trend toward increase in skin resistance through the night so that the largest values were recorded during the last 2 to 3 hours of sleep, the period when stage 1 eye activity was also at a maximum. On awakening, skin resistance fell precipitously in all cases, usually returning to pre-sleep level.

Instead of the expected fall in basal skin resistance during periods of stage 1 eye activity, we found paradoxically that the usual event was a rise. In the typical case this was followed by a decrease in skin resistance as the EEG record returned to stages 2–4.

Two subjects showed a pattern in which skin resistance rose rapidly to a high level which was then maintained as a plateau during the rest of the night.

Typically, the skin resistance and level of EEG activity both began to change following a gross body movement occurring after a period of relative stability. In one subject this change was accompanied by brief precipitous drops in skin resistance indistinguishable from the pattern seen when the subject was awakened. The EEG pattern in this instance was not one of waking, however. The basal skin resistance then rose in the usual fashion.

Figure 1 is a graph of a typical night's events. This subject was a 28-year-old white married male medical student. His records on other nights were similar.

There is evident a general trend in elevation of basal skin resistance during the night; it reaches a maximum in the early morning hours. There are superimposed cycles of rising and falling which parallel the change in EEG level. At approximately 1:30, 3:00, and 4:20, the basal skin resistance starts to rise, as the EEG level shifts from stage 3 or 4 to stage 1. These beginning changes were usually accompanied by a large body movement. To the observers monitoring the recording devices these changes were quite striking and they were able to anticipate a period of stage 1 activity with rapid eye movements as the basal skin resistance started to rise after a level period.

These findings suggest that either the hypothesis of an inverse relationship between arousal and basal skin resistance is incorrect or the concept that stage 1 represents a light stage of sleep needs revision. We favor the latter view.

Jouvet has demonstrated two phases of sleep in the cat which are relatively independent of each other (*6*). The first phase, which occupies most of the sleeping time, is characterized by high-voltage, slow-wave activity in the EEG, the persistance of some tonus in the spinal musculature, and an absence of rapid eye movements. The second phase occurs after the slow EEG phase and is of brief duration. It has been termed by Jouvet the paradoxical or rhombencephalic phase and is characterized by asynchronous low-voltage, fast EEG activity, the complete disappearance of muscle tonus, and the presence of rapid eye movements. The slow-wave phase requires the presence of the neocortex. The paradoxical phase is dependent on a totally different system situated at the level of the pontile reticular formation. This phase can be triggered in sleeping cats by stimulating the lower part of the brain stem. During this phase all muscular activity disappears though there may be variation in respiratory and cardiac rhythms. The threshold of awakening is increased in comparison with that of the slow-wave phase of sleep.

Horovitz and Chow have confirmed Jouvet's findings (*7*). They showed that the paradoxical sleep stage is characterized by an increase in reticular stimulation arousal threshold. Moreover, sufficient stimulation to produce minimal behavioral arousal produced 5-to-6-per-second activity quite different from the electroencephalographic pattern found on arousal from slow-wave sleep. This definitely indicates that the asynchronized sleep stage is deeper in some ways than the stage of synchronized sleep patterns.

A number of observers including ourselves have found that while body movements ushered in and out stage 1 activity there was a relative lack of body movement during this phase. Berger has demonstrated that there is a striking decrease in tonus of the extrinsic laryngeal muscles during stage 1 sleep (*8*).

The evidence suggests that emergent stage 1 sleep is not simply a lighter stage of sleep, but represents a neurophysiologically unique phase, quite likely similar to the rhombencephalic phase of sleep in the cat. Our findings with regard to the basal skin resistance are confirmation of this (*9*).

DAVID R. HAWKINS
HERBERT B. PURYEAR
CHARLES D. WALLACE
WILLIAM B. DEAL
EDWIN S. THOMAS

Department of Psychiatry, University of North Carolina School of Medicine, Chapel Hill

References and Notes

1. E. Aserinsky and N. Kleitman, *J. Appl. Physiol.* **8**, 1 (1955).
2. W. Dement and N. Kleitman, *J. Exptl. Psychol.* **53**, 339 (1957); *Electroencephalog. and Clin. Neurophysiol.* **9**, 673 (1957).
3. D. Goodenough and A. Shapiro, *J. Abnormal Social Psychol.* **59**, 295 (1959).
4. C. W. Darrow, *Psychol. Bull.* **33**, 73 (1936); E. Duffy, *Psychol. Rev.* **64**, 265 (1957); H. Schlosberg, *ibid.* **61**, 81 (1954); A. B. Carron, *Science* **129**, 784 (1959); A. J. Silverman, S. I. Cohen, B. M. Shmavonian, *J. Psychosomat. Research* **4**, 65 (1959).
5. G. H. Wang, P. H. Stein, V. W. Brown, *J. Neurophysiol.* **19**, 350 (1956).
6. M. Jouvet, in *Symposium on the Nature of Sleep*, G. E. W. Wolstenholme and M. O'Connor, Eds. (Little, Brown, Boston, 1961), p. 88.
7. Z. P. Horovitz and M. Chow, *Science* **134**, 945 (1961).
8. R. J. Berger, *ibid.* **134**, 840 (1961).
9. This work was supported by grant M-5543(A) from the National Institute of Mental Health.

1 December 1961

Estrogen-Sensitive Neurons and Sexual Behavior in Female Cats

Abstract. The stimulation of mating behavior by means of the stereotaxic introduction of small implants of solid C^{14}-diethylstilbestrol di-n-butyrate to the hypothalamus of ovariectomized cats is described. Autoradiographic examination of the brains of mating animals reveals that certain neurons in the region of these implants show a selective affinity for labeled estrogen.

It is known that estrogens are essential both for the expression of estrous behavior and for the state of sexual receptivity in female cats, since mating never occurs either during the anestrous phase of the cycle or after bilateral ovariectomy unless estrogens are administered. The clear-cut dependence of the behavioral pattern on hormone, together with the conspicuous and highly stereotyped character of the behavior itself, makes this species particularly suitable for the investigation of the possible sites of action of estrogens in the brain (*1*).

By using a technique by which small implants (0.1 to 0.3 mg) of solid estrogen are placed stereotaxically in different regions of the brain, it has been found that states of sustained sexual receptivity, lasting from 50 to 60 days, can be produced in ovariectomized animals carrying an implant in the hypothalamus (in different series, 60 to 90 percent positive results). The introduction of similar implants of estrogen to other sites in the brain (cerebellar hemisphere, frontal white matter, preoptic region, head of caudate nucleus, dorsomedial nucleus of thalamus, amygdala, and so forth) or the introduction of blank implants of control substances (paraffin wax or pro-

gesterone) to the hypothalamus itself, fails to result in any changes in behavior which remains, in these cases, typical of the ovariectomized animal (an angry, refusal reaction instead of the estrous, receptive reaction). In these experiments the behavioral responses were assessed daily in timed mating tests conducted with trained males, and the response of the genital tract to the presence of the brain implant was measured by daily examination of vaginal smears and periodic uterine biopsy. In several instances where females were continuously receptive and showed sustained mating behavior, examination of the data for the genital tract revealed that the vagina and the uterus had remained throughout in the anestrous condition. That is to say, behavioral heat was coexisting with atrophy of the genital tract (*2*). Observations of this kind led to the development of a hypothesis that the active sexual behavior resulting from hypothalamic implantation might be due to the *local* action of hormone upon some nervous mechanism in the region of the implant (*3*).

To test this notion experimentally, the techniques for behavioral testing and brain implantation outlined above have been further developed by employing C^{14}-labeled estrogen (C^{14}-diethylstilbestrol di-n-butyrate, specific activity 2.86 μc/mg) with the aim of measuring the extent of local spread of material in brain about the site of implantation. After removal of radioactive implants from brains and fixation with formol-saline by carotid perfusion, frozen sections were cut at 15 μ, and autoradiographs were prepared by dipping in Eastman Kodak emulsion type NTB 3 at 41°C. After incubation for 2 months at −10°C, the serial sections were developed (Kodak D72) and stained with buffered cresyl violet acetate.

The blackening was found to be localized to the immediate neighborhood of the implant, and the concentration of labeled material was found to fall very sharply with increasing distance from the site of implantation. Grain counts were no longer significantly above background 400 to 600 μ in any direction from the edge of the implant site. Thus it can be seen that, by this technique, a shell of neural tissue immediately adjacent to the implant is exposed for prolonged periods to a high concentration of hormone. The distribution of labeled material was found to be equally restricted in both mating and nonmating animals (*3*).

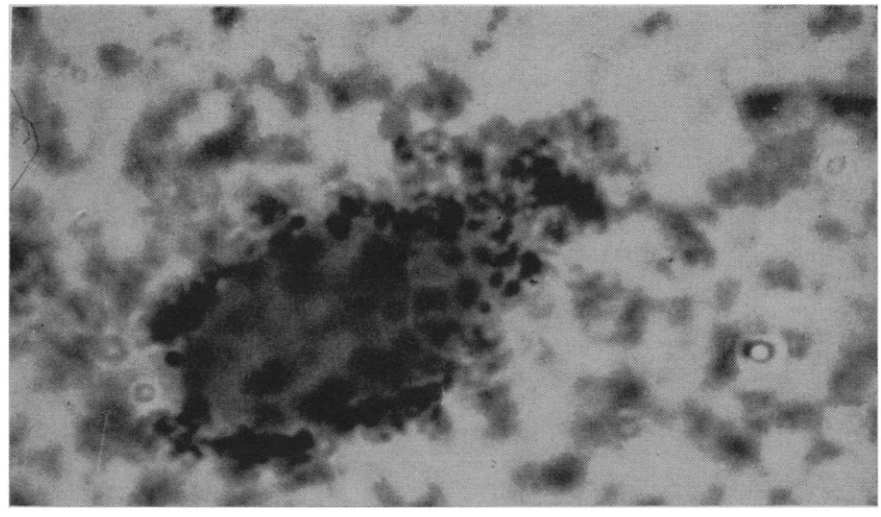

Fig. 1. Hypothalamic neuron in the region of brain implant showing selective affinity for C^{14}-labeled estrogen (autoradiograph, × 2750).

Detailed scrutiny of these autoradiographs has now revealed an association between areas of denser blackening and individual cells. Immediately adjacent to the implant site there is a region of intense blackening and glial reaction which obscures the normal histology and makes interpretation impossible. Beyond this zone of radiation damage the brain appears normal and grain counts are lower (10 to 20 grains per 100 μ^2 above background). It is in this region, 150 to 350 μ from the implant site, that unmistakable neurons can be observed which are associated with markedly increased grain counts. Figure 1 shows such a neuron which appears to be outlined by grains although the grains are, in fact, clustered over the entire surface of the cell. Because they are not all in the same plane, it is impossible to bring all the grains into focus at the same time. The phenomenon illustrated here occurs infrequently and is not very easy to observe clearly. Such cells are seen in both mating and nonmating animals but with greater frequency in the mating group. One particular brain, implanted in the mid-hypothalamus in the mid-line, provided eight good examples. The difficulty in obtaining clear resolution may be partially attributed to the range of the C^{14} beta particles in soft tissue (about 300 μ) which is great compared with the size of a neuron. Numerous nerve cells in the region of the implant appear completely unaffected, and no localization of isotopic estrogen has been observed in association with purely glial elements. It would seem that, of the vast population of nerve cells in the vicinity of an implant, relatively few possess this special capacity for accumulating and retaining hormone.

Discounting for the present the possibility of artifact, it is possible to interpret these observations as providing evidence that certain neurons in the region of an implant possess a selective, biochemical affinity for estrogen. It can further be postulated that these cells form part of a neuronal apparatus, specifically sensitive to hormone, which mediates the expression of sexual behavior (*4*).

RICHARD P. MICHAEL
Institute of Psychiatry,
Maudsley Hospital, London, England

References and Notes

1. R. P. Michael, *Nature* **181**, 567 (1958); *Behaviour* **18**, 1 (1961).
2. G. W. Harris, R. P. Michael, P. P. Scott, *Ciba Symposium on the Neurological Basis of Behaviour* (Churchill, London, 1958), p. 236.
3. R. P. Michael, in *Regional Neurochemistry* (Pergamon, London, 1961), p. 465.
4. This study was initiated at the Clinical Neuropharmacology Research Center, National Institute of Mental Health, U.S. Public Health Service. It is supported by a grant from U.K. Medical Research Council.

14 November 1961