Regardless of the mechanisms responsible for the multispiked action potentials, spikes probably are conducted along the IO axon, since the interval between spikes agrees with the delay between excitatory postsynaptic potentials (2 msec) evoked by the climbing fibers in Purkinje cells (Fig. 1) (2).

A graded depolarizing synaptic potential followed by a graded hyperpolarizing potential occurred with orthodromic stimulation (Fig. 2B), and rebound firing was often present after the hyperpolarizing potential (Fig. 2, C and D). These synaptic potentials will be discussed in detail in a later report. It is important to note here that a similar depolarizing-hyperpolarizing potential (with mean latency of 5 msec) was frequently observed following cerebellar stimulation (Fig. 2D), in addition to the all-or-nothing spike complex. Usually the threshold for the orthodromic action potentials from cerebellum was higher than that for antidromic firing. In nine cells, however, the orthodromic discharge was evoked at a lower stimulus intensity (Fig. 2I); the mean latency for this group was 9.5 ± 4.2 msec. If the current of the stimulus was increased the variable latency of these cells to the constant value characteristic of an antidromic response (Fig. 2J) was suddenly shortened. Thus, cerebellar stimulation can evoke a transynaptic response in an IO cell by a route other than antidromic excitation of its own axon. This is supported by Eccles' data showing that the "reflex climbing fiber response" of a Purkinje cell may be evoked without exciting the climbing fibers to that cell (2).

This study has shown that the mechanism of the IO cell unitary repetitive discharge seen after orthodromic, antidromic, or direct intracellular stimulation does not involve reactivation by way of recurrent collaterals. It cannot yet be stated whether transynaptic excitation of IO cells after cerebellar stimulation occurs by way of recurrent IO axon collaterals, by way of axon collaterals from mossy fibers, or possibly by way of a separate orthodromic cerebello-olive pathway.

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14 June 1967

Chemical-Cue Preferences of Inexperienced Snakes: Comparative Aspects

Abstract. Different species of new-born, previously unfed snakes will respond with tongue flicking and prey-attack behavior to water extracts of the skin substances of various small animals. However, there are clear species differences in the type of extract responded to by previously unfed snakes, even within the same genus. These differences correspond to the normal feeding preferences shown by the various species.

It has often been noted that animals can selectively respond to certain highly specific perceptual cues without the benefit of previous experience with those cues (1). The stimuli involved usually represent but a small fraction of the entire stimulus situation and are termed sign stimuli or releasers. In many instances the resulting response is also quite specific and stereotyped. For instance, newborn, previously unfed garter snakes (Thamnophis s. sirtalis) will respond with prey-attack behavior to extracts of the surface substances of normally eaten prey when these extracts are presented on cotton swabs (2). Similar specificity to chemical cues has been demonstrated in many forms of invertebrates and, to a lesser extent, in vertebrates (1).

Beyond the existence and analysis of such stimulus–response relations in a particular species looms the broader evolutionary implications. I here report the chemical perception aspects of feeding behavior in a number of species of neonate colubrid snakes.

I presented a variety of extracts from the surface substances of small animals to litters of individually isolated newborn snakes. The animals used in preparing extracts for the testing were: nightcrawler (Lumbricus terrestris), leafworm (Lumbricus rubellus), redworm (Eisenia foetida), turtle leech (Placobdella parasitica), slug (Deroceras gracile), cricket (Acheta domestica), minnow (Notropis atherinoides acutus), guppy (Lebistes reticulatus), goldfish (Carassius auratus), larval salamander (Ambystoma jeffersonium), metamorphosed salamander (Ambystoma jeffersonium), cricket frog (Acris crepitans blanchardi), and newborn mouse (Mus musculus). An extract was made by placing one or more of the intact animals in distilled water (10 ml of water per 1.5 g of body weight) at 50°C for 1 minute and stirring the water gently. The animal was then removed and the resulting liquid centrifuged and refrigerated until use. Extracts for any one experiment were always prepared on the same day.

The snakes were from litters or eggs borne by gravid females captured in the field and maintained in captivity until parturition or egg-laying. Shortly after birth or hatching the snakes were weighed, measured, and then isolated in glass tanks measuring 23 by 14 by 17 cm. Each tank was placed on white shelf paper and the four outside walls were covered with white partitions. The floor of the tank was bare except for a small plastic petri dish containing water. Except for testing periods, the aquaria were covered with glass tops. The temperature of the room in which the snakes were housed never varied more than between 22° to 26°C; during testing the temperature was maintained at 24° to 25°C.

Each member of a given litter of newborn snakes was tested only once on a series of extracts of the surface substances of potential prey. Usually twelve or thirteen different extracts were used. Distilled water was the control. Each subject received a different ordering of the test extracts, systematically balanced insofar as possible for each litter. Testing was carried out over 2 or 3 successive days beginning on the 3rd or 4th day of life and always before any previous feeding or exposure to the extracts (3). The testing procedure consisted of dipping a 15-cm

cotton swab into the extract or control, slowly introducing it into the tank, and bringing it within about 2 cm of the snake's snout. If the swab was not attacked within 30 seconds, it was moved closer until it touched the snout gently three times, as actual contact with the lips of the snake is sometimes necessary to elicit an attack. If no attack was made at the end of 1 minute, the swab was removed and the total number of tongue flicks emitted in the 1-minute interval recorded. If the swab was attacked, the elapsed time, measured to the nearest 0.1 second, was recorded (4).

An extract, when not actually eliciting a prey-attack response, would often elicit a large number of tongue flicks over and above that elicited by distilled water. It appeared as though the frequency of tongue flicking was correlated with the intensity of arousal by or interest in the swab. Since previous experiments indicated that the prey-attack response in snakes is mediated by the tongue–Jacobson's organ system (3, 5), tongue-flick data can reasonably be considered along with the attack data in assessing the relative releasing value of various extracts on different species of snakes. The control swab (distilled water) never elicited a prey-attack response in an inexperienced snake, although sometimes a large number of flicks would occur. In most of the species studied here, aggressive behavior was never shown by the newborn snakes; and, indeed, it was impossible to provoke such behavior. A conservative scoring system was used to score the extract given to each snake. The scoring system was based

on the assumptions that an actual attack is more definitive than any number of tongue flicks and that a more potent stimulus will lead to an attack with a shorter latency than will a weaker stimulus. 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 given to a swab containing an extract). A snake which did not attack was given a score identical with the number of tongue flicks it emitted in the 1-minute test period. If the snake did attack, it was given a score identical with the base unit (for that litter) plus one point or fraction for every second or fraction less than 1 minute that it responded. The score for an attacking subject can be represented by base unit $+$ (60 $-$ response latency), measured in seconds.

Figure 1 shows an example of the type of profile obtained when a litter is presented with a series of extracts. The species represented is the eastern plains garter snake, *Thamnophis r. radix*. The 22 living young from a litter of 24 born to a female captured at the Palos Forest Preserve were tested. Each of the previously unfed snakes was presented once with extracts prepared from earthworms, leeches, slugs, crickets, fish, amphibians, and baby mice. Responses to all extracts were significantly higher than those to the control except for those to the baby mouse, slug, cricket, and metamorphosed salamander extracts ($P < .01$, t-test). Although no extensive ecological studies have been done on this species, it appears that earthworms, amphibians, fish, and leeches are readily eaten, with

worms being probably most common in the natural diet (6). The present results with inexperienced newborn snakes on isolated chemical cues are in remarkably close agreement. Worms as a class were more effective than fish as a class, as there was no overlap in the mean scores for the various worm and fish species. The increased releasing value of the larval salamander over the metamorphosed form ($P < .0005$, t-test) is a relationship that has been found frequently in most species of newborn snakes tested that include amphibians in their normal diet. A chemical change in the skin during metamorphosis is probably responsible for the difference.

In sharp contrast to these results were results obtained from the western smooth green snake, *Opheodrys vernalis blanchardi*. This species is oviparous, and five young from a clutch of seven eggs laid by a female captured on the Palos Forest Preserve were tested. The eggs hatched on the same day that the plains garter snakes were born. The green snakes were tested along with the plains garter snakes on the same extracts at the same time. Although each green snake received a different ordering of the extracts, each sequence was identical to one used with a plains garter snake. In Fig. 2 the results for the green snake are presented for the same extracts as shown for the plains garter snake. The cricket extract was the most potent; indeed, it was the only extract to which actual attacks were made and the only one with a score significantly higher than that of the control ($P < .004$, Mann-Whitney U test, one-tailed). This result becomes
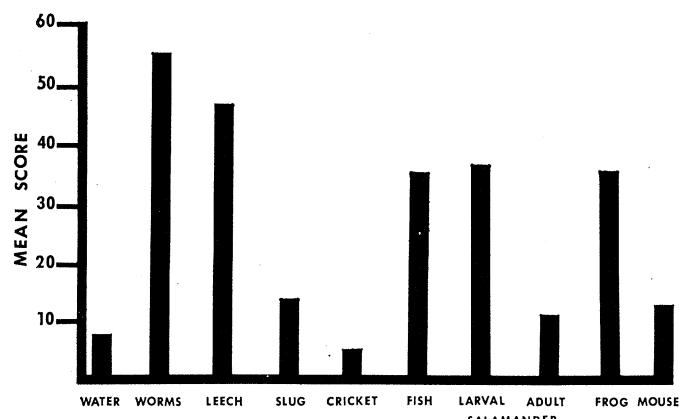
Fig. 1 (left). Response profile of 22 newborn, previously unfed eastern plains garter snakes (*Thamnophis r. radix*) to water extracts from the surface substances of various small animals. The results for the three species of earthworms and the three species of fish have been averaged together.   Fig. 2 (right). Response profile of five newly hatched, previously unfed western smooth green snakes (*Opheodrys vernalis blanchardi*). The snakes were the same age as those in Fig. 1 and were tested at the same time and with the same extracts.
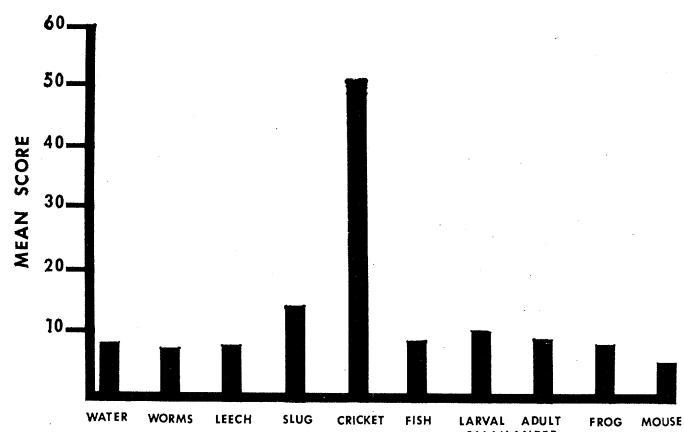
more meaningful when it is realized that the cricket extract is the only one which represents an organism eaten by the green snake. In fact, this species apparently will eat only insects, spiders, and perhaps small soft-bodied arthropods.

With literally every procedural detail controlled, clear differences were found in the chemical perception of food objects in the two species of inexperienced snakes. In contrast to the green snake, the plains garter snake does not show any interest in insects as food and the cricket extract received the lowest score of all the extracts. Likewise the green snake was uninterested in those extracts which elicited significant responses in the plains garter snake.

To test these results further, three more closely related forms were studied in the same manner, although only differentiating results for the three earthworm, three fish, and slug extracts will be presented. The eight surviving young of a litter of ten born to a midland brown snake (*Storeria dekayi wrightorum*) captured on the Palos Forest Preserve were tested. Of the above three classes of prey—earthworms, fish, and slugs—this species is known to eat only worms and slugs. The second species tested was Butler's garter snake (*Thamnophis butleri*). A litter of 15 was obtained from a female caught in south-ern Michigan. In captivity, Butler's garter snake readily eats worms and fish but not slugs. The third species was the aquatic garter snake, *Thamnophis elegans aquaticus*. A litter of nine was tested which were born to a female found in southern California. Of the three classes of prey, this species is known to eat only fish.

The inexperienced young of the three species were tested on the seven extracts representing worms, fish, and slugs (Fig. 3). All scores for the midland brown snake (*Storeria*) were doubled so as to bring them up to the same scale as the two species of *Thamnophis*. For a given species of snake, all scores above 25 are significantly higher than scores below 25 ($P < .05$, t-test). The responses of the different species of inexperienced snakes to skin extracts parallels the feeding habits of specimens captured in the wild. The generally lower scores shown by the midland brown snake are due mainly to the fact that the frequency of tongue flicking was lower than for the two *Thamnophis* species. This may be an important difference also.

These results clearly indicate that chemical perception in newborn young is species-specific. That these are related to the natural feeding ecology of the species is equally clear. But an inexperienced snake will respond to chemical cues that cannot or do not figure in the normal feeding behavior of the species. For instance, the aquatic garter snake (*T. elegans aquaticus*) rarely, if ever, encounters the guppy in nature, yet the inexperienced young readily responded to the guppy extract. Since the aquatic garter snake normally eats fish, however, it is probable that the guppy possesses chemical cues similar or identical to those found in fish which the snake normally eats. In Butler's garter snake (*T. butleri*), the situation is a little more complex, for fish do not constitute any part of the species' normal diet (6). Yet specimens readily eat fish in captivity and newborn young, as shown here, respond significantly to fish extracts. It is, therefore, apparent that the normal feeding habits and ecology of a species are not sufficient to explain the response to chemical cues in newborn young. In this case Butler's garter snake may retain the perceptual side of releasing mechanism that appears to be of no selective advantage in its present mode of life. Of course, retention of the potential to respond to chemical cues from fish by inexperienced snakes would be advantageous if a change in the environment occurred such that fish became a necessary or more easily obtainable food source. The same situation is found with amphibians in this species. Amphibian extracts are responded to by newborn snakes but amphibians also do not form a part of the normal diet of Butler's garter snakes. Therefore, in relation to the extracts used, Butler's garter snake would seem to possess more innate perceptual responsivity than does the aquatic garter snake, which did not respond to any of the worm extracts. Butler's garter snake is generally considered as having evolved from the plains garter snake which not only innately responds to extracts from fish and amphibians, but also normally eats them (7).

The results are open to an evolutionary interpretation. Highly specific stimulus-response information is probably genetically coded in the organism and must in part, at least, be expressed by an innate filtering mechanism at the level of Jacobson's organ or even within the central nervous system itself. This does in no way, of course, rule out the possibility that subsequent feeding experiences (or perhaps even maternal feeding) can influence the feeding preferences of newborn snakes. Indeed, something akin to food imprint-
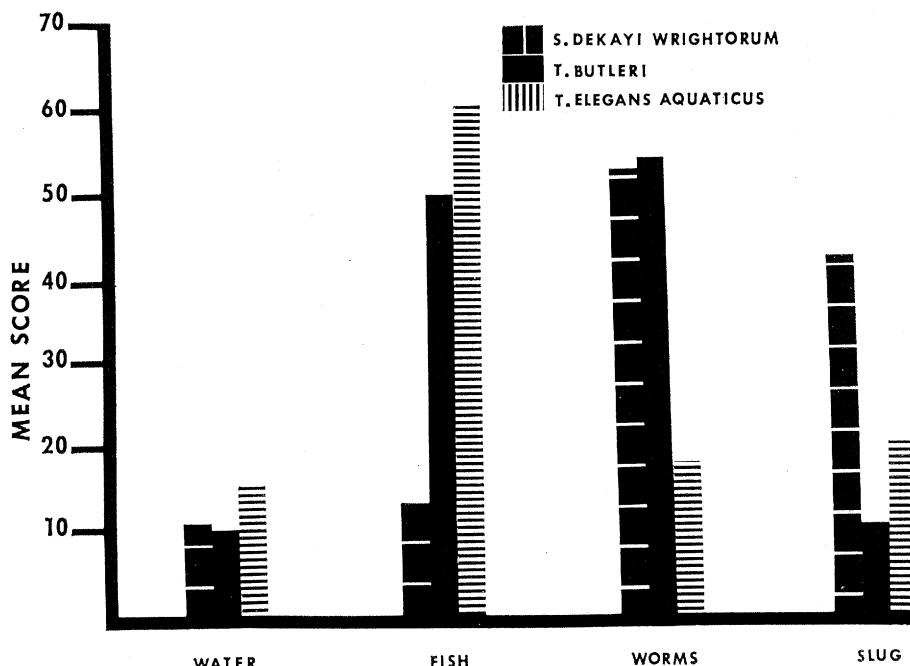

Fig. 3. Differential results for three species of newborn, previously unfed snakes with extracts from the surface substances of three classes of potential prey. Three species of worms and three species of fish were used. All scores for the midland brown snake (*Storeria dekayi wrightorum*) have been doubled to bring them up to the same scale as the two species of *Thamnophis*.

ing, such as already demonstrated in turtles, may take place (8). In any event, the present data show that innate perceptual differences can be useful in the study of closely related as well as more distantly related forms, and that the analysis of the chemical perceptual mechanisms involved should consider evolution and ecology.

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13 April 1967   ■

Effect of Neuraminidase on Isozymes of Alkaline Phosphatase and Leucine Aminopeptidase

Law's report (1) on the effect of neuraminidase on isozymes of alkaline phosphatase and leucine aminopeptidase would be of greater interest if sufficient details had been given to allow the reader to evaluate the validity of the data presented. The effects of neuraminidase on the fast-moving alkaline phosphatase isozyme of human serum are well known from the work of Robinson and Pierce (2), a paper not cited by Law. These authors found that the degree of retardation of the fast-moving phosphatase isozyme is dependent upon neuraminidase concentration. With high concentrations of neuraminidase, the migration of the fast component was retarded to a point much closer to the origin than that of the normal slow-moving isozyme.

Law reports the effects of only one concentration of neuraminidase and contends that the presence of neuraminic acid accounts for the difference between the isozymes. This conclusion is dubious unless it can be shown that higher concentrations of neuraminidase or treatment for a longer time do not further alter the mobility of the fast-moving isozyme.

Although Law mentions in a footnote the activity at pH 5.1 of the neuraminidase preparation he used, he fails to mention the pH he used in his experiments on the serums. [Neuraminidase preparations from Sigma Chemical Co. are either from *Vibrio cholera* or *Clostridium perfringens* (3); the enzymes from both sources are most active in a pH range of approximately 4.5 to 6 (4).] Assuming that Law treated the serums at a pH appropriate for effective neuraminidase activity, one would like to know the effect of this pH on the mobility of the enzymes not treated with neuraminidase. In view of the fact that Law mentions no control of normal serum treated with the buffer used for neuraminidase treatment, how may one determine whether the alteration of mobility is due to the action of neuraminidase or to the effect of lowered pH on the enzymes? One might also wonder whether there is any protease activity in the neuraminidase preparation.

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5 June 1967

The report of Robinson and Pierce, noted by Etzler, points out that genetically determined protein variants can differ by carbohydrate components—neuraminic acid, for example. My report of studies of chickens supports their suggestion. Under the conditions of my experiments with neuraminidase, two genetically controlled isozymes of both alkaline phosphatase and leucine aminopeptidase were affected differently.

The fast-moving forms were retarded in mobility to a point similar to the slow forms which were unaffected by the neuraminidase treatment. Addition of 0.5 mg of neuraminidase directly to 1 ml of plasma did not appreciably alter the pH of the plasma; thus all the change in migration of the fast forms of the enzymes was ascribed to the neuraminidase treatment. The amount of neuraminidase present was sufficient to provide conditions for complete reaction with all the enzymes in 1 ml of plasma even if the pH was not within the approximate range of 4.5 to 6, as mentioned by Etzler. Other electrophoresis experiments with buffers covering a range of pH from 4.0 to 9.5 have shown that the fast form is always anodal to the slow form. With appropriate low pH buffers in the gels, the slow forms migrate toward the cathode, and the fast forms migrate toward the anode. This further shows the net charge differences between the two molecular forms of both enzymes.

A thorough study of the number of neuraminic acid residues on each of the enzyme molecules and comparison with the similar enzymes in man would be rewarding. One contrast between chickens and man is that no secretion of blood group substance has been reported in chickens. The relationship of alkaline phosphatase in man and the secretion of blood group substance is well known, and it has been cited by Robinson and Pierce.

The main points of my report were that genetically determined variants of both enzymes were directly related in all samples tested and that the neuraminidase treatments have led to the suggestion that genetic control of a common carbohydrate component of the enzymes is responsible for the relationship.

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10 July 1967