of North America, Nashville TN. The salamanders were kept in plastic boxes lined with paper towels moistened with dechlorinated tap water and maintained in a refrigerator at an ambient temperature of $6 \pm 2°$ C.

Four compounds were chosen as stimuli [amyl acetate (AA), cyclohexanone (CH), n-butanol (BU), d-limonene (LI)]. These compounds were selected to represent stimuli commonly used in olfactory research. To humans the four compounds smell like bananas (AA), peppermint or acetone (CH), alcohol (BU), and lemon (LI) and at high concentrations are reported as irritating (Doty et al. 1978). The stimuli were presented via an air dilution olfactometer similar to that described by Walker et al. (1979) and employed in our previous studies (Silver and Moulton 1982; Silver et al. 1985). An airstream flowing at a known rate and saturated with the stimulus was mixed with a dilution stream of filtered air. The final delivery rate to the salamander was 100 ml/min. Dilution was controlled by flowmeters which regulated the ratio of flow between odor-saturated and dilution streams. Battery powered solenoid valves were used to switch from the filtered background air stream to the test stimulus.

Olfactory preparation. Salamanders were immersed in an ice water bath for 10 min, doubly pithed and secured by a clip attached to the lower jaw. The skin and cartilage on one side of the head between the naris and eye was removed. The dorsal surface of the olfactory epithelium was then removed exposing the ventral surface. The electro-olfactogram (EOG), a slow (DC) potential change elicited by the chemical stimulation of the olfactory epithelium was recorded by placing a Ringer-agar filled glass capillary, bridged to a calomel electrode directly on the olfactory mucosa (Arzt et al. 1986). A similar (reference) electrode was placed elsewhere on the skin. Electrodes were DC coupled to an amplifier and the signal displayed on an oscilloscope and pen recorder.

Stimuli were delivered to the ventral surface of the nasal cavity containing the olfactory epithelium. Concentration-response curves were obtained for each of the four compounds. Stimuli were presented in increasing concentrations (series began with stimulus concentrations below threshold, defined as the first concentration which elicited a response larger than the background activity) for 10 s periods with 3 min interstimulus intervals (ISI). The EOG response magnitude was measured as the deflection of the pen recorder from baseline to the peak of the response and is reported in mV. An arbitrarily chosen standard stimulus, approximately 1100 ppm CH, was presented at the beginning and end of each concentration series to check the reliability of the preparation, i.e., as long as the responses to cyclohexanone did not differ by more than 10%, the data from that particular series were included in the analyses. Control (clean air alone) stimuli also were used to check the reliability of the preparation.

Trigeminal preparation. Salamanders were prepared as described above. The left eye was excised, and the ophthalmic branch of the trigeminal nerve exposed and freed of the surrounding connective sheath. A small bundle of the branch was teased free and activity was recorded by placing the bundle on a pair of platinum-iridium electrodes. The electrode at the cut end served as the indifferent lead, and the animal was grounded through the clip attached to its lower jaw. Mineral oil was pipetted into the orbit covering the nerve, to prevent the nerve from drying out, and to ensure electrical insulation. The multiunit activity was amplified, and monitored with an oscilloscope and audio monitor. The amplified activity was also passed through an averaging circuit (see e.g. Kiyohara and Tucker 1978) with a rise time of 1 s and displayed on a pen recorder.

Stimulation of the ventral surface of the nasal cavity elicited weak responses from the trigeminal nerve. Stimulating the skin covering the dorsal surface of nasal cavity elicited much more vigorous responses. For trigeminal recordings then, the nasal cavity was left intact and stimuli were delivered directly to the skin between the naris and the eye ipsilateral to the nerve.

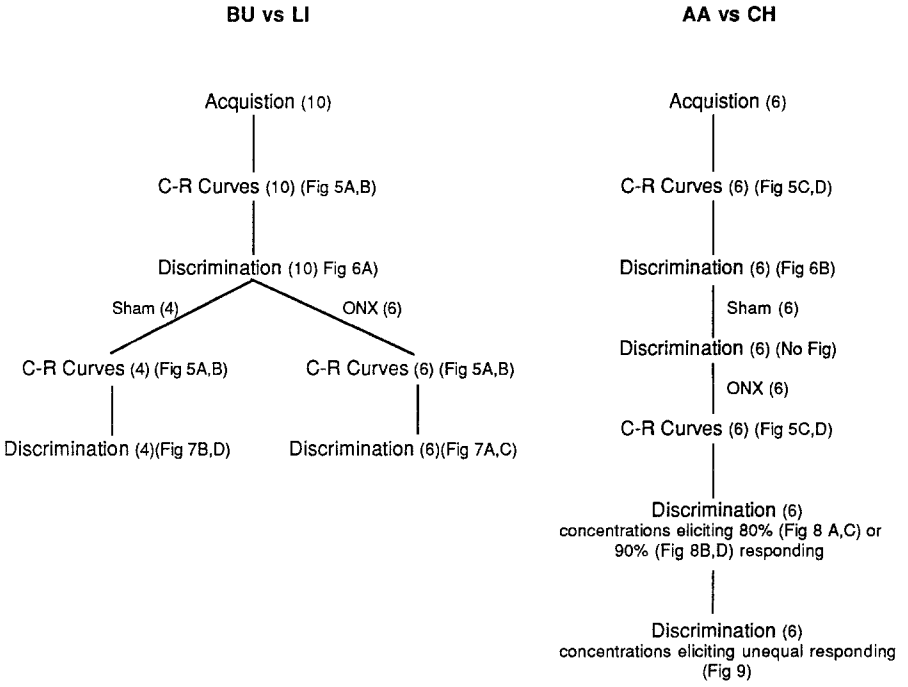

Fig. 1. Schematic representation of the behavioral procedures used on the two cohorts of salamanders (BU vs LI = butanol vs d-limonene; AA vs CH = amyl acetate vs cyclohexanone). Numbers in parenthesis are the number of animals used in each procedure. Five animals achieved criterion for BU as did five for LI (10 salamanders total) while criterion was achieved by only three animals each for AA and CH (6 animals total). Figures referred to are where the results of the tests can be found. See text for details

Concentration-response curves were obtained for each of the four compounds as described above. The integrated trigeminal response magnitude was measured in arbitrary units from baseline to the peak of the phasic response. Responses are reported as a percent of the response to a standard, approximately 1100 ppm CH. Control and standard stimuli were interspersed regularly as described above to check the reliability of the preparation.

An electrophysiological cross-adaptation paradigm was used to examine whether trigeminal chemoreceptors might possess different 'receptor mechanisms' (at least for the compounds tested). Cross-adaptation occurs when the response to a test stimulus decreases in magnitude following adaptation to another stimulus (relative to the response to the test stimulus following adaptation to air). Two compounds that cross-adapt are thought to share common receptor mechanisms (see e.g. Caprio and Byrd 1984). Concentration-response curves generated as described above were used to determine the concentration of each compound necessary to produce an equivalent response (150% of the CH standard). This concentration was used as the background (cross-adapting) stimulus in the cross-adaptation experiment (see Baylin and Moulton 1979). By using background concentrations which elicit equivalent responses, differences due to intensity can be eliminated.

Animals were tested in two groups. One group was presented with BU as the cross-adapting stimulus and LI as the test stimulus (or vice versa). For the second group, the stimuli were AA and CH. The procedure was similar to that used to obtain the concentration-response curves described above. The only difference was that the background (cross- or self-adapting) stimulus replaced the clean air presented to the animals during the ISI's. The selection of the stimulus pairs, BU and LI and AA and CH, for both the electrophysiological and behavioral experiments was arbitrary.

Behavior. Salamanders were purchased and maintained as described above. Different groups of salamanders were trained to respond to AA and CH (cohort 1), or BU and LI (cohort 2). After training, both cohorts were given a series of similar, but not identical, behavioral tests. Figure 1 is a schematic representation of the procedures used on both cohorts.

Acquisition. The apparatus and procedures used to train both cohorts have been reported previously (Mason et al. 1980). Stimuli were delivered to the whole animal via an olfactometer similar to the one used in the electrophysiological experiments. Relatively high concentrations of the four compounds, 1194 ppm AA, 980 ppm CH, 1274 ppm BU, and 427 ppm LI, were chosen for training to ensure detection by the salamanders. Salamanders were placed in a conditioning chamber so that their heads rested in a sniffing port, and stimuli were delivered to the port via separate Teflon tubing lines. During training, presentations of the chemical stimulus (S+) were followed by presentations of bright light which salamanders normally avoid. The light remained on for 20 s, or until the animal backed away from the sniffing port. If backing away occurred during presentation of the S+ stimulus, an avoidance response was scored. Interspersed among reinforced S+ presentations were an equal number of presentations of clean (blank) air which were not followed by the bright light (So). An attempt was made to train ten animals for each of the four compounds (40 animals total). Each animal was given 20 acquisition trials per day for ten days. Only those salamanders which reached criterion, i.e. avoided 80% of the odorant trials, by the tenth day were used in the tests described below.

Pre-surgical testing. Immediately after training, animals were given concentration-response tests with their respective training stimulus. A temporal forced-choice ascending method of limits was used to present the stimuli (Engen 1972). That is, increasing concentrations of the chemical stimulus were presented to animals in the conditioning chamber following randomly varied intervals during which filtered air alone was presented. BU and LI animals were tested for 4 days, while AA and CH animals were tested for 6 days. Stimulus presentations during concentration-response tests were reinforced, as they had been during acquisition.

Following concentration-response tests, BU and LI ani-

mals were given discrimination trials between BU and LI for 3 days (Mason et al. 1981). Likewise, AA and CH animals were given discrimination trials between AA and CH for 5 days. For both cohorts, stimulus concentrations were chosen that elicited 80% avoidance during concentration-response tests.

Post-surgical testing. Following discrimination testing, treatment of the two cohorts diverged (Fig. 1). AA and CH animals were given sham surgeries, followed by an additional 5 days of discrimination tests between stimulus concentrations that had elicited 80% avoidance prior to surgery. Afterwards, these animals were given bilateral olfactory nerve cuts (described below), and following recovery from surgery, 6 additional days of concentration-response trials. After concentration-response tests, AA and CH animals were given discrimination tests between stimulus concentrations that elicited either 80% (3 days) or 90% (3 days) avoidance. Discrimination trials between 'matched' stimulus concentrations were followed by discrimination trials in which the concentrations of the S+ and So stimuli were varied asymmetrically (4 days), i.e. AA concentrations which elicited 80% avoidance were compared with CH concentrations which elicited 90% avoidance and vice versa.

BU and LI animals were randomly assigned to sham ($n=2$ per compound) or bilateral olfactory nerve cut ($n=3$ per compound) subgroups following discrimination trials. Following recovery from surgery, these animals were given 4 days of concentration-response tests, followed by 4 days of discrimination trials between stimulus concentrations that had elicited 80% (2 days) or 90% (2 days) avoidance.

Surgery. For bilateral olfactory nerve cuts (ONX), salamanders were anesthetized by immersion in a 0.5% aqueous solution of MS-222 (tricaine methane sulfonate). A flap was made in the skin between the eyes, and a small portion of the bone overlying the olfactory bulb was drilled away. Olfactory (as well as vomeronasal and nervus terminalis) nerves were cut where they emerged from the cribriform plate. A small piece of Gelfoam was then placed in the cavity and the skin was closed with cyanoacrylate glue. Sham animals were treated similarly, except that the nerves were not cut. Verification of ONX and sham surgeries were made in post-mortem gross examinations immediately after testing.

Results

Electrophysiology

Olfactory and trigeminal responses to increasing concentrations were obtained for all four compounds. Olfactory and trigeminal concentration series of CH are shown in Fig. 2. The shape of the concentration-response curves for the four compounds differed for trigeminal and olfactory responses (Fig. 3). Trigeminal concentration-response curves continued to rise even at the highest concentrations tested (vapor saturation). EOG concentration-response curves, however, plateaued before vapor saturation was reached. For all four compounds, thresholds were higher for trigeminal chemoreceptors than for olfactory receptors (Table 1).

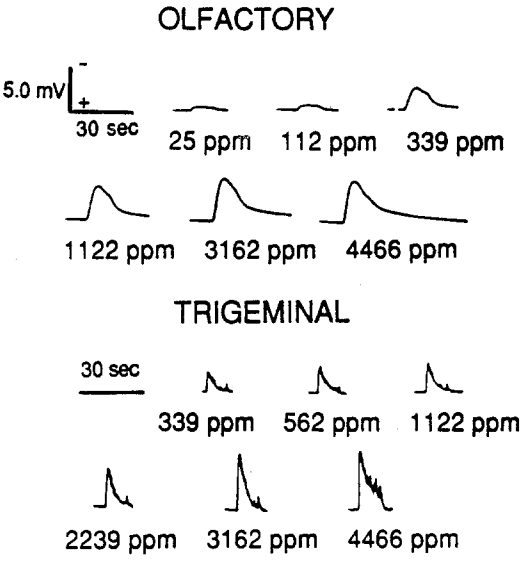

Fig. 2. Olfactory (EOG) and trigeminal (integrated multiunit) responses to increasing concentrations (in ppm) of cyclohexanone. Records were obtained from different salamanders. For this olfactory preparation concentrations below 25 ppm were not tested. For this trigeminal preparation concentrations below 339 were not tested

A cross-adapting stream of ≈1600 ppm BU or ≈800 ppm LI severely reduced responsiveness to both BU and LI, respectively (Fig. 4A, B). These cross-adapting concentrations, as well as those for AA and CH, were chosen because they elicit equivalent response magnitudes (150% of the CH standard; see Methods). At concentrations below background, responding was eliminated. Similar results were obtained for AA and CH background stimuli of ≈1500 ppm CH or ≈2600 ppm AA (Fig. 4C, D). Only concentrations above background increased neural activity above baseline.

Behavior

Acquisition. Five of 10 BU animals achieved criterion (avoided 80% of the odorant trials by the tenth day of training), as did 5 of 10 animals given LI. Of the 10 animals trained with AA and CH only 6 ($n=3$/group) achieved criterion levels of avoidance. Only the 16 animals that met the performance criteria were tested further.

Pre-surgical concentration-response tests. For all groups, mean avoidance of S+ presentations increased in a gradual fashion as stimulus concentrations increased (Fig. 5). The slopes for these curves, obtained from log-log plots, are given in Table 1. For all compounds except BU, threshold values, defined as concentrations of the stimulus

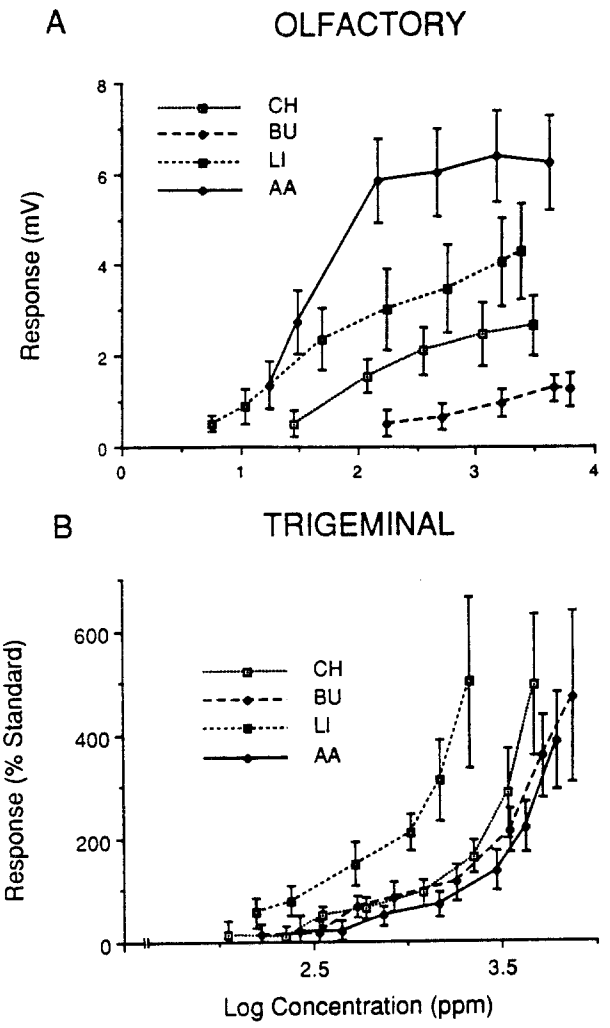

Fig. 3. **A** Mean integrated multiunit responses from the trigeminal nerve of 6 tiger salamanders in response to increasing concentration of amyl acetate (*AA*), cyclohexanone (*CH*), butanol (*BU*), d-limonene (*LI*). **B** Mean electro-olfactogram responses obtained from the olfactory epithelium of 6 different salamanders to the same stimuli. Responses in **A** are reported in arbitrary units as a % of the response to the standard, approximately 1100 ppm CH. Responses in **B** are reported in absolute units as mV. Capped vertical lines denote standard errors

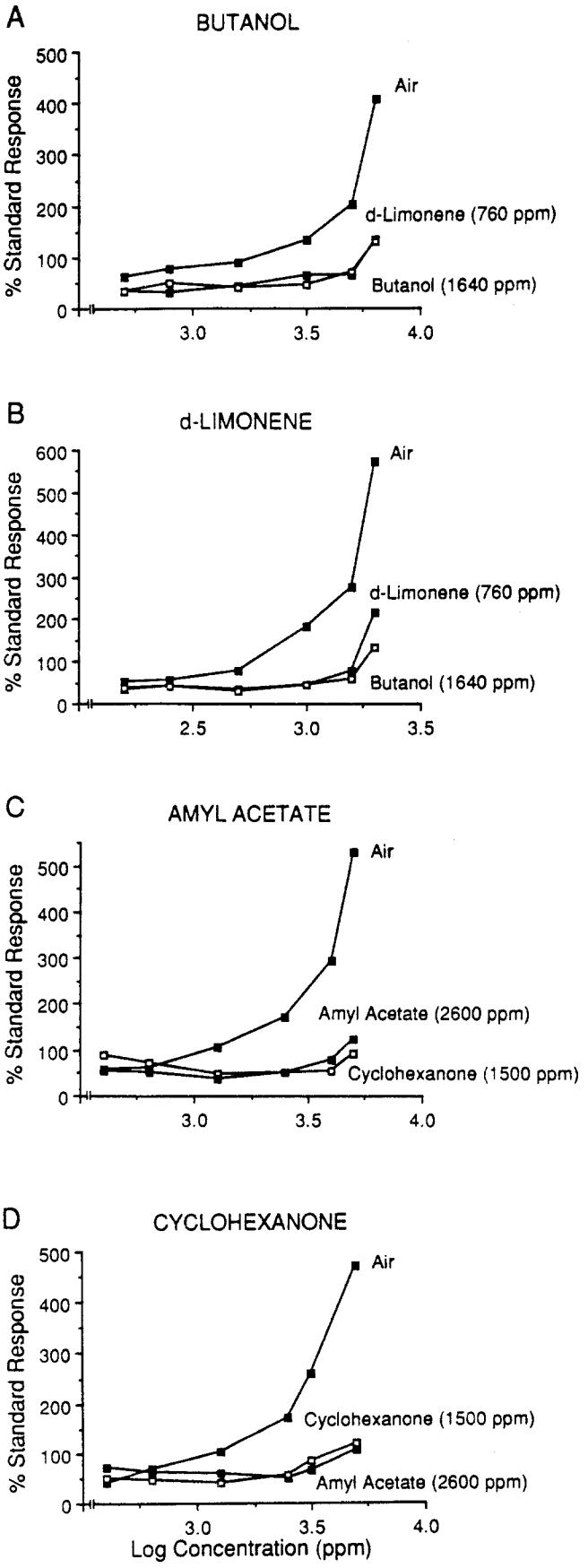

Fig. 4. Mean integrated multiunit responses from the trigeminal nerves of six salamanders to increasing concentrations of (**A**) butanol, (**B**) d-limonene, (**C**) amyl acetate, and (**D**) cyclohexanone. Response magnitude is presented as a % of the response to the standard stimulus, approximately 1100 ppm cyclohexanone. In **A** and **B** stimuli were presented against a background of either air, d-limonene, or butanol. The background concentrations of d-limonene (760 ppm) and butanol (1640 ppm) elicited responses of equal magnitudes as determined from Fig. 3. In **C** and **D** stimuli were presented against a background of either air, amyl acetate, or cyclohexanone. The background concentrations of amyl acetate (2600 ppm) and cyclohexanone (1500 ppm) elicited responses of equal magnitudes as determined from Fig. 3

Table 1. Slopes and thresholds estimated from electrophysiological and behavioral concentration-response curves for four chemical stimuli

Electrophysiology

Olfaction			Trigeminal		
Odorant	Slopes	Estimated threshold (ppm) ±SD	Odorant	Slopes	Estimated threshold (ppm) ±SD
AA (4)*	0.25	12±1.0	AA (5)	0.93	450± 74
CH (4)	0.33	16±1.0	CH (5)	0.97	348± 80
BU (4)	0.27	114±1.0	BU (5)	0.92	671±281
LI (4)	0.32	6±0.0	LI (5)	0.78	261± 69

Behavior

Pre-surgery (olfactory)			Post-surgery (trigeminal)		
Odorant	Slopes	Estimated threshold (ppm) ±95% confidence interval	Odorant	Slopes	Estimated threshold (ppm) ±95% confidence interval
AA (3)	0.20	56±1.5	AA (3)	0.61	631±1.6
CH (3)	0.15	32±1.5	CH (3)	0.62	562±1.4
BU (5)	0.14	45±1.7	BU (3)	0.45	501±1.5
LI (5)	0.46	22±1.6	LI (3)	2.08	355±2.0

Numbers in parenthesis refer to the number of animals tested
AA amyl acetate; *CH* cyclohexanone; *BU* butanol; *LI* d-limonene

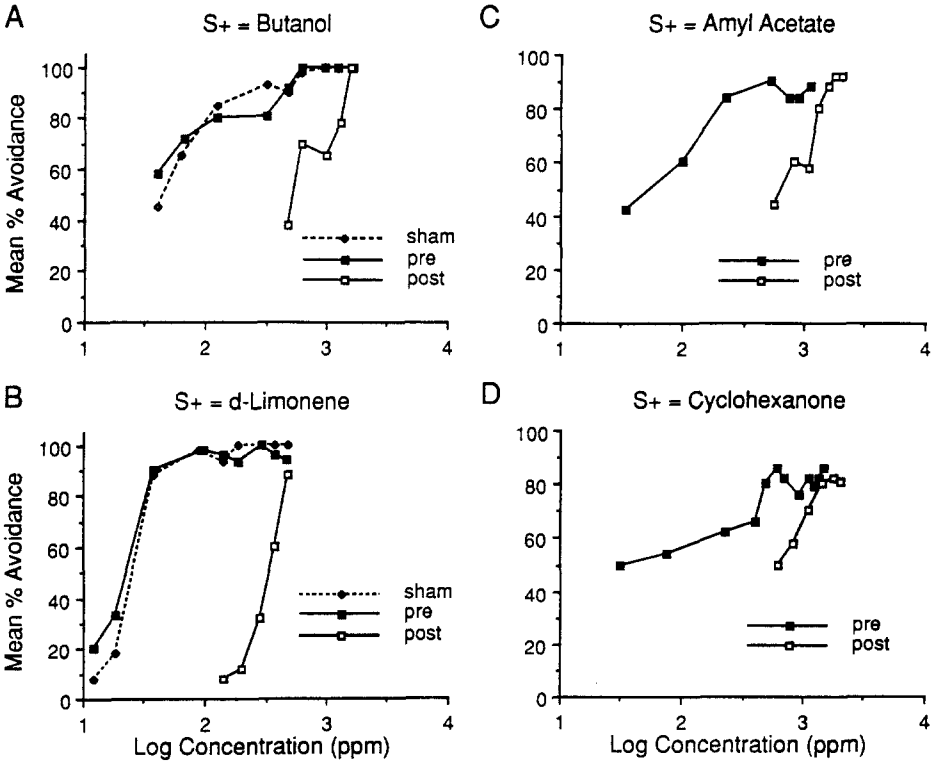

Fig. 5. Mean avoidance responding to presentations of (**A**) butanol, (**B**) d-limonene, (**C**) amyl acetate, and (**D**) cyclohexanone, before (*pre*) and after (*post*) bilateral nerve cuts. Animals also were tested with d-limonene and butanol after sham surgeries. Butanol and d-limonene were each tested on five animals while amyl acetate and cyclohexanone were each tested on 3 animals

that elicited responses at least 50% of the time, were calculated (Table 1), along with 95% confidence intervals around these thresholds. Thresholds ranged from 22 ppm (LI) to 56 ppm (AA).

Pre-surgical discrimination tests. For both cohorts, 3 factor ANOVAs (odor, day, S+/So) were used to assess the results of pre-surgical discrimination tests. This analysis demonstrated that intact salamanders discriminated BU from LI and AA from CH at behaviorally equivalent concentrations eliciting 80% avoidance (Fig. 6A). For BU and LI animals, there were significant differences in responding between S+ and So ($F(1,8)=248.4$, $P<0.00001$): both groups showed higher avoidance of S+ (7.96 ± 0.21) than of So (2.33 ± 0.22). For AA and CH animals, the respective S+ also elicited greater avoidance (8.13 ± 0.23) than So (2.27 ± 0.28) ($F(1,4)=619.5$, $P<0.0004$) (Fig. 6B).

Post-sham surgery discrimination tests (AA/CH). A 3 factor ANOVA also was used to assess the results of post-surgical discrimination tests. Sham surgery had no apparent effect on the discrimination between AA and CH: both groups exhibited reliably higher avoidance of S+ (8.0 ± 0.39) than So (1.8 ± 0.45) ($F(1,4)=1048.4$, $P<0.0003$).

Post-ONX surgery concentration-response tests. Following bilateral olfactory nerve cuts, higher stimulus concentration ranges were necessary to elicit avoidance responding for all four compounds (Fig. 5). Also, threshold and slope values were higher (Table 1), and concentration-response curves accelerated more rapidly.

Post-ONX surgery discrimination tests. Salamanders were unable to discriminate between BU and LI at concentrations which elicited 80% avoidance after sectioning of their olfactory nerves (Fig. 7). A 4 factor ANOVA (compound, surgical condition, day, S+/So) was used to assess post-surgical responding by animals in the BU/LI cohort. There were significant differences between surgical conditions ($F(1,6)=15.2$, $P<0.008$): ONX animals exhibited higher overall levels of responding than did sham animals. Also, there were significant interactions between surgical condition and S+/So ($F(1,6)=500.4$, $P<0.00001$) and day and S+/So ($F(3,18)=3.4$, $P<0.04$). Post-hoc assessment of these effects revealed that while sham animals continued to discriminate S+ (7.6 ± 0.75) from So (1.7 ± 0.95) (Fig. 7B, D), ONX animals did not (S+: 4.5 ± 2.14; So: 4.5 ± 1.3) (Fig. 7A, C).

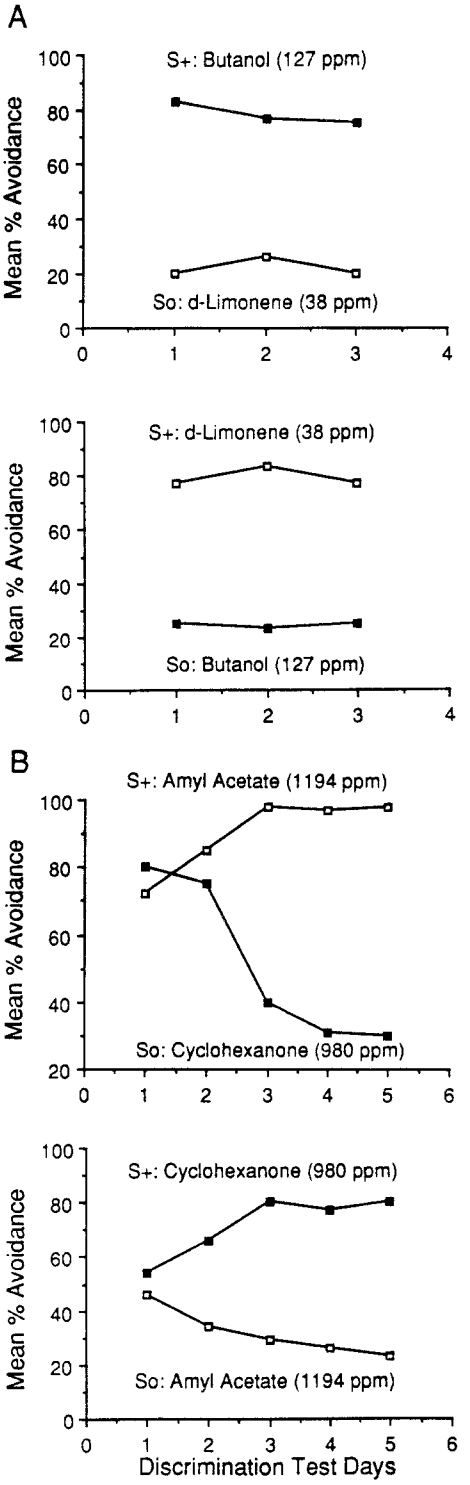

Fig. 6. Discrimination between (**A**) butanol and d-limonene and (**B**) amyl acetate and cyclohexanone in intact salamanders. Concentrations discriminated were 'equal', in that they elicited 80% avoidance as determined from Fig. 5. In the top panel of **A** salamanders were trained to avoid butanol but not d-limonene. In the bottom panel of **A** salamanders were trained to avoid d-limonene but not butanol. In the top panel of **B** salamanders were trained to avoid amyl acetate but not cyclohexanone. In the bottom panel of **B** salamanders were trained to avoid cyclohexanone but not amyl acetate

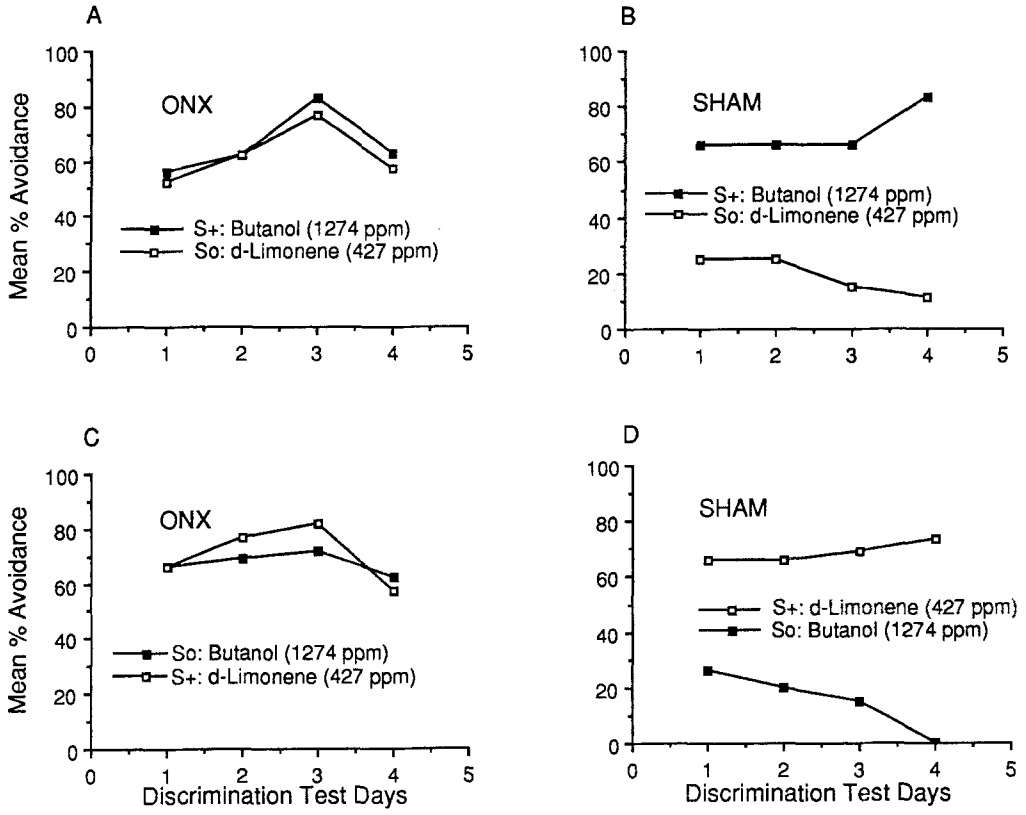

Fig. 7. Discrimination between butanol and d-limonene in salamanders given olfactory nerve-cuts (ONX) or sham surgeries. Concentrations discriminated were 'equal', in that they elicited the same % avoidance as determined from Fig. 5. In panel **A**, although ONX animals were trained to avoid butanol but not d-limonene they could not discriminate between concentrations which elicited 80% avoidance. Animals given sham surgeries, panel **B**, continued to discriminate between the two compounds. In panels **C** and **D** similar results were seen for salamanders trained to avoid d-limonene but not butanol

Salamanders also were unable to discriminate between AA and CH at concentrations eliciting either 80% (Fig. 8A, C) or 90% (Fig. 8B, D) avoidance after their olfactory nerves were cut. A 3 factor ANOVA (compound, days, S+/So) was used to assess post-ONX 'matched concentration' discrimination by animals in the AA/CH cohort. The only significant difference was among days ($F(6,24)=8.2$, $P<0.0002$).

Salamanders were able, however, to discriminate between unequal concentrations of AA and CH (Fig. 9). A 4 factor ANOVA (compound, days, S+ concentration, S+/So) was used in this analysis. There was a significant interaction between S+ concentration (high or low, relative to So) and avoidance of S+ and So ($F(1,4)=480.5$, $P<0.0004$). Post-hoc assessment of this effect revealed that when the concentration of S+ was higher than that of So, responding to S+ was significantly higher (4.54 ± 0.08) than responding to So (0.83 ± 0.21). Conversely, when the concentration of So was higher than that of S+, responding to So was higher (4.1 ± 0.21) than responding to S+ (1.4 ± 0.16). Therefore, it appears that the salamanders avoided the more intense stimulus, regardless of whether it was S+ or So.

Discussion

The salamander experiments discussed in this paper demonstrate that for the compounds tested, trigeminal chemoreceptors cannot discriminate between chemical stimuli on the basis of quality and that trigeminal chemoreceptors are less sensitive than olfactory receptors.

The electrophysiological cross-adaptation results demonstrate perfect symmetrical cross-adaptation between stimulus pairs. This suggests that the stimulus pairs tested share the same receptor mechanisms (see Caprio and Byrd 1984). These chemical stimuli may thus stimulate trigeminal receptors nonspecifically. An implication of these results is that the animal may not be able to discriminate behaviorally between two of these stimuli that are matched for equal intensity.

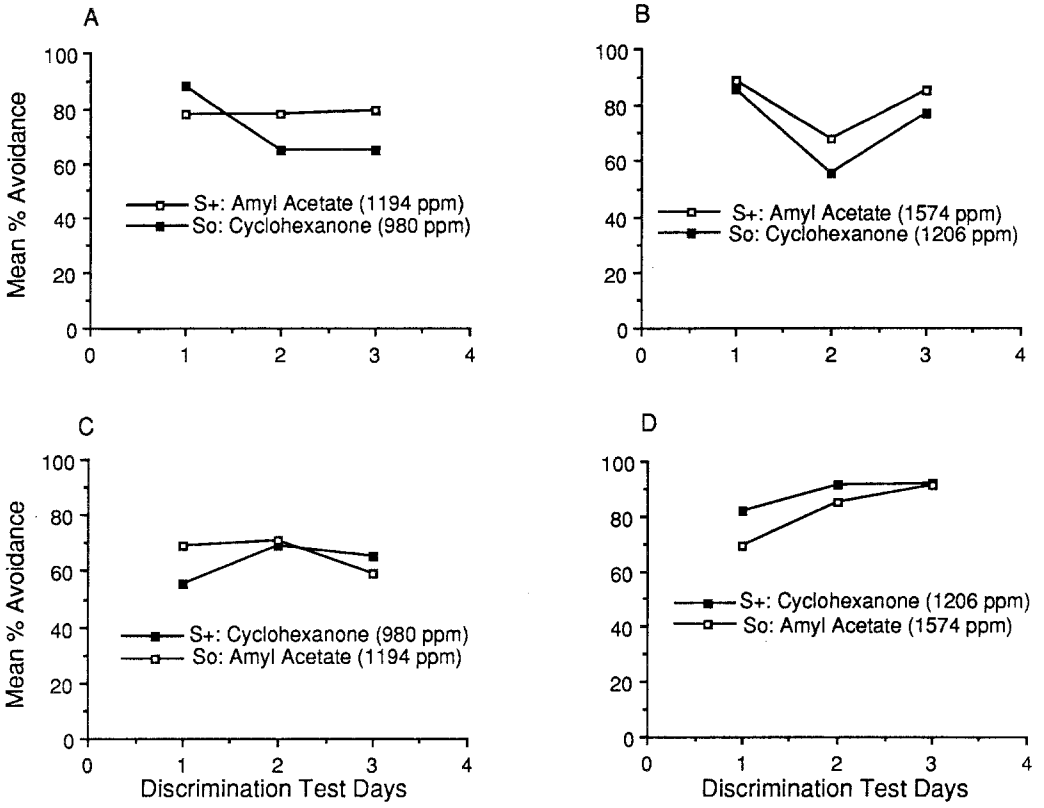

Fig. 8. Discrimination between amyl acetate and cyclohexanone in olfactory nerve-cut (ONX) salamanders. Concentrations discriminated were 'equal', in that they elicited the same % avoidance as determined from Fig. 5. ONX animals trained to avoid amyl acetate could not discriminate between concentrations of amyl acetate and cyclohexanone which elicited 80% (panel **A**) or 90% (panel **B**) avoidance. Similar results are seen in panels **C** and **D** for salamanders trained to avoid cyclohexanone

The results from the behavioral discrimination experiments provide evidence that salamanders with intact olfactory nerves can discriminate between behaviorally equivalent stimulus concentrations, i.e. concentrations which have the same perceptual intensity. In contrast, animals with bilateral olfactory nerve cuts cannot. However, when behaviorally unequal stimulus concentrations are presented, animals with bilateral olfactory nerve cuts still exhibit discrimination, but always avoid the relatively more 'intense' stimulus regardless of which stimulus it was trained to avoid. This also demonstrates that olfactory nerve cuts per se, do not render salamanders incapable of making any kind of discrimination. We suspect that this ability to avoid the relatively more 'intense' stimulus stems from the 4 days of testing when S+ was presented at the 'high' concentrations and presentations were reinforced. Animals had learned to respond to stimulus cues in terms of quantity (i.e. intensity) and no longer discriminated between chemical stimuli on the basis of quality. This finding is consistent with the results of Walker et al. (1979) in which the responding of olfactory nerve-sectioned pigeons in discrimination test sessions appeared to be only a function of stimulus intensity. Therefore, at least for two species, salamanders and pigeons, the trigeminal system appears to be able to discriminate chemical stimuli on the basis of intensity rather than quality.

Electrophysiological thresholds were obtained from both trigeminal and olfactory receptors. Salamander trigeminal thresholds for the compounds tested (AA: 450 ppm; CH: 348 ppm; BU: 671 ppm) were similar to those reported previously for the rat (AA: 465 ppm; CH: 175 ppm; BU: 400 ppm; Silver and Moulton 1982), rabbit (AA: between 170 and 520 ppm; Tucker 1963), and tortoise (AA: between 170 and 520 ppm; Tucker 1963). Trigeminal thresholds reported in the present paper, ranged from 6 (butanol) to 44 (limonene) times higher than olfactory thresholds. Tucker (1963) reported that trigeminal amyl acetate thresholds in the rabbit and tortoise were as much as 4 logs units higher than olfactory thresholds obtained from nerve twig recordings. The relatively

small differences between electrophysiological trigeminal and olfactory thresholds may be peculiar to the salamander or may be influenced, in part, by the use of the EOG to measure thresholds. Olfactory thresholds determined with the EOG have been reported to be about 2 log units higher than thresholds obtained from integrated neural recordings (Silver 1982).

In human psychophysical studies, concentration-response curves are often described as power functions with the exponent of the function characterizing the growth of the response magnitude (perceived intensity) with increasing concentrations (Murphy 1987). These functions are straight lines in log-log plots with the slope of the line equal to the exponent of the power function. Psychophysical functions for irritation (trigeminal stimulation) grow at a higher rate than those for odor (olfaction) (Cain 1976). For olfaction, response magnitude grows slowly as a function of concentration with exponents in the range of 0.2 to 0.7 (Murphy 1987). Exponents for irritants and painful stimuli typically are greater than 1.0 (Cain and Murphy 1980; Lawless and Gillette 1985). The steeper the slope the smaller the increase in concentration necessary to produce large increases in response magnitude, an important function of a pain detecting system. This same relationship for olfactory and trigeminal concentration-response functions also is seen in the present electrophysiological results. When the data in Fig. 3 are plotted on log-log coordinates, the resulting trigeminal curves have slopes ranging from 1.05 to 1.13 while the olfactory curves have slopes from 0.26 to 0.37 (Table 1). Although the measures obtained from the salamander electrophysiological experiments and the human psychophysical experiments are obviously quite different, the analyses of their concentration-response functions are consistent with the trigeminal nerve's possible role as a protection system.

Behavioral results appear similar to the electrophysiological results in terms of thresholds and concentration-response curves. Thresholds for the four compounds are 11 to 18 times lower in intact salamanders than in salamanders with their olfac-

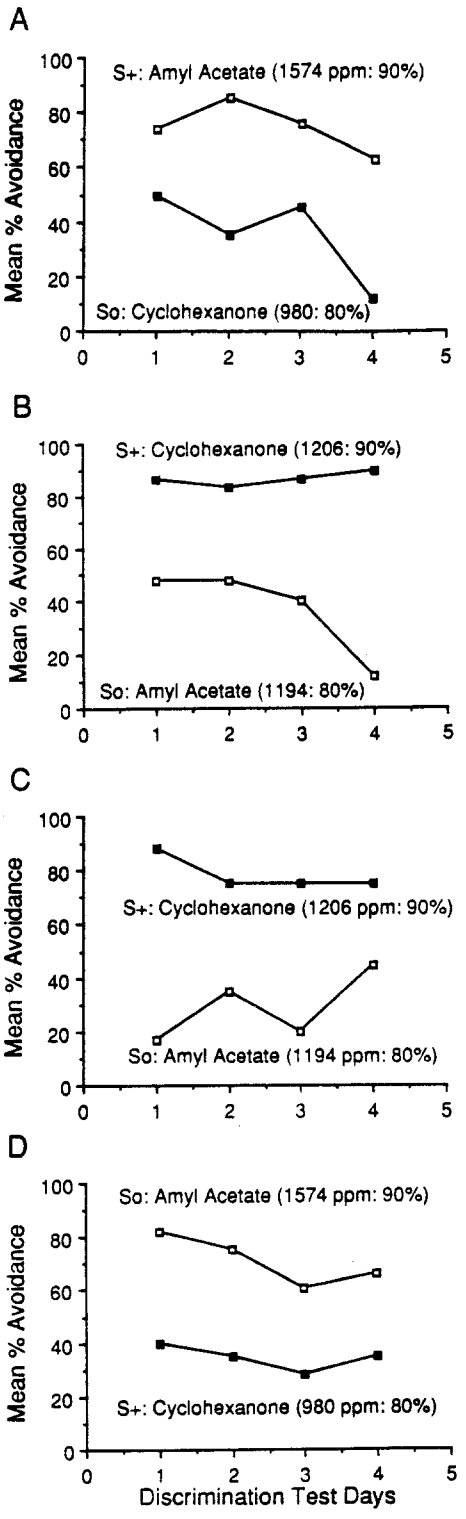

Fig. 9A–D. Discrimination between behaviorally 'unequal' concentrations of amyl acetate and cyclohexanone in olfactory nerve-cut (ONX) salamanders. Concentrations were determined from Fig. 5. Salamanders trained to avoid amyl acetate (**A**) but not cyclohexanone, or trained to avoid cyclohexanone but not amyl acetate (**B**) discriminated between the two when the reinforced stimulus (S+) was presented at a concentration that elicited 90% avoidance (amyl acetate 1574: cyclohexanone 1206) and the nonreinforced stimulus (So) was presented at a concentration that elicited 80% (amyl acetate 1194 ppm: cyclohexanone 980 ppm) avoidance. When the reinforced stimulus (S+) was presented at a concentration that elicited 80% avoidance and the nonreinforced stimulus (So) was presented at a concentration that elicited 90% avoidance, the salamanders responded to the nonreinforced stimulus (So) as if it were the reinforced stimulus (S+). This is seen in **C** and **D**

tory nerves cut. Although salamanders with their olfactory nerves cut may be using receptors other than trigeminal receptors to detect the stimuli (e.g. taste receptors), we assume that the detection is primarily mediated by the trigeminal system. In a previous report (Mason and Stevens 1981) a behavioral threshold of ≈ 2 ppm for BU was obtained in intact salamanders. This compares to a behavioral threshold of 45 ppm determined in the present study. Walker et al. (1979) examined AA thresholds in pigeons before and after olfactory nerve removal. The threshold decreased by a factor of ≈ 600 (from 0.6 ppm to 370 ppm). The pigeon trigeminal threshold for AA is similar to the present value for salamanders (370 ppm compared to ≈ 630 ppm), however, the intact pigeon is considerably more sensitive to AA than the intact salamander (0.6 ppm compared to ≈ 5 ppm). Using a cardiac acceleration paradigm, Walker et al. (1986) also reported a 2 to 4 log unit difference in AA, BU, butyl acetate, and benzaldehyde thresholds in olfactory nerve-sectioned and intact birds.

Behavioral concentration-response curves also were steeper in ONX animals than intact animals. That is, the curves for ONX animals grew at a higher rate than for intact animals. This is seen in Fig. 5. If the data are plotted on log-log coordinates, the resulting postsurgical curves have slopes ranging from 0.45 to 2.08 (mean $\pm$ SD $= 0.24 \pm 0.13$) while the presurgical curves have slopes from 0.14 to 0.46 (mean $\pm$ SD $= 0.94 \pm 0.66$) (Table 1). Again this corresponds to the observation that psychophysical functions for irritation (trigeminal stimulation) grow at a higher rate than that for odor (olfaction) (Cain 1976).

On the basis of the present electrophysiological and behavioral findings, we conclude that trigeminal chemoreceptors are unable to discriminate between chemical stimuli matched for equal intensity and that trigeminal chemoreceptors are less sensitive than olfactory receptors. At least for the four compounds tested on salamanders, trigeminal chemoreceptors discriminate chemical stimuli on the basis of intensity, not quality. We propose that qualitatively, for the trigeminal system, chemical stimuli may be similar. Previous experiments (Silver et al. 1985) have suggested that nasal trigeminal chemoreceptors may be similar to a class of pain receptors (i.e., polymodal nociceptors). Perhaps chemical stimuli that stimulate these receptors are not differentiated from other stimulus sources (e.g., heat, pressure). An interesting experiment in this regard would be to test whether heat, for example, might cross-adapt trigeminal responsiveness to a chemical stimulus.

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