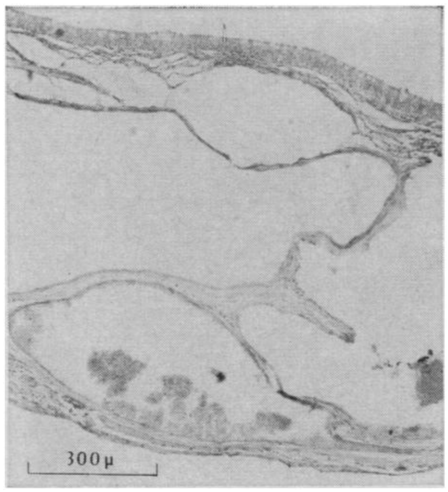

Fig. 1. The mucosa of the middle ear of a California sea lion (*Zalophus californianus*). Epithelial layer above and distended venous sinuses below; some of the sinuses contain red blood cells.

middle ear cavity of the sea lions is significantly different from that of terrestrial mammals. The mucosa is apparently attached to the underlying bone only in the area of the epitympanic recess, which houses the middle ear ossicles. Elsewhere it is unattached except where the many veins pass through the bone. The mucosa consists of three distinct layers which are visible grossly as well as microscopically. The inner layer is ciliated pseudostratified columnar epithelium interspersed with mucus-secreting goblet cells. The outer layer consists of thin, exceedingly tough, dense connective tissue in which the fibroblasts are tightly packed together. The much thicker middle layer consists of a highly complex network of venous channels and sinuses embedded in a matrix of loose connective tissue. This venous network is freely collapsible and distendable.

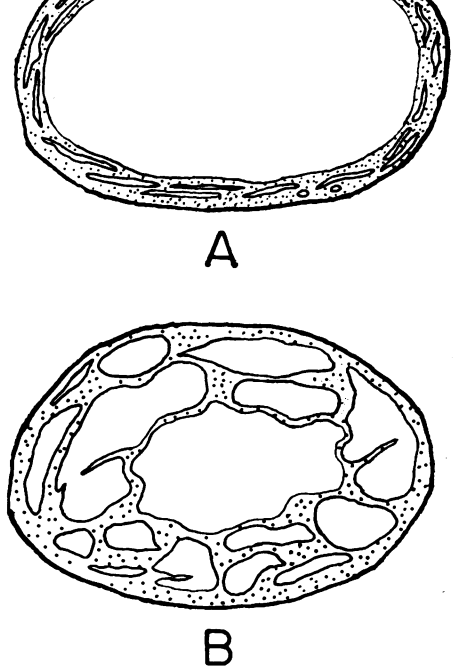

Fig. 2. Diagram of the mucosa of the middle ear of sea lions. (*A*) The venous sinuses are collapsed, and the mucosa is flat. (*B*) The venous sinuses are distended with blood which reduces the lumen of the middle ear cavity.

The blue latex injected into the posterior vena cava of the first sea lion had not penetrated the venous sinuses of the middle ear mucosa, probably because the flow was impeded by the valves of the jugular vein. The mucosa was relatively flat, and the venous network was collapsed. The latex was injected through the external jugular vein of the second sea lion, and sufficient pressure was exerted so that the latex colored the entire mucosa blue. Microscopic examination revealed large, dilitated sinuses, and the mucosa was greatly enlarged (Fig. 1).

The physiological significance of the mucosa of the middle ear is apparent if one considers the environment in which these animals live. During a dive the pressures exerted on sea lions are much greater than those usually experienced by terrestrial mammals. Also the rate of change of pressure occurs much more rapidly in water. A positive pressure in the middle ear is easily adjusted while a negative pressure is much more difficult to overcome (*4*). In adapting to small negative changes in the pressure of the middle ear in air, man swallows, which increases the pressure in the pharynx and helps force air up the Eustachian tube. In the water, scuba divers must resort to the valsalva maneuver (forced exhalation against a closed mouth and nostrils) in order to equalize the pressure. If the pressure is not equalized, a mere change of 3 m in depth can result in hemorrhage into the middle ear and sometimes in rupture of the ear drum (*2*).

The anatomical facts suggest that sea lions do not have to use such mechanisms as the valsalva maneuver to equalize pressure in the middle ear. When the sea lion is on the surface, the mucosa of the middle ear is flat, and the venous sinuses are collapsed (Fig. 2*A*). During a dive, as a negative pressure develops, the mucosa is sucked inward and the sinuses fill with blood (Fig. 2*B*). This probably causes the mucosa to expand, and it distends into the cavity, compressing the air until the pressure becomes equal to that on the outside of the body. During deeper dives, the remaining air space is probably restricted to the small epitympanic recess. Thus the tympanic membrane may remain functional and the ossicular chain of conduction of sound may maintain its integrity under water. This is significant since these animals use an active sonar under water (*5*). It is likely that the regulation of pressure in the middle ear of sea lions by the mucosa can operate purely passively and that it need not require nervous regulation. As a positive or negative pressure starts to develop, the venous sinuses could automatically collapse or distend with blood until equilibrium was established.

STEWART ODEND'HAL

Department of Anatomy,
School of Veterinary Medicine,
University of California, Davis

THOMAS C. POULTER

Biological Sonar Laboratory,
Stanford Research Institute,
Menlo Park, California, 94025

References and Notes

1. E. Thompson, *Amer. J. Physiol.* **110**, 312 (1934).
2. ———, *Spectrum* **13**, 42 (1965).
3. M. Lawrence, *Arch. Otolaryngol.* **71**, 133 (1960).
4. H. A. F. Dishoeck, *Acta Oto-Laryngol.* **35**, 317 (1947); H. S. Wigodsky and J. H. Tillisch, *Amer. J. Med.* **4**, 629 (1948).
5. T. C. Poulter, *Inst. Electrical Electronic Eng. Trans. Ultrasonics Eng.* **UE-10:3**, 109 (1963); T. C. Poulter, *Science* **139**, 753 (1963); T. C. Poulter, *J. Aud. Res.*, in press.
6. Supported in part by PHS grant NB 04736-03. We thank L. M. Julian and M. L. Perry for their encouragement and suggestions.

28 March 1966

Sexual Behavior in the Male Rat

An article by R. D. Lisk (*1*) entitled "Inhibitory centers in sexual behavior in the male rat" contains the following statement which represents the major finding: "Small lesions placed near the diencephalic, mesencephalic junction, in either the lateral or medial mammillary region, resulted in an increase of copulatory behavior." I believe that this conclusion probably is valid, but, because the experimenter failed to observe the behavior of his animal subjects, other interpretations cannot be excluded. Male rats were housed with females treated with estrogen and the level of copulatory activity was inferred from a daily count of vaginal plugs recovered from beneath the floor of each male's cage. The number of plugs found under the cages of males with mammillary lesions markedly exceeded that discovered beneath the cages of unoperated subjects or of males with lesions in other parts of the brain, and on this basis alone it was concluded that, "lesions in the mammillary region lead to increased total copulatory performance."

Orbach (*2*) reported that male rats maintained in individual cages "spontaneously" produce seminal plugs which can be recovered if the animal is prevented from eating them. This finding has been confirmed in our laboratory by W. Westbrook (*3*). Schwartz and Kling (*4*) found that the number of plugs recoverable from the cages of isolated male rats is increased by bilateral damage to the amygdala and hippocampus and, to a lesser degree, by lesions in the septum, olfactory bulbs, hypothalamus and cortex.

In the light of these studies Lisk's findings seem open to at least three interpretations: (i) Mammillary lesions have no effect upon the number of plugs formed in 24 hours but do markedly depress the tendency of the male to consume his own seminal products. (ii) Mammillary lesions have no effect upon the tendency of the male to copulate with the female but do increase the number of plugs formed within 24 hours. (Specifically in Lisk's experiment the lesions prevent a gradual decline in the rate at which plugs are produced over an observation period of 42 days.) (iii) Mammillary lesions increase the frequency with which males copulate with females treated with estrogen, and this frequency is positively and reliably related to the number of plugs found beneath the cage. On the basis of the evidence presented it is impossible to eliminate any one of these choices.

My intuitive conclusion is that Lisk's interpretation is the correct one. That male rats with lesions in the junction of the diencephalon and mesencephalon showed an increase in the number of ejaculations delivered in a 30-minute mating test with receptive females lends support to his conclusion (*5*). This letter is not written to deny or to minimize the importance of Lisk's findings, but to call the attention of subsequent investigators of similar problems to the obligation to directly observe the behavior of their experimental subjects.

FRANK A. BEACH

Department of Psychology, University of California, Berkeley

References

1. R. D. Lisk, *Science* **152**, 669 (1966).
2. J. Orbach, *ibid.* **134**, 1072 (1961).
3. F. A. Beach, W. H. Westbrook, L. G. Clemens, *Psychosom. Med.*, in press.
4. N. B. Schwartz and A. Kling, *Fed. Proc.* **20**, 335 (1961).
5. L. Heimer and K. Larsson, *Experientia* **20**, 1 (1964).

3 June 1966

In reply to F. A. Beach's argument that there are other explanations for my findings (*1*), I would like to suggest the following reasons for the validity of my conclusions. Beach bases his entire argument on my failure to observe the animals copulating in daily mating tests. As evidence for other possible interpretations he cites an abstract by Schwartz and Kling (*2*) and a report by Orbach (*3*). The abstract gives no data for comparison, only the statement that after amygdalectomy some males showed frequent, spontaneous ejaculations. Orbach used the same technique as I did; that is, he checked for plugs on the paper beneath the male's living quarters. Then he kept the animal from licking itself by fitting it with a plastic collar or taping its mouth shut. Nearly all the restrained animals produced a plug; the record for 1 day only is shown however. Any material from the size of a pin head to that of a grain of corn was called a plug.

These precautions were taken in my experiments. First, I noted that the majority of plugs found were pinkish or red in color and cone-shaped. This appeared to be due to irritation of the vagina by the spines on the penis. Plugs were classified as red or white, cone-shaped or irregular in shape, large or small in size. Females were checked for sperm in the earlier part of my experiments. Whenever a red plug was found, sperm were always present in the vagina. When an opaque, white plug was found, I also observed sperm in the vagina. Whenever a small, white, translucent plug or a large, corkscrew-shaped, white plug was found, sperm were not present in the vagina. My records are based on plugs having features which I know are characteristic of plugs formed during copulation.

Further evidence for the specificity of my results, is provided by these control experiments: (i) Nine pairs of males were maintained for a 7-week observation period. During this period only four white plugs, produced by three of the male pairs, were found. (ii) Ten of these rats were then placed with castrated females, and the male-female pairs were observed for 6 weeks. During this period only one white plug was found. (iii) Lateral mammillary lesions were made in five of these males, and medial mammillary lesions in the other five. I then returned the males to their home cages with the castrated females and kept a record of the plugs produced in 4 weeks. I found two plugs. (iv) Then I replaced the castrated females with females treated with estrogen and kept a record of the plugs produced in 4 weeks. Sixteen plugs were found which had been produced by five of the males. (v) Seven of the males were separated from their partners on a Friday. Starting the following Monday 5 days of observed mating tests were carried out. All seven males mounted and showed pelvic thrusts and intromissions on all five test days. All of the treated females showed lordosis. After a male had mounted a lordotic female ten times, the test on that male was concluded, and a new male was put through a similar test. All seven males were tested on each of 5 days. Even in a test this short in duration, two males ejaculated and left plugs in the female.

Thus we have demonstrated that males with mammillary lesions do show copulatory behavior in the presence of females treated with estrogen and seminal plugs are left in the females. Furthermore the sexual behavior of estrogen-treated females and males with mammillary lesions as measured by plug records increased 500 percent over that of pairs of castrated females and males with mammillary lesions, of pairs of normal males and castrated females, or of pairs of normal males. The same male partners were used for all these experiments.

ROBERT D. LISK

Department of Biology, Princeton University, Princeton, New Jersey

References

1. R. D. Lisk, *Science* **152**, 669 (1966).
2. N. B. Schwartz and A. Kling, *Fed. Proc.* **20**, 335 (1961).
3. J. Orbach, *Science* **134**, 1072 (1961).

28 June 1966