Increased Turnover of Cerebral Norepinephrine during Rebound of Paradoxical Sleep in the Rat

Abstract. *Changes in turnover of cerebral norepinephrine, as measured after intracisternal administration of the H^3 amine, have been studied in rats during selective paradoxical sleep deprivation and its following rebound. Experiments were performed under neurophysiological control. A marked increase of turnover of norepinephrine is associated with the augmentation of paradoxical sleep characteristic of the rebound period.*

During the last few years, growing experimental evidence has demonstrated the alteration of both slow-wave and paradoxical sleep by drugs which are known to affect one or many aspects of the metabolism or disposition of norepinephrine and serotonin (*1, 2*) or by destruction of some norepinephrine- or serotonin-containing neurons (*3, 4*). Thus it is possible to suggest that the succession of events leading to the different states of sleep and wakefulness are partly dependent on variations in the metabolism or disposition of norepinephrine and serotonin in some central neurons and synapses. In order to test this hypothesis we have examined the change in norepinephrine turnover in the central nervous system of rats during deprivation of paradoxical sleep and during the rebound phase of increased paradoxical sleep which immediately follows such deprivation (*5*).

Male Charles River rats (250 to 280 g) were deprived of paradoxical sleep by being placed upon supports surrounded by water, in metallic boxes (*5*). The diameter of the support (4.5 cm) was chosen to avoid the total relaxation of the body musculature which occurs in paradoxical sleep and which would result in the animals falling into the surrounding water; control animals were disposed individually in cages and kept in the same room.

The animals were kept in these respective experimental situations for 91 hours beginning at 3 p.m. of one day and terminating at 10 a.m. of the 4th day. Groups of 6 to 20 rats were injected intracisternally under short-lasting ether anesthesia at the end of the 91-hour period with 20 μl of Merles' solution containing 3 to 7 μc of 7-H^3-DL-norepinephrine (5 to 8 c/mmole; New England Nuclear Corp., Boston, Mass.) by a modification (*6*) of the intraventricular injection technique of Glowinski *et al.* (*7*). The animals of the control and experimental groups were killed by decapitation at 0.5 or 5 hours after the intracisternal injection. Their brains were quickly removed and dissected in the cold. The cerebellum was discarded and the brain was separated in two regions by a transverse section made in front of the superior colliculus. The rostral part included the telencephalon and diencephalon and the caudal part, the lower brain stem, and mesencephalon. Tissues were treated for determination of tritiated and endogenous norepinephrine following a procedure previously described (*8, 9*).

For a neurophysiological control in the experiments, three groups of rats were chronically implanted under Nembutal anesthesia with electrodes on the cortex and into the neck muscles. Three days after the intervention the animals were disposed under similar experimental conditions as described for the biochemical studies and continuous (24 hours per day) recording was done to permit quantitative estimation of the two sleep states during an overall 115-hour period (*10*). As in the biochemical experiments the rats were anesthesized 91 hours after the beginning of the experiment and received similar amounts of H^3-norepinephrine intracisternally; recordings continued for an additional 24 hours.

During the first 91 hours, animals disposed upon small supports in metallic boxes were completely deprived of paradoxical sleep by the impossibility of total and persistent relaxation of their bodies. This deprivation of paradoxical sleep was more pronounced than the effect observed on slow-wave sleep (paradoxical sleep = 0 percent; slow-wave sleep = 58.4 ± 8 percent of the control values). The total and almost selective effect of deprivation of paradoxical sleep later produced a marked increase of this state of sleep, as was demonstrated during the five experimental hours which followed the end of the deprivation period (Table 1). This enabled us to study the variation in the turnover of norepinephrine and in the initial accumulation of the exogenous amine which may occur in well-defined and quantified situations of sleep.

It has been previously shown that the introduction of tritiated norepinephrine in the lateral ventricle results in a specific labeling of the central catecholamine-containing neurons (*11, 12*). However, after intracisternal administration, norepinephrine accumulates in negligible amounts in the dopamine-containing endings of the striatum (*13*). Thus it is possible to assume that the data obtained in the rostral part of the brain result from a practically exclusive labeling of norepinephrine-containing axones and endings. The global estimation of norepinephrine turnover in different parts of the brain can be made by following the changes in the specific activity of H^3-norepinephrine between 0.5 and 5 hours after the injection (*12, 9*). The early 0.5-hour time was chosen to appreciate also changes in the initial accumulation of the amine which perhaps could be modified by physiological processes.

A statistically significant increase in this initial accumulation was observed in the animals deprived of paradoxi-

Table 1. Paradoxical sleep and synthesis of norepinephrine (NE). C, control rats; R, rats which present a rebound of paradoxical sleep (Fig. 1B); D, rats which present no rebound of paradoxical sleep (Fig. 1C).

Item	Brain stem and mesencephalon			Telencephalon and diencephalon		
	C	R	D	C	R	D
PS/RT* (%)	0.1 ± 0.1	11.7 ± 3	0	0.1 ± 0.1	11.7 ± 3	0
K per hour†	0.27	0.48	0.29	0.22	0.46	0.36
Mean NE (ng/g)	550	565	465	355	370	380
Rate of NE synthesis ($ng\ g^{-1}\ hr^{-1}$)	148	270 (+89%)	135 (−9%)	78	170 (+119%)	137 (+75%)

* PS/RT: the data represent the absolute value of paradoxical sleep (PS) during the 5 hours following the intracisternal injection of H3-NE in each experimental situation, in relation to recording time (RT). † The computation of our K values was based upon our 0.5 hour and 5 hours compared with control values for K performed in the same laboratories under similar conditions. The endogenous values are the mean of the 0.5-hour and 5-hours determinations presented in Fig. 1.

cal sleep and only in the part corresponding to the diencephalon and telencephalon (Fig. 1A). Similar observations were made on the total radioactivity detected in the tissue. It is noteworthy that these 91 hours of deprivation did not lead to any appreciable modification of the endogenous content of norepinephrine in the two brain regions examined.

In the animals exhibiting marked rebound of paradoxical sleep, the H^3-norepinephrine in both brain regions was markedly decreased at 5 hours, whereas the endogenous content of the amine was not modified. Thus the specific activity of norepinephrine, like the H^3-norepinephrine content, was decreased significantly as compared with values in control animals (Fig. 1B).

Such changes in the specific activity of norepinephrine 5 hours after the beginning of this sleep recovery process could have resulted from modifications of norepinephrine metabolism and utilization previously induced by the deprivation period itself. In order to test this possibility, a group of sleep-deprived animals injected as in the previous experiment were again subjected to sleep deprivation by putting them back on the water-surrounded pedestals, after a period of 45 minutes necessary for the injection and recovery from ether anesthesia. They were killed 5 hours after the beginning of the injection of the labeled amine, during which sleep deprivation was maintained. Over the 45 minutes after the injection, there was no paradoxical sleep as revealed by our polygraphic recording. As shown in Fig. 1C, no significant variation of H^3-norepinephrine could be detected in either region examined, but a small and significant decrease of endogenous norepinephrine was observed in the brain stem and mesencephalon. The specific activity of brain norepinephrine in deprived and control animals was not significantly different 5 hours after the injection of the labeled amine.

As opposed to pharmacological approaches which have been used for the estimation of turnover rates of norepinephrine, the method we have employed which involves labeling rather than altering the endogenous pools enables us to study these processes under the different physiological conditions examined with no likelihood of specific interference with them.

After a period of total deprivation of paradoxical sleep lasting more than 3 days, there is a marked and significant reduction in the norepinephrine specific activity at the end of the 5 hours' experiments in the group of animals which has been allowed to experience a rebound of paradoxical sleep. This effect is visible in the two parts of the brain examined and suggests an increase of turnover of norepinephrine, which likely indicates an augmentation of synthesis and utilization of the amine, leading possibly to an increased availability of physiologically active norepinephrine in some central synapses during this period of rebound of paradoxical sleep.

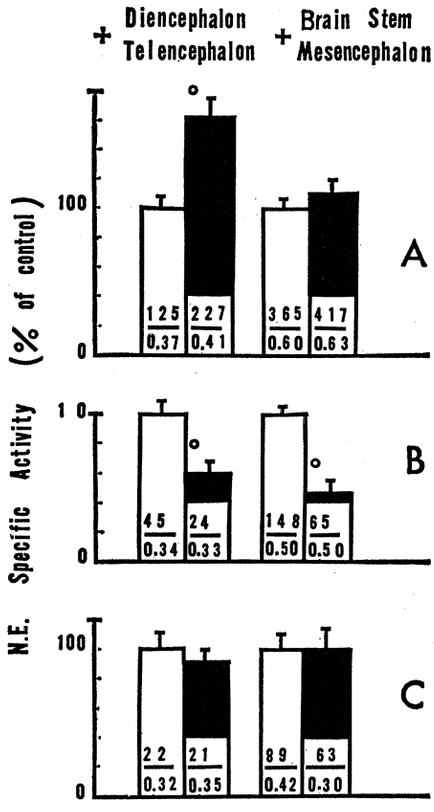

Fig. 1. Changes in specific activity of H^3-norepinephrine in the rat brain after 91 hours of paradoxical sleep deprivation. (A) Rats are killed half an hour after intracisternal injection of the labeled amine. (B) After intracisternal injection rats are put in isolated boxes for sleep recuperation and killed 5 hours after the beginning of the injection. (C) Forty-five minutes after intracisternal injection rats are again put on their respective supports surrounded by water, and killed 5 hours after the beginning of the injection. Each bar represents the specific activity of H^3-norepinephrine expressed in percent of control ± standard error of the mean. The control and experimental groups are respectively represented by white and black bars. The underlined numbers in the bars represent the H^3-norepinephrine concentration in nanocuries per gram, and the other numbers represent the endogenous concentration of norepinephrine in micrograms per gram. The white points represent the P values for the experimental group calculated as compared with controls. $P < .001$.

By comparing the relative changes in the specific activity of norepinephrine between ½ and 5 hours a global estimate of the turnover constant of norepinephrine efflux and of rate of synthesis of norepinephrine was made by assuming a single compartmental model for the distribution of norepinephrine in the two gross regions of the brain considered. There is almost a twofold increase in the synthesis of norepinephrine in the brain stem and mesencephalon during the rebound of paradoxical sleep, and no modification of this process in the animals which were kept from having paradoxical sleep (Table 1). This demonstrates a direct relationship between paradoxical sleep and increased synthesis and utilization of norepinephrine.

The correlation between paradoxical sleep and increased norepinephrine turnover is in agreement with the finding that α-methyl-dopa and α-methyl-*m*-tyrosine, which act indirectly as false neuro transmitters (*14*), suppress paradoxical sleep specifically and for a long period of time even after its previous selective deprivation (*2*). This could also explain the fact that some norepinephrine-containing neurons of the pontine tegmentum are necessary for the normal occurrence of paradoxical sleep as shown in the cat (*4*).

In the telencephalon and diencephalon, the marked increase of norepinephrine synthesis observed in the group of animals which had a recuperation of paradoxical sleep may result in a cumulative effect induced by paradoxical sleep and by a physical stress possibly due to the previous state. Physical stress has in fact been shown to increase the turnover of norepinephrine (*15*). In this region the synthesis of norepinephrine is still greater in the group which had a period of rebound of paradoxical sleep than in the group which was maintained in deprivation.

The reuptake process is an important means of inactivation of free norepinephrine in synapses (*16*). It is possible that the increased initial accumulation of exogenous norepinephrine observed after deprivation of paradoxical sleep in the part of the brain

in which are found mainly norepinephrine-containing nerve endings and axons is due to a change in some regulatory mechanism acting upon the uptake process. This effect seems to be specific for norepinephrine; we have not been able to detect it with H^3-serotonin under similar experimental conditions (*8*).

JEAN FRANÇOIS PUJOL
Unité de Neuropharmacologie Biochimique, Collège de France, Paris V^e, France

JACQUES MOURET, MICHEL JOUVET
Laboratoire de Pathologie Générale et Expérimentale, Faculté de Médecine, Lyon, France

JACQUES GLOWINSKI
Unité de Neuropharmacologie Biochimique, Laboratoire de Neurophysiologie Générale, Collège de France

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Littorina littorea: Occurrence in a Northern Newfoundland Beach Terrace, Predating Norse Settlements

In recent years several articles have dealt with the time of appearance and the apparent diffusion of the marine gastropod *Littorina littorea* Linné in eastern North America. It has been suggested that the species could have been introduced accidentally in Norse vessels during their voyages of around 1000 A.D., or subsequently (*1*). Because the species is well established on the beaches adjacent to the site of a Norse settlement near L'Anse aux Meadows on the Newfoundland side of the Straits of Belle Isle and because archeological examples antedating the European settlement of New England have been found, the suggestion of introduction by the Norsemen was not unreasonable. Now, however, we have evidence that the introduction occurred at a time appreciably earlier than that at which the Norsemen occupied this area.

During the summer of 1964, in the course of conservation work at the site at L'Anse aux Meadows, I encountered two examples of *L. littorea* (*2*) within the raised marine beach terrace on which the Norse houses had been constructed. To minimize flooding during the spring thaw, a 24-m drainage trench was dug transversely across the terrace, the surface of which is about 3.6 m above highwater mark. The trench, with a maximum depth of 2.4 m, revealed typical wave-washed sand and gravel capped with about 9.6 cm of dark brown fibrous turf. Below the influence of soil acids, there were some scattered fragments and occasional small concentrations of water-worn marine shells, mainly *Mytilus*, in friable condition. From the trench walls were obtained two examples of *L. littorea* and one specimen of *Buccinum undatum* Linné. They lay several feet below the surface, clearly in the undisturbed, wave-deposited material; these date from the period of terrace formation.

No determinations of age have been made for the marine terraces in northern Newfoundland and adjacent Labrador. In his detailed study of the region, Tanner (*3*) was reluctant to estimate the ages of the lower terraces. He attributed them to post-glacial times and remarked on the unmodified, fresh appearance of some terraces in certain situations; he also noted that the uplifting is seemingly still in progress. From my own observations in Newfoundland and Labrador I concur that many situations suggest that land rise continues at the present.

Those concerned with how a European gastropod reached America should check data on movements of hydrographic drift bottles in the northern Atlantic waters. The Labrador Eskimos in the vicinity of Hopedale, like their relatives in northeastern Greenland, were familiar with iron, in the form of nails, long before they established direct contact with Europeans. The main source of such iron was driftwood, presumably from European sources. If this source is born out by specific drift data, some examples of *L. littorea* might have traveled the same route on driftwood.

JUNIUS B. BIRD
Department of Anthropology, American Museum of Natural History, New York 10024

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