

# CAPSAICIN AND ITS EFFECTS ON OLFACTION\* AND TRIGEMINAL CHEMORECEPTION

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Received October 2, 1985  
Accepted October 24, 1985

Capsaicin injections severely reduced or eliminated nasal trigeminal responses to 3 odorants (Experiment 1). However, capsaicin treated animals exhibited no deficits in locating buried food, in odor avoidance learning, or in operant odor detection and discrimination (Experiments 2 and 3). In addition, capsaicin desensitization did not affect responsiveness to salty or sour, but may have raised rejection thresholds for bitter (Experiment 4). Finally, while desensitized animals rejected menthol solution, they consumed relatively more than controls, suggesting that capsaicin may have menthol sensitivity. The present results suggest that substance P-containing fibers mediate trigeminal responsiveness to odorants and irritants but that the loss of this responsiveness does not appreciably affect smell or taste, per se.

**Keywords:** Capsaicin, trigeminal, olfaction, gustation

While most research in the chemical senses has focussed on olfaction and gustation, the trigeminal system also detects environmental chemicals [23]. Trigeminal chemoreception is not restricted to irritants, and non-irritating odorants (e.g., phenethyl alcohol, benzyl amine) are capable of stimulating naso-trigeminal chemoreceptors at concentrations below those which elicit olfactory responses [26, 27, 28]. However, the role that trigeminal chemoreception plays in odorant or taste perception remains unclear. In odor perception, for example, trigeminal involvement could occur via (a) reflexive modification of respiration, nasal secretion, and/or nasal patency, (b) modulation of olfactory bulb activity via centrifugal input, or (c) combination with olfactory stimulation to produce overall sensation. We have initiated electrophysiological and behavioral studies to explore the relative importance of olfaction, gustation, and trigeminal chemoreception to the perception of odorants and tastes.

In many of our studies, capsaicin has been used as a tool to eliminate trigeminally-mediated responding. Capsaicin is believed to act through deple-

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\* Supported in part by NIH grant No NS-23326 t W. L. S.

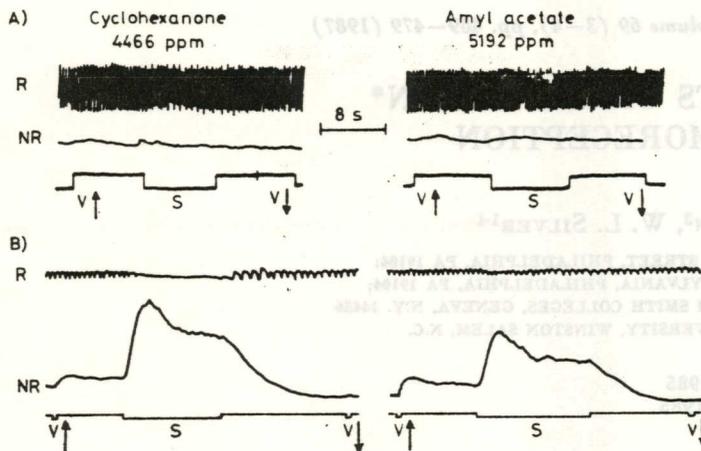


Fig. 1. Integrated recordings from the rat ethmoid nerve and respiratory rate in capsaicin desensitized and control animals. R = respiration; NR = neural response; V↑ = vacuum on; V↓ = vacuum off; S = stimulus duration. Eight second time bar applies to all records in the figure. Amplitude of respiration differs due to different amplifier settings at the time of recording. Although respiratory rates vary from animal to animal, it is evident when apnea occurs.

(A) Respiration and neural responses to saturated cyclohexanone and amyl acetate in capsaicin treated rats. There were no neural responses nor were there changes in respiration in these animals.

(B) Respiration and neural responses to saturated cyclohexanone and amyl acetate in control animals. Note that although there was the typical apnea in response to cyclohexanone, no change in breathing rate was seen to amyl acetate.

in 3 of the 6 experimental rats, although these responses appeared to adapt more rapidly than those in controls (Fig. 2).

High concentrations of amyl acetate failed to alter respiratory rate in either group (Fig. 1a). However, concentrations of cyclohexanone and propionic acid that elicited apnea in controls did not affect respiratory rate in desensitized animals (Figs 1b and 2).

## Experiment 2

### Method

Experiment 2 was performed to assess the effects of capsaicin desensitization on 2 olfactorially mediated tasks: finding buried food and odor avoidance learning. Adult male Sprague Dawley rats were randomly assigned to 6 groups ( $n = 8/\text{group}$ ). Three groups were given 8 injections of capsaicin solution as in the previous experiment. The other 3 groups were given 8 injections of vehicle alone. To test whether the capsaicin injections had produced desensitization, all groups were given 60 minute, 2-choice preference tests for 10 days between 1% capsaicin solution and vehicle [21]. Then, 1 experimental and 1 control group was food-deprived for 12 hours on each of 4 days, and given the standard cookie test for anosmia [1].

The other 4 groups (2 capsaicin and 2 control) were used in an odor avoidance paradigm. They were adapted to a water-deprivation regime for 7 days by presenting water for 15 min during the first hour of light, and 30 min during the tenth hour of light [19]. On the day of conditioning, 1 experimental and 1 control group were presented with a drinking bottle having a sipper tube that impaled a 2-cm piece of filter paper. The filter paper was wetted with 25% (vol/vol) phenethyl alcohol in corn oil [13]. The other 2 groups were presented with similar

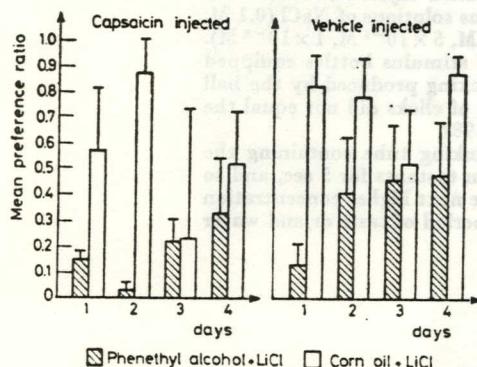


Fig. 3. Mean preference ratios for consumption of water paired with phenethyl alcohol. Ratios were calculated by dividing consumption of water paired with phenethyl alcohol by total consumption. Capped vertical lines represent standard errors of the means

( $F(9, 38) = 5.6, p < 0.05$ ), and post-hoc comparisons [29] revealed that while the odor avoidance exhibited by vehicle injected animals extinguished by the third test day, capsaicin injected animals continued to show strong avoidance on all test days ( $p_s < 0.05$ ).

### Experiment 3

#### Method

Experiment 2 demonstrated that capsaicin treated animals showed no deficits in two tests of olfactory performance. In Experiment 3, more sensitive olfactometric assessments were made of the effects (if any) of capsaicin treatment on olfactory performance. Ten adult male Long-Evans rats were adapted to a 23 hr water deprivation schedule and trained over several months to detect successively lower concentrations of amyl acetate presented olfactometrically [16]. Detection training involved shaping animals: (a) to sample from the 3 stimulus ports; (b) to identify the port containing odorant; and (c) to respond by pressing the lever beneath that port. Correct choices triggered the delivery of water reward and caused the door to remain open for 5 sec. A response at either of the 2 ports not containing the odorant constituted an error and triggered immediate closure of the access door. Following door closure, there was a 20 s intertrial-interval (ITI). On any given trial, 1 port contained the odorant while the other 2 contained clean air. Stimulus positions were switched at the beginning of the ITI, permitting odorant and clean air equilibration before the start of a trial. Training was performed with amyl acetate at a concentration of approximately 5-ppm.

Daily training was continued until each animal met a criterion of  $90 \pm 5.0\%$  correct during 5 consecutive sessions. Concentrations then were gradually reduced to approximately 0.5-ppm, while maintaining a performance of  $74 \pm 8.8\%$ . After stable detection performances were achieved, rats were assigned to experimental and control groups that were balanced with respect to mean detection performance. The experimental animals were then given injections of capsaicin, and the control animals injections of vehicle, as previously described.

Amyl acetate detection trials continued throughout the injection period, and for 5 days thereafter. Saturated formaldehyde vapor was then substituted for 0.5-ppm amyl acetate, and 3-dys of test trials were given. After formaldehyde testing, the animals were given another 4-dys of amyl acetate detection trials, followed by 4-dys of discrimination testing between amyl and propyl acetate. Discrimination tests differed from detection trials only in that approximately 40-ppm propyl acetate replaced blank air. The choice of 40-ppm propyl acetate was based on data for rats [14] indicating that the detectability of this propyl acetate concentration was similar to that for 0.5-ppm amyl acetate.

Testing was performed approximately 14-dys after the last capsaicin injection. Rats were adapted to 23-hr water deprivation, and then presented with aqueous solutions of NaCl (0.1 M, 0.3 M), HCl (0.1 N, 0.03 N, 0.01 N), and QSO<sub>4</sub> ( $5 \times 10^{-5}$  M,  $1 \times 10^{-5}$  M,  $5 \times 10^{-4}$  M,  $1 \times 10^{-3}$  M).

Presentation of taste stimuli was by small (15-ml capacity) stimulus bottles equipped with straight drinking tubes containing a ball check-valve. The clicking produced by the ball during licking served as the dependent measure. While the number of clicks did not equal the number of licks, the two measures were highly correlated ( $r = +0.98$ ).

Each test session began by presenting subjects with a drinking tube containing the lowest concentration of tastants for 5 sec, then water for 5 sec, then tastants for 5 sec, and so on for a total of 15 observations at each test concentration. Then the next higher concentration of tastants was presented and click counts recorded for each 5-sec period of taste or/and water presentation.

## Results

There were no differences between experimental and control animals in responding to NaCl or HCl. Both groups exhibited continuous responding towards NaCl, and both showed increasing rejection of HCl, as concentrations increased ( $F(8,48) = 25.6$ ;  $p < 0.001$ ). However, there were differences between groups in their responses toward QSO<sub>4</sub> ( $F(8,48) = 3.0$ ;  $p < 0.05$ ; Table II). Post-hoc comparisons [56] revealed that this between-group difference occurred only at the highest concentration tested ( $1 \times 10^{-3}$  M;  $p < 0.05$ ). While both groups exhibited sharp declines in the mean number of clicks to this concentration ( $ps < 0.05$ ), the experimental group responded at a higher rate than the control group, as though they found the solution relatively less aversive.

Table II

Timed Counts of Clicking (Mean  $\pm$  S.D.) for Experimental and Control Groups Presented with Increasing Concentrations of NaCl, HCl, and Quinine sulfate

Stimulus	Experimentals	Controls
NaCl .1M and .3M	continuous (20)	continuous (20)
HCl .01N	17.2 $\pm$ 3.0	13.4 $\pm$ 4.4
.03N	5.9 $\pm$ 1.2	7.4 $\pm$ 3.9
.1N	1.3 $\pm$ 0.6	2.0 $\pm$ 1.1
Quinine $5 \times 10^{-5}$ M and $1 \times 10^{-5}$ M	continuous (20)	continuous (20)
$5 \times 10^{-4}$ M	16.9 $\pm$ 4.1	10.3 $\pm$ 6.2
$1 \times 10^{-3}$ M	4.5 $\pm$ 0.3	2.5 $\pm$ 0.3

Individual measurements for each test concentration were obtained by averaging the number of clicks per 5-sec stimulus presentation interval, over five presentations, repeated three times (for a total of 15 counts per test concentration, per subject).

When clicking was "continuous" (no bouts of  $< 5$  sec were observed), the assignment of "20", as a nominal figure, represented an average continuous rate of about 4 licks/sec.

neurons [5, 6]. This suggests that these fibers, presumably polymodal nociceptors [3] may be responsible for the ethmoid nerve response to odorants. The fact that we obtained trigeminal responses to high concentrations of propionic acid, albeit diminished and more rapidly adapting than in controls cannot be explained at this time. Such responses probably do not reflect recovery from capsaicin treatments, as they occurred in the second, fourth, and fifth animals used in the experiment. These data are consistent with previous reports of rapidly adapting responses in animals not completely desensitized [10].

Olfactory sensitivity appeared to be unaffected by capsaicin treatments. Experimental animals did not exhibit deficits in olfactory detection, transfer or discrimination performance. Capsaicin treatment may have had an effect on rats given odor avoidance conditioning, insofar as learned avoidance by desensitized animals was more durable than avoidance by control animals. However, because there were no differences in olfactory sensitivity in Experiment 3, it is also possible that in Experiment 2, capsaicin affected learning, *per se*, rather than sensory capability.

Experiment 1 revealed that capsaicin desensitization eliminated the powerful reflex apnea usually associated with the inhalation of irritants. Even the highest concentration of propionic acid, which elicited neural responses, did not affect respiration. These data, together with the formaldehyde results of Experiment 3 suggest capsaicin desensitization may provide a method to assess behaviorally an animal's olfactory sensitivity to strong irritants.

The effects of capsaicin desensitization on gustation are unclear from our data (Experiment 4). While some evidence indicates the trigeminal system can influence taste responses [11], the present results may be due, in part, to our method of assessment. Also, the apparent insensitivity to QSO4 after desensitization reflects changes in taste, rather than trigeminal sensitivity. Specifically, the rat glossopharyngeal nerve, which is relatively more sensitive to bitter stimuli than is the chorda tympani [20], contains substance P which is depleted by capsaicin treatment [18].

The results of Experiment 5 suggest capsaicin desensitization may have affected the perceptibility of menthol, although sensitivity to this stimulus was not abolished. Reasons for this partial desensitization are unclear, but we speculate that the capsaicin injections may have selectively affected only a portion of the fibers mediating menthol perception. Douglas et al. [4] and Hensel and Iggo [8] have reported that in cats and monkeys, respectively, cool sensations are mediated by trigeminal A fibers and C fibers. Because menthol produces chemogenic cooling, and is not merely a tastant, *per se* [7, 9], it may sensitize both A and C fibers. If capsaicin selectively affected only the C fibers, the remaining A fibers, which are myelinated and of relatively larger diameter, may have mediated the response to menthol. Alternatively, because menthol at the concentration used in Experiment 5, has a noticeable (to hu-

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