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Effects of Light on the Morphology of Subcommissural Organ (SCO) in *Xenopus laevis*[1)]

By

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(With 4 Text-figures, 1 Table and 1 Plate)

It is now well established that in the lower vertebrates the subcommissural organ (SCO), lying subjacent to the pineal organ in the diencephalic roof, secretes into the brain ventricle its material which later becomes the Reissner's fiber (Reichold, 1942; Mazzi, 1954). For instance, a number of investigators have confirmed its secreting activity histochemically (for reference, see Palkovits, 1965) and Sterba *et al.* (1967) observed precisely the processes in which the Reissner's fiber is elaborated.

Lately, much interest has been devoted to the study of the relationship of each organ constituting the dien- and mesencephalic roof (Mautner, 1965). Although the SCO is connected with the pineal organ through the *tractus pinealis* (Oksche, 1962; Wakahara, 1968), physiological significance of the nervous connection between the two organs remains still to be elucidated. In the present investigation, therefore, a series of experiments were undertaken to establish whether the SCO displays morphological changes under different lighting conditions, using the African clawed toads, *Xenopus laevis*, as material.

Materials and Methods

All the toads, *Xenopus laevis*, used in this investigation were obtained through induced mating and reared thereafter in our laboratory, until they reached 20 mm in body length (about 2.0 gm in wet weight) 2 months after metamorphosis. Totally 178 such animals were selected and used as follows.

In the first series of experiments 28 toads put in an outdoor pond and subjected to natural day-night condition for a week before they were sacrificed for examination. They were killed by decapitation and fixed at 12:00, 16:00, 20:00, 24:00, 4:00, 8:00 and 12:00, 4 animals each time. The sun rose at 4:10 and set at 19:10 when this series of experiments was performed. Water temperature was not regulated.

In the second series of experiments 132 animals were divided into three groups: 60 animals were reared in complete darkness, 36 animals under continuous light (200 lux) and another 36 animals as controls under usual day-night condition in the laboratory. They were fixed on 1st, 2nd, 4th, 8th, 16th and 32nd day, each time 10 dark-adapted animals and 6 animals in other two groups, respectively. Feeding of animals, water exchange

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and killing and fixation of the specimens were performed under the corresponding conditions to the experiments. All the animals were killed and fixed always at 14:00. During the experiments the temperature was kept at 20°–22°C by thermoregulator.

Eighteen animals in the third experimental series were blinded by eye removal. The operation was accomplished with a pair of fine scissors while the animals were anesthetized with MS–222 (1/2000). After the operations the blinded animals were divided into three and kept in three lighting conditions: complete darkness, continuous light, and usual day-night condition. After 32 days in such experimental conditions, they were fixed in the same way as other groups.

For histological examination, all specimens were fixed in Helly's fixative for 3 hours, embedded in paraffin and serial sections were cut sagittally at a thickness of 5μ. All the sections were stained with Gabe's (1953) aldehyde fuchsin (AF) with preoxidation by acidified permanganate. To obtain the conformity in stainability rate the staining solution was used only on the fourth day after prepared. The affinity to AF in the SCO was determined as follows: by exactly clocking the shutter speed of the microphotograph auto-exposure apparatus (PMS-II, Olympus Co., Tokyo) the optical density at a median sagittal section of the SCO was obtained. In order to get correct values the voltage of the input current to the light-source was stabilized by means of a slidac and an ammeter. Measurements were made at 12 points successively along the antero-posterior axis of the SCO and the mean of ten values without the maximal and the minimal values was obtained as an actual value of an animal. The relative optical densities in the same experimental series were calculated by making the maximum of the actual value as 100. The microphotograph of a median sagittal section of each SCO was weighed to compare the size of the SCO in different groups.

Results

1. *Circadian rhythmicity in the SCO*

It was confirmed that the affinity to AF in the SCO evidently exhibited circadian rhythm in the intact animals which had been reared in the natural condition (outdoors) for a week (Text-figure 1). Vertical lines on points in the figure indicate one standard deviation ($\pm$1.0 SD). The affinity in animals fixed in day-time (8:00, 12:00 and 16:00) was about 80 per cent of that in animals fixed during night-time (20:00, 24:00 and 4:00). Further, the size of the SCO and the position of the AF positive substances located in the SCO cells were compared but little change could be demonstrated among the animals sacrificed at various periods of a day. These results seem to indicate that the SCO has an overt circadian rhythm in its chemical nature, if not secretory.

2. *Experiments in intact animals*

The changes in morphological properties of the SCO and the Reissner's fiber were studied of the intact animals that had been kept in complete darkness, continuous light or as control, in usual day-night condition for a period as long as 32 days. In the animals kept in either complete darkness or continuous light the affinity in the SCO decreased abruptly, whereas in the control the value was maintained rather constant (Text-figure 2). After 8 days the affinity to AF in the

two experimental groups went down below 50 per cent of the mean control value, and after 32 days to about 30 per cent.

The Reissner's fibers arising from the SCO extended through the mesencephalic aqueduct and central canal of the spinal cord, ranging 0.8–4.5μ in diameter when measured. As indicated in Table 1, however, little difference in thickness which measured at the site just beneath the tectum (Fig. 1) could be observed of the fiber with respect to the three lighting conditions.

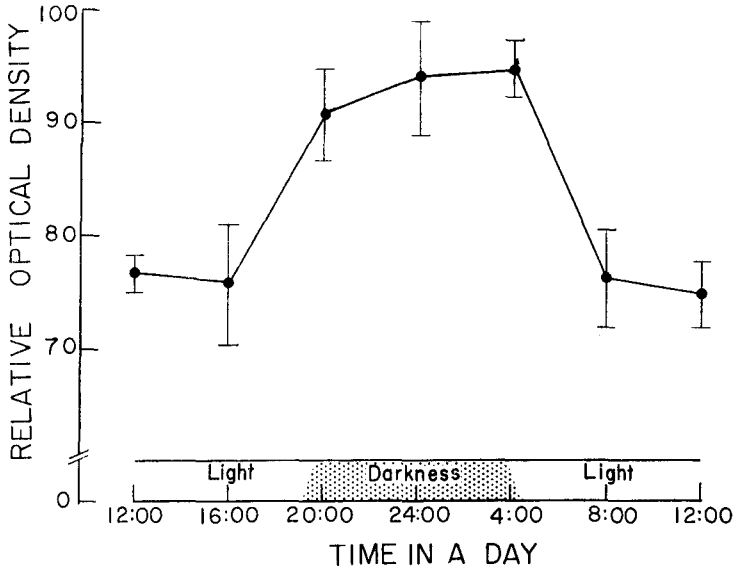

Fig. 1. Circadian change in the affinity to AF of the SCO at a median sagittal section of *Xenopus laevis*. Vertical lines on points indicate one standard deviation.

It was suspected that these experimental conditions of complete darkness and continuous light might affect the secretory activity of the SCO. It was found that the brain area consisting of the SCO and posterior commissure in all the animals examined increased in size. Therefore, only the size of the SCO in a median sagittal section was compared among the three groups (Text-figure 3). Only the SCO of the control animals increased in size possibly due to the growth, whereas both in the dark- and the light-adapted animals the organ decreased in size. When the experiments were started the SCO in the light-adapted toads decreased in size more greatly than that of the dark-adapted ones, but after 8 days the rate of decrease in size of the SCO in the two experimental groups was about the same.

Cytological changes could be induced in the SCO cells by different lighting conditions. In the normal control animals, the SCO cells had a strong affinity to

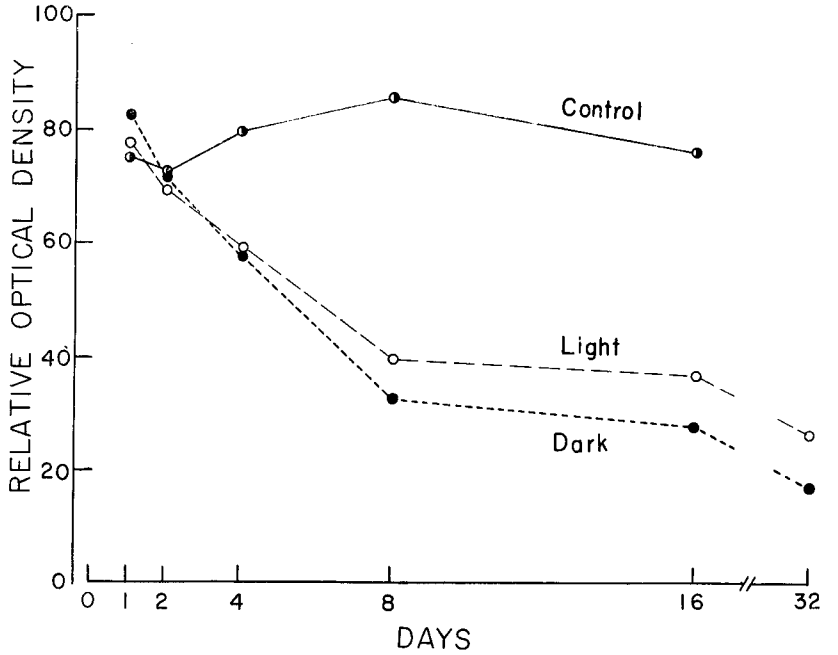

Fig. 2. Change in the affinity to AF of the SCO at a median sagittal section of *Xenopus laevis* that had been kept in continuous light (circles) or complete darkness (dots) as long as 32 days. Half-filled, controls.

Table 1. The thickness (μ) of the Reissner's fiber just beneath the tectum of toads kept in three different lighting conditions

Conditions / Duration (Days)	Complete darkness		Continuous light		Control	
	No.	Diameter	No.	Diameter	No.	Diameter
1	8	1.88±0.12*	6	2.17±0.19	5	2.12±0.17
2	6	2.08±0.22	5	1.92±0.11	5	2.10±0.20
4	9	1.86±0.21	6	2.14±0.17	6	1.99±0.18
8	9	2.03±0.08	6	1.75±0.18	4	2.21±0.20
16	10	1.85±0.12	4	1.81±0.15	1	2.5
32	7	1.96±0.15	5	2.12±0.18		

* Mean ± standard deviation

AF in the sites of the basal process, basal part, apical part and microvilli or cilia (Figs. 2 and 3). The basal process of the SCO cells which extended through the hypendymal layer seemed to swell up with an AF positive substance in it and attained to the capillaries distributed over the area (Figs. 3 and 4). The basal part was represented as a triangle densely positive to AF. The AF positive

substance was granulated in the apical part. After 4 or 8 days in the two experimental conditions, the AF positive substance in swollen basal process and the basal part was diminished. However, the substance was still rich in the apical part and the microvilli or cilia (Figs. 5 and 6). In the SCO cells after 32 days in experimental conditions, the AF positive substance was demonstrated to be packed only

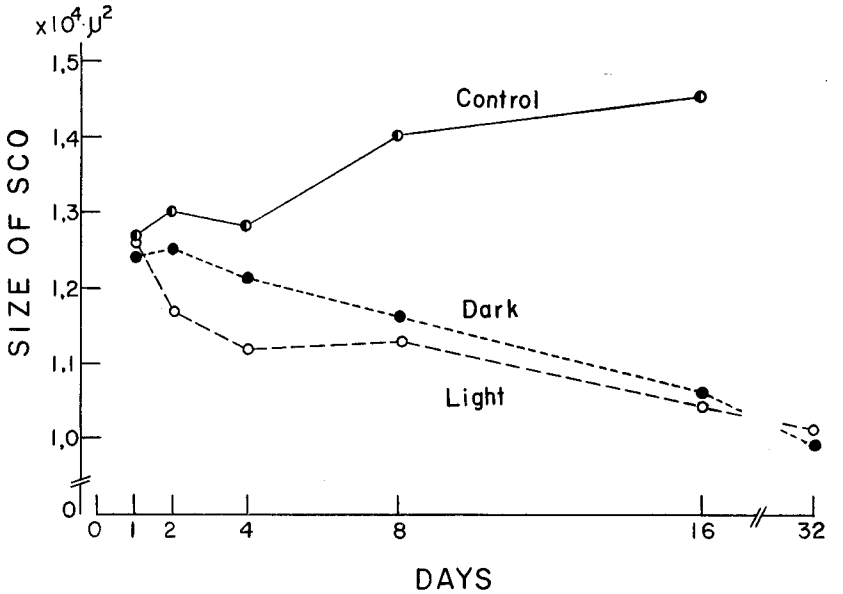

Fig. 3. Change in size of the SCO of toads kept in different lighting conditions. For more explanation, see text.

in the apical surface and scarcely distributed in the cytoplasm (Fig. 7). In the ventricle subjacent to the SCO, a lot of AF positive substance which elaborated many coarse granules and thin fibers called "Ursprungsfäden" (Sterba *et al.*, 1967) were observed in all specimens examined in this work (Figs. 8 and 9). The amount of the AF positive substance and "Ursprungsfäden" in the ventricle seemed to be unaltered by lighting conditions.

3. *Experiments in blinded animals*

In the third series of experiments, the difference in the affinity to AF in the SCO of the blinded animals which had been kept in complete darkness, continuous light and usual day-night conditions as control for the duration of 32 days was observed (Text-figure 4). In the animals kept in the two experimental conditions, complete darkness and continuous light, the affinity went down about 60–70 per cent of those in the blinded control animals. It seemed valid, therefore, to con-

sider that the two experimental conditions exerted certain effects upon the SCO's affinity to AF *via* non-retinal pathway.

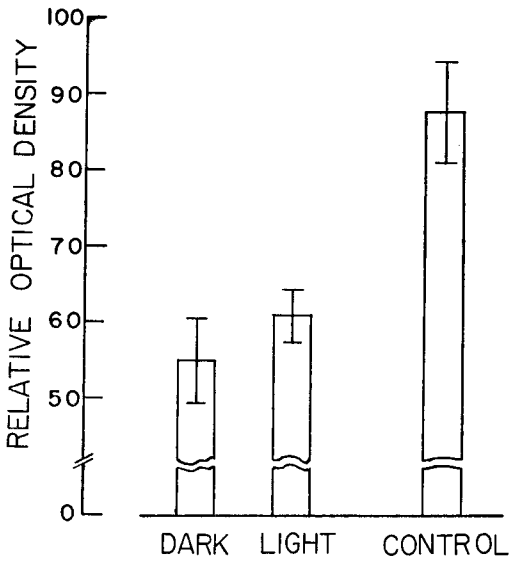

Fig. 4. Difference in the affinity to AF of the SCO at a median sagittal section of blinded *Xenopus* that had been kept in different lighting conditions.

Discussion

Positive affinities of the SCO cells to Gomori's chrome hematoxylin in frog (Stutinsky, 1950) and to AF in mammals (Wislocki and Leduc, 1953) have been considered as an evidence of the secretory activity of the cells. Moreover, recent investigators have revealed that the secretory material is a neutral polysaccharide-protein complex very rich in cystine, tyrosine and tryptophan (Naumann, 1968). Therefore, provided that the SCO's AF positivity described in this paper is due to the secretory material of such chemical nature, the change in the affinity of the SCO would indicate a parallel change in the amount of secretory material within the organ. Thus in *Xenopus laevis* the amount of secretory material in the SCO was relatively larger in night-animals than in day-animals. This shows a close resemblance to the biochemical rhythm in the mammalian pineal gland, in which the melatonin-forming enzyme (HIOMT) activity is greatest at midnight (Wurtman and Axelrod, 1965). However, the amount of secretory material in the SCO cells decreased in intact animals placed in either complete darkness or continuous light. A similar result was obtained even in blinded animals kept in the same conditions. These results led us to conclude that the circadian rhythmicity in the SCO was maintained by a factor other than the light through the lateral eyes,

whereas in the mammalian pineal glands the HIOMT cycle was completely dependent on the environmental lighting condition, because it was abolished when animals were blinded. As a number of investigators have reported that the pineal organ in amphibians posesses photoreceptor cells (Kelly, 1962; Eakin *et al.*, 1963; Oksche and Vaupel-von Harnack, 1963) and further is connected through the nerve with the SCO (Oksche, 1962; Wakahara, 1968), it seems reasonable to suppose that the circadian rhythmicity in the SCO in *Xenopus* is maintained by a stimulus from the pineal organ.

The SCO cells are believed to secrete their products into two directions: on their apical surface into the third ventricle the material which later becomes the Reissner's fiber, and through their basal processes the material into the capillaries (Oksche, 1961; Palkovits, 1965). Of these the apical secretion seemed to be not affected by lighting condition, because both the thickness of Reissner's fiber and the amount of the coarse granules of the secretory material or "Ursprungsfäden" were unaltered by the two experimental conditions. Furthermore, the fact that the growth of the SCO was suppressed more or less in the two experimental conditions appeared to present a favorable evidence to the hypothesis that these particular conditions inhibit the synthesis but not the release of secretory material. The more rapid decrease in size of the SCO under continuous light than under complete darkness may add the data concerning the mechanism of production and release of a chemical substance. In this respect, it is worth noting that the sustained discharge of action potentials was recorded in the dark from the epiphyseal stalk of *Rana* in which the lateral eyes, the frontal organ and the *tractus frontalis* were removed (Dodt and Jacobson, 1963). Moreover, these authors stated that illumination of the region of the diencephalon from which the responses were recorded by light of all wavelengths produced inhibition of activity. Morphological data concerning the basal secretion, or the release of the secretory material into the circulatory system through the basal process of the SCO cell, were so little as to suppose any change among the groups in different lighting conditions.

Summary

1. The subcommissural organ (SCO) was investigated of young toad, *Xenopus laevis*, about 2 months after metamorphosis, which had been kept in different lighting conditions.

2. It was found that in normal animals the organ has an overt circadian rhythm of affinity to aldehyde fuchsin (AF): the affinity was greater in night-animals than in day-animals.

3. The SCO's affinity to AF was decreased greatly in animals that had been kept in either complete darkness or continuous light more than 8 days.

4. In these two experimental conditions, the SCO was decreased in size, where-

as the Reissner's fiber secreted from the SCO cells seemed to show little change in thickness.

5. Among the blinded animals, those kept in experimental lighting conditions had the SCO weaker in its affinity to AF than those subjected to normal light and dark cycle.

6. These results are compared with the data hitherto obtained by other investigators on this organ, and the significance of its circadian rhythmicity is discussed.

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References

Dodt, E. and M. Jacobson 1963. Photosensitivity of a localized region of the frog diencephalon. J. Neurophysiol. **26**: 752–758.

Eakin, R.E., W.B. Quay, and J.A. Westfall 1963. Cytological and cytochemical studies on the frontal and pineal organ of the tree frog, *Hyla regilla*. Z. Zellforsch. **59**: 663–683.

Gabe, M. 1953. Sur quelques applications de la coloration par la fuchsine-paraldéhyde. Bull. Micr. appl. **3**: 153–162.

Kelly, D.E. 1962. Pineal organs: photoreception, secretion, and development. Am. Scientist **50**: 597–625.

Mautner, W. 1965. Studien an der Epiphysis Cerebri und am Subcommissuralorgan der Frösche. Mit Lebendbeobachtung des Epiphysenkreislaufs, Totalfärbung des Subcommissuralorgans und Durchtrennung des Reissnerschen Fadens. Z. Zellforsch. **67**: 234–270.

Mazzi, V. 1954. Alcune osservazioni intorno al sistema neurosecretorio ipotalamo-ipofisario e all'organo sottocommissurale nell'ontogenesi di *Rana agilis*. Monit. Zool. Ital. **62**: 78–82.

Naumann, W. 1968. Histochemische Untersuchungen am Subcommissuralorgan und am Reissnerschen Faden von *Lampetra planeri* (Bloch). Z. Zellforsch. **87**: 571–591.

Oksche, A. 1961. Vergleichende Untersuchungen über die sekretorische Aktivität des Subcommissuralorgans und die Gliacharakter seiner Zellen. Z. Zellforsch. **54**: 549–612.

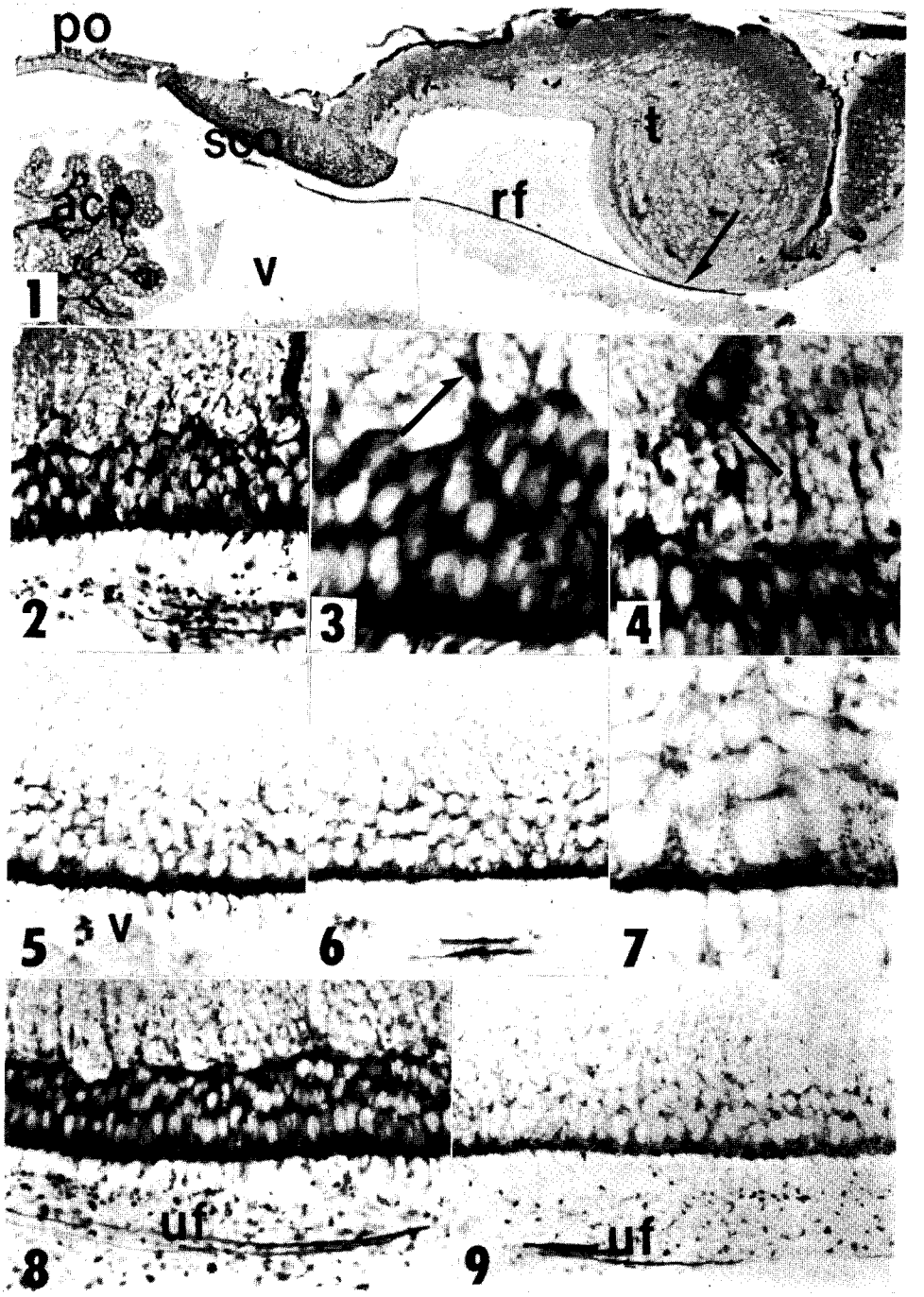
———— 1962. Histologische, histochemische und experimentelle Studien am Subcommissuralorgan von Anuren (mit Hinweisen auf den Epiphysenkomplex). Z. Zellforsch. **57**: 240–326.

———— and M. Vaupel-von Harnack 1963. Elektronenmikroskopische Untersuchungen an der Epiphysis Cerebri von *Rana esculenta* L. Z. Zellforsch. **59**: 582–614.

Palkovits, M. 1965. Morphology and function of the subcommissural organ. Stud. Biol. Hungr. vol. 4, Akadémiai Kiadó, Budapest.

Reichold, S. 1942. Untersuchungen über die Morphologie des subfornicalen und subcommissuralen Organs bei Säugetieren und Sauropsiden. Z. mikr. -anat. Forsch. **52**: 455–472.

Sterba, G., H. Müller, and W. Naumann 1967. Fluoreszenz- und elektronenmikroskopsiche



M. Wakahara: *Effects of Light on SCO in* Xenopus

Untersuchungen über die Bildung des Reissnerschen Faden bei *Lampetra planeri* (Bloch). Z. Zellforsch. **76**: 355–376.

Stutinsky, F. 1950. Colloide, corps Hering et substance Gomori positive de la neurohypophyse. C.R. Soc. Biol. **144**: 1357–1360.

Wakahara, M. 1968. Observations on developing pineal organ in the African clawed toad, *Xenopus laevis* D. J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. **16**: 346–352.

Wislocki, G.B., and E.H. Leduc 1953. The cytology of the subcommissural organ, Reissner's fiber, perivascular glial cells, and posterior collicular recess of the rat's brain. Anat. Rec. **117**: 610.

Wurtman, R.J., and J. Axelrod 1965. The pineal gland. Sci. Amer. **213**: 50–60.

Explanation of Plate XIII

Figs. 1–9 are microphotographs at median sagittal sections of brains of young toads, *Xenopus laevis*. Gabe's aldehyde fuchsin stain; anterior to the left.

Fig. 1. A montage microphtograph from two successive sections showing the dien- and mesencephalic roof. The thickness of the Reissner's fiber (rf) was measured at the site (arrow) just beneath the tectum (t). acp, anterior choroidal plexus; po, pineal organ; sco, subcommissural organ; v, third ventricle. ×90.

Fig. 2. Section through the SCO of normal control animal showing the basal process, basal part, apical part and microvilli or cilia of the AF-positive SCO cells. ×400.

Figs. 3 and 4. Higher magnification (×800) of sections through the SCO of normal controls. The basal process is sometimes swollen (arrow, Fig. 3) or is extended to reach a nearby capillary (arrow, Fig. 4).

Fig. 5. A sagittal section through the SCO of animal kept in complete darkness for 8 days. Compare with Fig. 2. v, third ventricle. ×400.

Fig. 6. Continuous light for 32 days. Compare with Figs. 2 and 5. ×400.

Fig. 7. The SCO cells of a toad kept in complete darkness for 32 days. The cells show lesser affinity to AF than normal control (Fig. 3) but the granular nature of AF positive substance is evident on apical portion. ×800.

Fig. 8. Section through the SCO and subjacent ventricle of normal control showing the AF positive coarse granules and "Ursprungsfäden" (uf). ×400.

Fig. 9. Section through the SCO and subjacent ventricle of an animal subjected to continuous light for 32 days. Comparing to normal control (Fig. 8), the animal had the SCO cells with lesser amount of AF positive material. However, in both cases the ventricle contained many coarse granules and similarly developed "Ursprungsfäden" (uf). ×400.