Journal of Comparative and Physiological Psychology
1968, Vol. 66, No. 2, 289–295

FACTORS INFLUENCING THE CHEMICAL RELEASE OF PREY ATTACK IN NEWBORN SNAKES[1]

GORDON M. BURGHARDT[2] AND ECKHARD H. HESS
University of Chicago

Attack behavior of inexperienced garter snakes to water extracts of normally eaten prey was investigated. Since eliminating vision and olfaction in newborn snakes did not alter response to chemical cues, Jacobson's organ is considered to be the receptor most involved. Slight variations in age and concentration did not affect potency of an extract, but, over a wider range of concentration, the relationship between extract concentration and releasing value appeared logarithmic. Raising newborn snakes on an artificial force-fed diet for over 6 mo. did not alter the prey-attack behavior itself or the relative effectiveness of extracts from a variety of organisms. This "releasing mechanism" is resistant to stimulus and response deprivation.

Much of the serpent's behavior involves the use of chemical senses, especially the olfactory and vomeronasal organs. The constellation of activities involved in finding and ingesting food depends particularly upon chemical stimulation (Baumann, 1929; Naulleau, 1966; Noble & Clausen, 1936; Wilde, 1938). It has been found that inexperienced newborn garter snakes (*Thamnophis s. sirtalis*) will attack objects which contain chemical stimuli from the skin of normally eaten prey, such as earthworms or fish (Burghardt, 1966b). In fact, the only way to elicit this prey-attack response in naive snakes is through chemical stimulation. Different species, even within the same genus, will attack different extracts corresponding to their species characteristic feeding habits (Burghardt, 1967).

The relations between stimulus and response in this behavior make it closely akin to the "releasing mechanism," both as classically conceived and as reevaluated (Schleidt, 1962). The behavior involves a chemical sign stimulus in an advanced vertebrate, and the innate component appears to be of great importance. The present experiments were designed to elucidate some of the parameters governing attack on prey extracts in inexperienced snakes. The factors investigated include the critical sensory modality involved, extract age, extract concentration, and the effects of prolonged periods of deprivation before the first opportunity to attack.

[1] This report is based on a dissertation submitted in partial fulfillment of the requirements for the PhD degree at the University of Chicago by the first author. The research was supported by Grants MH-00776 and MH-15707 from the National Institute of Mental Health. Naturalists at the Cook County Forest Preserve (Palos) kindly provided most of the gravid snakes.

[2] Now at the University of Tennessee.

METHOD

Newborn young of three species of garter snakes were used in the experiments: *Thamnophis sirtalis*, *Thamnophis radix*, and *Thamnophis butleri*. Wild-caught gravid snakes were kept in 4-gal. aquaria and offered minnows and earthworms until parturition. The young were individually housed after birth. Descriptions of the extract technique, stimulus presentation, and scoring are found in Burghardt (1966a, 1967).

In Experiments 2 and 3, *S*s were tested on a series of extracts for comparative purposes on the third and fourth day after birth before the present experiments.

The tongue-flick attack score was the sum of the base unit and the quantity (60 - response latency in seconds). The base unit was the maximum number of tongue flicks given by any individual of the litter tested to any of the test stimuli; the maximum was invariably to a noncontrol swab. Those *S*s which did not attack were given scores identical with the number of flicks they emitted toward the swab in the 1-min. test period.

EXPERIMENT 1

Burghardt (1966b) showed that in the absence of chemical cues, visual stimuli from live normally eaten prey did not elicit attack behavior in newborn snakes. Per-

TABLE 1

EFFECTS OF SENSORY ELIMINATION ON RESPONSE TO PREY EXTRACTS IN NAIVE SNAKES

Group	Total attacks	*M* attack latency (sec.)	*M* tongue-flick attack score	*M* control score
Test day				
1	6	34.1	64.1	11.1
2	4	19.0	61.7	8.4
3	4	16.7	58.1	10.5
4	4	26.1	50.7	7.1
Control day				
1	1	54.2	17.1	3.1
2	2	18.9	29.3	5.2
3	2	33.6	24.0	2.5
4	5	19.2	64.0	5.4

haps, however, visual information of some kind was necessary in order for an attack response to be made when chemical cues were present. Moreover, any of several chemical senses may have been involved in the response to the chemical cues themselves. By severing the olfactory and vomeronasal nerves in adult *Thamnophis sirtalis*, Wilde (1938) showed that Jacobson's organ was most important in the actual attack on prey. Is Jacobson's organ also the critical chemical receptor in naive newborn snakes? Gustation seems to be ruled out as a possibility since the taste buds are restricted to the rear of the mouth and none appear on the tongue (Oliver, 1955). In Experiment 1, ingestively naive and previously untested young had vision and olfaction eliminated.

Method

Subjects. Twenty *Thamnophis radix* were born of a female which came into our possession 2 mo. earlier. The mother refused to eat the entire time she was in captivity, but did not show any signs of weight loss, disease, or sluggishness.

Procedure. The 20 *S*s were placed in the standard experimental situation and randomly divided into four groups of five each. In Group 1, olfaction was eliminated; in Group 2, vision was eliminated; in Group 3, both vision and olfaction were eliminated; and *S*s in Group 4 remained normal. The two sense organs were eliminated by covering the eyes and nostrils with a mixture of collodion and lampblack. Enough lampblack was added to the collodion so that the thinnest coating possible with the brush used would make clear plastic opaque. The mixture was applied in two coats 20 and 15 min. before testing began. A small photographer's sable hair touch-up brush was used (No. 0). The eye of a snake does not have a movable eyelid but is covered with a transparent scale which makes the present procedure feasible.

The testing was done on the seventh day after birth. The stimuli were presented in the standard manner on cotton swabs. The *S*s were tested on a distilled water control swab, then on a nightcrawler (*Lumbricus terrestris*) extract prepared in the usual manner (3 gm. per 20 cc of 50°C. distilled water). The control and nightcrawler extracts were then presented to *S*s again. The *S*s in each of the four groups were tested successively so that about 30 min. elapsed between tests for individual *S*s. The collodion mixture was removed immediately after the last test by applying alcohol to soften the dried covering and then gently lifting off the plug with a fine-nosed forceps.

The following day the same testing procedure was repeated with the 20 *S*s without the application of the collodion mixture. All testing was done in the early afternoon.

Results

Table 1 gives the mean score for each group. Both trials for each snake on a given day were combined as no consistent order effects were evident. It is rather clear that normal *S*s did not differ from any of the other three groups on the day when collodion was present. A one-way analysis of variance of Day 1 scores minus control scores showed virtual equality among the groups ($F = .07$, $df = 3/16$). On the following day, when all the snakes were normal, all the groups showed a decrease in mean score except the normal group, which showed an increase. Analyses of variance on Day 2 scores minus control scores ($F = 1.71$, $df = 3/16$) and on the differences between Days 1 and 2 ($F = 2.70$, $p < .10$) failed to reveal significant differences.

Although Day 1 scores were similar for all groups, observations of *S*s' behavior in the test situation revealed differences. The *S*s with covered eyes appeared to be functionally blind (Groups 2 and 3); waving a hand in front of them did not result in the flight response usually elicited in normal awake snakes. Introduction of the swab resulted in the usual tongue flicking, but there was no quick following of movement of the stimulus. Attacks, when they occurred, were undirected, i.e., *S* lunged forward slightly and opened its mouth but did not aim the attack at the swab itself. Therefore, although vision was not necessary for an attack to occur, the differences from normal *S*s were quite dramatic.

Covering the nostrils did not seem to cause such gross differences. However, it seemed that the anosmic *S*s did not begin tongue flicking as quickly.

Discussion

The present experiment eliminates olfaction and vision as critical cues in the innate elicitation of the prey-attack response. It can be concluded that Jacobson's organ is the necessary and sufficient modality. In this, the more direct experiments by Wilde (1938) on adult snakes are supported. The study further demonstrates that vision is not necessary even as a supportive factor. Visual aspects of prey are, therefore, less important than in lizards, especially iguanids (Burghardt, 1964).

Although the decreased Day 2 scores of those *S*s which had collodion applied on the previous day cannot be satisfactorily explained, several possible reasons suggest themselves. An emotional or fear reaction may have resulted from the handling of the *S*s while applying and removing the collodion on Day 1. The application of the collodion itself may have had a painful or irritative effect, which lowered the reactivity of *S*s on Day 2.

The finding that olfaction was not necessary when the source of chemical stimulation was close to *S* does not prove that olfaction is not involved in feeding behavior. A series of early experiments by Noble and his associates (Noble & Clausen, 1936; Noble & Kumpf, 1936) showed that in the trailing and locating of prey, olfaction, rather than Jacobson's organ, was the important chemical receptor. Olfaction may well serve as the chemical distance receptor by which snakes sense prey and other stimuli. Recent work on rattlesnakes by Cowles and Phelan (1958) supports this view.

Experiment 2

An important aspect of the prey-attack response to chemical stimuli is the concentration of chemical needed to elicit the response. In this experiment, the concentration of the releasing substance was varied both by changing the weight of prey used per given amount of extracting medium and by dilution of the extracts themselves. Since extracts were used for several days after preparation, an older extract was also compared to a freshly prepared one.

Method

Subjects. The *S*s were 20 ingestively naive young *Thamnophis sirtalis semifasciata.*

Procedure. The standard extract was made in the usual manner with 3 gm. of leafworm (*Lumbricus rubellus*) per 20 cc of distilled water (1A); some of this extract was diluted with distilled water to one-half (1B) and some to one-fourth (1C) the original strength. Another solution was prepared using 6 gm. of washed worms in 20 cc of water (2A); this, too, was diluted in making two more extracts to one-half (2B) and one-fourth (2C) of its original strength. An extract (3) made by using 1.5 gm. of leafworm to 20 cc of distilled water was used only at full strength. In addition to these seven extracts prepared on the day of testing, a standard 3 gm/20 cc extract had been prepared 3 days earlier and kept refrigerated until the day of testing.

In the usual manner each *S* was given 8 tests with the extracts and 2 control tests with distilled water presented on the first and last trial of the 10. The order of the eight test extracts was systematically varied so that each *S* received a different sequence. All 10 trials were run consecutively with approximately 40 min. between tests for each *S*.

Results

A summary of scores for each extract is presented in Table 2. A two-way analysis of variance showed no significant difference between any of the eight test extracts; all test extracts produced significantly higher mean scores than the controls ($p < .01$, Mann-Whitney U test, one-tailed). However, the diluted extracts always had slightly lower scores than the same extract less diluted.

No consistent correlation was apparent between age or concentration of extracts and attack frequency or latency. Since

TABLE 2

RESPONSE SCORES OF 20 NEWBORN SNAKES TO WORM EXTRACTS DIFFERING IN AGE AND CONCENTRATION

Extract		Total attacks	*M* attack latency (sec.)	*M* score
Preparation	Concentration			
H_2O	—	0	—	16.2
H_2O	—	0	—	14.9
3 gm/20 cc	1	13	11.7	103.8
3 gm/20 cc	½	12	19.8	98.1
3 gm/20 cc	¼	10	11.7	85.2
6 gm/20 cc	1	12	12.9	100.5
6 gm/20 cc	½	10	6.8	94.7
6 gm/20 cc	¼	11	14.5	93.8
1.5 gm/20 cc	1	9	8.4	87.8
3 gm/20 cc[a]	1	12	13.4	102.0

[a] Three days old.

changes correlated with extract concentration were in the expected direction when the tongue-flick data were utilized, the validity of the standard scoring procedure over the mere consideration of attack data was supported.

Discussion

This experiment showed that within the limits of extract variability normally encountered, age and concentration of the extract are of little importance.

EXPERIMENT 3

The previous experiment showed that variations in preparation and dilution of an extract had little effect if limited to a factor of 2. Experiment 3 was concerned with the strength of the response when the standard extract was diluted by successive factors of 10. Although the exigencies of time and space precluded the use of completely naive young in this study, the *S*s had never eaten the prey object used for making the test solutions.

Method

Shortly after birth, 12 Butler's garter snakes (*Thamnophis butleri*) were tested on a series of different extracts. After they had been maintained communally and raised on an all-horsemeat diet for 7 mo., they were placed individually in the standard test situation for these tests.

A nightcrawler extract was made in the standard manner on the day of testing. Through dilutions with distilled water, five solutions were prepared having the following concentrations of the standard extract: 1, .5, .1, .01, .001. Since each *S* was also given one control swab of distilled water, six tests were run on each *S*. The basic order, 1, .1, H_2O, .001, .5, .01, was sequentially rotated so that two *S*s were run with each extract first. The half-strength extract (.5) was included in the series for another purpose and will not be discussed further as our interest is in the more dilute extracts. About 25 min. elapsed between each test for an *S*.

Results

Figure 1 shows the results for the test extracts. The attack rate to distilled water (28.7) was a fraction higher than that to the .001 strength extract (27.8). While the .1% solution resulted in no attacks, the tongue-flick data permit the computation of an attack-rate score. The solution, when diluted to 1% of its original strength, produced one attack with a latency of 25 sec. However, the .1 (10%) solution had an overwhelming superiority and elicited five attacks with a mean latency of 43.4 sec. ($p < .001$, Mann-Whitney *U* test, one-tailed). Full strength, the solution yielded nine attacks with a mean latency of 11.8 sec. A logarithmic relationship between concentration and effectiveness seems indicated.

Discussion

These results clearly illustrate the sensitivity of snakes to meaningful chemical stimuli. In addition, a tentative relationship between concentration and potency is

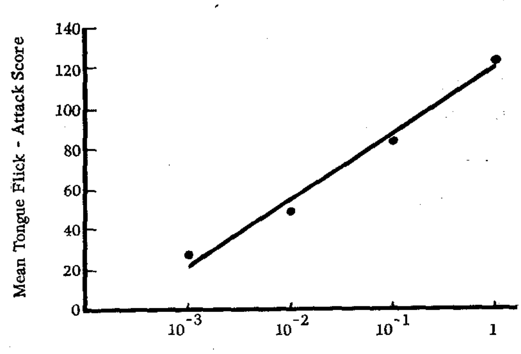

FIG. 1. Results for 12 Butler's garter snakes tested on nightcrawler extracts of varying concentrations.

indicated. Until the active substances are identified, however, this relationship cannot be expressed in precise chemical or quantitative terms, as can be done with known odiferous substances (Engen, 1964).

Since the response to the full-strength extract was close to the maximum as measured by the tongue-flick attack formula, and the response to the .001 extract was almost identical with the controls, it is probable that the range of concentration used covered the range over which changes in response level with changes in concentration, as measured by the present method, will be seen.

Experiment 4

Although there have been many attempts to raise animals without certain sensory experiences, no experiments appear to have been done involving long-term deprivation of a chemical sense. While there is presently no way in which Jacobson's organ can be reversibly eliminated, it is possible to restrict the animal's experience to specific and behaviorally crucial stimuli.

An important question concerning the prey-attack response and its innate perceptual schema or releasing mechanism is how dependent the behavior pattern is upon performance of the response and perception of the sign stimulus. In other words, does instinctive regression occur if the animal is deprived of certain experiences (Thorpe, 1956)? It is known that certain behaviors, just as physical structures, can atrophy if not used or exercised.

Adult snakes maintained in captivity readily ate food which they would be unlikely to have experienced in nature. For instance, many of the *Thamnophis* ate guppies. Experience with one type of fish might generalize to all kinds of fish via a common chemical factor. However, some snakes ate several types of fish even though they were captured in areas distant from bodies of open water containing any species of fish. It is conceivable that experience with any type of prey releasing the innate prey-attack response was sufficient to keep the response activated to all prey objects to which the young snake responds at birth.

The present experiment was designed to assess the importance of experience to the newborn snake in the elicitation of prey attack and the form of the attack itself. Will the prey-attack response and the releasing mechanism be present, altered, or absent in an *S* raised from birth on an artificial diet which it would not eat by itself and tested at an age it could not have reached in nature without feeding? Will there be any change in the response to the artificial food?

Method

Subjects. The surviving 43 young from a litter of 46 *Thamnophis sirtalis semifasciata* were used. Fifteen were chosen at random to undergo testing shortly after birth on 12 different animal surface extracts and a water control. The remaining 28 young began the experimental regime described below.

Procedure. All *S*s were isolated in a room in which the 12 animals used to provide extracts for the testing of their littermates were never present. They were kept communally in two 2-gal. aquariums. At 2 mo. of age, *S*s were moved into the testing room and transferred to individual testing tanks covered with plate glass tops.

Beginning when *S*s were 30 days old, they were force fed at approximately 4-day intervals. The food was Gerber's strained liver baby food which, besides beef liver, contains only water and salt. The *S*s ignored it when offered to them. The food was occasionally mixed with .5 cc of Poly-Vi-Sol liquid vitamin drops per 3½-oz. jar. The liver was kept refrigerated between feedings.

The *S*s were fed with a syringe to which was attached a 7.5-cm. section of plastic tubing 3 mm. in diameter. A short section of rubber tubing was used to attach the less flexible and narrower plastic tubing to the base of the syringe. The syringe was filled and the tubing gently forced down *S*s throat to a distance of approximately 4 cm. Then the appropriate amount of food was injected.

Many of the snakes died before reaching the age at which they were to be tested. Some died of skin diseases, others because they were given too much food at a given time. Through trial and fatal errors, .4 cc was found to be the optimum amount of food to be given in one injection. Others may have died of internal factors connected with the forcing of the tube down the throat. It was necessary to force the tube down a comparatively long way to prevent regurgitation of the liver. Also, since snake raising is not yet an exact science, several may have died from causes completely unrelated to the specific experimental situation.

TABLE 3

EXTRACT TEST RESULTS FOR NEWBORN AND DEPRIVED GROUPS OF GARTER SNAKES

Stimulus[a]	Days deprived					
	4		64		191	
	Total attacks	*M* score	Total attacks	*M* score	Total attacks	*M* score
H_2O	0	17.3	0	16.9	0	27.5
NC	11	93.9	3	76.3	2	101.2
LW	10	84.2	2	65.8	2	99.6
RW	4	60.7	3	79.4	2	100.6
SA_l	9	83.4	2	67.4	2	78.6
SA_a	2	27.5	1	40.8	0	16.5
FR	3	50.3	0	33.4	2	79.6
M	7	60.7	0	41.4	1	46.9
G	4	48.0	0	24.2	0	40.0
GF	1	37.4	1	47.9	1	40.9
LE_p	5	65.6	—	—	—	—
LE_m	—	—	1	42.9	1	50.3
SL	0	25.5	0	12.4	0	12.5
BM	0	21.3	0	16.4	0	38.0
MT	—	—	—	—	0	17.5
LI	—	—	—	—	0	21.0

[a] LW—leafworm (*Lumbricus rubellus*); NC—nightcrawler (*Lumbricus terrestris*); RW—redworm (*Eisenia foetida*); G—guppy (*Lebistes reticulatus*); M—minnow (*Notropis atherinoides acutus*); GF—goldfish (*Carassius auratus*); FR—cricket frog (*Acris crepitans blanchardi*); SA_a—salamander adult (*Ambystoma jeffersonianum*); SA_l—salamander larva (*Ambystoma jeffersonianum*); LE_p—turtle leech (*Placobdella parasitica*); LE_m—giant leech (*Macrobdella decora*); SL—slug (*Deroceras gracile*); BM—baby lab mouse (*Mus musculus*); MT—horsemeat (*Equus caballus*); LI—beef liver (*Bos taurus*).

At 64 and 65 days of age, five *S*s were tested on their reaction to a set of 12 stimuli similar to those used to test their littermates at 4 and 5 days of age. Two other *S*s were tested similarly at 191–192 days after birth. The extracts (see Table 3), presented on swabs in the standard manner, were made from frozen or substitute prey organisms when living specimens were not available. No two *S*s in a group received the stimuli in the same order. In the groups tested at birth and after 64-days deprivation, a six-control technique was used with a distilled water swab being presented before the first test extract, after the third test extract, and after the sixth. The following day the remaining six-test extracts were presented with water swabs similarly interspersed. The 191-day deprived *S*s were tested using a one-control technique where the water swab was only presented once, but interspersed at a different point in the order for each *S*. In this group, eight tests were given on Day 1 and seven tests were given on Day 2.

Results

The results for the two deprived groups are summarized in Table 3. The important point to be made is that *S*s tested at ages 64–65 days and 191–192 days showed an attack response toward the test swabs that was indistinguishable from that of the newborn *S*s. This held across all classes of prey objects effective in the newborn snakes. Maximum tongue-flick scores were 85, 59, and 49 for the 4-, 64-, and 191-day groups, respectively.

Some *S*s in the 64-day deprived group were not as reactive as others. These *S*s were getting ready to shed their skins, as evidenced by their cloudy eyes; at such a time little, if any, feeding occurs. Nevertheless, their scores were averaged along with the rest. The rank correlations of the 13 stimuli common to the two deprived groups was highly significant ($rs = .83$, $p < .01$). Correlations between the deprived groups and the newborn group were based on 12 stimuli since the same species of leech was not used throughout. The correlations of the 64- and 191-day deprived *S*s with the newborn *S*s were .83 and .88, respectively ($p < .01$). No increased interest was shown in the extract from the liver that had been the snakes' total diet, nor would they eat it when placed in front of them.

Discussion

Changes due to deprivation did not occur either in the prey-attack response nor in the innate schema within 6 mo. after birth. Indeed, the rank ordering of the releasing values of the various stimuli remained quite constant. It is reasonable to assume that longer deprivation would have had little effect unless, perhaps, continued for years. Further, no learned association between the odor of the force-fed food and the food reinforcement was demonstrated.

This experiment does not rule out the possibility that experience with attacking and eating foods may alter snakes' preferences as measured by either food-choice techniques or response to chemical extracts in a manner similar to that shown to obtain in turtles (Burghardt & Hess, 1966). What has been shown here is that in the absence of any experience of this type, the perceptual and motor patterning of this behavior remains intact. It is therefore clear that in snakes the innate releasing mechanism, as exhibited by the relative preferences for food-related chemical stimuli, is stable and does not need reinforcement during ontogeny.

REFERENCES

Baumann, F. Experimente über den Geruchssinn und den Beuteerwerb der Viper (*Vipera aspis L.*). *Z. vergl. Physiol.*, 1929, **10,** 36–119.

Burghardt, G. M. Effects of prey size and movement on the feeding behavior of the lizards *Anolis carolinensis* and *Eumeces fasciatus*. *Copeia*, 1964, 576–578.

Burghardt, G. M. Prey attack in naive snakes: A comparative and analytic study on the role of chemical perception. Unpublished doctoral dissertation, University of Chicago, 1966. (a)

Burghardt, G. M. Stimulus control of the prey attack response in naive garter snakes. *Psychon. Sci.*, 1966, **4,** 37–38. (b)

Burghardt, G. M. Chemical-cue preferences of inexperienced snakes: Comparative aspects. *Science*, 1967, **157,** 718–721.

Burghardt, G. M., & Hess, E. H. Food imprinting in the snapping turtle, *Chelydra serpentina*. *Science*, 1966, **151,** 108–109.

Cowles, R. B., & Phelan, R. L. Olfaction in rattlesnakes. *Copeia*, 1958, 77–83.

Engen, T. Psychophysical scaling of odor intensity and quality. *Ann. N. Y. Acad. Sci.*, 1964, **116,** 504–516.

Naulleau, G. *La biologie et le comportement prédateur de* Vipera aspis *au laboratoire et dans la nature*. Paris: Fanlac, 1966.

Noble, G. K., & Clausen, H. J. The aggregation behavior of *Storeria dekayi* and other snakes with especial reference to the sense organs involved. *Ecol. Monogr.*, 1936, **6,** 269–316.

Noble, G. K., & Kumpf, K. F. The function of Jacobson's organ in lizards. *J. genet. Psychol.*, 1936, **48,** 371–382.

Oliver, J. A. *The natural history of North American amphibians and reptiles.* Princeton: Van Nostrand, 1955.

Schleidt, W. Die historische Entwicklung der Begriff "Angebornes auslösendes Schema" und "Angeborener Auslösemechanismus" in der Ethologie. *Z. Tierpsychol.*, 1962, **19,** 697–722.

Thorpe, W. H. *Learning and instinct in animals.* Cambridge: Harvard University Press, 1956.

Wilde, W. S. The role of Jacobson's organ in the feeding reaction of the common garter snakes (*Thamnophis sirtalis sirtalis*). *J. exp. Zool.*, 1938, **77,** 445–465.

(Received March 24, 1967)