transmission through the synapse. The record in Fig. 2A2 was taken 10 seconds after an antidromic train of impulses in the postsynaptic axon and shows that the reduction in transmitter release produced by the postsynaptic train was sufficient to prevent activation of spike generation in the postsynaptic axon. Figure 2A3 shows recovery of synaptic transmission and impulse generation 3 minutes after the end of the postsynaptic train.

The modulation of transmitter release by repetitive postsynaptic action potentials can function as a type of negative feedback. Figure 2A shows that this feedback is effective in modulating transmission through the synapse. Previous investigations on the mammalian central nervous system (CNS) have shown different types of negative feedback. For example, recurrent inhibition can be mediated by pathways through axon collaterals and interneurons, as in the case of the motoneuron (*18*) and hippocampal pyramidal cell (*19*), or through dendrodendritic interactions with interneurons, as in the case of the olfactory mitral cell (*20*). The main feature of these negative feedback systems is that they feed back synaptic inhibition on the output neuron. However, a direct feedback inhibition of transmitter release from nerve terminals impinging on a neuron has not, to our knowledge, been previously reported. This investigation provides evidence for a direct inhibitory feedback from the postsynaptic structure that modulates transmitter release from the presynaptic terminal.

Figure 2B illustrates schematically how such a feedback inhibition might function in the CNS. Repetitive action potentials in one excitatory synaptic pathway (2 in Fig. 2B) would result in repetitive firing of the neuron. The accumulation of extracellular K resulting from the repetitive neuronal activity could, in turn, result in feedback inhibition of synaptic transmitter release from other synaptic pathways (1 and 3 in Fig. 2B) impinging on the neuron (*21*). In this way, the accumulation of extracellular K may modulate synaptic transmission and function as an integrating mechanism in the nervous system.

FORREST F. WEIGHT
*Laboratory of Neuropharmacology, National Institute of Mental Health, St. Elizabeths Hospital, Washington, D.C. 20032*

S. D. ERULKAR
*Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia 19174*



**References and Notes**

1. E. R. Kandel and W. A. Spencer, *Physiol. Rev.* **48**, 65 (1968); V. F. Castellucci and E. R. Kandel, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 5004 (1974).
2. F. F. Weight, in *The Neurosciences: Third Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1974), p. 929.
3. T. P. Feng, *Chin. J. Physiol.* **15**, 367 (1940).
4. J. C. Eccles, B. Katz, S. W. Kuffler, *J. Neurophysiol.* **4**, 362 (1941).
5. A. Lundberg and H. Quilisch, *Acta Physiol. Scand. Suppl. 111* **30**, 111 (1953).
6. T. P. Feng, *Chin. J. Physiol.* **16**, 341 (1941); A. W. Liley and K. A. K. North, *J. Neurophysiol.* **16**, 509 (1953).
7. F. Frank and M. G. F. Fuortes, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **16**, 39 (1957); J. Dudel and S. W. Kuffler, *J. Physiol. (London)* **155**, 543 (1961); J. C. Eccles, R. M. Eccles, F. Magni, *ibid.* **159**, 147 (1961).
8. E. Kandel and L. Tauc, *J. Physiol. (London)* **181**, 28 (1965); R. Epstein and L. Tauc, *ibid.* **209**, 1 (1970).
9. S. D. Erulkar and F. F. Weight, *Soc. Neurosci. Fourth Annu. Meet. Abstr.* (1974), p. 202.
10. Lowering the temperature of the preparation prevented EPSP initiation of the postsynaptic action potential and then progressively decreased the amplitude of the EPSP (F. F. Weight and S. D. Erulkar, *Nature (London)* **261**, 720 (1976).
11. S. D. Erulkar and F. F. Weight, in preparation.
12. The term after-hyperpolarization is used synonymously with "positive phase" (*13*) or "undershoot" (*14*).
13. B. Frankenhaeuser and A. L. Hodgkin, *J. Physiol. (London)* **131**, 341 (1956).
14. D. A. Baylor and J. G. Nicholls, *ibid.* **203**, 555 (1969).
15. See also R. K. Orkand, J. G. Nicholls, S. W. Kuffler, *J. Neurophysiol.* **29**, 788 (1966); Baylor and Nicholls (*14*).
16. The small depolarization of the postsynaptic membrane resulting from the increase in $[K]_o$ would reduce the EPSP by only 0.2 to 0.6 mv per millivolt change in membrane potential [S. Hagiwara and I. Tasaki, *J. Physiol. (London)* **143**, 114 (1958); R. Miledi, *Nature (London)* **223**, 1284 (1969); R. Llinas, R. W. Joyner, C. Nicholson, *J. Gen. Physiol.* **64**, 519 (1974)]. On the other hand, if the EPSP is due to an increased Na and K conductance, increasing $[K]_o$ would decrease the K equilibrium potential, which would be expected to increase the amplitude of the EPSP. Separate analysis (*11*) indicates that the reduction in EPSP amplitude is associated with and can be accounted for by a decrease in presynaptic spike height.
17. The possibility that synaptic transmitter release might be reduced by a synaptic pathway impinging on the presynaptic terminal seems most unlikely in this experiment for several reasons. First, there is no known anatomical pathway from the postsynaptic nerve with synapses on the giant terminal [J. Z. Young, *Philos. Trans. R. Soc. London Ser. B* **229**, 465 (1939); K. Hama, *Z. Zellforsch. Mikrosk. Anat.* **56**, 437 (1962); O. J. Castejon and G. M. Villegas, *J. Ultrastruct. Res.* **10**, 585 (1964); J. Z. Young, *Brain Res.* **57**, 457 (1973)]. Second, continuous recording in the presynaptic terminal (Fig. 1B) does not reveal synaptic potentials in the presynaptic terminal upon antidromic stimulation of the giant axon. Third, the reduction in the presynaptic spike AH indicates an accumulation of extracellular K (*13, 15*).
18. J. C. Eccles, P. Fatt, K. Koketsu, *J. Physiol. (London)* **126**, 524 (1954).
19. E. R. Kandel, W. A. Spencer, F. J. Brinley, *J. Neurophysiol.* **24**, 225 (1961); P. A. Andersen, J. C. Eccles, Y. Loyning, *ibid.* **27**, 592 (1964).
20. W. Rall and G. M. Shepherd, *ibid.* **31**, 884 (1968); R. A. Nicoll, *Brain Res.* **14**, 157 (1969).
21. The effect would presumably be restricted to synaptic terminals on regions of the neuron with postsynaptic impulse activity, as at the cell body, axon hillock, initial segment, and excitable regions of dendritic membrane.
22. We thank G. R. Siggins and B. J. Hoffer for critical review of the manuscript. This investigation was conducted at the Marine Biological Laboratory, Woods Hole, Mass., and was supported in part by PHS grant NS 12211.

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# Monoamine Changes in the Brain of Cats During Slow-Wave Sleep

Abstract. *We have found that the metabolism of 5-hydroxytryptamine increases in the hippocampus and that the metabolism of dopamine decreases in the striatum and thalamus during slow-wave sleep, and we suggest that these changes are related to this stage of sleep. We have also found that the concentration of dopamine increases in the hippocampus during slow-wave sleep, and suggest that this may be related to the subsequent appearance of paradoxical sleep. These data raise new questions on the hippocampal role in the sleep-wakefulness cycle.*

The hypothesis that monoamines participate in the regulation of sleep has been supported by the findings that lesion of the raphe neurons containing 5-hydroxytryptamine (5-HT) (*1*) leads to a state of permanent desynchronization with behavioral arousal (*2*) and that bilateral destruction of the nucleus locus coeruleus, rich in catecholamines (*1*), is followed by a total disappearance of paradoxical sleep (*3*). While behavioral, electroencephalographic (EEG), and pharmacologic evidence also substantiates the role of monoamines in sleep, chemical correlates have been lacking because of the difficulty of obtaining brain tissue for chemical analysis during sleep. By using a brain biopsy technique, we have found that the metabolism of 5-HT and the concentration of dopamine (DA) increases in the hippocampus, while the metabolism of DA decreases in the striatum and thalamus during slow-wave sleep (SWS). We suggest that the increased 5-HT metabolism in the hippocampus and decreased DA metabolism in the striatum and thalamus is related to SWS, and that the increased concentration of DA in the hippocampus is related to the subsequent appearance of paradoxical sleep.

Adult cats were used as the experimental subjects. Under pentobarbital anesthesia, the left parieto-temporal bone of the skull and underlying dura mater were removed (*4*). After screw electrodes for EEG recording were implanted into the frontal cortex, electrode wires were sol-

dered to an Amphenol plug which was fixed to the skull by dental cement. The exposed left brain hemisphere was covered by skin, and the incision was made airtight by suturing. Two days after surgery, the cats were restrained in bags with EEG cables attached to their electrode plugs and were given 3 to 4 days in which to adjust to these conditions. Five to 6 days after surgery, the stitches were removed under local anesthesia and the left hemisphere was exposed. The EEG was monitored and the animals were observed. In one group of animals, after 20 minutes of spontaneous SWS, the left hemisphere was ablated by a scalpel in a procedure that lasted from 4 to 6 seconds. Desynchronization in the EEG appeared when the ablation was completed and at this time the animals were killed by intracardial injection of saturated KCl solution. Because of circadian changes in brain monoamine content, all animals were killed between 12:00 noon and 3:00 p.m. The left hemisphere was washed in ice-cold saline and specific brain sites were dissected on ice. In the other group of animals, the left hemisphere was removed after 30 minutes of wakefulness, and the same procedure was performed as before.

Dissection of the various brain regions lasted from 8 to 10 minutes. The compounds 5-HT, DA, norepinephrine (NE), 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, and homovanillic acid (HVA), a metabolite of DA, were determined in the cerebral cortex, the head of the caudate nucleus, the hippocampus, and the striatum-thalamus (*5*). Monoamines and their metabolites in single samples of each brain region were determined spectrophotofluorometrically by described methods (*6*).

Table 1 shows that the concentrations of 5-HT and 5-HIAA increased significantly in the hippocampus during SWS. The increase over the concentration in cats during wakefulness was 143 percent for 5-HT, and 289 percent for 5-HIAA, the amount during wakefulness being taken as 100 percent. We have previously observed in cats an increased 5-HIAA concentration in the cerebrospinal fluid of the cisterna magna during SWS (*7*). Since cisternal cerebrospinal fluid contains neuronal metabolites of different brain structures, particularly those lining the ventricular and subarachnoid space, it was not possible to trace the origin of the site which contributed to the increased cisternal 5-HIAA concentration. Based on our new evidence of increased 5-HT metabolism in the hippocampus during SWS, and also on the proximity of the hippocampus to the lateral ventricles, the increased concentration of 5-HIAA in the cisterna magna might reflect increased serotonergic activity in the hippocampus. Other brain structures that might contribute to the increase in cisternal 5-HIAA concentration in SWS are the raphe nuclei in the brainstem. Their composition (rich in 5-HT neurons), location (close to ventricular and subarachnoid space), and possible involvement in the onset and maintenance of SWS (*3*) would support such an assumption. Because our biopsy technique did not reach as far as brainstem, we could not determine the metabolism of monoamines in the brainstem structures including the raphe system. However, in animals killed by intravenous infusion of saturated KCl during wakefulness, SWS, and paradoxical sleep, Sinha *et al.* (*8*) measured regional brain monoamines and found no changes in 5-HT metabolism in the midbrain, pons, and medulla.

The data in Table 2 show that during SWS the DA concentration increased by 178 percent in the hippocampus and decreased by 13.5 percent in the striatum-thalamus (the amount of DA during wakefulness being taken as 100 percent). The amount of HVA in the striatum and thalamus decreased to values below the sensitivity of the method (33.6 ng per sample). This difference in the activity of DA in the two regions may indicate a double role for catecholamines in sleep-waking mechanisms. One function of catecholamines might be to promote wakefulness, because lesions in the pontine tegmentum of cats, in areas with DA-containing neurons, produced decreased behavioral arousal, reduced concentrations of DA in the brain, and normal waking patterns in the EEG (*9*). The decrease in DA metabolism in the striatum and thalamus during SWS (Table 2) supports this postulate. Another suggestion is that catecholamines are essential for the appearance and maintenance of paradoxical sleep (*3*). Because a certain amount of SWS normally precedes paradoxical sleep, the increased DA concentrations in the hippocampus after 20 minutes of SWS (the average duration of an initial episode of SWS in the cat) may be related to the onset of paradoxical sleep. Also, if a function of paradoxical sleep is to restore catecholamine activity (through

Table 1. The concentrations of 5-HT and 5-HIAA in specific brain structures removed from cats during wakefulness (W) (six animals) and during SWS (six animals). The results are means ± standard error (S.E.). The mean weight of tissue for each brain region is for 12 cats.

| Brain region | Concentration (ng/g wet weight) | | | | Tissue mean weight (mg ± S.E.) |
|---|---|---|---|---|---|
| | 5-HT | | 5-HIAA | | |
| | W | SWS | W | SWS | |
| Hippocampus | 503 ± 31 | 722* ± 51 | 616 ± 81 | 1786* ± 125 | 714 ± 8 |
| Striatum and thalamus | 668 ± 20 | 802 ± 144 | 917 ± 181 | 619 ± 134 | 1025 ± 17 |
| Cortex | 463 ± 88 | 477 ± 127 | 229 ± 68 | 258 ± 45 | 1320 ± 59 |
| Caudate (head) | 1430 ± 168 | 1418 ± 176 | 1179 ± 42 | 1072 ± 95 | 379 ± 15 |

*Difference is significant by Student's *t*-test (unpaired) (*22*): $P < .005$.

Table 2. Concentrations of DA, HVA, and NE in specific brain structures removed from cats during wakefulness (W) (six animals) and SWS (six animals). The results are means ± S.E. The mean weight of tissue for each brain region is for 12 cats.

| Brain region | Concentration (ng/g wet weight) | | | | | | Tissue mean weight (mg ± S.E.) |
|---|---|---|---|---|---|---|---|
| | DA | | HVA | | NE | | |
| | W | SWS | W | SWS | W | SWS | |
| Hippocampus | 407 ± 27 | 728* ± 91 | 815 ± 87 | 1856 ± 423 | 351 ± 89 | 291 ± 55 | 714 ± 8 |
| Striatum and thalamus | 4246 ± 661 | 574† ± 101 | 824 ± 195 | ‡ | 360 ± 84 | 426 ± 111 | 1025 ± 47 |
| Cortex | 492 ± 74 | 448 ± 88 | 546 ± 104 | 457 ± 96 | 308 ± 56 | 358 ± 69 | 1320 ± 59 |
| Caudate (head) | 10428 ± 2836 | 9636 ± 826 | 1834 ± 204 | 1776 ± 364 | 1965 ± 293 | 1742 ± 248 | 379 ± 15 |

*Difference is significant by Student's *t*-test (unpaired) (*22*): $P < .05$. †$P < .025$. ‡Less than 33 ng per sample.

increased catecholamine metabolism) in the central nervous system (*10*), the hippocampus could be one of the sites of this restoration process.

The electrical activity of the hippocampus is unusual. While the rest of the brain shows a desynchronized EEG pattern during attention, learning, and paradoxical sleep, the hippocampus displays a synchronized electrical activity of four to seven cycles per second (theta rhythm) (*11*). It is suggested that the hippocampus, through synchronizing influences on the neocortical and subcortical structures, counteracts the effects exerted by the ascending reticular-activating system, and that the functional interplay of the hippocampus and reticular system seems to be of importance with regard to the rhythm of sleep and wakefulness (*12*). Spontaneous activity of the hippocampal pyramidal cells in cats with permanently implanted electrodes sharply decreased in SWS (*13*), a phenomenon observed in other brain areas (*14*). It may be that the electrical activity of specific brain structures during SWS is keyed to local chemical processes. Our data regarding the chemical changes in the hippocampus during SWS indicate that the 5-HT and DA systems become activated at this time. This is of interest because current hypotheses propose an increased activity in the 5-HT system of the raphe nuclei during SWS and an increased activity in the catecholamine system of the nucleus locus coeruleus during paradoxical sleep (*3*). Because of the reciprocal connection between limbic-forebrain structures (including the hippocampus) and the raphe nuclei, the latter are potentially modulated by limbic-forebrain mechanisms (*15*). When 80 to 90 percent of the raphe system is destroyed, animals enter a state of permanent arousal that lasts 3 to 4 days; SWS returns partially within a 3-week period (*2*), and a near normal sleep profile (*16*) appears by day 30. This indicates that other brain sites functionally compensate for the loss of 5-HT neurons in the raphe. Furthermore, since hippocampectomy was found to reduce significantly both SWS and paradoxical sleep, the hippocampus has been implicated in the facilitation of both SWS and paradoxical sleep (*17*). Although hibernation differs from normal sleep in many respects (*18*), it has been reported that when animals are entering hibernation, the concentration of 5-HT increases in the hippocampus several times more than it does in other brain areas (*19*). Also, when 5-hydroxytryptophan, an immediate precursor of 5-HT, is administered to rats, the highest concentration of 5-HT is found in the hippocampus (*20*); when administered to rabbits, 5-hydroxytryptophan results in the most marked rate of increase in 5-HT being found in the hippocampus (*21*). All these findings point to the importance of a serotonergic mechanism in the hippocampus, and a possible role of this area in SWS. The specific increases in the metabolism of 5-HT and the concentration of DA in the hippocampus during SWS indicate that the hippocampus functions as a subsidiary sleep structure to the raphe system and the nucleus locus coeruleus in the brainstem. We also suggest that the obtained decrease in DA metabolism in the striatum and thalamus during SWS may be related to the sleep-generating mechanisms.

RUŽICA KOVAČEVIĆ*
MIODRAG RADULOVAČKI†
*Department of Pharmacology, College of Medicine, University of Illinois at the Medical Center, Chicago 60612*

### References and Notes

1. A. Dahlstrom and K. Fuxe *Acta Physiol. Scand.* **62**, Suppl. 232 (1964).
2. M. Jouvet, P. Bobillier, J. F. Pujol, J. Renault, *C. R. Seances Soc. Biol. Paris* **160**, 2343 (1966).
3. M. Jouvet, *Science* **163**, 32 (1969).
4. Since dura mater contains pain receptors, removal of the dura prior to the experimental procedure was an important part of the technique. It enabled painless cutting of the brain tissue and ablation of the left hemisphere with the least disturbance to the animal.
5. The striatum-thalamus included the putamen, globus pallidus, tail of caudate nucleus, claustrum, and thalamus.
6. C. C. Chang, *Int. J. Neuropharmacol.* **3**, 643 (1964); F. A. Gerbode and M. B. Bowers, *J. Neurochem.* **15**, 1053 (1968); J. Korf and T. Valkenburgh-Sikkema, *Clin. Chim. Acta* **26**, 301 (1969); A. S. Welch and B. L. Welch, *Anal. Biochem.* **30**, 161 (1969); H. S. Alpers and H. E. Himwich, *J. Pharmacol. Exp. Ther.* **180**, 531 (1972).
7. R. L. Buckingham and M. Radulovački, *Brain Res.* **99**, 440 (1975).
8. A. K. Sinha, S. Henriksen, W. C. Dement, J. D. Barchas, *Am. J. Physiol.* **224**, 381 (1973).
9. B. E. Jones, P. Bobillier, C. Pin, M. Jouvet, *Brain Res.* **58**, 157 (1973).
10. E. L. Hartmann, *Int. Psychiat. Clin.* **7**, 308 (1970); W. C. Stern and E. L. Hartmann, *Proc. Am. Psychol. Assoc.* **7**, 308 (1971).
11. E. Grastyan, K. Lissak, I. Madarasz, H. Donhoffer, *Electroencephalogr. Clin. Neurophysiol.* **11**, 409 (1959); W. R. Adey, D. O. Walter, C. E. Hendrix, *Exp. Neurol.* **7**, 282 (1961); E. N. Sokolov, *Annu. Rev. Physiol.* **25**, 545 (1963); M. Radulovački and W. R. Adey, *Exp. Neurol.* **12**, 68 (1965); M. Jouvet, *Prog. Brain Res.* **18**, 20 (1965); A. Routtenberg, *Psychol. Rev.* **73**, 481 (1966); W. R. Adey, *Prog. Brain Res.* **27**, 228 (1967).
12. P. L. Parmeggiani, *Prog. Brain Res.* **27**, 413 (1967).
13. H. Noda, S. Mahonar, W. R. Adey, *Exp. Neurol.* **24**, 217 (1969).
14. E. V. Evarts, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **19**, 828 (1960); P. R. Huttenchlocher, *J. Neurophysiol.* **24**, 451 (1961); E. V. Evarts, *ibid.* **27**, 152 (1964); E. Bizzi, O. Pompeiano, I. Somogyi, *Arch. Ital. Biol.* **102**, 308 (1964); H. Sakamura, *Jpn. J. Physiol.* **18**, 23 (1968).
15. P. J. Morgane and W. C. Stern, in *Serotonin and Behavior*, J. Barchas and E. Usdin, Eds. (Academic Press, New York, 1973), pp. 427–442.
16. P. J. Morgane, in *Sleep and the Maturing Nervous System*, C. D. Clemente, D. P. Purpura, F. E. Mayer, Eds. (Academic Press, New York, 1972), pp. 141–162.
17. C. Kim, H. Choi, C. C. Kim, J. K. Kim, M. S. Kim, H. J. Park, B. T. Ahn, *Electroencephalogr. Clin. Neurophysiol.* **38**, 235 (1975).
18. L. M. N. Bach, in *Hibernation and Hypothermia, Perspectives and Challenges*, F. E. South, J. P. Hannon, J. R. Willis, E. T. Pengelley, N. R. Alpert, Eds. (Elsevier, Amsterdam, 1972), pp. 535–550.
19. N. K. Popova and N. N. Voitenko, *Dokl. Akad. Nauk SSSR* **218**, 1488 (1974).
20. F. Okada, Y. Saito, T. Fujeida, I. Yamashita, *Nature (London)* **238**, 355 (1972).
21. E. Costa and F. Rinaldi, *Am. J. Physiol.* **194**, 214 (1958).
22. W. J. Dixon and F. J. Massey, Jr., *Introduction to Statistical Analysis* (McGraw-Hill, New York, 1969), p. 119.
23. This work was supported by PHS NS 10921.
* Present address: Institute of Physiology, Faculty of Medicine, Belgrade, Yugoslavia.
† Reprint requests should be sent to M.R.

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## Island Biogeography and Conservation: Strategy and Limitations

As human destruction of remaining natural habitats accelerates, biologists have felt intuitively that most existing wildlife refuges are too small to avert extinctions of numerous species. However, because there has been no firm basis for even approximately predicting extinctions in refuges, biologists have had difficulty convincing government planners faced with conflicting land-use pressures of the need for large refuges. Recently several workers have recognized that a predictive understanding of extinction might be obtained from island biogeography, since refuges of natural habitat in a sea of human-altered environment behave as islands for species dependent on natural habitat (*1–6*). All these investigators attempting to understand implications of the "island dilemma" for conservation strategy have concluded that some large refuges are essential to minimize extinction rates and to ensure certain species any chance of survival at all. These conclusions are based not only on studies of oceanic islands but also of habitat "islands" on mainlands, as well as of refuges themselves.

Simberloff and Abele (*7*) argue that these applications of biogeographic theory to conservation practice are premature and are based on insufficiently validated theory and possibly also on idiosyncratic results. These authors show that, given certain assumptions, several small refuges may contain more species than a single large refuge of equivalent area. Their reasoning from their assumptions is correct but minimizes or ignores much more important