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CHEMORECEPTION IN THE MIGRATORY SEA TURTLE, *CHELONIA MYDAS*

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It has long been suspected on the basis of neuroanatomical (Papez, 1961), neurophysiological (Tucker, 1963; Tucker and Shibuya, 1965) and behavioral evidence (Boycott and Guillery, 1962), that fresh water and land turtles have a sense of smell. Nothing is known of chemoreception in sea turtles, although loggerhead turtles (*Caretta caretta*) have been observed underwater with their nostrils open and the floor of the mouth moving up and down, possibly engaged in chemical sampling (Walker, 1959).

The green turtle (*Chelonia mydas*), whose life cycle has been studied intensively, is known to migrate long distances through the open sea. Tagging studies have demonstrated that populations of Atlantic green turtles usually leave their year-round feeding grounds to mate and breed on beaches that are hundreds of miles away (Carr, 1967). For example, the population that nests on Ascension Island feeds near the coast of Brazil, a distance of 1400 miles. Their method of navigation is unknown. Orientation by visual cues alone seems unlikely, moreover these turtles have been shown to be myopic when their eyes are out of water (Ehrenfeld and Koch, 1967). It has been suggested recently that the detection of chemicals entering the South Equatorial Current from Ascension Island might aid in the navigation of the Brazilian migrants (Koch, Carr and Ehrenfeld, 1969). Carr (1972) has called attention to evidence that olfactory cues might also be available to migrants to a mainland nesting shore.

In this study we used operant conditioning techniques to examine the ability of the green turtle to detect various chemical substances dissolved in water. In addition we tested a method of reversibly interrupting olfaction for a period of days by treating the olfactory epithelium with a 0.35 M solution of $ZnSO_4$.

Materials and Methods

The experimental subjects were four immature Caribbean green turtles. At the start of the experiment they were 6 months old and weighed 300 to 450 g. They lived in recirculating artificial sea water and were tested in fresh running water (green turtles are osmotically highly adaptable). The turtles had been hatched and reared in the laboratory from eggs obtained in Costa Rica, and were previously untested. They were kept on a 23-hr food deprivation schedule during the experiments.

The apparatus used for training and testing is diagrammed in Figure 1. The experimental chamber was a tank 30 cm wide, 45 cm long and 30 cm deep containing water at a depth of 7 cm (8 l) which flowed continuously through the

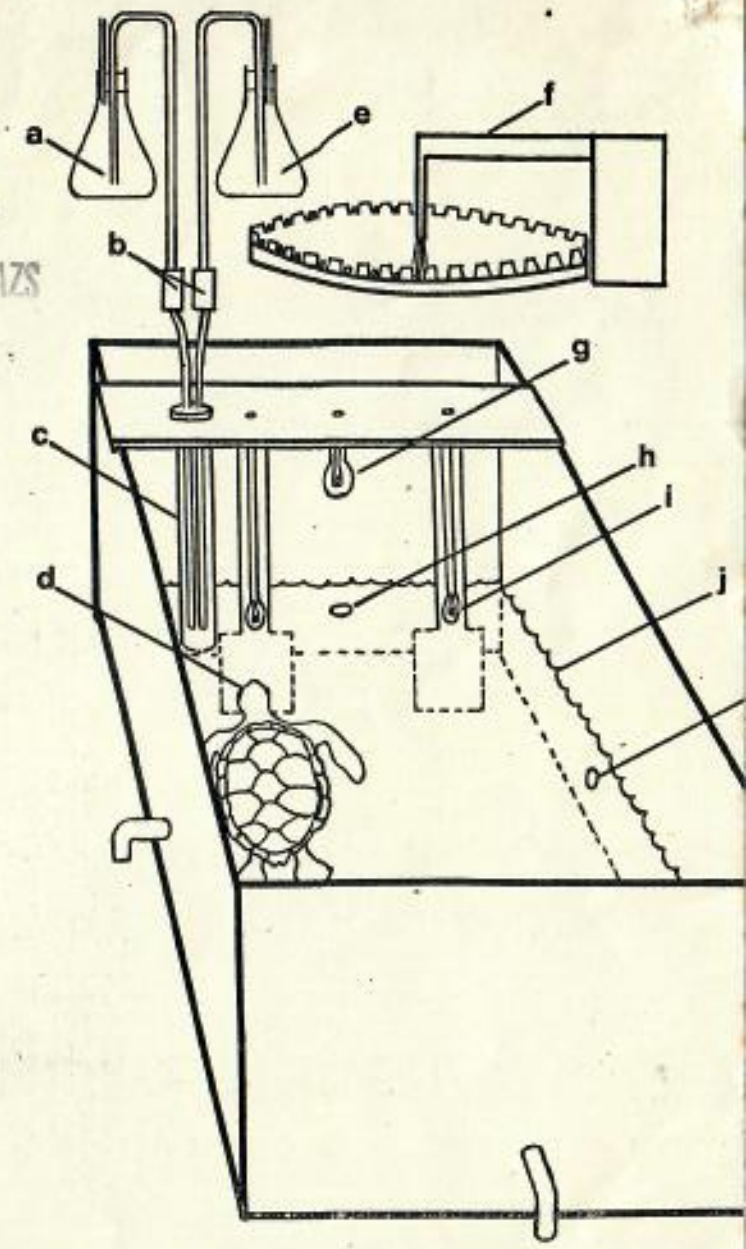

Figure 1. Diagram of the experimental tank; (a) chemical or water release valves; (c) glass conduit housing delivery tubes; (d) turtle pressing (production) key; (e) second reservoir; (f) automatic feeder; (g) overhead light inlet; (i) key light; (j) water level; (⊢≺) one of the three water outlets.

presentation, a response to the right key was scored as a correct report and reinforcement was delivered. During the 20 sec after water presentation, a response to the right key was scored as a false report and no reinforcement was delivered. The false reports were used as controls to sample the tendency to respond to the reinforcement key when no signal was present.

The first test substance was 0.05 M β-phenethylalcohol. This chemical has been used in olfactory threshold studies with teleost fish (Teichmann, 1959) and is non-toxic, non-irritating and colorless at the concentration used. The interval between chemical trials varied from 1 to 4 min during a session. A minimum interval of 1 min was chosen because tests with an indicator dye added to the tank water in the same concentration as the test chemical, showed a 95% reduction from the initial concentration during the first min after dye addition.

The other organic chemicals tested were also selected on the basis of their use in previous experiments on chemoreception. They were, in order of their presentation: 0.05 M iso-pentyl acetate, 0.01 M triethylamine, 0.01 M cinnamaldehyde, 0.1 M L-serine and 0.1 M glycine. (Calculations of actual concentrations in the tank at the time of chemoreception are presented in "Discussion.") The procedure was identical in all cases. Data were collected from a minimum of 10 consecutive sessions for each test chemical.

The experiment, as outlined, cannot differentiate chemical discrimination mediated by olfaction from that mediated by taste. Therefore the method developed by Alberts and Galef (1971) for producing temporary anosmia in rats by bathing the olfactory mucosa with $ZnSO_4$ solution, was modified for use with these marine turtles. Reagent grade $ZnSO_4 \cdot 7H_2O$ was used in making solutions. After testing several concentrations we selected 0.35 M $ZnSO_4$ for use.

Before treatment with either $ZnSO_4$ or a control solution (NaCl or $MgSO_4$) each turtle was removed from the home tank for at least one hour to permit drying of the nasal cavities and mouth. The turtle was then placed on its carapace with its head tilted downwards. The mouth was held open, and the tongue was screened from contact with the $ZnSO_4$ during treatment. The solution was injected, using a recurved and blunted syringe needle, directly into the internal nares. Approximately 0.3 cc was introduced on each side; drops were observed to run out of the external nares. The area around the internal nares was aspirated to remove any excess $ZnSO_4$ and the turtle was kept in the same position for a few minutes to prevent solution from draining back into the mouth. The turtle was returned to the home tank an hour after treatment. Two turtles (Nos. 2 and 3) were treated with 0.35 M $ZnSO_4$ solution in this manner. The other pair received an identical intranasal injection of either 0.35 M NaCl (No. 1) or 0.35 M $MgSO_4$ (No. 4) as controls.

Turtles were run in their usual chemical discrimination test sessions on the same day as treatment and daily thereafter until behavior returned to the pretreatment baseline.

The treatment was then repeated with the modification that the intranasal injection was made through the external nares. Care was taken to ensure that the head tilted downwards throughout the treatment to minimize flow to the mouth; the mouth was opened, and the solution was injected until it welled up in the internal nares and in the nostril not being injected. The previous control animals

TABLE I

Mean responses (%) to 4 chemicals and to water, averaged over 15 sessions

Turtle No.	β-phenethylalcohol		iso-pentyl acetate		triethylamine		cinnamaldehyde	
	Mean correct detection (%)	Mean false reports (%)	Mean correct detection (%)	Mean false reports (%)	Mean correct detection (%)	Mean false reports (%)	Mean correct detection (%)	Mean false reports (%)
1	75.7	32.3	86.1	45.3	87.5	42.1	81.6	28.5
2	93.1	42.1	96.3	65.6	94.9	57.3	94.4	47.2
3	85.9	23.2	92.3	52.5	95.7	49.6	92.5	45.9
4	77.3	14.4	85.3	39.2	93.1	44.0	92.0	37.1
Average	83.0	28.0	90.0	50.7	92.8	48.3	90.1	39.7

were injected with $ZnSO_4$ solution (Nos. 1 and 4) while the other pair, which had recovered full olfactory function, received identical injections of NaCl (No. 2) and $MgSO_4$ (No. 3).

RESULTS

Once trained, the green turtles maintained a steady base rate of response to the left (signal production) key with an average of 10 responses/min. Nevertheless, the tendency to respond occasionally to the food key when no signal was present was never completely eliminated.

Approximately 30 sessions/turtle were required to effect the transfer of the learned operant behavior from the progressively reduced light signal to the first chemical signal employed (0.05 M β-phenethylalcohol). By the time the light was dimmed to almost zero intensity the turtles had ceased to look at it, as was their previous habit. The results from 15 consecutive sessions with phenethylalcohol after signal pairing was discontinued are graphed in Figure 2A. The open circles of each graph show the % correct reports after chemical release and the open squares show the % false reports after water release. The turtles all responded to the right (food) key in the presence of the phenethylalcohol solution with a consistently higher probability than to the water control. The mean performance for the phenethylalcohol trials for all 4 turtles, averaged over the 15 sessions (a total of 1500 trials), was 83% correct detection. The mean performance for the same number of water control trials during the same 15 sessions was 28% false reports. The individual means are included in Table I.

Similar results were obtained when the test chemicals were 0.05 M isopentyl acetate, 0.01 M triethylamine and 0.01 M cinnamaldehyde. In these cases, the turtles readily generalized the experimental function of "chemical" and retraining was not necessary when a new test substance was introduced. The per cent correct detection of the test chemical and the per cent false reports to the water tended to vary together. This covariance indicates that although absolute detection varied, relative detection was fairly stable. This was largely a function of a turtle's general activity level on a particular day.

Table I summarizes the data for the first 4 chemicals tested. Each entry in Table I reports the per cent response by an individual turtle to 375 chemical or water trials during 15 consecutive sessions.

TABLE II

Mean responses (%) to 2 amino acids and to water, averaged over 10 sessions

Turtle No.	L-Serine		Glycine	
	Mean correct detection (%)	Mean false reports (%)	Mean correct detection (%)	Mean false reports (%)
1	65.7	71.6	80.8	80.0
2	84.4	84.8	85.6	86.0
3	85.2	82.4	86.0	84.6
4	74.8	75.2	79.2	68.0
Average	77.5	78.5	82.9	79.7

Direct observation of the turtles during their daily sessions revealed a distinctive change in their otherwise leisurely behavior, upon release of the chemical. Flipper movements markedly increased and approaches to the reinforcement key were often quite violent. At times, after chemical release, the turtles became too excited to push the key within the 20 sec limit allotted for correct detection. This frenzied behavior was not observed during water trials. The entire behavioral sequence of pressing on the left key, swimming over to the right key and pressing it, invariably occurred with the head completely under water. During chemical release the turtles directed their nostrils downward and appeared to be pumping water through the nasal cavities by means of throat movements. Breathing pauses, during which the nostrils were above water, were infrequent and were only made during well defined breaks in responding.

As a session progressed we were able to detect a slight odor of the test chemical above the experimental tank. However, it appears virtually impossible that the turtles were using this odor as a cue since the correlation between airborne odors, reinforcement availability and the emergence of the nostrils above water was necessarily random.

The results of the tests of the two amino acids, L-serine and glycine, are given in Table II, and the L-serine results are graphed in Figure 2B. Trials were stopped after 10 consecutive sessions because the learned response pattern was disintegrating in the absence of any stimulus that the turtles could discriminate. It was necessary to retrain the turtles to baseline performance after each amino acid was tested. Phenethylalcohol, earlier shown to be detected, was used for this purpose.

$ZnSO_4$ solution temporarily interrupted chemical discrimination for periods lasting from 1 to 5 days. Recovery of function occurred gradually over a period of several days. Chemoreception before and after $ZnSO_4$ treatment can be compared for all 4 turtles in Figure 3A. Turtles 1 and 4 received $ZnSO_4$ through the external nares and the resulting anosmia was brief. The intranasal injections of saline (turtles 1 and 2) and $MgSO_4$ solution (turtles 3 and 4) had virtually no effect on the performance of the chemical discrimination, as shown in Figure 3B. None of these localized chemical treatments caused any other observed behavioral changes. Turtles treated with $ZnSO_4$ swam and fed normally immediately after treatment. The discriminative test chemical was 0.01 M cinnamaldehyde.

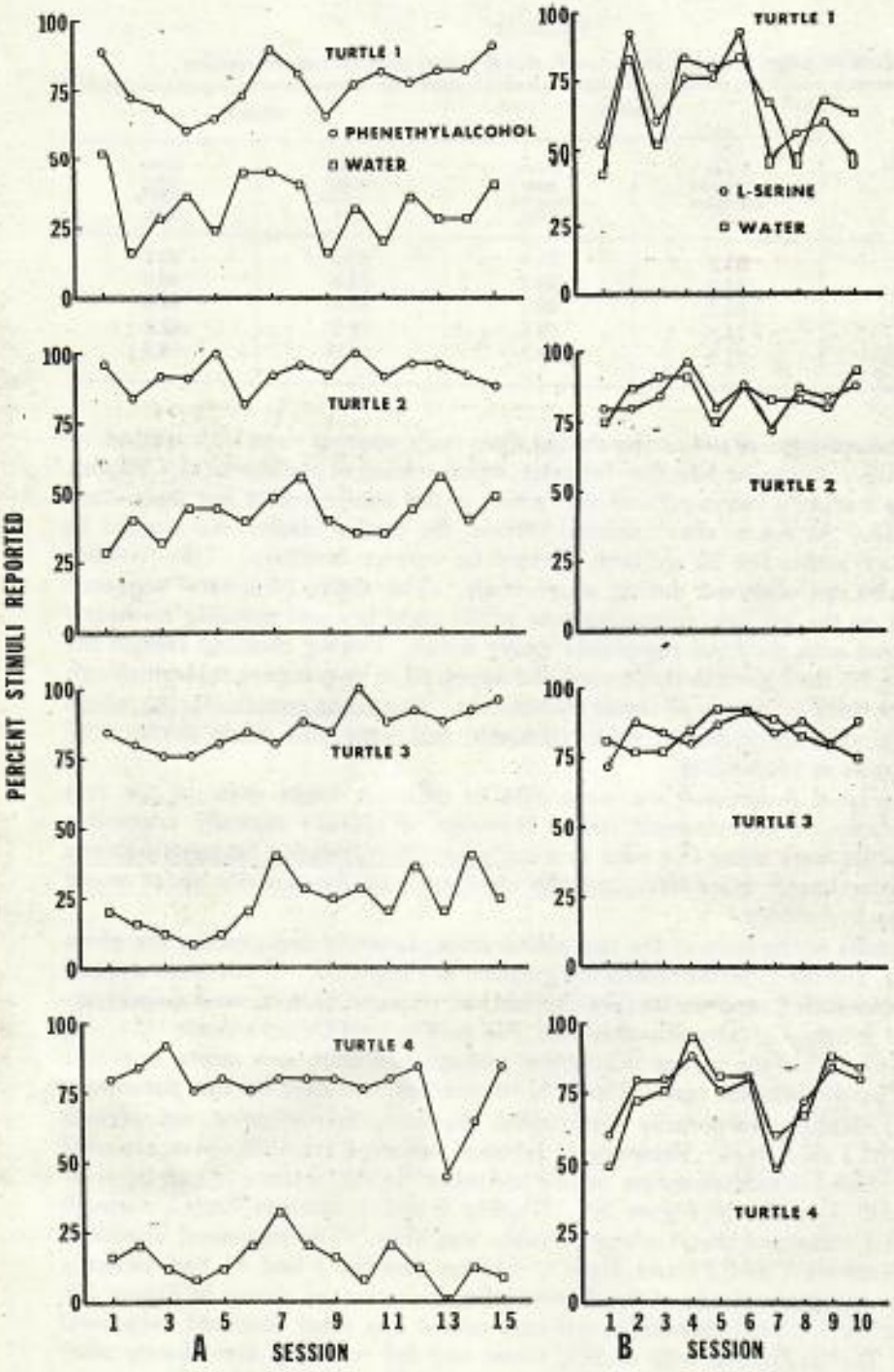

FIGURE 2. The per cent correct and false reports for 4 green turtles during presentation of phenethylalcohol (A) and L-serine (B). The open circles show the per cent correct reports after chemical release. The open squares show the per cent false reports after water release.

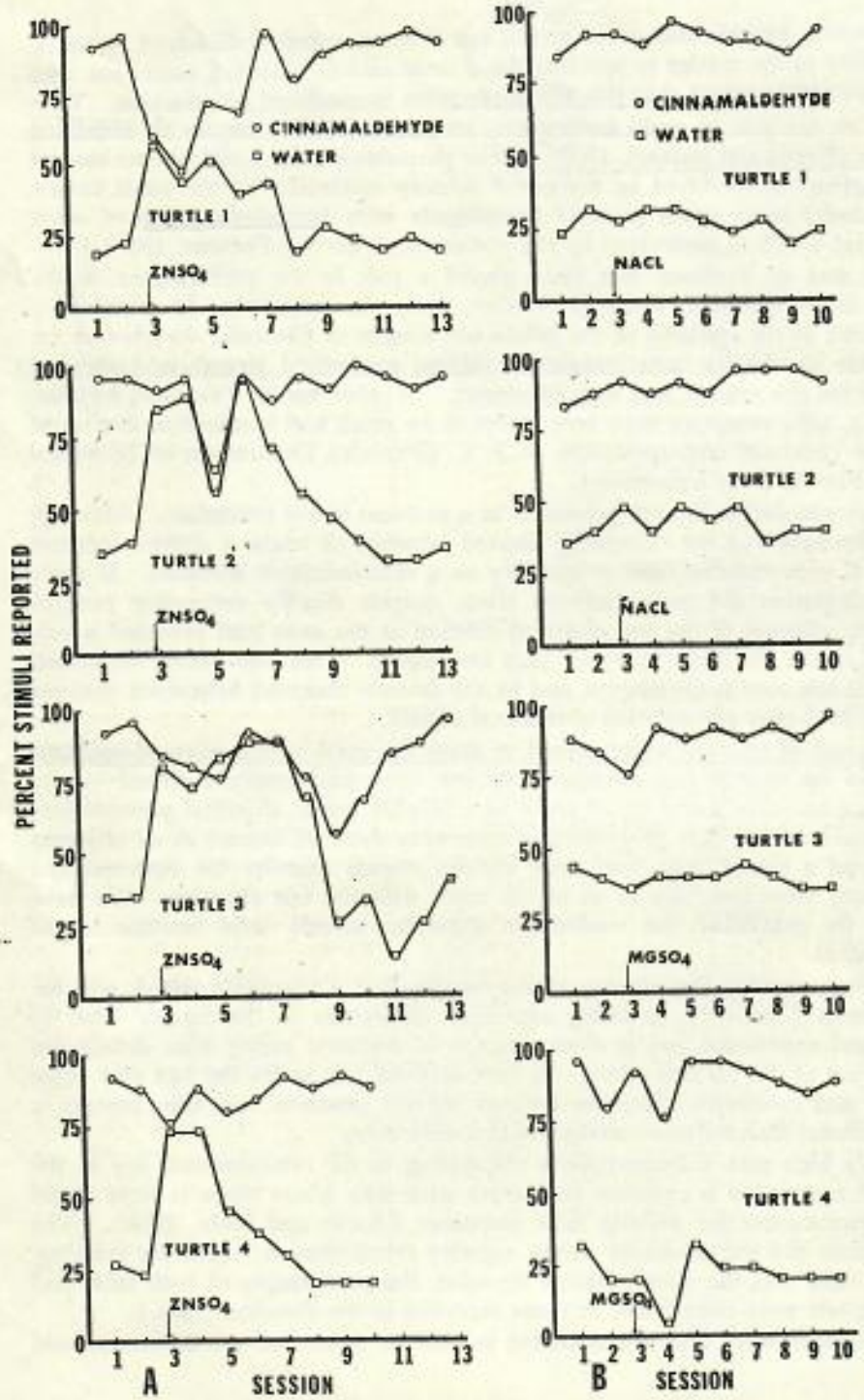

FIGURE 3. The per cent correct (open circles) and false (open squares) reports for 4 green turtles before and after intranasal treatment (arrow) with $ZnSO_4$ (A) or either NaCl or $MgSO_4$ (B). See text for further details.

Discussion

Our results indicate that green turtles can detect a chemical dissolved in water. The inability of the turtles to perform the discrimination following treatment with $ZnSO_4$ solution suggests that this chemoreception is mediated by olfaction. Thus these turtles are able to smell underwater, an unusual ability for an air-breathing vertebrate (Evans and Bastian, 1969). [For purposes of this discussion we assume that "olfaction" is mediated by the entire sensory epithelium of the nasal cavity, which includes some tissue possibly homologous with Jacobson's organ of other reptiles, and which is innervated by the vomeronasal nerve (Parsons, 1967).]

There was no evidence that taste played a role in the performance of the chemical discriminations, although further study is indicated. In histological examinations of the epithelia of the palate and tongue of *Chelonia*, we have so far been unable to identify taste receptors, whereas anatomical structures associated with olfaction are present and well developed. In other turtles (the land tortoise, *Gopherus*), taste receptors have been found to be small and localized to the tip of the tongue (personal communication, P. P. C. Graziadei, Department of Biological Sciences, Florida State University).

Sensory adaptation has not proven to be a problem in our procedure. Although the test chemical was not completely cleared between all trials, a sudden increase in chemical concentration was satisfactory as a discriminative stimulus. If some sensory adaptation did occur between trials, despite steadily decreasing concentration, the addition of the test chemical solution at the next trial provided a sufficient change in the stimulus to act as a new signal. This was clearly confirmed both by the test scores, themselves, and by the directly observed behavioral changes which occurred after presentation of chemical stimuli.

The sound of running water served to mask the noise of the solenoid-operated valves, and the control and chemical solutions were occasionally switched in any case so that no valve sound could serve as a reliable cue to chemical presentation. Relays, counters and tape programming apparatus were all located in an adjacent room behind a closed door, and their various sounds (similar for chemical and water trials) were inaudible to us in the room with the test chamber. We have dismissed the possibility that inadvertant apparatus sounds could function in the discrimination.

Differences among the average scores for the first 4 chemicals tested, and for their controls (Table I), probably represent differences in the turtles' level of training and experience and in their strategy of response rather than differences in perception of the various odors. In each of these test series the fact that there is a large and consistent difference between correct detection and false reports is more significant than the exact amount of that difference.

A fairly high rate of inappropriate responding to the reinforcement key in the absence of a stimulus is common in operant situations where there is little or no effective punishment for making false responses (Azrin and Holz, 1966). The 2-sec blackout did not constitute strong negative reinforcement. (During training, when the light was the discriminative stimulus, the percentages of both false and correct reports were comparable to those recorded in the chemical trials.)

The transfer from one test chemical to another presented no difficulties and

gave clear evidence of stimulus generalization. A single session was usually sufficient to establish correct responding to the new odor. On the other hand, when the stimulus presented could not be detected, behavior was disrupted. The behavior during the amino acid test sessions was characterized by alternation between the keys, frequent pauses between bouts of responding and occasional defecation in the experimental tank. (Salmon, unlike *Chelonia,* are reported to be able to detect L-serine in extremely low concentrations (Idler, Fagerlund and Mayoh, 1956).)

It is possible to make an estimate of the sensory acuity demonstrated in this experiment. From the relative positions of the turtle (at the left key) and the chemical release point we can assume that the delivered chemical is diluted, at the time of sampling, in a volume of water equivalent to $\frac{1}{3}$ of the tank volume (1000 ml). Detection therefore occurs at approximate concentrations of from 5×10^{-6} M to 5×10^{-5} M depending on the solution used. Dye tests confirm the assumption underlying this calculation. (The undetected amino acids were presented at an approximate concentration of 10^{-4} M.)

The mechanism of the $ZnSO_4$-induced anosmia is as yet unknown. The data from the saline and $MgSO_4$-treated controls appear to rule out osmotic shock or trauma following treatment. The role of Zn^{++} seems crucial. The present method of $ZnSO_4$ application produces considerable variation in the period of anosmia. One turtle showed definite signs of the return of olfaction after 24 hr, while the longest period of complete anosmia was 5 days. Factors such as the mode of administration and the degree of dryness of the nasal passages probably influenced the effectiveness of the treatment.

Since the animals used in this study are difficult to obtain, Zn^{++}-induced peripheral anosmia has certain advantages over olfactory bulb ablation or olfactory nerve sectioning. The Zn^{++} effect is both reversible and relatively non-traumatic. Furthermore, the present technique provides the opportunity for field studies of the role of olfaction in the orientation of green turtles, without causing permanent loss of functional individuals from an endangered population. The possible role of olfaction in both open sea navigation and in site selection at the nesting beach can be experimentally studied with this approach. In general, the use of Zn^{++}-induced anosmia offers promise of opening new areas of investigation of the interaction between olfaction and behavior among a wide range of vertebrates; and it may provide an additional tool for the study of the mechanism of olfaction itself.

Our experimental procedure is a sensitive behavioral assay for underwater chemoreception in aquatic vertebrates. It further demonstrates the utility of operant conditioning methods in the study of reptilian sensory physiology. Our findings show that the migratory sea turtle, *Chelonia mydas,* can smell a variety of chemicals dissolved in water in moderately low concentrations. Such detection is a prerequisite sensory capability if, as has been suggested, chemical cues borne in ocean currents play a role in navigation.

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Summary and Conclusions

1. The ability of the green turtle (*Chelonia mydas*) to detect various chemical substances dissolved in water has been investigated using operant conditioning techniques. The turtles pressed underwater keys to obtain food reinforcement in the presence of a chemical stimulus.

2. The turtles were capable of underwater chemoreception of β-phenethylalcohol, iso-pentyl acetate, triethylamine and cinnamaldehyde at approximate concentrations of 5×10^{-6} M or 5×10^{-5} M, but not of L-serine or glycine at an approximate concentration of 10^{-4} M.

3. Stimulus generalization occurred when turtles were shifted from one test chemical to another.

4. Intranasal injection of 0.35 M zinc sulfate solution interrupted olfaction for periods of from 1 to 5 days. Treatment with 0.35 M saline or magnesium sulfate had no effect on the performance of the chemical discrimination. It was concluded on the basis of these experiments that chemoreception in *Chelonia* is largely or entirely mediated by olfaction rather than by taste.

5. The advantages of the zinc-induced anosmia over surgical techniques and the possible use of the zinc treatment in field studies of orientation are discussed.

6. Our results provide evidence to support the current theory that soluble compounds entering ocean currents from the vicinity of nesting sites might be detected by green turtles, and that this could aid in navigation.

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