

Conditioned Aversions to an Intravascular Odorant

J. A. MARUNIAK

Department of Physiology, Medical School, and Clinical Smell and Taste Research Center, University of Pennsylvania, Philadelphia, PA

AND

J. RUSSELL MASON AND J. G. KOSTELC

Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104

Received 18 December 1982

MARUNIAK, J. A., J. R. MASON AND J. G. KOSTELC. *Conditioned aversions to an intravascular odorant*. *PHYSIOL BEHAV* 30(4) 617-620, 1983.—The odorant allyl sulfide (essence of garlic) dissolved in a corn oil vehicle was injected into rats to induce a conditioned aversion. In subsequent two-choice drinking tests, rats injected with odorant and lithium chloride, and rats injected with odorant and saline avoided drinking from a water bottle paired with the odorant. Because allyl sulfide and saline injections produced symptoms of malaise, we suspect that the odorant served as its own unconditioned stimulus. Rats injected with vehicle and saline showed no differential behavior. In a second experiment, gas chromatography indicated that allyl sulfide was present on the rat's breath within 3 minutes of injection, and was detectable for up to 5 hours post-injection. We conclude that conditioned aversions can be obtained to an intravascular odorant and that one route by which such odorants reach the nose is the breath.

Intravascular odors Conditioned aversions Allyl Sulfide Gas chromatography Olfaction

ODORANTS are normally carried to the olfactory receptors in the air currents associated with breathing, sniffing and eating. However, they can also reach the receptors after being injected into the circulatory system [3]. There is considerable evidence from human and animal studies that injected odorants are carried to the olfactory receptors in the exhaled air after diffusing out of the bloodstream in the lungs [1, 2, 6, 7, 11, 13, 21]. In addition, it has recently been shown that odorants in the circulatory system can still reach olfactory receptors when tracheotomy prevents the exhaled air from passing to the nose [13]. In such animals it is hypothesized that injected odorants reach the receptors by diffusing out of the blood stream in the nasal capillary bed [13].

There is some question concerning the quality of the odorous sensations arising from the perception of injected odorants. The literature is unclear about whether odorants "smell" the same when reaching the nose during normal breathing or sniffing as when reaching the nose via the intravascular route [7]. Conceivably, animals might perceive an odorant to have different qualities when experiencing it during normal breathing and sniffing and when experiencing it via the intravascular route. To answer the analogous question for intravascular taste, Bradley [4] injected rats with saccharin in a conditioned aversion paradigm and concluded that saccharin must have a very similar or identical taste when delivered to the tongue by the bloodstream or by direct application to the surface.

In this report, the results of a procedure conceptually similar to that used by Bradley [4] are described. Rats were

injected with an odorant and then with lithium chloride, and later were tested for aversion to the odorant. We chose conditioned aversion learning as our behavioral measure because there is evidence that aversions can be obtained to compounds that stimulate only chemosensory systems sensitive to volatiles [8, 10, 18, 19]. Since odorant aversions appear to be relatively stimulus specific [8, 10, 18, 19], if an odorant that is delivered to an animal by the intravascular route induces an aversion that is expressed when the animal later sniffs it in a test situation, then it is likely it recognizes the odorant as similar despite the different modes of delivery.

A second experiment used gas chromatography to monitor the concentrations of sulfur-containing odorants in the breath of rats following injection of the odorant allyl sulfide. We further investigated the variety and concentrations of sulfur-containing breakdown products.

METHOD

All animals were adult male Sprague Dawley rats housed individually in 28×20×20 cm metal cages in a room with a 12:12 light cycle and temperature of 22±2°C. Food (Wayne Lab Blox) and water were provided ad lib except as described below.

Intravascular Aversion

Beginning 7 days before treatment (Day 1), 24 rats were given water daily for only 15 minutes during the first hour of

the light cycle, and 30 minutes during the tenth hour of the light cycle [15, 16, 17]. Water was presented in 50 ml graduated drinking bottles fitted with metal sippers. The animals were ranked according to mean drinking during the 15 min periods and assigned to 3 groups (n=8) that were balanced with respect to water intake.

On the day of treatment (Day 8), all animals were given access to water during the 15 min morning period (mean consumption = 8.0 ± 0.8 ml). Then Group 1 animals were given a 0.1 ml intraperitoneal (IP) injection of 25% (vol/vol) allyl sulfide (garlic essence, Eastman Kodak, AR grade) in corn oil solution followed 60 min later by an IP injection of 0.15 M lithium chloride at 2% body weight. The animals in Group 2 were given an IP injection of allyl sulfide solution followed 60 min later by an IP injection of 0.9% saline at 2% body weight. Group 3 animals were given an IP injection of oil alone followed 60 min later by an injection of 0.9% saline at 2% body weight. All animals in Groups 1 and 2 exhibited symptoms of malaise (e.g., sprawling at the rear of the cage) within 15 minutes of the allyl sulfide injection. Conversely, no animal in Group 3 exhibited such signs. On the day following conditioning, and during the light period of the next day, all animals were given food and water ad lib. Water deprivation was begun again during the dark period of the second post-treatment day (Day 10).

During the 15 minute drinking period on each of the next 5 days (Days 11–15), all animals were given counterbalanced two-choice tests in which they were presented with 2 drinking bottles mounted 5 cm apart on the front of each cage. A 2 cm² piece of filter paper was impaled approximately 2 cm below the stopper on the sipper tube of each bottle. The paper on one tube was wetted with 0.01 ml of the allyl sulfide injection solution while the paper on the other bottle was wetted with oil alone. Care was taken that neither allyl sulfide solution nor oil reached the surface of either sipper tube, and the filter papers were positioned so rats could not physically contact them. At the end of each test period consumption from each bottle was recorded and the bottles were removed from the cages. All animals were permitted 30 min access to water (not paired with allyl sulfide or corn oil) during the tenth hour of the light cycle on each of 5 test days.

The data were converted to suppression ratios, and analyzed using a two way analysis of variance with repeated measures on one factor. The independent factor was groups, while the repeated factor was days. Tukey b tests [23] were used to isolate significant differences among means.

Odorant Concentrations in the Breath

Three rats of the same age group as those in the first experiment and housed, fed, and watered as stated in the general methods, were anesthetized with nembutal, (50 mg/kg). Fifteen minutes later 10 ml breath samples were collected by aspirating lung air through the external nares via a teflon/tygon sampling tube system which completely covered the nares. The first sample was used to obtain a profile of background levels of sulfur compounds in the rat's breath. All samples were injected immediately after collection into a Perkin-Elmer 3920B gas chromatograph equipped with a flame photometric detector and adapted for selective detection and quantitation of nanogram quantities of sulfur-containing compounds. Breath samples were aspirated from the rats directly into a 10 ml sample loop in the chromatograph which allowed pneumatic injection into the column. Upon sampling, the contents in the loop were transferred via a six-port valve onto a 11 m \times 2 mm ID fluorinated ethylene

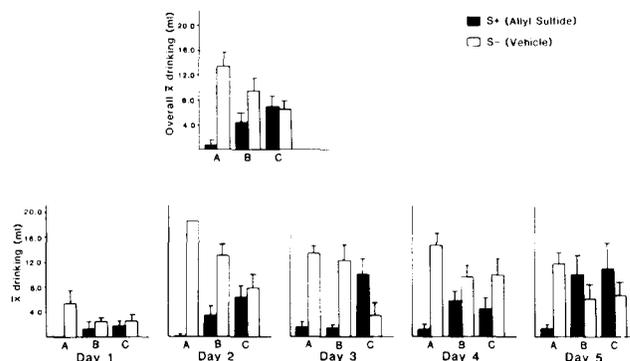


FIG. 1. Consumption of water paired with allyl sulfide (dark bars) or vehicle (open bars) by Group 1 rats injected with odorant and lithium chloride (A); Group 2, odorant and saline (B); and Group 3, vehicle and saline (C). The top panel represents overall drinking. The bottom panel represents daily consumption. Capped vertical bars represent standard errors of the means.

propylene (FEP) teflon column packed with 5% polyphenyl ether and 0.5% phosphoric acid on 40/60 mesh Chromosorb T. The sample was chromatographed using the following temperature program: 55°C for 1 min, 55°C to 167°C at a rate of 32°/min and 167°C for 4 min. Preliminary experiments revealed that under such conditions, allyl sulfide had an elution time of 7.1 minutes. The flame photometric detector was 130°C and the carrier gas, ultra pure air, had a flow rate of 25 ml/min.

Volatile sulfur compounds were quantitated from standard graphs derived from analysis of known concentrations of test compounds [22]. A standard calibration curve for allyl sulfide was prepared by dilution of neat allyl sulfide with methylene chloride.

After the first breath samples were taken, the rats were injected IP with 0.1 ml of 25% (vol/vol) allyl sulfide dissolved in corn oil. Breath samples were subsequently taken and immediately injected into the column at 3, 15, 30, 45, 60, 120, 180, 240, and 300 min after allyl sulfide injection.

RESULTS

Intravascular Aversion

There were significant differences among groups in suppression of drinking from the tube associated with allyl sulfide, $F(2,18)=23.4$ $p<0.000001$, and within each group, the degree of suppression was constant across the 5 test days ($p<0.05$). The results of the Tukey tests indicated that both Group 1 (allyl sulfide and lithium chloride injections) and Group 2 (allyl sulfide and saline injections) exhibited suppression of drinking from the tube associated with allyl sulfide ($p<0.01$; Fig. 1). Examination of the data across days revealed that Group 1 showed strong suppression on all test days, while Group 2 showed apparently (though not significantly) weaker suppression over the tests.

Odorant Concentrations in the Breath

Allyl sulfide was detectable in the breath of all animals 3 min after injection. Concentrations of the odorant increased until 60 minutes post-injection, and then decreased to undetectable levels over 4–5 hours (Fig. 2). Interestingly, when the gas chromatograph showed allyl sulfide to be present in a

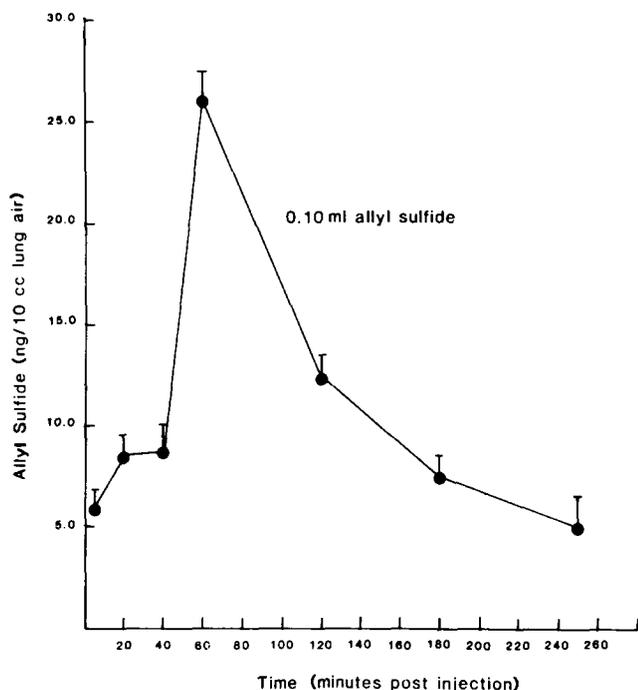


FIG. 2. Allyl sulfide concentrations in lung air of rats following intraperitoneal injections of 0.1 ml of 25% allyl sulfide/oil solution. Capped vertical bars represent standard errors of the means.

sample, the experimenters were also able to smell the characteristic garlic odor in the rats' breath. Hydrogen sulfide was the only consistent sulfur containing breakdown product observed.

DISCUSSION

The present results demonstrate that conditioned aversions can be obtained in rats to an injected odorant, insofar as such aversions are expressed when the rat subsequently sniffs the odorant in a preference test. Because odor aversions are stimulus specific [8, 10, 18, 19], this strongly suggests that allyl sulfide reaching the nose after injection was perceived to be similar (if not identical) to allyl sulfide encountered during normal sniffing in the preference test. As such our findings are consistent with those of Bradley [4], who concluded that saccharin was perceived to be very similar by rats when it was delivered by the intravascular route or by normal licking behavior.

Both Groups 1 and 2 showed conditioned aversion learning. This was surprising in that we had no a priori reasons to suspect that allyl sulfide by itself would act as an unconditioned stimulus. On the other hand, all animals injected with allyl sulfide exhibited symptoms of malaise. However, aversions exhibited by Group 2, which were injected with only allyl sulfide and saline, appeared to weaken more rapidly than aversions exhibited by animals in Group 1, who

received both allyl sulfide and lithium chloride injections (Fig. 1). The differences between the groups were not significant, but we believe they would have become so had testing continued several additional days. As such, we propose that the durability of the aversions was influenced by the degree of malaise experienced on the day of treatment. This proposition is consistent with results collected by others showing that the degree of malaise experienced on the day of treatment influences the strength of taste aversions [14]. Because Group 3 (which received only oil and saline injections) did not behave differentially toward allyl sulfide, we infer that allyl sulfide was neither inherently attractive nor repellent to the rats before conditioning (Fig. 1). This inference is consistent with previously published work in which allyl sulfide was used as a stimulus (e.g., [5]).

We believe that one or both of two possible mechanisms are responsible for transporting an odorant in the bloodstream to the olfactory receptor sites: (1) It can diffuse out of the bloodstream in the lungs and pass to the receptors in the exhaled air; and/or (2) diffuse out of the circulation in the nasal capillary bed to the receptor sites [13]. The data from the second experiment demonstrate that the first hypothesis is certainly tenable. Breath concentrations of allyl sulfide rose quickly after injections so that between 3 min and 4 or 5 hr post-injection the levels were high enough to cause olfactory responses. During this time period the odor of garlic in the rats' breath was easily detected by the experimenters. Laing [9], in a study comparing human and rat thresholds for odorants, suggested that this is a good indication that an awake rat could also easily detect the odorant. Nonetheless, our data do not exclude a contribution by the direct diffusion of injected odorant from the nasal capillaries to the acquisition of the aversion.

It is interesting to speculate what the effects of adaptation were on our experimental rats' perception of the odor of injected allyl sulfide. When humans eat foods containing garlic, the odor of garlic (mainly allyl sulfide) similarly appears in the breath for hours. However, because of adaptation, humans generally do not perceive the odor of garlic on their own breath. Our gas chromatographic data indicated that concentrations of allyl sulfide in the rats' breath were increasing until 60 min post-injection (Fig. 2). Thus allyl sulfide breath concentrations should have been high enough to be perceived during the onset of both allyl sulfide and LiCl induced malaise. However, it is known that the strength of aversion learning is enhanced by the discreteness of the conditioned and unconditioned stimuli [12], and additionally that anesthetized animals can acquire conditioned taste aversions [19]. This leads us to speculate that, in our animals, perception of the garlic essence, allyl sulfide, after injection may have been very short and/or the rats may not have required sensory awareness of the odorant in order to acquire an aversion to it.

ACKNOWLEDGEMENTS

This work was partially supported by grant NS 16365 from the National Institute of Health.

REFERENCES

1. Antonelli, A. A. Electrophysiologische untersuchungen am Olfaktionssystem der Katze während intrahamatischer injektion von Essenzlösungen. *Laryngol Rhinol Otol (Stuttg)* **41**: 822-828, 1962.
2. Bartalena, G. L'olfatto nei laringectomizzati. *Boll Mal Orecchio-Gola-Naso* **4**: 362-365, 1958.
3. Bednar, M. and O. Langfelder. Über das intravenöse (hamatogene) Riechen. *Monatsschr Ohrenheilk Laryngo-Rhinol* **64**: 1133-1139, 1930.

4. Bradley, R. M. and C. M. Mistretta. Intravascular taste in rats as demonstrated by conditioned aversion to sodium saccharin. *J Comp Physiol Psychol* **75**: 186-189, 1971.
5. Capretta, P. J. and L. H. Rawls. Establishment of flavor preference in rats: Importance of nursing and weaning experience. *J Comp Physiol Psychol* **86**: 670-673, 1974.
6. Dishoeck, H. A. E. Van and N. Versteeg. On the problem of hematogenic olfaction. *Arch Otolaryngol (Stockholm)* **47**: 396-401, 1957.
7. Guttich, H. Intravenos verabreichte Riechstoffe: gustatorisches Riechen. *HNO* **13**: 42-45, 1964.
8. Hankins, W. G., J. Garcia and K. W. Rusiniak. Dissociation of odor and taste in baitshyness. *Behav Biol* **8**: 407-419, 1973.
9. Laing, D. G. A comparative study of the olfactory sensitivity of humans and rats. *Chem Senses Flavor* **1**: 257-269, 1975.
10. Lorden, J. F., M. Kenfield and J. J. Braun. Response suppression to odors paired with toxicosis. *Learn Motiv* **1**: 391-400, 1970.
11. Marco, J., H. Morera and J. Gimenez. Olfaction in laryngectomized patients. *Acta Oto-Laryngol* **46**: 114-126, 1956.
12. Mackintosh, N. J. *The Psychology of Animal Learning*. New York: Academic Press, 1974, pp. 66-70.
13. Maruniak, J. A., W. L. Silver and D. G. Moulton. Olfactory receptors respond to bloodborne odorants. *Brain Res.* in press.
14. Nachman, M., J. Rauschenberger and J. H. Ashe. Stimulus characteristics in food aversion learning. In: *Food Aversion Learning*, edited by M. W. Milgram, L. Krames and T. M. Alloway. New York: Plenum Press, 1977, pp. 105-130.
15. Nowliss, G. H. and M. Frank. Qualities in hamster taste: behavioral and neural evidence. In: *Olfaction and Taste IV*, edited by J. LeMagnen and P. MacLeod. Washington, DC: Information Retrieval Inc., 1977, pp. 241-248.
16. Nowliss, G. H., M. Frank and C. Pfaffman. Specificity of acquired aversions to taste qualities in hamsters and rats. *J Comp Physiol Psychol* **94**: 932-942, 1980.
17. Nowliss, G. H. and C. Pfaffman. The compound taste of saccharins. *Am Chemorecept Soc Abstr* **2**: 1980.
18. Panhuber, H. Odor quality and intensity effects on odor aversion learning in the rat. *Olfaction and Taste VII*, edited by N. van der Starr. London: IRL Press, 1980, p. 428.
19. Panhuber, H. Effect of odor quality and intensity on conditioned odor aversion learning in the rat. *Physiol Behav* **28**: 149-154, 1982.
20. Roll, D. L. and J. C. Smith. Conditioned taste aversion in anesthetized rats. *The Biological Boundaries of Learning*, edited by M. E. P. Seligman and J. L. Hager. New York: Appleton-Century-Crofts, 1972.
21. Teatini, G. P. and G. Pincini. Estimulacion olfatoria por via hematica. *Acta Oto-Rino-Laringol Ibero-Am* **12**: 417-431, 1961.
22. Tonzetich, J. Direct gas chromatographic analysis of sulfur compounds in mouth air in man. *Arch Oral Biol* **16**: 587-597, 1971.
23. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962.