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10. Supported by grant A-3678 from the U.S. Public Health Service and by a grant from the Ministry of Education of Japan. We thank Dr. Noumura and Mr. Hirano for their valuable help.

10 June 1963

Olfactory Receptor Response to the Cockroach Sexual Attractant

Abstract. *The recently isolated sex attractant of the female American cockroach elicits an electrical response in the antennae of males, females, and nymphs of this species. These electroantennograms are known to be summated receptor (generator) potentials of many olfactory sensillae stimulated simultaneously. Many other odorous substances also elicit such responses in the cockroach antenna.*

The isolation and identification of a sexual attracting substance from the female American cockroach, *Periplaneta americana* (L.), has been reported recently (*1*). The substance was found to be 2,2-dimethyl-3-isopropylidenecyclopropyl propionate. Its biological efficiency has been ascertained by use of a characteristic behavior of the males of this species (*2*) as bioassay (*1*).

Because it is known that the *Periplaneta* males detect this attractant with olfactory sense organs in the antennae (*2*), it was of interest to test the response electrophysiologically. This was done successfully with the electroantennogram (EAG)-method which proved useful in earlier investigations on olfaction in moths (*3*).

The thread-like antenna of the cockroach is not too well suited to recording summated olfactory receptor potentials known as "EAG's." Branched antennae of other insects give greater responses, thus permitting the testing of smaller amounts of odorous substances. As with moths, recording was either made from the isolated antenna or from the antenna of a living cockroach which had been mechanically fixed with adhesive tape and wire hooks on a cork plate so that the antenna could not move. Contact was made with glass capillary Ag-AgCl electrodes inserted into the distal and proximal ends of the antenna. The rather wide electrodes were filled with insect-Ringer solution and connected to a conventional d-c oscilloscope with a cathode-follower. EAG's were recorded on moving film. While isolated antennae survived amputation and EAG's could be recorded for some hours, it was possible to record such responses from the antennae of living insects for many days.

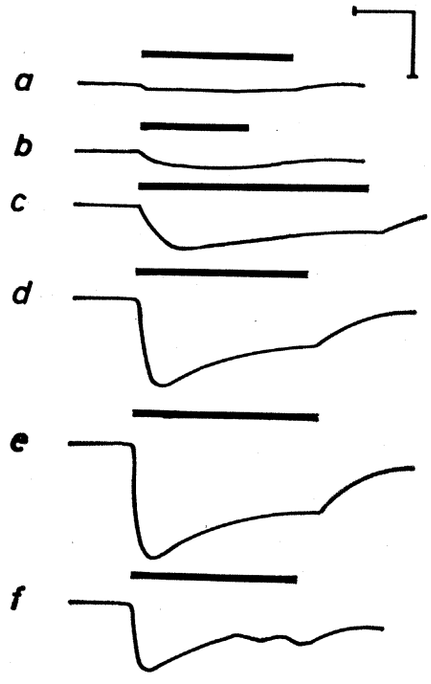

Fig. 1. Electroantennograms recorded from *Periplaneta americana* adult males in response to the chemically isolated sex-attractant odor of the female. Black bars above each trace indicate the duration of the odorous air current. Amount of natural product per cartridge: *a*, control (air without odor); *b*, 0.1 μg; *c*, 1 μg; *d*, 10 μg; and *e*, 100 μg; the cartridge *f* contained amyl acetate, 0.2 ml. Calibration: 1 mv, 1 sec.

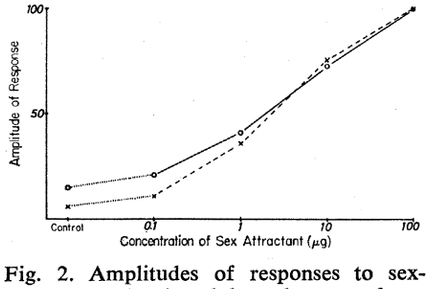

Fig. 2. Amplitudes of responses to sex-attractant odor in adult males as a function of stimulus concentration. Solid line: mean values of nine experiments, 100 μg amplitude set at 100 percent; broken line: amplitudes of the experiment demonstrated in Fig. 1.

Stimulation was accomplished by currents of air containing the odor. To achieve quantitatively constant stimuli, pieces of fluted filter paper were impregnated with a known amount of the substance to be tested. This paper was placed in short glass tubes (cartridges) mounted on the outlet of the air system in such a way that they could be easily exchanged. Air currents were controlled with a flowmeter and an electric valve, and were aimed at the whole antenna.

Figure 1 shows the result of such an experiment. In control experiments the cartridge contained only untreated paper. The amplitude of the response to the sex attractant increased in approximate proportion to the logarithm of the intensity of the stimulus (Fig. 2). By this method, the threshold concentration was found to be close to 0.1 μg per paper, when the air current was passed at a rate of 5 liter/min and the diameter of the tube was 6 mm. The distance between the air outlet and the antenna was 50 mm.

A number of other substances elicited similar responses. Amyl acetate, which, for the human nose, has an odor similar to that of banana, is known to attract cockroaches and always gave good responses.

The maximum amplitude of an EAG recorded from a female stimulated with its own sexual attractant was approximately 50 percent of that recorded from the male. However, both male and female antennae reacted very similarly to other odorous stimuli. In some instances, female antennae gave no response to the sex attractant but reacted well to other odors. Although there was some correlation between the length of the antennae and the amplitude of response, this can only be partly responsible for the weaker response of the female antenna to its own lure.

Antennae of nymphs of both sexes gave lesser responses than antennae of adults. However, the response to the concentrated sexual attractant, even in female nymphs, was still significantly greater than in the controls. This was also true for the antennae of older males in the larval stage. In female larvae the response was so small that no significant difference from the controls could be found.

A strong EAG response of a male insect antenna stimulated with the sexual attractant of its female was expected, and it was therefore rather astonishing that in the cockroach the female antenna likewise responds with

an EAG to this substance. In the moth, the female does not even possess the olfactory receptor specialized to detect its own lure substance (*3*). While it has recently been shown that sensilla responsible for sex-odor perception in saturniid moths can be identified (*4*), preliminary investigations have not yet shown any morphological differences in the sensilla inventories of male and female cockroach antennae.

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8 July 1963

Teratogenic Effect of 5-Hydroxytryptamine in Mice

Abstract. *The subcutaneous injection of a single dose of 5-hydroxytryptamine into pregnant mice produced a large number of fetal abnormalities, mostly of the eyes, limbs, and tail; the skull and central nervous system were also sometimes affected. These effects could result from the action of the drug on placental function and blood supply.*

Injection of 5-hydroxytryptamine (5-HT) into mice during the second half of pregnancy rapidly causes death of the fetuses and also leads to degenerative and hemorrhagic changes in the placenta (*1*). There is strong evidence that the fetus dies because the drug interferes both with the functional activity of the placenta and with its blood supply (*2*). Since it is well known that nutritional changes may cause fetal abnormalities (*3*), we were interested in investigating the possibility that administration of 5-HT in doses insufficient to kill the fetus would produce abnormalities by interfering with the function of the placenta and thus with the nutrition of the fetus. While the main purpose of our experiments was to find out if a single injection of 5-HT into pregnant mice would produce abnormalities in those fetuses which survived, we also hoped to discover whether the kind of abnormality produced could be influenced by the time at which the injection was given.

We used for our experiments a nonpure line of white mice maintained (but not inbred) at this hospital for the last 12 years. The drug was given during the 5th to 12th day of pregnancy, counting the 1st day as that on which the vaginal plug was found. The animals were divided into three groups. Those in groups I and II received one subcutaneous injection of 2.0 mg of synthetic 5-hydroxytryptamine creatinine sulfate (May and Baker Ltd.). Those in group II were also injected subcutaneously with 2.0 mg of progesterone in oil, starting from the day before the injection with 5-HT and continuing daily until the mice were killed. By this experiment we hoped to confirm the results of Lindsay *et al.* (*4*) who found that progesterone would not reverse the lethal action of 5-HT. The mice in group III received one subcutaneous injection of 0.5 mg of 5-HT. It has been shown previously (*4*) that this lower dose is effective in interrupting pregnancy and, furthermore, has no obvious toxic effects on the mother except for transient diarrhea. A greater number of survivors was expected in group III.

The results obtained in groups I and II were not significantly different and have therefore been combined in the tables of results. Table 1 shows that injection of 2 mg of 5-HT had little effect on litter survival up to day 7 of pregnancy, but administration after this stage caused a progressive rise in fetal mortality so that by the 10th day most of the fetuses were killed. As expected, a single injection of 0.5 mg of 5-HT did not produce the same high proportion of deaths (Table 2) and some 70 percent or more of the fetuses survived the treatment at all the stages of pregnancy which we investigated. A high incidence of abnormalities was found in the surviving fetuses from all three groups of mice; comparing the same days of treatment (days 6 to 11), this was greater in the animals treated with the larger dose of 5-HT (18/136 compared with 19/424; $P < 0.001$). On the other hand, when the number of abnormalities are calculated as a function of the number of mothers used (comparing the same days of treatment), there was no difference between the two groups (15/74 as compared with 19/74; $P < 0.5$).

Table 1. The effect of a single injection of 5-HT (2 mg) given at various stages of pregnancy. Some of these animals also received progesterone.

Day of injection	Number of mice	Number of fetuses		
		Dead or resorbed	Live	Abnormal*
5	6	4	37	0
6	4	5	24	1
7	6	9	36	1
8	17	78	37	2
9	20	95	35	12
10	14	115	4	2
11	13	64	0	0
12	13	87	6	1

* The number of surviving fetuses showing abnormalities.

The abnormalities recorded were all visible on careful macroscopic examination of the intact fetus. The results of more specialized examinations, histological for example, are not yet available. The abnormalities included brain and skull deformities; absence of one or both eyes; one cyclops; absence of eyelids; polydactyly and syndactyly; phocomelia—wasting of limbs and absence of tail; gastroschisis; and absence of the outer ear. The eyes, limbs, and tail were the most frequently affected, and it appears that administration of the drug on day 8, 9, or 10 of pregnancy gives rise to the greatest number of fetal abnormalities.

The incidence of spontaneous abnormalities is extremely low in the line of mice used in our experiments. As a control experiment we examined, in the same way and over the same period, 459 fetuses from untreated mothers. The only deviation from normal which we observed was failure of development of the eyelids in three of the fetuses.

These results show that 5-HT is a powerful teratogen. Although the mode of action may be by interference with placental function, this has by no means

Table 2. Effect of a single injection of 5-HT (0.5 mg) given at various stages of pregnancy.

Day of injection	Number of mice	Number of fetuses		
		Dead or resorbed	Live	Abnormal*
6	7	2	50	0
7	9	4	67	2
8	16	20	55	5
9	17	51	95	4
10	11	22	64	5
11	14	21	93	3

* The number of surviving fetuses showing abnormalities.