SLEEP-PROMOTING EFFECTS OF CEREBROSPINAL FLUID FROM SLEEP-DEPRIVED GOATS*

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In 1913 Legendre and Pieron[1] reported that injection of cerebrospinal fluid (CSF) from a sleep-deprived dog into the cisterna magna of a normal animal induced sleep in the recipient for 2–6 hours following the injection. Recipients of fluid from normal dogs remained alert. The "hypnotoxic" factor was said to be non-dialyzable and thermolabile. The Pieron phenomenon was reinvestigated in 1939 by Schnedorf and Ivy,[2] who reported positive results in 9 out of 20 trials. The experimental conditions involved severe stress to both donor and recipient animals. Donor animals were deprived of sleep for 7–16 days and the technique of transferring relatively large quantities of CSF from donor to normal recipient without anesthesia undoubtedly involved severe trauma to the experimental animals. For these and other reasons, Schnedorf and Ivy questioned the relevance of the Pieron phenomenon to normal sleep, although they were convinced that the phenomenon was real.

Monnier et al.[3, 4] have recently reported that electrical stimulation of "sleep centers" in the thalamus of rabbits causes release of a dialyzable sleep-promoting factor in cerebral venous blood. The role of this substance in the initiation of normal sleep is unknown, as is its relation to the nondialyzable material described by Pieron. A humoral factor inducing sleep has also been postulated by Hayashi who reported that CSF obtained during the period of depression following convulsive seizures contains a factor which inhibits convulsions[5] and promotes sleep[6] when injected intraventricularly. Hayashi suggested that the active principle might be 4-amino-2-hydroxybutyrate or possibly 4-OH-butyrate.

We have now conducted a new investigation of the Pieron phenomenon, taking advantage of new techniques which enable experiments to be carried out under more physiological conditions than were possible in the past. Crucial to our experiments is an abundant supply of CSF from goats which are provided with nylon guide tubes permanently implanted in occipital bone above the cisterna magna.[7, 8] CSF can be withdrawn repeatedly from each animal at the rate of 0.1 ml/minute for many hours without the use of anesthesia and without causing the animals any apparent discomfort. For initial trials we prepared five cats with chronically implanted intraventricular cannulas of the Feldberg-Sherwood type.[9] The ventricles of these animals were infused slowly (0.1 ml/min) with 1–3 ml of CSF from a normal goat or from the same goat which had been deprived of sleep for 72 hours. Cats which received fluid from sleep-deprived goats fell into a profound sleep or torpor which lasted 12–24 hours; the "sleep" appeared to be natural in the sense that the cats could be awakened by noise or handling, but reverted to sleep when left undisturbed. Some animals fell asleep during the infusion. Similar behavior was not observed when the same cats were infused with control CSF from the same (non-sleep-deprived) goats. These results supported Pieron's observations and indicated that the phenomenon was not species-specific. However, laboratory

cats usually sleep during most of each 24 hours and are therefore ill suited for the biological assay of sleep-promoting factors. Thus we were led to systematic studies on rats, making use of alterations in their regular day-night cycles of sleep and activity to obtain a quantitative biological assay for the hypothetical sleep factor.

Methods.—(1) *Intraventricular infusions in rats:* Extradural guide tubes were implanted chronically in parietal bone over one lateral ventricle. The ventricle was punctured through the guide tube at the time of each infusion by a sterile no. 26 probe attached via 40 cm PE 20 tubing to a motor-driven syringe. Direction of the probe was determined by the guide tube; depth of penetration into brain was set by a collar soldered to the shank of the probe. Infusion pressure was recorded from a T connection between the syringe and probe. All infusions were at the rate of 3.3 µl/min for 30 min (0.1 ml total). An infusion pressure of less than 10 cm H_2O relative to the auditory meatus was one indication that the tip of the probe was free in the ventricle. During each infusion the rats were unrestrained in 15- × 25-cm open cages. Details of the technique of implantation of guide tubes, postoperative care, infusion procedures, distribution of materials in ventricles, and functional tests for adequate infusion are available.[10]

(2) *Measurement of day-night activity cycles:* Rats were kept in individual cages at 18 ± 2°C. Room lights were turned on at 7 A.M. and off at 7 P.M. The room was entered only between 3 and 6 P.M. to clean cages, provide food and water, and clean the guide tubes. Infusions were always conducted in this period. Each of 12 cages was traversed by two light beams from GE 46 bulbs operated at 4.2 volts and focused through red filters on Clairex CL 503 photoconductive cells. The electrical circuit was arranged to activate a relay momentarily whenever a photocell was exposed to light after a 2-sec interruption by the rat. Relays controlled electromagnetic counters, one per cage, located in an adjoining room. The bank of 12 counters was photographed automatically at intervals of 3 or 6 hr.

(3) *Sleep deprivation and withdrawal of CSF (goats):* Goats with cisternal guide tubes were kept awake for 72 hr, this duration being commonly used in experiments on sleep deprivation in human volunteers.[11] After 72 hr the cisterna magna was punctured and fluid withdrawn into a sterile, iced syringe at the rate of 0.1 ml/min for 5 hr. Each 10-ml aliquot was deposited in a sterile container and frozen until subsequent use. All fluids were passed through sterile Millipore bacterial filters immediately prior to infusion.

Results.—(1) *Daily activity cycle of rats:* Three to four days are required for rats to become adapted to their cages and the routine. After this time the activity cycle is reasonably constant as shown by the control data of Table 1. During the period 9 P.M. to 9 A.M. the rate of activity (interruption of light beams) is in the range 70 to 90 counts/hr per rat; from 9 A.M. to 3 P.M. during the light cycle, the animals spend much of the time sleeping and the counts are in the range 10 to 30/hr per rat.

TABLE 1

ACTIVITY OF RATS BEFORE AND AFTER INTRAVENTRICULAR INFUSION OF 0.1 ML CSF FROM GOAT Z-5

		Average Counts/Hr/Rat	
		Group I*	Group II†
Control period (8-day mean ± SEM‡)	9 P.M.–3 A.M.	79 ± 8	79 ± 12
	3 A.M.–9 A.M.	82 ± 13	78 ± 12
	9 A.M.–3 P.M.	30 ± 8	48 ± 16
Postinfusion first 24 hr	9 P.M.–3 A.M.	67	26
	3 A.M.–9 A.M.	77	36
	9 A.M.–3 P.M.	30	35
Postinfusion second 24 hr	9 P.M.–3 A.M.	117	106
	3 A.M.–9 A.M.	106	99
	9 A.M.–3 P.M.	50	36

* Group I (5 rats) receiving normal CSF.
† Group II (5 rats) receiving CSF withdrawn after 72 hr of sleep deprivation.
‡ The standard errors are calculated from the daily variations of the group averages about the 8-day mean, i.e., $n = 8$.

(2) *Effects of infusing CSF from normal and from sleep-deprived goats:*   A typical experiment is summarized in Table 1.   The activity counts of ten rats were recorded for 8 consecutive 24-hour cycles.   Five rats (group I) were then infused with CSF from a normal goat and five rats (group II) were infused with CSF taken from the same goat after it had been deprived of sleep for 72 hours.   Rats were selected for each group so that the average of their individual nightly activity counts was approximately the same in each group during the eight-day control period (i.e., 78 to 82 counts/hr/rat) as shown in the first two rows of Table 1.   There was little change in the activity counts of rats in group I during the night subsequent to infusion as compared with the eight previous control nights.   In contrast, the activity of rats receiving fluid from the sleep-deprived goat (group II) was reduced from 79 ± 12 to only 26 counts/hr/rat in the period 9 P.M. to 3 A.M., a depression equivalent to that of the normal daytime sleeping level.   Activity was still substantially reduced from 3 A.M. to 9 A.M.   On the following night (second 24 hr) the activity of both groups was slightly higher than normal; this supernormal phase is variable in rats receiving control fluid but is a characteristic and significant feature of the response in rats that have been depressed for one night as a result of receiving the "sleep factor."   The normal cycle of activity is resumed about 48 hours after an infusion, as shown in Table 2, which summarizes the average activities of ten rats for several days before and after infusion of CSF from a sleep-deprived goat.

TABLE 2

EFFECTS OF INFUSING SLEEP CSF* ON TEN RATS

	Preinfusion 4-day mean and extremes	—Group Averages (Counts/Hr/Rat)— Postinfusion		Subsequent 4-day mean and extremes
Time period		1st 24 hr	2nd 24 hr	
7 P.M.–1 A.M.	71 (66–77)	29	80	66 (60–71)
1 A.M.–7 A.M.	89 (85–98)	61	102	88 (86–91)
10 A.M.–1 P.M.	22 (17–29)	21	27	17 (15–18)

* From goat B6 after 72 hr sleep deprivation.

Not all rats receiving CSF from sleep-deprived goats showed the striking response illustrated by the mean values of Tables 1 and 2.   It was not uncommon, especially in our early experiments, to find that one to three out of six rats failed to respond significantly.   Conversely, the activity of an occasional rat receiving control fluid or no fluid at all was markedly depressed.   Failure to respond may result from failure of the infusate to reach the third or fourth ventricles; a large proportion of animals develop noncommunicating hydrocephalus after three infusions and positive responses were never obtained in these animals.   In recent experiments, utilizing rats which had not been infused more than twice previously, the incidence of failure to respond was reduced to 7 out of 51 trials.

Table 3 summarizes responses to infusion with artificial CSF (15 rats), CSF from normal goats (52 rats), and CSF from goats deprived of sleep for 72 hours (80 rats). These average results are qualitatively similar to those illustrated in the single experiments of Tables 1 and 2.   The mean activities of the 67 rats infused with control solutions were slightly less than normal in the 9 P.M. to 3 A.M. interval, unchanged from 3 A.M. to 9 P.M., and slightly above normal on the following night. In contrast, the mean activities of rats receiving fluid from sleep-deprived goats

TABLE 3

EFFECTS OF ARTIFICIAL CSF, CSF FROM NORMAL GOATS, AND CSF FROM GOATS
DEPRIVED OF SLEEP FOR 72 HR ON MOTOR ACTIVITY OF RATS

	Activity, Relative to Preinfusion Values (Mean ± SEM)		
	Artificial CSF	CSF from normal goats	CSF from sleep-deprived goats
Postinfusion (1st 24 hr)	$n = 15$	$n = 52$	$n = 80$
9 P.M.–3 A.M.	0.87 ± 0.08	0.83 ± 0.07	0.59 ± 0.03
3 A.M.–9 A.M.	1.05 ± 0.012	1.02 ± 0.07	0.87 ± 0.05
Postinfusion (2nd 24 hr)	$n = 12$	$n = 42$	$n = 75$
9 P.M.–3 A.M.	1.25 ± 0.09	1.26 ± 0.06	1.30 ± 0.065
3 A.M.–9 A.M.	1.10 ± 0.08	1.22 ± 0.08	1.48 ± 0.09

was 59 ± 3 per cent of normal in the first six-hour interval and 86 ± 5 per cent of normal in the second. There was a significant overshoot in the first night of recovery, especially from 3 to 9 A.M.

CSF from several sleep-deprived goats was filtered through Dialflo UM2 gel membranes (Amicon Corp., Cambridge, Mass.) which are said to retain molecules of molecular weight greater than 1000–2000. CSF proteins estimated by the Lowry technique[12] were concentrated three- to fivefold in the filtrand and were less than 5 µg/ml in the filtrates. Both the filtrates and filtrands decreased the 9 P.M.– 3 A.M. activities of 20 rats to less than 50 per cent of normal. Similar results were found in dialysis experiments, using Visking tubing.

Discussion and Conclusions.—Our experiments leave little doubt that slow intraventricular infusions of small amounts of CSF from sleep-deprived goats have a profound depressant effect on the motor activity of rats for several hours subsequent to the infusion. From a behavioral point of view the rats appear to sleep; they curl up in a corner of the cage in the same manner as they do during their normal daylight sleep period, waking occasionally to obtain food or water. They can be wakened at any time by noise or handling but revert to sleep when left undisturbed. Many different types of measurements (EEG, EMG, temperature, etc.) will have to be made to determine the nature of this sleep-like behavior. Nevertheless, the essential observation made by Pieron can be regularly confirmed using objective methods under relatively physiological conditions involving moderate sleep deprivation, minute amounts of CSF, and little or no trauma to either the recipient or donor animals. The fact that fluid from sleep-deprived goats is active in cats and rats suggests that we are dealing with a humoral factor of general and fundamental importance to the sleep mechanism. In preliminary experiments, performed in collaboration with Dr. M. L. Karnovsky, it has been possible to concentrate the active factor several-fold after partial removal of ionic constituents from protein-free ultrafiltrates. These concentrates are considerably more active than the original CSF and encourage us to believe that highly concentrated material can be obtained for chemical studies as well as for physiological experimentation in large animals.

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