Reports and Letters

Latent Learning in Earthworms

It has been suggested that any attempt to explain the complex kind of purposive action made possible by a highly developed central nervous system may be premature as long as we do not possess an adequate biological theory of the comparatively simpler kinds of purposive functioning (*1*). If, therefore, our purpose lies in understanding the functional relationship that exists between a complex nervous system and its environment, we must start by obtaining information about the behavior of nervous systems belonging to simpler organisms (*2*). The present study considered the problem of learning for a comparatively "simple" animal, the earthworm.

We selected the earthworm because it is one of the lowest invertebrates in which modifiability has been demonstrated and also to allow for subsequent experimentation attempting to show that higher nervous centers are necessary for the formation of cognitive maps—that is, without the supra-esophageal ganglion, latent learning is not possible, but not in the simpler situation of the reinforced type. In addition, we agree with those workers who have emphasized the importance of analytic studies of learning in very different animal types, for these investigations should reward us with important evidence and theoretical insight.

Investigations on the behavior of the earthworm date back to the classic paper of Darwin (*3*), who went so far as to ascribe "some degree of intelligence" to it. The studies of Yerkes (*4*) and von Heck (*5*) showed that earthworms are capable of learning a simple T-maze and that removal of the first five segments (cephalic ganglia) will not result in a loss of habit until the new segments have been regenerated. The work of Swartz (*6*) has shown that the behavior of the earthworm in a T-maze is random until training is started. Robinson (*7*) and Schmidt (*8*) have recently investigated learning in the same animal, with a view toward proving and disproving, respectively, the existence of a two-factor theory of learning.

The specific problem we investigated was that of latent learning. Our hypothesis was that the animals confined to the maze for 20 hours prior to training with reinforcement would reach the criterion of seven successive correct responses (or more) out of ten trials in fewer runs than the animals that had had no previous maze experience.

Six earthworms (*Lumbricus terrestris* L.) were used, three in the latent (L) group and three in the reinforced (R) group. The T-maze was constructed as follows: The arms were constructed of glass tubing 25.4 cm long and 2 cm in diameter. The vertical arm was constructed so that it could fit into the horizontal arm, but it was detached therefrom. The position of the junction between the two arms was such that the horizontal arm was divided into a left arm of 10.1 cm and a right arm of 15.3 cm. These arms represented the negative and positive goals, respectively. At the end of the left arm (negative goal) very rough sandpaper was placed. This was followed by electrodes that delivered a shock of 1 volt. At the end of the right arm (positive goal) was a glass beaker filled with moist earth and moss. The beaker was covered with paper in order to reduce the light. The floor of the maze was lined with moist blotting paper, which was changed frequently to prevent tracking.

The following procedure was used. Group 1 (R): the animals were run with positive and negative reinforcement. Group 2 (L): the animals were allowed to run in a closed T-maze for a cumulative period of 20 hours. Negative or positive reinforcements were used during this period. At the end of this time, the animals were removed from the maze and run with positive and negative reinforcement. A correct choice was defined as turning to the right and going halfway down the right arm. The animals were run five times a day, when possible. Time between trials varied from 50 seconds to 20 minutes. A flashlight and a small camel's hair paint brush were used whenever necessary to start the animals moving when they halted for any length of time. After they had completed their runs in the maze, the animals were put in large Petri dishes, which were then put in the refrigerator, since the animals seemed to thrive best under these conditions.

The number of trials necessary for the animals in the R group to reach the required criterion was 37, 45, and 47, respectively. For the animals in the L group, the number of trials was 21, 22, and 23, respectively. The t test was employed to test the hypothesis that the mean number of trials required for learning by the R and L groups are equal against the alternative that the means are unequal. The t test substantiated (at the 0.01 confidence level) our initial hypothesis that the L group would learn more rapidly than the R group. An analysis of the complete data was done, utilizing the theory of stochastic processes. A Markov chain model was developed; its applicability to learning experiments has been discussed (*9*).

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References and Notes

1. F. A. Hayek, *The Sensory Order* (Univ. of Chicago Press, Chicago, 1952).
2. I have also considered the problem of using the ameba in T-maze situations and studying the effects of learning, and retention if any, in its progeny. However, this problem was temporarily abandoned because of experimental difficulties.
3. C. Darwin, *On the Formation of Vegetable Mould through the Action of Worms, with Observations on Their Habits* (Appleton, New York, 1898).
4. R. M. Yerkes, *J. Animal Behavior* **2**, 332 (1912).
5. L. von Heck, *Lotos* **68**, 168 (1920).
6. R. Swartz, *J. Comp. Psychol.* 9, 17 (1929).
7. J. S. Robinson, *J. Comp. Physiol. Psychol.* 46, 262 (1953).
8. H. Schmidt, *Science* **121**, 341 (1955).
9. R. P. Bharucha, unpublished master's thesis, Illinois Institute of Technology, 1953.
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Fresh-water Sawfishes and Sharks in Netherlands New Guinea

A short notice on the occurrence of sharks and sawfishes in Lake Sentani, Netherlands New Guinea, has recently been published [*Science* **121**, 759 (1955)]; about the same remarks can be found in the 18 February 1955 issue of the Australian newspaper *Daily Telegraph*. Because the data, which are said to be provided by H. Van Pel, fisheries officer for the South Pacific Commission, contain several errors, a rectification in this place seems advisable.

Lake Sentani is situated in the extreme northeastern part of Netherlands New Guinea; the eastern shore is within 10 miles southwest of Hollandia at an altitude of only about 250 feet above sea level. There is an effluent river of length about 40 miles that is, according to local (and rather unreliable) information, hardly passable in its upper reaches even for native proas (canoes). According to current theory, which is sustained by considerable evidence, this lake has been formed out of an open bay through upheaval of this part of New Guinea by tectonic forces. Volcanism is not known to have occurred in this region.

During our recent visit to Netherlands New Guinea (October 1954–May 1955), we succeeded in obtaining from Lake Santani two large sawfishes that measured 9½ and 11 feet. With the invaluable help of the Royal Netherlands Navy, we were able to ship these voluminous specimens to the Rijksmuseum van Natuurlijke Historie at Leiden. Because only small specimens or just saws have generally been collected and preserved, this was a very rare and valuable addition to our collections, much more remarkable than the occurrence of these specimens in fresh water.

After a provisional examination, we decided that both specimens seem to belong to *Pristis microdon* Latham, a species known to be at home in fresh water as well as in brackish and salt water. Specimens may occur far upstream in large rivers, and probably not only as occasional stragglers. For some species, there are strong indications of breeding in fresh water, a well-known habit of the specimens in Lake Nicaragua. For further data I refer to a previous rectification by A. W. C. T. Herre [*Science* **122**, 417 (1955)].

Because we did not have the opportunity to investigate closely the conditions in the upper reaches of the effluent river, it still remains uncertain whether sawfishes may venture so far upstream as to reach Lake Sentani. However, although conditions may prevent the intrusion of specimens of the size we collected, it seems likely that small examples can make the journey, at least under favorable circumstances—for example, in the rainy season.

Reconsidering the adaptability to salt and fresh water, and the probable existence of a passable connection between Lake Sentani and the sea, the occurrence of sawfishes in this lake can easily be explained without using the theory of gradual upheaval and gradual replacement of salt water by fresh water. I should be more inclined to adopt this theory for the explanation of the occurrence of various other species that belong to essentially marine groups—for example, jacks (Carangidae)—which do not usually invade fresh water by free will. On the other hand, fishes from marine groups with little adaptability for fresh water were found—for example, in the Digoel River near Tanah Merah, about 450 miles from the sea, in a region where obviously no gradual upheaval and consecutive gradual replacement of previously salt water has taken place.

Small sawfishes are also said to occur in the rivers near Genjem, about 15 miles west of Lake Sentani, but none could be obtained. Further specimens belonging to a different species were collected in the Digoel River near Tanah Merah.

Sharks were not collected in, or reported from, Lake Sentani, and they probably do not exist there. The only fresh-water sharks we obtained were found in Lake Jamoer, a rather large and almost circular lake (diameter approximately 5 miles) situated on the narrow neck of the Vogelkop Peninsula (longitude 135°E). The altitude is about 200 feet. The physical characteristics of the effluent river Omba are insufficiently known, which makes it at present impossible to establish with certainty whether the species is landlocked. The collected examples measure up to 5 feet and, according to a superficial examination, are closely related to the landlocked shark from Lake Nicaragua and to the Ganges shark.

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Inhibition of Audiogenic Seizures by Carbon Dioxide

In previous publications, it has been shown that the acid-base balance plays a role in the susceptibility of rats and mice to audiogenic seizure (*1, 2*). Acidosis, as determined by blood *p*H measurements (*1*) was produced by a carbonic anhydrase inhibitor (acetazoleamide). This effect was correlated with diminished seizure susceptibility. Alkalosis, produced by injections of subtetanic doses of sodium bicarbonate, conversely increased the number of seizures. Presumably, the observed inhibition of audiogenic seizures was due to the accumulation of carbon dioxide and the presence of acidosis in the animals (*3*). However, since acetazoleamide is a drug with rather diffuse actions, it seemed desirable to determine the effect of carbon dioxide without any injected agent on the susceptibility of rats to audiogenic seizures.

The audiogenic seizure apparatus used has been described elsewhere (doorbell method, *1*). A rubber tubing inlet was provided in the lid of the seizure chamber to allow the introduction of gases. Mixtures of gases prepared consisted of carbon dioxide and air, with enough oxygen added to raise the oxygen concentration to between 20 and 21 percent. The gas delivery rate into the seizure chamber was approximately 8 lit/min.

Rats were placed in the seizure chamber 2 minutes before the bell was turned on, and the various mixtures of CO_2 were run into the chamber during this time. Actual CO_2 levels at the end of 2 minutes in the seizure chamber were determined by the Haldane method (*4*). Stimulation time was 1.5 minutes, with CO_2 administration being continued throughout.

Table 1. Inhibition of audiogenic seizures by various levels of carbon dioxide. There were 24 trials in each series.

CO_2 treatment (%)	Seizures	Percentage of seizures (CO_2 rats/ controls)
2.09	14	77.7
Controls	18	
4.79	8	42.1
Controls	19	
8.91	2	11.8
Controls	17	
13.55	0	0
Controls	20	

The rats were males of the Wistar strain weighing 200 to 400 g.

All rats used in this study were originally chosen for their seizure susceptibility. The rats were divided into two groups for testing the effects of the individual levels of CO_2. One group received CO_2 at a given level on the first trial, and the other group served as controls; air was delivered into the chamber for an equal period of time. Five days later, the groups were reversed for the second trial, the former controls receiving CO_2 at the same level and the former CO_2 rats acting as controls.

A seizure was taken as a convulsion, usually clonicotonic. If a convulsion did not occur, a seizure was not counted.

Table 1 shows the gas level-response data that were obtained by administering various concentrations of CO_2 to seizure-susceptible rats and testing for seizures after 2 minutes. The CO_2 levels represent actual concentrations in the seizure chamber as determined by Haldane analysis. The various levels from top to bottom were obtained by running 2.5, 5, 10, and 20 percent CO_2 into the seizure chamber for the specified time interval. For the control rats, it was found that 0.235 percent CO_2 was present in the seizure chamber. It is apparent that CO_2 levels as low as 2.09 percent exhibit a slight effect and that 13.55 percent CO_2 resulted in complete inhibition of seizures. It is interesting to note that 8.91 percent CO_2 resulted in exactly the same values as injected acetazoleamide at 200 mg/kg; the latter values were obtained from a previous study (*1*).

In other studies on the effect of acid-