serum used for resuspending the mixture were artificially increased by introducing sugar from exogenous sources. It was of interest that no matter how poor had been the control of the diabetes, patients with ketoacidosis and complicating infection were excluded from the study, no evidence was found for an impaired function by these cells.

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Induction of Paradoxical Sleep by Lights-Off Stimulation.* (31571)

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Sleep cycles have been studied in man and a number of other mammalian species, including the rat(1). Each cycle consists of 3 discrete phases classified on the basis of electroencephalographic (EEG) activity: alertness (A), slow wave sleep (SS) and paradoxical sleep (PS). The paradoxical phase is a recent discovery, and much effort has been expended to unravel its physiology. That PS is required for the homeostasis of the organism appears well established, since depriving the animal of PS leads to a compensatory increase in time spent in this sleep state(2) and extreme deprivation of PS can lead to hallucinations(3). When the pontine center which appears to initiate PS is destroyed in the cat(4), the animal may be left with a 2-stage sleep cycle (A and SS) or it may suffer insomnia to the point of death. However, almost nothing is known concerning discrete functional mechanisms which may be regulated *via* PS. This report presents evidence that PS in the rat can be triggered by change in lighting, and that the PS so induced may be involved in the maintenance of various physiological mechanisms which are of a circadian nature and light sensitive.

Methods. Mature cycling female Sprague-Dawley rats maintained on a diurnal light cycle of 14 hours light and 10 hours dark were used in our experiments. Both cortical and subcortical electrodes were implanted chronically, the latter in the following brain regions: preoptic, arcuate and ventromedial hypothalamus, amygdala, reticular formation of hippocampus. Each animal received 3 deep concentric bipolar electrodes and 2 superficial silver ball electrodes on the frontal and parietal cortex. Recording was done with Grass EEG equipment with the subjects in a sound-proof recording room, in which short (30 minutes) light cycles were controlled automatically with a programmable Gerbrand timer. For most experiments a cycle of 25 minutes "lights-on" and 5 minutes "lights-off" was employed. Recordings were made simultaneously from 2 rats in cylindrical glass chromatography jars separated by an opaque partition. The rats did not appear to influence each other. Each experimental session lasted approximately 8 hours, 10:00 a.m. to

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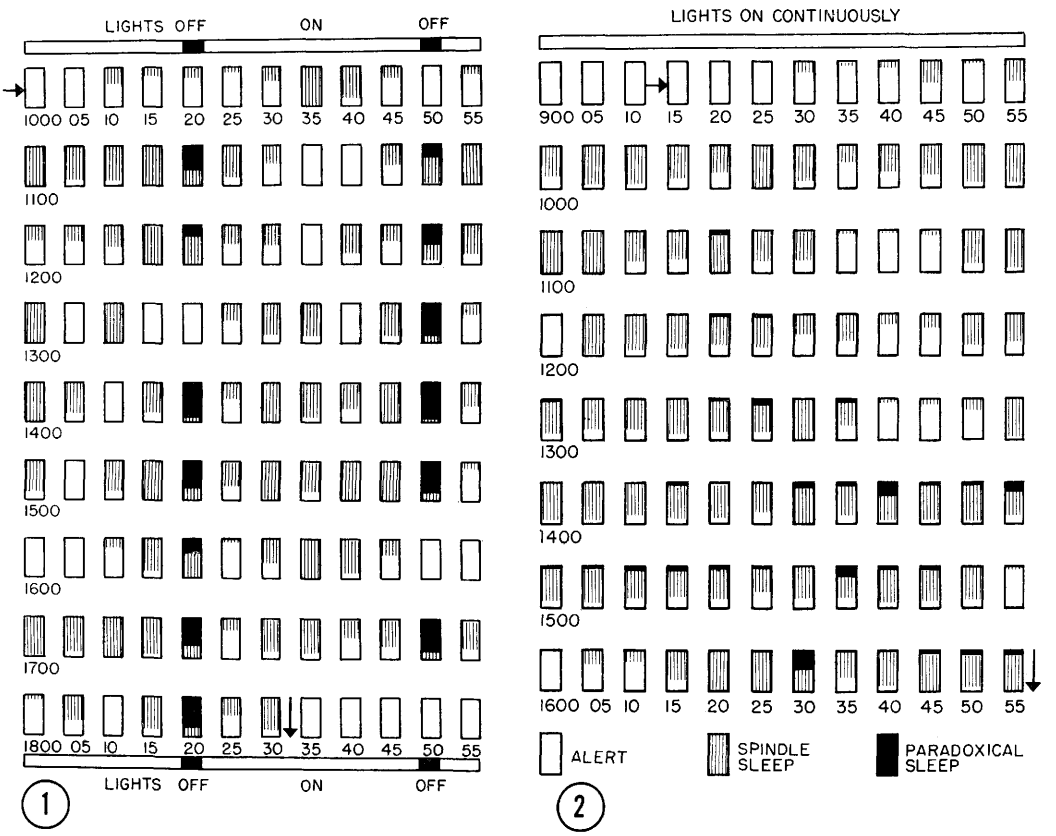

FIG. 1. Analysis of EEG tracing by 5-minute intervals for rat 6. Animal was on a 30-min cycle of 25 min "lights on" and 5 min "lights off." Note paradoxical sleep occurs only during "lights off." Recording was started at horizontal arrow and terminated at vertical arrow. See Fig. 2 for symbols.

FIG. 2. Analysis of EEG tracing by 5-minute intervals for rat 6. Illustration shows a typical control record with lights on constantly.

6:00 p.m. with the animals maintained under diurnal conditions in which the light periods continue from 5:00 a.m. to 7:00 p.m. A one-way mirror window allowed direct observation of the subjects. EEG records were analyzed and plotted by 5-minute intervals for percentages of A, SS and PS. A total of 33 days on 9 rats was analyzed and plotted.

Results. In the experimental 30-minute light cycles all of 9 rats tested showed the bulk of PS during the 5 minutes "lights-off" interval and very little or none during the intervening 25-minute period of "lights-on" irrespective of the phase of the estrous cycle. One animal showed PS only during "lights-off" intervals (Fig. 1). An analysis of the EEG records, for the same animal, during a control recording period ("lights-on" all the time) is shown in Fig. 2. The two figures clearly illustrate the profound effect of "lights-off" as a stimulus to PS.

With a few exceptions, after the EEG tracing had 5 minutes of SS prior to "lights-off," PS always occurred within 33 seconds of "lights-off." Ordinarily the PS episode did not last through the entire 5-minute dark period. If the rat was fully alert at "lights-off," it would often remain alert or achieve PS only after a mean latency of 110 seconds. Seven of the nine animals showed at least one EEG shift to PS with a zero latency interval after "lights-off." Data for the 9 animals have been summarized in Table I. The stimulus provided by "lights-on" did not appear to affect, or alter, ongoing brain activity in the rat, as judged by the EEG tracings.

TABLE I. Mean Responses for Individual Animals and for the Group, Showing Latency to PS and Duration of PS in Seconds Following "Lights Off."

Animal No.	No. of test days	Animals in SS at "lights off"		Animal in A at "lights off"			No. of episodes of PS with zero latency
		Latency SS	Duration PS	Latency A	Latency SS	Duration PS	
		Sec					
1	4	41	148	29	81	134	4
2	3	29	181	10	88	90	2
3	3	43	148	10	87	134	1
4	6	24	135	22	62	119	6
5	1	38	119	30	116	54	1
6	5	48	192	12	95	157	1
7	5	32	161	18	87	144	2
10	5	9	160	29	101	110	0
12	1	—	—	18	88	78	0
Total animals	Mean	33	156	20	89	113	

Discussion. Sleep cycles during the daylight hours have been investigated for the rat by Roldan *et al*(5), who noted a 13-minute cycle made up of 10 minutes SS, 2 minutes PS and 1 minute alert. Recent work by Yokoyama *et al*(6) indicates that 40 to 50% of the PS episodes are separated by A-SS intervals of 10 to 20 minutes. The 30-minute cycle we employed is therefore not directly reinforcing to these naturally occurring sleep cycles in the rat. However, we were able to induce PS in almost 100% of the "lights-off" stimuli when the animal was in SS. In a few experiments which were run with a 15-minute cycle of 10 minutes "lights-on" and 5 minutes "lights-off," the animals again responded by showing the bulk of the PS during the "lights-off" phase.

The rat is a nocturnal animal, and in recent long term EEG studies employing continuous 24-hour recordings from subjects with electrodes chronically implanted in the brain we have observed that even under controlled lighting in the laboratory the subject sleeps a great deal more during the day ("lights-on") than during the night ("lights-off") (Lisk, *et al,* unpublished). This was true not only for the more common slow wave or spindle sleep but also for the activated paradoxical or REM sleep in which the EEG pattern resembles arousal. A bustle of activity is commonly noted in a light-controlled rat colony as the lights go off in the evening. This observation raised the question of whether switching off the lights might serve as a generalized alerting stimulus. Much to our surprise we found that if the rat was in SS "lights-off" generally triggered a period of PS. The present experiments are the result of investigating this phenomenon further. The immediate bustle of activity in a rat colony at "lights-off" may be caused by a relatively small number of animals that happen to be alert at the time.

What is the utility of the "lights-off" signal to the rat? The ability to respond to light cycles is of fundamental importance to many animals. In a nocturnal animal absence of light must be an important alerting stimulus *per se.* Actually "arousal type" stimulation of the reticular formation has been reported to induce PS under certain conditions in the sleeping cat(7,8). The rat is representative of a large group of mammals which employ data from light-dark cycles to regulate such basic rhythms as the estrous cycle(9). If the animal is placed in constant light this cycle ceases and ovulation fails. Everett and Sawyer(10) have shown that under conditions of cyclic lighting (14L-10D) a specific signal for ovulation occurs between 2:00 to 4:00 p.m. on the day of proestrus, an interval which they called the "critical period." If brain function is inhibited with an anesthetic during the critical period ovulation will not occur that night. One can wait until the critical period next day and again block brain function and inhibit ovulation. Thus a 24-hour rhythm has been demonstrated. Recent experiments in-

volving changes in length of daily light-darkness intervals suggest to us that the critical period is more closely related to the time of "lights-off" than to when the lights come in, *i.e.*, starts 5 to 6 hours before "lights-off" rather than a definite interval after "lights-on"(11). The control of reproductive function in the rat by light-dark cycles serves as an example of the many functions which appear to be light regulated and have a circadian nature in this species. Thus a general brain response to "lights-off" may play an important regulatory function for control of cyclic phenomena in this species.

Preliminary observations on the rabbit, a species which does not have a spontaneous ovulation cycle and does not appear to employ light cycles to regulate function to the same extent as the rat, indicate no marked influence of a "lights-off" signal for regulation of brain function in relation to sleep-wakefulness intervals.

Summary. Paradoxical sleep occurred in rats in response to a 5-minute period of "lights-off" stimulation. Usually the paradoxical sleep episode had ended before the lights came on again. The "lights-on" stimulus did not appear to produce any changes in the sleep-wakefulness cycle.

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Effect of Polyvinyl Pyrrolidone on Plasma Coagulation Factors.* (31572)

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Polyvinyl pyrrolidone (PVP) provides effective protection for human red blood cells during frozen storage in liquid nitrogen. Hemolysis during processing and loss of ability to survive on subsequent transfusion remain at an acceptably low level(1,2). PVP does not enter the red cells; thus there is no need to wash it out of the cells by the series of complicated maneuvers required for the endocellular protective agents, glycerol and dimethyl sulfoxide(3,4). The principal obstacle to the use of PVP has been evidence that the transfused polymer is stored in the reticuloendothelial tissues of the recipients for prolonged periods(5). It seemed possible that this obstacle might be largely overcome by centrifugation of the thawed blood, discarding the plasma with its contained PVP before transfusion of the red blood cells. In the course of experiments to evaluate this approach, it became obvious that gross precipitate formation occurred in the plasma of blood to which PVP had been added, associated with a marked effect on plasma coagulation factors. The mechanism of the interaction and quantitative effects were therefore explored.

Materials and methods. 1. Blood for the

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