drites and cell body colored lavender and the axonal pole of the neuron a deep purple (Fig. 1). Both of the Mauthner's cells in the nonstimulated fish had a rather homogeneous lavender coloration (Fig. 2). In all fish, both experimental and controls, the neuronal elements such as the nucleus appeared to be essentially normal. The surface of the dendrites, the cell body, and the axon hillock region were literally covered with synaptic endings.

In previous neurophysiological experiments it was shown that the observed response of the fish to unilateral stimulation of roots of the VIIIth nerve was a powerful flexion of the tail to one side only, in a one-to-one stimulus-response pattern (*2*, *3*). Also, it was found that bilateral simultaneous stimulation of roots of the VIIIth nerve could not induce the two cells to discharge at the same instant, as was evidenced by recording the evoked potentials from the two Mauthner's axons (*3*).

Histological studies have shown that the distribution of the VIIIth nerve synaptic endings is to the dendrites and cell body of the homolateral Mauthner's cell and to the axon pole of the contralateral cell. This pattern is in accord with the concept of polar function of the neuron, in which it was postulated that the afferent fibers which end on the dendrites and the cell body function to excite neuronal discharge, while those ending on or near the axon hillock serve to inhibit neuronal discharge (*1*, *3*).

A system of axoaxonal collaterals has been found to interconnect the two Mauthner's cells. These branchings arise from the axon of one Mauthner's cell, and after forming a spiral around the proximal part of the axon of the other Mauthner's cell, they end on its axon hillock. These collaterals may function as a feedback loop to augment the direct inhibitory effect of the VIIIth nerve synaptic endings, which also terminate on or near the axon pole of the Mauthner's cell contralateral to the side of their origin (Fig. 3).

These findings of the existence of axoaxonal collaterals which interconnect the two Mauthner's cells and the differential staining reaction of the two cells, which is alterable by afferent fiber stimulation, seem most significant. Inasmuch as the VIIIth nerve afferents are distributed to rather specific regions on the two cells and since it has not been possible to induce both cells to discharge simultaneously even though the entering roots of the VIIIth nerve of both sides are stimulated at the same time, it seems likely that the distinctive cell coloration is indicative of neuronal excitation and inhibition.

It is proposed that in this VIIIth nerve Mauthner's cell system the two cells function as reciprocating units and that the synaptic effect is to alter the intracellular chemical state which results in excitation or inhibition as the case may be. This, of course, implies that the dendrites, the cell body, and the axon pole are of prime functional importance in nervous integration, while the synaptic endings serve as activators rather than as specific excitors or inhibitors (*4*).

ERNEST RETZLAFF
JOAN FONTAINE
Research Division, Columbus Psychiatric Institute and Hospital—Ohio State University Health Center, Columbus

References and Notes

1. E. Retzlaff, *J. Comp. Neurol.* **101**, 407 (1954).
2. ———, *Federation Proc.* **14** (1955).
3. ———, *J. Comp. Neurol.* **107**, 209 (1957).
4. This report was presented in part at the 21st International Congress of Physiological Sciences, Buenos Aires, Argentina, 9–15 Aug. 1959, by Chauncey D. Leake. In the discussion which followed he suggested that the Cl^- ion shift mechanism might be responsible for the differential staining reaction described. It should be noted that G. J. Romanes [*J. Anat.* **84**, 104 (1950)] proposed that Cl^- ions are essential for the effective staining of neurons by the reduced silver method.

16 September 1959

Orientation of Migratory Restlessness in the White-Crowned Sparrow

Abstract. Individuals of two migratory races of white-crowned sparrows (*Zonotrichia leucophrys*) caged under an open sky showed a pronounced orientation in their night restlessness during normal periods of migration for the species. In August and September 1958 most birds showed a southerly orientation at night; daytime activity was random to somewhat northerly. In April and May 1959 most birds showed a strong northerly orientation at night; daytime activity was random to somewhat southerly (*1*).

Several species of caged passerine birds, which normally migrate at night, exhibit night restlessness (*Zugunruhe*) during the season of migration (*2*). This night activity provides a useful device for investigating the energy requirements of migration in these species (*3*).

In Europe several species of birds have been demonstrated to show a seasonal and predictable orientation with respect to migratory restlessness. These birds apparently navigate by the sun if they are day migrants and by the stars if they are night migrants (*4*).

White-crowned sparrows (*Zonotrichia leucophrys gambelii and Z. l. pugetensis*) captured on their winter range in the vicinity of San Jose, Calif., were kept captive in an outdoor aviary on the roof of the Natural Sciences Building on the campus of San Jose State College in mid-town San Jose. Conditions in the aviary allowed the birds to maintain reasonably natural patterns of seasonal weight change, molt, and gonadal development. Birds captured in early 1958 were first tested in August 1958. Most birds tested in early 1959 had been captured in late 1958 and early 1959.

Our activity-orientation cage is a modification of that used by Kramer (*5*) and is designed for continuous automatic recording of activity. Orientation of restlessness was obtained by placing an individual bird in a circular cage 36 in. in diameter and 10 in. high (Fig. 1) under an open sky. All later tests were made with a 24-in. masonite screen around each cage to block out most surrounding "landmarks." Each cage has a central circular perch surrounding its food and water cafeteria, and has around its periphery eight separate activity-sensitive perches. Each perch occupies just under 45° of the 360° circle. Perches are monitored electrically on remotely located Esterline-Angus (20-pen) recorders. One activity-orientation cage was used in late 1958 and early 1959, and four were in operation as of April 1959.

In April and May (1959), birds which had completed their prenuptial molt and which had gained weight to more than 30 gm (normal weight at other periods is 25 to 27 gm) were active at night. This night restlessness was strongly oriented to the north and northwest (see Table 1). Daytime activity tended to be random or to show a slight southerly orientation. The increasing trend toward a northerly orientation during the first two or three nights in which significant activity occurred suggests that restlessness (i) may develop before a sense of

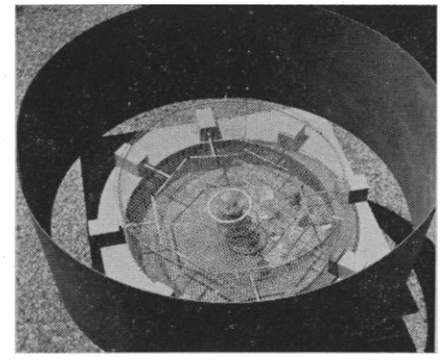

Fig. 1. Activity-orientation cage showing proportions of parts and the position of the 24-in. masonite screen.

Table 1. Numbers of times each directional perch was used by a caged white-crowned sparrow (*Zonotrichia leucophrys gambelii*) by day and by night during the period of development of vernal migratory restlessness. The sparrow was a male (28 gm) captured 5 Feb. 1959 near Gilroy, about 30 miles southeast of San Jose State College. Prenuptial molt was completed about 27 April. Critical weights were as follows: 12 April, 31 gm; 18 April, 32 gm; 25 April, 38.5 gm; and 3 May, 38.5 gm. All nights were partly cloudy except that of 25-26 April, which was cloudy and yielded 0.7 in. of rain.

Date (1959)	Orientation of daylight activity								Orientation of nighttime activity							
	E	SE	S	SW	W	NW	N	NE	E	SE	S	SW	W	NW	N	NE
23 Apr.	37	45	54	76	61	49	56	51	1	0	5	3	5	2	1	0
24 Apr.	73	109	154	155	148	131	113	109	0	0	0	5	0	0	0	0
25 Apr.	238	183	241	210	203	234	211	167	0	0	2	1	0	4	0	0
26 Apr.	130	385	314	481	390	295	437	220	31	7	13	31	26	28	66	82
27 Apr.	32	68	53	68	53	55	76	41	17	1	6	19	23	199	477	499
28 Apr.	46	55	121	141	41	78	138	55	4	5	7	12	9	58	1620	186
29 Apr.	24	31	105	373	67	52	242	35	10	12	98	57	15	80	1005	73
30 Apr.	16	39	41	88	55	51	66	30	22	6	5	26	11	18	945	135
1 May	25	64	39	84	48	50	59	49	8	2	2	6	6	22	1185	78
Total	621	979	1122	1676	1066	995	1398	757	93	33	138	160	95	411	5299	1053
Percentage	7	11	13	20	12	12	16	9	1	0	2	2	1	6	73	15

orientation has materialized or (ii) may be necessary to permit the development of orientation. The fall-off in the intensity of daytime activity when nocturnal activity becomes strong also appears to be characteristic of individual birds which exhibit the clearest patterns of orientation.

In August, September, and October (1958), birds which had completed their postnuptial molt and which had gained sufficient weight exhibited significant night restlessness. They showed a significant tendency to move toward the south, southeast, or southwest during the hours of darkness when the sky was clear. Daytime activity tended to be random or somewhat northerly in orientation. It should be noted that these birds were already within a few miles (10 to 50) of their natural winter home, and that this would tend to lead to a more diffuse pattern of activity. The greater strength of the northerly orientation in the vernal period than of the southerly orientation in the estival period was to have been expected, for the birds were many hundreds of miles south of their breeding range.

The presence or the absence of the masonite screen seemed to have little effect on the orientation of day or night activity. Rotation of the screened cage in which a bird showed strong orientation of activity revealed some influence of points of reference in the cage. After a 90° rotation, nearly complete correction was accomplished the first night if the night was clear. If, however, the sky was overcast or partly cloudy, correction was not accomplished as readily.

These results and additional preliminary findings from manipulation of daily photoperiods suggest that the activity-orientation cage provides a useful tool for the study of the physiology of the orientation of migration with birds of the genus *Zonotrichia*.

L. RICHARD MEWALDT
RICHARD G. ROSE
Department of Biological Sciences, San Jose State College, San Jose, California

References and Notes

1. This study was supported in part by an anonymous grant and by a grant from the National Science Foundation (NSF-G-7137). The technical advice of Lester Brubaker in developing orientation cages is gratefully acknowledged.
2. F. Sauer, *Z. Tierpsychol.* **14**, 29 (1957); ——— and E. Sauer, *Rev. suisse zool.* **62**, 250 (1956); D. S. Farner, L. R. Mewaldt, J. R. King, *J. Comp. and Physiol. Psychol.* **47**, 148 (1954); M. B. Eyster, *Ecol. Monographs* **24**, 1 (1954); G. Kramer, *Ibis* **94**, 265 (1952); P. Palmgren, *ibid.* **91**, 561 (1949); F. W. Merkel, *Ber. Verhandl. Schles. Ornithol.* **25**, 1 (1938); H. Schildmacher, *Vogelzug* **9**, 7 (1938); H. O. Wagner, *Z. vergleich. Physiol.* **12**, 703 (1930).
3. J. R. King and D. S. Farner, *Proc. Soc. Exptl. Biol. Med.* **93**, 354 (1956); J. Aschoff, *Studium Generale* **12**, 752 (1955); D. S. Farner, *Proc. Intern. Ornithol. 12th Congr.*, in press.
4. G. Kramer, *Ibis* **94**, 265 (1952); F. Sauer, *Z. Tierpsychol.* **14**, 29 (1957); ———, *Sci. American* **199**, 42 (1958).
5. G. Kramer, *Ibis* **94**, 265-285 (1952).

24 August 1959

State of Dynamic Equilibrium in Protein of Mammalian Cells

Abstract. Labeled strain L cells in suspension tissue culture showed no degradation of protein when maintained in logarithmic growth. Although the protein of these cells was not in dynamic equilibrium, the conclusions cannot be transferred to the intact mammalian organism.

The concept of "dynamic equilibrium" of cellular proteins has been accepted since the investigations of Schoenheimer and his colleagues (*1*). Recently this concept has been challenged by workers studying adaptive enzyme formation in bacteria (*2*). They found that preformed cellular protein did not contribute to a newly induced adaptive enzyme. Furthermore, once an adaptive enzyme was formed and the inducer was removed, that particular enzyme did not incorporate labeled amino acid into its structure. Similar results on the lack of protein turnover were obtained with yeast cells maintained in logarithmic growth (*3*). However, using mammalian tissue culture cells, one group found a turnover of 0.85 to 1.0 percent per hour (18.5 to 21.3 percent per day) (*4*), and other investigators reported a turnover of 12.9 percent per day (*5*).

It is now possible to grow mammalian cells in a manner very similar to that in which bacteria are grown (*6*). By maintaining L cells in logarithmic growth, it has been shown that both deoxyribonucleic acid and ribonucleic acid undergo no turnover in rapidly growing cultures (*7*). These facts have made it desirable to reinvestigate protein degradation in mammalian cells maintained in strict logarithmic growth.

Strain L cells were grown in 250-ml erlenmeyer flasks placed on a rotary shaker in an incubator maintained at 37.5°C. Each flask contained cells (200,000 per milliliter), Eagle's basal medium (72 ml), horse serum (8 ml), penicillin (100 units/ml), streptomycin (100 μg/ml), and leucine-1-C^{14} (3 to 4 $\times$ 10^6 counts per culture, specific activity 1.4 mc/mm) (*8*).

The culture was allowed to grow for 3 days until a high cell number (1 $\times$ 10^6 cells per milliliter) was reached. All counts were made in duplicate on a standard hemocytometer. At this time, one-third of the cells were removed and washed 3 times by centrifugation in 50 ml of Krebs-Ringer phosphate buffer. After resuspension in fresh unlabeled medium, the cells again grew logarithmically and showed a generation time of 26 hours. During this time the cells eliminated almost all the free labeled amino acid from their free intracellular pool. At the end of 3 days, 20 ml of this culture, containing approximately 1.5 $\times$ 10^6 cells per milliliter, was poured into a new flask containing 60 ml of fresh medium. This fresh medium had previously been warmed to 37.5°C, and the *p*H adjusted to 7.1. It was essential that the transfer be completed quickly, carefully, without change in *p*H or temperature, and without other disturbances which might result in compensatory equilibratory reactions and interfere with the delicate autoregulatory processes associated with logarithmic growth of the cells.