which have a bearing on Barber's question.

In order to study the flash-communicative systems of fireflies it is essential to have females of the species being studied. Unfortunately, these are usually at a premium. An hour or two of searching may yield but one, more frequently none. The best method is to walk about the area flashing a pocket flashlight in a manner which simulates the flash-pattern of the males of that particular species. Although in competition with dozens or hundreds of male fireflies, the flashlight will often draw flash-responses from females 6 to 12 m away, while male fireflies are seldom answered at distances greater than 3 m. While searching in this manner for female *Photinus* fireflies, I have on five occasions received flash-responses from *Photuris* females.

1) *Fife, Goochland County, Virginia, 13 June 1963.* While searching in the site of *Photinus ignitus* Fall, I received a single flash-response to a quick flash of the flashlight after a delay of 5.5 seconds at 14°C. This is the delay time and flash of *P. ignitus* females. When collected after several more similar flash-responses, this female was found to be *Photuris* (*2*).

2) *Red Hills State Park, Lawrence County, Illinois, 24 July 1963.* During the early period of activity of *Photinus pyralis* (Linné) I located a *Photuris* female in a *P. pyralis* site by her flash given 2.2 seconds after a flash from my flashlight at 21°C. This is the time delay Buck (*3*) found for *P. pyralis*.

3) *Gainesville, Alachua County, Florida, 24 May 1964.* In the site of a large population of a species in the *Photinus collustrans* LeConte complex, two *Photuris* females repeatedly answered my single flash with a single long pulse, 1 second in duration after a delay of about 1 second (the flash-and-delay-characteristics of this *Photinus* species). No *Photuris* males were seen.

4) *Gainesville, Florida, 6 April 1965.* The flash pattern of males in one species of the *Photinus consanguineus* LeConte complex consists of two short pulses separated by about 2 seconds. This phrase is repeated every 4 to 7 seconds. While searching for females I received a response from the direction of a low weed along a stream. The flash appeared greener and brighter than usual and upon investigation I found a large (14 mm), black *Photuris* female. One 11-mm black *Photuris* male was later caught which emitted single, ragged, flickering flashes at intervals from 3 to 5 seconds in duration. I watched this female for the next half-hour, and during that time she responded to twelve passing males of the *Photinus* species with a single flash-response similar to that of the females of this species—a single pulse about 1 second after the second male pulse. All of these males were at least partially attracted to her. One flew into the stream. Two flew into the grass below her and then she stopped answering them; presumably she couldn't see their flashes. Eight of the males were attracted to within 1 m of her and then she stopped answering them. While answering, she would occasionally flash after the first male pulse and then again after the second pulse. Usually she answered only after the second pulse. I also noted that, as the males neared her, she greatly reduced the intensity of her flashes. The last male attracted, after three or four flash-exchanges, landed about 7 cm from her. After another flash sequence I turned on my light and found him 15 cm from her. Following the next flash exchange, after a pause of 10 to 15 seconds, I checked and found she was clasping him and chewing on his pronotum.

5) *Gainesville, Florida, 15 April 1965.* The flash pattern of the males in one species in the *Photinus consimilis* Green complex consists of two or three pulses delivered at 1.2- to 1.4-second intervals; the flash pattern is repeated every 10 to 14 seconds. Being unable to find females of this species, I tried unsuccessfully to attract the males, using a variety of different flashlight techniques. Later, while again searching for females, I received a response at 5.0 seconds delay after the last stimulus pulse at about 22°C. The response flash consisted of two, long, single pulses about two seconds apart. This female responded with a similar delay several times and, when collected, was found to be a *Photuris*. Using this flash-response I was able to attract several, although not all, of the *Photinus* males tested.

The answer to Barber's question has precipitated a deluge of new questions, not the least of which concerns the males of the genus *Photuris*. Is the female *Photuris* predaceous before she has mated? If so, how does her mate avoid the fate of attracted *Photinus* males? Also of interest is the question of how this interspecific signaling developed in evolution. The most logical beginning would have the female *Photuris* preying on *Photinus* males of species with signal systems similar to their own. Finally, can a single *Photuris* species prey upon more than one *Photinus* species with different signal systems? In other words, how many flash patterns do *Photuris* females have in their "repertoires," and is predation on *Photinus* fireflies in any sense obligatory? Certainly, this kind of predation must have had effects on the evolution of the signal systems and other behavior of members of the genus *Photinus*.

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Rhythmic Enzyme Changes in Neurons and Glia during Sleep

Abstract. *Rhythmic changes occur in the activities of enzymes in both the neurons and neuroglia isolated from the caudal part of the reticular formation of rabbits killed during sleep and wakefulness. During sleep, enzyme activity is high in the neurons and low in the glia; during wakefulness, this situation is reversed. In the oral part of the reticular formation rhythmic enzyme changes occur only in the neurons. No rhythmic changes occur in the hypoglossal and trigeminal mesencephalic neurons and glia. These findings indicate that the caudal part of the reticular formation reflects in metabolic changes the biological clock behind the sleep rhythm.*

We reported previously (*1*) that rhythmic changes in enzyme activity occur during sleep and wakefulness in both neurons and neuroglia in part of the brainstem. We studied the succinate-oxidizing enzyme system in the large nerve cells and glia of the

nucleus reticularis giganto-cellularis in the caudal part of the reticular formation of rabbits. Succinate has a unique position in the Krebs cycle, electrons being transferred directly to the cytochrome system of the respiratory chain. During sleep, enzyme activity was high in the neurons, and low in the glia. During wakefulness, this relation was reversed.

In the study reported here we extended our analyses to the neurons and neuroglia of the nucleus reticularis pontis oralis and the hypoglossal and trigeminal nuclei. We chose the oral part of the reticular formation of the brainstem because of its position as a target for the activity of the caudal part, as reported by Moruzzi (*2*). Moruzzi's electrophysiological studies indicate that the lower reticular formation has a damping influence on the upper part and produces the characteristic electroencephalographic pattern of sleep. We found that changes occur in the enzyme activities of the oral and caudal part of the reticular formation and that no such changes occur in areas outside the reticular formation during sleep and wakefulness.

White rabbits (165 in all) weighing 1.5 to 1.7 kg were used. They were divided into three groups. Those in group 1 (52) were trained to sleep in individual wooden boxes provided with a hole in the side, fitting around the neck. A training period of 5 hours each day was established between 10 a.m. and 3 p.m., the animals being kept in a quiet room with subdued light where they could be observed through a window in the door. After 12 days the animals were killed, without being awakened, 1.5 hours after being placed in the boxes. Using this type of arrangement, Sterner (*3*) studied the electroencephalograms (EEG) of rabbits and found that only four to five training periods were necessary for adapting the animals to the boxes, in which they soon showed the synchronous EEG of sleep. Using rabbits with implanted electrodes, he studied the changes of the sleep rhythm during different conditions; rabbits living in their home cages readily fell asleep between periods of feeding. For this reason we used control animals (45), designated group 2, which were kept active and awake by gentle handling for at least 1 hour and were killed as described earlier (*4*). Rabbits (68) in group 3 were kept in their home cages without being disturbed unnecessarily.

When a rabbit was killed the skull was rapidly opened and a section through the nucleus reticularis giganto-cellularis of the brainstem at the lower level of the acoustic tubercles was removed. The section was immediately placed in 0.25*M* sucrose or in Krebs-Ringer solution. The large nerve cells and the equivalent volume and dry weight of the glia surrounding the perikarya were isolated by freehand dissection as described previously (*4*). Each glia sample contained an average of eight glial nuclei.

The activity of the succinate-oxidizing enzyme system was determined by a modified microdiver technique (*5*), according to the Zeuthen method. The weight of each diver was from 0.2 to 0.3 mg, corresponding to a gas volume of 0.10 to 0.15 μl. Only one cell sample was used in each diver. The results are shown in Table 1.

In the nucleus reticularis giganto-cellularis caudalis, the enzyme activity of the nerve cells was almost 3 times higher during sleep than during wakefulness. In nerve cells from rabbits in group 3, the amount of activity was between that of nerve cells from rabbits in groups 1 and 2 (Table 1). This is interesting since the EEG recordings of such rabbits (group 3) showed both the synchronous rhythm of sleep and the arousal pattern (*3*). The enzyme activity of the glia cells was significantly lower during sleep than during wakefulness, the inverse of the result for the nerve cells.

Table 1. Succinoxidase activity of neurons and glia isolated from rabbits. The results are expressed as 10^{-4} μl of oxygen per sample per hour. Group 1, sleep; group 2, wakefulness; group 3, cage controls.

Group	Nerve cells		Glia	
	Activity (10^{-4} μl O_2)	No. of analyses	Activity (10^{-4} μl O_2)	No. of analyses
	Nucleus reticularis giganto-cellularis			
1	3.41 ± 0.51	29	2.34 ± 0.18	25
2	1.30 ± 0.25	24	3.06 ± 0.24	28
3	2.74 ± 0.21	39	2.16 ± 0.18	33
	Nucleus reticularis pontis oralis			
1	6.38 ± 0.58	35	3.50 ± 0.30	19
2	4.01 ± 0.52	15	2.94 ± 0.21	17
3	5.41 ± 0.39	32	3.72 ± 0.58	12
	Nucleus trigeminus mesencephalicus			
1	2.68 ± 0.29	9	1.46 ± 0.23	8
2	3.08 ± 0.83	5	1.11 ± 0.12	5
3	3.21 ± 1.16	5	1.15 ± 0.15	13
	Nucleus hypoglossus			
1	1.03 ± 0.17	13		
2	0.83 ± 0.08	13		
3	0.68 ± 0.13	3		

When wakefulness was compared to sleep, the difference between the enzyme values in nucleus reticularis giganto-cellularis proved to be significant both for nerve cells ($p < .001$) and glia ($p < .02$). Values for group 1 compared to those for group 3 were not significant. The difference between the values for the nerve cells in the nucleus reticularis pontis oralis of groups 1 and 2 was significant ($p < .01$).

If the quotients of the enzyme activities per nerve cell and glia sample are compared in the groups 1, 2, and 3, the values 1.46, 0.43, 1.27, are obtained, respectively. A diurnal rhythm is thus clearly reflected by the ratio of enzyme activity in the nerve cells to that in the glia during wakefulness and sleep.

In the nucleus reticularis pontis oralis, the enzyme activity of the neurons was about 60 percent higher during sleep than during wakefulness. The neurons of rabbits in group 3 showed activity values between those of rabbits in groups 1 and 2.

The enzyme activity of the glia was the same during sleep and wakefulness in all three groups. To test the lability of the cellular enzyme activities in the neurons of the reticular oral region, 13 of the animals in group 1 were placed in their boxes in a soundproof room during sleep. In the neurons of these animals the enzyme activity was remarkably high compared to that of the active controls ($9.02 \pm 0.72 \times 10^{-4}$ μl per cell per hour compared to 4.01 ± 0.52). The average activity of the nerve cells in group 2 was 6.38 ± 0.58; this shows that an arousal by weak sounds can be reflected in significantly decreased enzyme activity values.

For control purposes, we studied neurons and glia of a sensory type from an area of the brainstem not related to the reticular formation—the large neurons of the nucleus trigeminus mesencephalicus. No significant differences were apparent when the neuronal enzyme activities were compared during sleep and wakefulness. Similar results were obtained for the glia. The ratio between the neuronal and glial enzyme activities remained around 3. As control neurons of a motor type, we used the hypoglossal nerve cells. The major part of these neurons form a uniform group with respect to size, dry weight, protein, and RNA content (*6*).

In comparison with the trigeminal cells, the hypoglossal nerve cells are rather small, which is also reflected in the low oxygen consumption values per cell and per hour. There were no significant differences in the enzyme activities of these cells during sleep and wakefulness.

Our results indicate that the caudal part of the reticular formation is a center of the brainstem which reflects in pronounced metabolic changes the

biological clock behind the sleep rhythm.

Our data are too limited to provide an explanation of this mechanism, but we have drawn certain general conclusions. The neuron and glia form a functional unit with two parts which are energetically coupled and influence each other functionally (*5, 7*). When functional demands increase, inverse changes in enzyme activity occur in the neurons and their glia within hours. The total protein contents per neuron and glia follow these enzyme changes. This indicates that a synthesis of enzyme protein occurs and not only changes in enzyme activities. Furthermore, it seems significant that inverse RNA changes also occur concomitantly. A recent electrophoretic analysis of the RNA fractions which increased in the neuron and decreased in the glia showed an identical base ratio composition of the two RNA fractions. This suggested that RNA molecules or, alternatively, nucleotides (*8*) could be transferred from the glia to the neuron. In summary, such a two-cell collaboration would form a stable functional system from a cybernetic point of view. As a response to functional demands, primary processes in neurons and glia comprising RNA, enzyme, and other types of protein synthesis can evidently vary between two levels.

We suggest that the energy metabolism in the caudal part of the reticular formation oscillates with inverse changes between the neurons and the glia during the circadian sleep rhythm. This recalls the findings of Moruzzi (*2*) that an area within the lower reticular formation of the brainstem has a damping influence on the upper part of the reticular formation and produces the EEG pattern of sleep. The biochemical response of the neurons in the oral part of the reticular formation in the present study may therefore be interpreted as influenced by the caudal part.

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Transfer of a Response to Naive Rats by Injection of Ribonucleic Acid Extracted from Trained Rats

Abstract. *Rats were trained in a Skinner box to approach the food cup when a distinct click was sounded. Ribonucleic acid was extracted from the brains of these rats and injected into untrained rats. The untrained rats then manifested a significant tendency (as compared with controls) to approach the food cup when the click, unaccompanied by food, was presented.*

Although there has been abundant theorizing of late which implicates ribonucleic acid (RNA) in the process of memory storage (*1*), direct experimental evidence in support of this contention has been scanty. Hydén and Egyhazi (*2*) reported changes in the ratio of bases in the nuclear RNA of vestibular neurons of rats after the animals had performed a wire-climbing task. In further work (*3*), Hydén and his associates extended their biochemical analyses and studied an additional training paradigm involving reversal of handedness in rats. In the most recent of these experiments, they reported that different types of neural RNA are produced during the early, as compared with the later, stages of learning. Preliminary experiments with planarians (*4*) indicate that, if RNA from trained animals is injected into naive animals, the naive animals respond more than do controls upon subsequent testing. In the present study with rats we used a direct test similar to that used for the planarian experiments.

We used 50- to 60-day-old male Sprague-Dawley rats, each weighing approximately 250 g. Eight rats received magazine-training in a standard Grason-Stadler Skinner box—that is, they were trained to approach the food cup upon hearing the distinct click produced by operation of the pellet dispenser. Magazine training was accomplished as follows. On the first day, a rat deprived of food for 48 hours was placed in the Skinner box and allowed to eat two 45-mg Noyes pellets which had been placed in the cup. Then, while the rat was investigating the cup, the food magazine was operated a number of times in succession, producing each time a distinct click and delivery of a single 45-mg pellet. As training progressed, the click was withheld until the rat moved first a short, and later a longer distance from the cup. During this time, the rat was permitted a number of interspersed cup investigations which were not preceded by the click and hence were not rewarded with food.

Each of the rats was given 200 food-reinforced approaches to the food cup per day for 4 days and 100 such trials on the 5th day. No additional food was given to these rats. By the end of training, each rat approached the food cup promptly and swiftly from any part of the box when the click was sounded, and rarely or never approached the cup in the absence of the click. Control rats were fed daily an amount of Purina Lab Chow equivalent in weight to the amount of food received by the experimental animals.

On the day of completion of magazine-training, each of the eight experimental rats was killed with ether, and the brain was removed as quickly as possible. A posterior cut was made on a line joining the superior colliculus to the rostral end of the pons. An anterior cut was made which removed the frontal areas and the olfactory bulbs. The tissue posterior to the posterior cut and the tissue anterior to the anterior cut were discarded. The selection of this portion of the brain was based on preliminary work showing that (i) this portion was sufficient to give the effect observed in the present experiment, and (ii) this amount of tissue was convenient for our techniques. The average weight of the tissue retained was 1.0 g. We then extracted RNA from this tissue by the following procedure. The tissue was placed in a cold mortar with 5 ml of phenol (90 percent) and 5 ml of