

as the number of ova produced in superovulated rats (4). Although the reduction in the number of mated rats presenting implantation sites after treatment with chlorpromazine is not significant, the trend is present. This fact led to an experiment in which the left uterine horn was traumatized on day 6 after treatment with PMS (Table 2); 100 percent of the rats showed a decidual response when treated with PMS only and 88 percent responded when chlorpromazine also was given. However, the increase in the weight of the traumatized horn, as compared with the control horn, was 192 percent for the first group and 92 percent for the group receiving chlorpromazine, a difference significant at the 5-percent level. Thus the decreased sensitivity of the uterine endometrium may be a factor in the decreased incidence of implantation in the rats treated with chlorpromazine.

Parturition failed to occur earlier than day 23 of pregnancy; in some rats it occurred as late as day 25. The average period of gestation for our colony is 21 days. On autopsy the rats that failed to deliver by day 25 of gestation contained viable young *in utero*. Comparison of size of fetuses *in utero* from different animals on the same day of pregnancy, as well as the degree of resorption occurring, suggested delayed nidation. Prolongation of gestation by from 2 to 11 days has been reported in immature rats treated with PMS and human chorionic gonadotropin, and in 33-day-old rats treated with PMS alone (3). Thus there appears to be an initial hormonal imbalance that is not conducive to implantation at the normal time. Although it has not been ascertained whether the initial levels of estrogen and progesterone are too high or too low for promotion of nidation, available evidence suggests that production of estrogen may be prodigious (5). Treatment with PMS may cause a greater degree of thecal cell luteinization than is normal, and this possibility could account for greater production of estrogen by the corpora lutea.

These data indicate that the ovum produced by the immature rat after coitus can be fertilized and developed into a normal fetus. Thus reflex ovulation, in response to coitus, can result in pregnancy in a spontaneous ovulator such as the rat. This demonstration of coitus-induced ovulation followed by pregnancy supports our earlier pro-

posal (1) that ovulation and pregnancy may occur in the spontaneous ovulator after coitus. Such occurrence could explain the reports of women's conception just before, during, and immediately after menstruation (6).

M. X. ZARROW
P. S. CAMPBELL
J. H. CLARK

Department of Biological Sciences,
Purdue University, Lafayette, Indiana

References and Notes

1. C. Aron, G. Arch, J. Ross, *Intern. Rev. Cytol.* **201**, 139 (1966); R. R. Carlson and J. DeFeo, *Endocrinology* **77**, 1014 (1965); J. W.

- Everett, *ibid.* **80**, 145 (1967); F. E. Harrington, R. G. Eggert, R. D. Wilbur, W. H. Linkenheimer, *ibid.* **79**, 1130 (1966); M. X. Zarrow and J. H. Clark, *J. Endocrinol.*, in press.
2. M. X. Zarrow, A. L. Caldwell, E. S. E. Hafez, G. Pincus, *Endocrinology* **63**, 748 (1958).
3. H. H. Cole, *Amer. J. Physiol.* **119**, 704 (1937); *Science* **91**, 436 (1940); H. M. Evans and M. E. Simpson, *Endocrinology* **27**, 305 (1940).
4. V. J. DeFeo, *Anat. Rec.* **127**, 409 (1957); A. Psychoyos, *J. Endocrinol.* **27**, 337 (1963); M. X. Zarrow and K. Brown-Grant, *ibid.* **30**, 87 (1964).
5. D. T. Armstrong and R. O. Greep, *Endocrinology* **76**, 246 (1965); E. G. Rennels and G. G. Guillet, *Anat. Rec.* **145**, 274 (1963); H. M. Teel, *Amer. J. Physiol.* **79**, 184 (1926).
6. B. B. Rubenstein, *Ohio State Med. J.* **35**, 1066 (1959); S. Sevtit, *Lancet* **1946-II**, 448 (1946).
7. Aided by NIH grant HD-02068. The PMS was obtained by courtesy of J. B. Jewell, Ayerst Laboratories, New York.

16 October 1967

Intracerebral Saline: Effect on Memory of Trained Mice Treated with Puromycin

Abstract. *It was shown that puromycin administered to mice 1 or more days after maze-learning blocks expression of memory; the blockage can be removed by intracerebral injections of saline. We present evidence that intracerebral injections of saline are relatively ineffective in restoring memory when puromycin is administered either before or immediately after training; in these two situations puromycin appears to interfere with consolidation of memory.*

We have reported that expression by mice of memory of maze-learning, lost after treatment with puromycin, can be restored by small intracerebral injections of saline (1). More recently we have found sham injections to be ineffective in removing puromycin's blockage of memory. In all these experiments puromycin was injected 1 or more days after training in an amount demonstrated to cause essentially complete suppression of memory for at least 3 months—the longest duration of these retention tests. A single treatment with intracerebral injections of saline, as late as 60 days after the puromycin, proved capable of restoring expression of memory.

Our most recent experiments attempted to unmask whatever memory may be established when training is conducted in the presence of puromycin, or when puromycin is injected immediately after training. Barondes and Cohen (2) observed that when mice were trained to a Y-maze in the presence of puromycin they retained memory of the maze at a high level for less than 45 minutes, and that memory was virtually lost after 3 hours. Agranoff, Davis, and Brink (2) found memory of training in goldfish to be lost when puromycin was given immediately after training; they concluded that puromycin in this situation interferes

with consolidation of memory. There is the possibility in both these instances, as in those that we studied previously, that puromycin blocks expression of memory without alteration of the process that maintains the basic memory trace. We have used intracerebral injections of saline, given several days after training, to test this possibility.

All intracerebral injections, 12 μ l in volume, were made bitemporally (3) in albino mice weighing 28 to 32 g; each injection contained 90 μ g of puromycin or isotonic NaCl, and was made 5 days after the injection of puromycin.

Our usual behavioral procedure (3)

Table 1. Retention of memory by mice treated with puromycin and, except for controls, 5 days later with saline. Group A: puromycin given 5 hours before discrimination-training. Group B: puromycin given 4 to 8 minutes after training. Percentages of savings of trials and errors for the mice that retained memory were, respectively, 94 ± 7 and 96 ± 4 percent (means \pm S.D.); for those with impaired memory, 37 ± 26 and 57 ± 19 percent; for those that lost memory, 1 ± 4 and 1 ± 4 percent.

Group	Memories (No.)		
	Lost	Impaired	Retained
Control A	13	1	0
Saline A	9	5	3
Control B	7	0	0
Saline B	4	9	6

was used with mice treated with puromycin immediately (4 to 8 minutes) after training; they were trained in a Y-maze to a criterion of nine correct responses out of ten, shock being given for failure to move from the stem of the Y within 5 seconds and for errors in left-right discrimination. The same procedure was used in tests for retention of the training experience. The retention tests were given 5 days after treatment with saline; in control groups without saline, they were given at corresponding times.

In the presence of puromycin our mice, when naive to the maze, are exceptionally difficult to train. This difficulty was avoided by training in two stages: The mice were first trained to avoid to criterion in the maze, with free choice of either arm of the Y. Next day they were injected with puromycin and 5 hours later, when inhibition of protein synthesis was at its height (3), they were trained not to enter the preferred arm in the previous free-choice situation. During discrimination-training in this group, errors of avoidance were infrequent and, because of the plan of the experiments, percentages of savings in the retention tests were calculated on the basis of errors of discrimination. An insignificant difference in the results was introduced by the basing of savings on errors of both avoidance and discrimination.

Table 1 gives the results. Of the 14 control mice that received puromycin after avoidance-training and before discrimination-training (group A), but no saline subsequently, all but one failed to show memory of training when tested for retention. By contrast, eight of 17 mice treated with saline showed varying degrees of retention. Statistical analysis (*t*-test), however, gave no convincing evidence that in savings the saline-treated mice differed significantly from their controls: the percentages of savings of trials and errors for the controls were, respectively, 8.5 ± 5.6 and 7.1 ± 7.0 percent (means \pm S.E.); the corresponding figures for mice treated with saline were 27.8 ± 10.0 and 35.2 ± 10.3 percent. The difference in savings of trials between the two sets of mice was not significant ($P > .1$), while the difference in savings of errors was marginally significant ($P < .05$).

The mice of group B (Table 1) were injected with puromycin immediately after training. In this instance, all con-

trol mice (no saline) when tested for retention had lost memory of training, while 15 of 19 mice treated with saline showed varying degrees of retention. In the controls, the percentages of savings of trials and errors were, respectively, 1.2 ± 1.5 and 3.2 ± 2.3 percent (means \pm S.E.); in the mice treated with saline the corresponding figures were 43.8 ± 8.9 and 54.6 ± 7.9 percent. The difference between the two groups in savings of trials and errors was clearly significant ($P < .01$).

Our earlier results on the efficacy of saline in restoring memory after treatment with puromycin referred to 47 mice in which puromycin was given 1 or more days after training and was followed 30 hours to 60 days later by intracerebral injections of saline. In these mice the percentages of savings of trials and errors were, respectively, 66.2 ± 4.6 and 76.8 ± 3.2 percent (means \pm S.E.). These savings are marginally significantly greater ($P < .05$) than those obtained when puromycin, given immediately after training, was followed 5 days later by saline.

We conclude that treatment with puromycin either before or immediately after training interferes with the process responsible for establishing the basic memory trace. This effect is apparently stronger when puromycin is given before training, although, because of differences in the training procedures, comparison of the two groups is uncertain. One should note that, although puromycin was given immediately after training, this fact does not mean that interference with consolidation of memory started at this time; the mechanism of action of puromycin on memory must be better understood before the time available for consolidation can be estimated in such a situation.

LOUIS B. FLEXNER

JOSEFA B. FLEXNER

Department of Anatomy and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia 19104

References and Notes

1. J. B. Flexner and L. B. Flexner, *Proc. Nat. Acad. Sci. U.S.* **57**, 1651 (1967).
2. S. H. Barondes and H. D. Cohen, *Science* **151**, 594 (1966); B. W. Agranoff, R. E. Davis, J. J. Brink, *Proc. Nat. Acad. Sci. U.S.* **54**, 788 (1965).
3. J. B. Flexner, L. B. Flexner, E. Stellar, *Science* **141**, 57 (1963); L. B. Flexner, J. B. Flexner, R. B. Roberts, *Proc. Nat. Acad. Sci. U.S.* **56**, 730 (1966); *Science* **155**, 1377 (1967).
4. Supported by PHS grant MH12719.

11 December 1967

Auditory Sense in Certain Sphingid Moths

Abstract. *Moths of the genus Celerio, hovering and feeding from blossoms, have been observed to react vigorously to high-pitched sounds. The acoustic receptor appears to involve the labial palps. Its characteristics have been determined from responses made, when the moth is exposed to artificial sound pulses, by an interneuron originating in its head. These responses have been found only in 4 out of 19 sphingid species tested.*

Moths belonging to the family Sphingidae lack the thoracic or abdominal tympanic organs found in several other families (1). In Noctuidae there is evidence that these tympanic organs serve as receptors for the ultrasonic cries of insectivorous bats (2) and that they mediate various kinds of evasive behavior (3). These findings, together with the lack of any recent observations (but see 4) that sphingid moths react to high-pitched sounds, have given rise to the belief that sphingids lack auditory organs. Indeed, such a warning system might seem superfluous in those sphingid species that are large and powerful fliers and hence unlikely to fall prey to insectivorous bats.

In March 1967, one of us (K.D.R.) had the opportunity to watch about a dozen individuals of *Celerio lineata* Fabr. hovering, as they fed on nectar, in front of a trellis covered with jasmine. A slight "hiss" or a jingle of keys caused the moths to dash away at high speed, only to return and resume feeding in 10 to 15 seconds. This observation has been repeated with *C. euphorbiae* Linn. in captivity. The moths, confined in a cage, could be induced to hover and feed on the nectar in phlox blossoms. A high-pitched sound would abruptly terminate this feeding behavior and cause wild dashing flight lasting several seconds. When at rest, or when restrained for closer observation, the moths gave no behavioral response to ultrasound.

Neurophysiological studies carried out on these two species of *Celerio* show that there is a descending spike response in an axon in the cervical connectives when ultrasonic pulses from a loudspeaker are directed at the moth (Fig. 1). The latency of this spike response (6 to 8 msec) and other characteristics suggest that it belongs