of 10,12-hexadecadien-1-ol (*20*). Three of the synthetic isomers, those excluding *cis*-8, *trans*-10 (7.3 minutes at 173°C), possess identical retention times, 7.75 minutes at 173°C on the polar GC column. Retention time of the crude extract active component was again determined by measuring EAG responses of 1-minute collections and was found to be identical to that of the three synthetic isomers possessing a common retention time. This is strong evidence that one of the three isomers may be similar to the pheromone compound, since conjugation with the double bond in the 10-position would produce compounds with longer retention times than other doubly unsaturated C_{12} alcohols. The EAG responses to the four synthetic isomers for a series of concentrations give curves (Fig. 2) that are very similar to those described for the geometrical isomers of the silkworm attractant (*22*), except that in our study the *trans-trans* isomer produces the strongest responses. The *trans-trans* EAG response trace is also distinguished from the others by exhibiting a much slower return to baseline. Good EAG responses with the *trans-trans* isomer at a concentration of 10^{-10} g provide additional evidence that this compound may be the codling moth sex pheromone. The antennal responses are logarithmic with concentration, making it necessary to use over 2000 times more *trans*-9-dodecenyl acetate than compound **1** to elicit a 4-mv response (Fig. 2).

Intense sexual stimulation is elicited in laboratory bioassays with very low concentrations of the synthetic compound **1**, although many chemicals produce stimulation in the laboratory but do not attract males in the field. Field tests with synthetic compound **1** showed it to be very attractive to male codling moths. Preliminary tests were conducted by releasing male codling moths in an apple orchard. Test chemicals (1 μl) were placed on rubber septa located in Sectar insect traps (*23*). The *trans-trans* isomer was very attractive to male moths (19 males per trap per night), as was the reaction mixture containing *trans-trans* (75 percent) and *cis*-8, *trans*-10 (25 percent) (15 males per trap per night), while the *trans*-8, *cis*-10 isomer attracted no males. A mixture of synthetic isomers was acetylated and separated by thin-layer chromatography on silver nitrate–impregnated silica gel developed with benzene. Several fractions were scraped from the plate, hydrolyzed, and placed in a trap on dental wicking. Only the top scraping containing the *trans-trans* isomer was attractive to males in the field. Field studies in Australia showed that a rubber septum containing 1 μl of attractant was attractive for over a month and out-caught the usual port wine lure pots (58 males per trap to 4 males per pot).

WENDELL ROELOFS, ANDRÉ COMEAU
ADA HILL, G. MILICEVIC
Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva 14456

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Circadian Rhythm: Population of Interacting Neurons

Abstract. *The circadian rhythm in the frequency of compound action potentials recorded from the isolated eye of* Aplysia *is a consequence of interactions among the cells of the retinal population. As the population number is reduced to a critical 20 percent, progressively shorter circadian periods and ranges are expressed. Below the critical number, the population oscillates at ultradian frequencies.*

Many physiological and biochemical processes in both plants and animals exhibit fluctuations in output that occur with a circadian (about a day) periodicity (*1*). These circadian rhythms are not just a response to fluctuating environmental stimuli but are due to internal endogenous oscillators whose phases are influenced by environmental stimuli. Many circadian activity rhythms in animals are thought to be controlled by neuronal oscillators in the central nervous system, probably in the brain (*2*). One fundamental question regarding neuronal oscillators, and circadian oscillators in general, is whether the observed circadian oscillations are caused by a single master oscillator or by interactions within a population of oscillators (*3*). Two circadian rhythms in neuronal activity have been described in *Aplysia*. A single neuron within the central nervous system has been shown to have a circadian rhythm of spike frequency (*4*), and a circadian rhythm in the frequency of compound action potentials in the isolated eye of this animal has been characterized (*5*, *6*).

We now show that the circadian rhythm in the isolated eye of *Aplysia* is a consequence of interaction among a population of endogenously active neurons of the eye. Eyes removed from *Aplysia californica*, subjected to light-dark cycles of 12 hours of light (200

lumen/m^2) and 12 hours of darkness, and kept in artificial seawater (Instant Ocean) at 15°C were used in the experiments. The optic nerve was severed at the cerebral ganglion, and the isolated eye and optic nerve were placed in 100 ml of culture medium described previously (*5, 7*). The eyes were maintained in complete darkness and constant temperature (14° to 15°C) for several days while recordings were made from the whole optic nerve with a tubing electrode. The optic nerve was drawn into the submerged end of a polyethylene U-tube (PE 10 or 20), and the suction was released. Gravity kept the optic nerve submerged and in the tubing during the experiment. One end of the U-tube projected above the surface of the culture medium; a silver wire coated with silver chloride inserted into it permitted recording of the electrical activity of the optic nerve on a Grass polygraph. Both eyes from each animal, one experimental and the other the control, were tested in the same culture medium chamber. The experimental eye was cut so as to remove either the lens alone or the lens and various portions of the distal retinal population as shown in Fig. 1; the control was left intact. After the activity was recorded, normal and experimental eyes were fixed in alcoholic Bouin's fluid, cut at 4 μm, and stained with azure-eosin or silver, and cell counts were made.

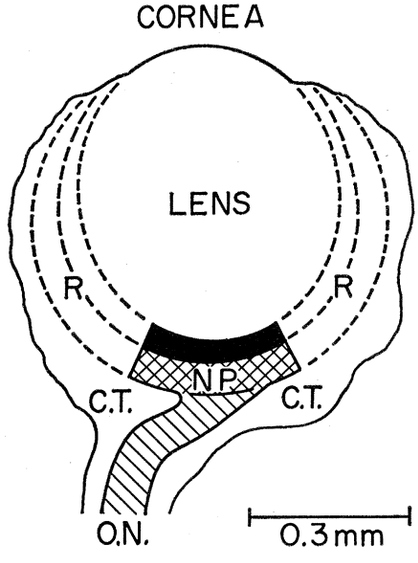

Fig. 1. Diagram of the structure of the eye. The retina (*R*) is composed of three populations of cells. Receptor cells and support cells have pigmented (black) distal segments that project to the central lens (lens). Secondary cells, fiber tracts, and neuropile are outside this layer (crosshatch). Connective tissue (*C.T.*) encapsulates the eye. The optic nerve (*O.N.*) goes to the cerebral ganglion. The dotted lines indicate the portions of the whole eye that were cut away in reducing the experimental eyes to the critical population level.

The retina of the eye of *Aplysia* is composed of three types of cells: receptor, support, and secondary (*8*). The receptors transduce light stimuli and show graded receptor potentials in response, but they do not produce action potentials in response to light or during spontaneous activity of the optic nerve. The support cells do not exhibit any known electrical activity. The secondary cells fire in synchrony with the optic nerve activity (*8*). There are 3700 receptors and 950 secondary cells in an intact eye as determined from an actual count. These cells can be distinguished by their location in the retina, their staining by azure-eosin, and the relative size of their nuclei. In an intact eye the ratio of secondary cells to receptor cells is 0.2; in an eye cut so that 0.15 of the eye remains the ratio is 0.4; and in an eye cut so that 0.02 of the eye remains the ratio is 1.7. The actual number of secondary cells in the 0.02 of an eye (Fig. 2) is 73. Thus, large numbers of the secondary cells are found throughout the retina but in highest density near the optic nerve. The optic nerve contains thousands of fibers, but some of them are efferents from the cerebral ganglion (*6*), so the exact correspondence of the optic nerve fibers to the retinal cells is uncertain. Recordings from the whole optic nerve of the isolated eye are from a population of fibers.

The isolated whole eye exhibits endogenous activity as a synchronous population firing in the form of a compound action potential (CAP) (*8*). The CAP's usually occur in bursts of two to six per minute (Fig. 3). During a circadian period, the CAP frequency and CAP burst frequency fluctuate from a minimum near zero through a maximum of as many as 300 CAP's per hour and return to near zero (Fig. 4). The whole eye also shows a change in CAP amplitude within a single CAP burst (Fig. 3, B and B′) and also over a circadian period (Fig. 4). The change in CAP amplitude was verified on the oscilloscope and is not due to the response characteristics of the pen recorder. Such changes in the CAP amplitude are seen in the light-evoked responses as well (*8*). In either case, the change in amplitude reflects the change in the number of the individual potentials in each CAP (*8*).

Removing the lens from the eye does not change the normal waveform, frequency, burst frequency, or amplitude of the CAP or the circadian period and range. Figure 4 shows the changes in CAP amplitude and CAP frequency exhibited by two eyes from the same animal for 3 days of continuous recording in the same culture solution. One eye is whole and shows the normal changes in CAP amplitude and frequency. The experimental eye is reduced to one-eighth of the population and shows very little change in the CAP amplitude or the CAP frequency which remains close to the maximum rate of the normal eye. The circadian range of CAP frequency is reduced, ultradian

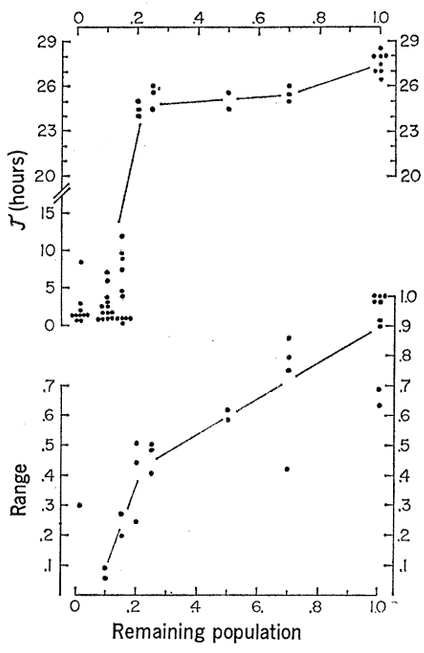

Fig. 2. Graph of period (τ) and "range" [1 — (minimum CAP frequency divided by maximum CAP frequency)] against the remaining population of receptor and secondary cells; 1.0 of the population is 3700 receptors and 950 secondary cells; 0.1 of the population is 380 receptors and 120 secondary cells; 0.02 of the population is 42 receptors and 73 secondary cells by actual count. Each point represents one complete period in culture; two periods from four eyes for 1.0 population, two periods from one eye and one period from one eye for 0.7 population, two periods from one eye for 0.5 population, two and one periods from two eyes are shown for 0.25 population; three periods for one eye for 0.2 population; successive periods from one eye each for 0.15, 0.1, and 0.02 population. For 1.0 population, nine ranges were measurable but only eight periods; for 0.7 population, four ranges were measurable but only three periods. The ranges for 0.15, 0.1, and 0.02 eyes were measured for circadian times rather than for each measured period.

frequencies are apparent, and the circadian period is shortened and appears only as a remnant. The relatively small change in CAP average amplitude for one-eighth of an eye compared to that for the normal eye shows that the variation in number of cells contributing to the CAP over circadian periods is reduced in the former.

Twenty percent of the population is the critical size for the expression of circadian periods since below this level periods from 1 to 12 hours are expressed. Figure 2 shows the changes in period (τ) and the range of CAP frequency of each period for normal and experimental eyes. As a preliminary step in determining the period, a midline was fitted to the data for each eye. The midline is a continuous line with a single convexity fitted by eye and drawn through the middle of all the oscillations for each eye (over at least 48 hours and up to 96 hours of continuous recordings). Each successive upward crossing of the midline by the frequency curve was used to determine the period. A Fourier frequency analysis was performed on some of the data with a Univac 1108 computer. This analysis shows that whole eyes have a dominant mode of oscillation at periods of 26 to 28 hours and very little energy at shorter periods. The 0.2 eye (Fig. 2) has a dominant mode at a period of 25 hours and a minor mode at 20 to 22 hours. The 0.1 eye has a dominant mode at 21 hours and minor modes at 10 and 7.5 hours. The 0.02 eye has a major mode at 7.5 hours and minor modes at 12 to 16 hours and 18 to 20 hours. The Fourier analysis substantiates our other estimates of the period and demonstrates the shift of the modes of oscillation to higher frequencies (shorter periods) as the eye population is reduced. The range of each oscillation is measured as the ratio of the minimum CAP frequency over the maximum CAP frequency subtracted from 1 for each period. Since the minimum CAP frequency for normal eyes is near zero, the range for normal eyes is very close to 1. It is apparent from Fig. 2 that as the population is reduced the range is reduced proportionately until the critical 20 percent of the population remains, when the range drops off sharply. The period of the oscillation is also reduced significantly from an average of 27.5 hours for normal eyes to an average of 24.5 hours at the critical population. After this point, the period also drops off sharply. Reducing the population to

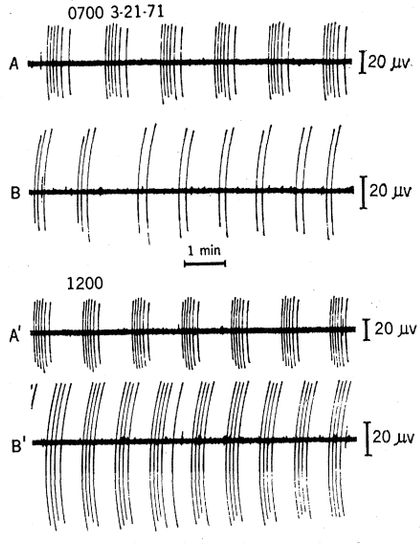

Fig. 3. Sample polygraph records of compound action potentials (CAP) from the optic nerve at a 5-hour interval (0700 and 1200 E.S.T.) to show the change in CAP frequency, CAP burst frequency, and the CAP amplitude. Each vertical excursion of the pen is a CAP. The CAP are clustered into bursts of two to six. Records are from the same preparation shown in Fig. 4. A and A′ from one-eighth eye; and B and B′ from whole eye.

25 percent by cutting the eye transversely or sagitally makes no difference in the changes in period or range. Therefore, the changes appear to depend only on how much of the distal population remains rather than on what portion of the distal population remains.

Since both experimental and normal eyes remain viable and respond to photic stimuli after 3 days in the culture medium, the experimental eyes are not damaged by the surgical manipulations. The CAP frequency of the experimental eyes is about equal to the maximum CAP frequency of the normal eyes, thus the functioning of the remaining neuronal population is not seriously distorted either; instead it appears to lack the pronounced circadian depression of the CAP frequency of the normal eye (Fig. 4). A population of neurons is involved in the CAP production since the amplitude changes within a CAP burst and during a circadian period. The CAP amplitude is also a function of the strength of the stimulus voltage if the optic nerve is stimulated electrically (*8*). The population of secondary cells is probably responsible for the CAP since intracellular recordings show that they do fire action potentials during CAP firing (*8*). The receptors are probably not responsible since intracellular recordings from their cell bodies show that no action potentials occur in them during CAP firing (*8*). When the total population of 950 secondary cells are active and interacing, the result is the circadian rhythm and when the population is reduced to the critical level of 20 percent or less, the output of the system is not circadian. Some substantiation for this reasoning comes from theoretical studies by Pavlidis (*3*) on populations of coupled oscillators where the period of

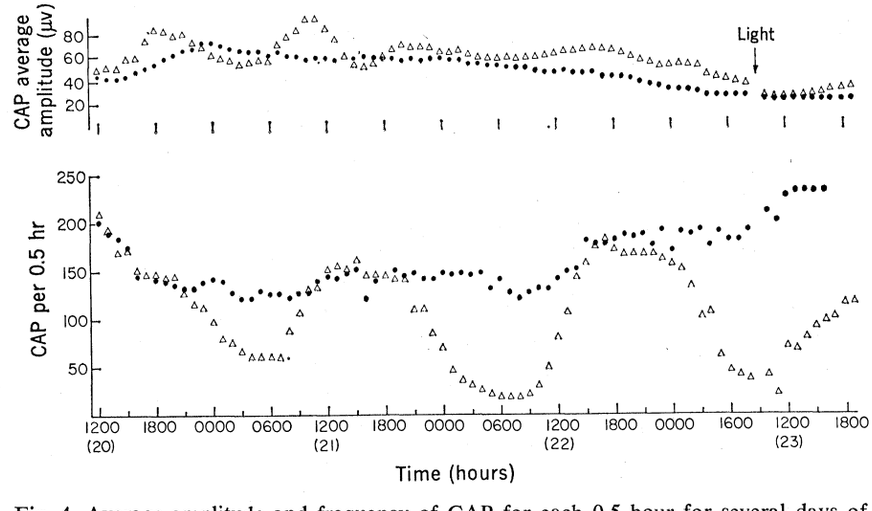

Fig. 4. Average amplitude and frequency of CAP for each 0.5 hour for several days of continuous recording from both eyes from the same animal, tested in the same culture medium in constant darkness. The whole eye has clear circadian periodicity in CAP frequency; the one-eighth eye does not. The intact *Aplysia* was kept on a daily cycle with light from 0800 to 2000 previous to 20 March 1971. The eyes were then cultured at 14°C in culture medium II. The period of the whole eye (τ) was 28 hours. Times are Eastern standard time. Triangles, whole eye; circles, one-eighth eye. Light refers to a brief light used to test responsiveness of the eye.

the population oscillation is much longer than that of any individual in the population. The period of these oscillations is a function of the size of the population and strong inhibitory coupling among the individuals of the population. The organization of the population of oscillators in the *Aplysia* eye is probably more complicated than the model by Pavlidis since both excitatory and inhibitory interactions occur in the eye (*8*). If we assume that the interaction between neurons (coupling) remains constant after the eye is cut, then the changes in circadian period and range must be due to the reduction in the number of interacting neurons in the population.

There are at least three alternative models for the organization of the endogenously active neuron (oscillator) population of the eye: (i) a population driven by a master circadian oscillator, (ii) a population of circadian oscillators, and (iii) a population of noncircadian oscillators that together produce a circadian rhythm. If a hypothetical master oscillator does not receive feedback from its followers, the circadian period should stay constant at 27.5 hours until the master oscillator is cut away. Since this does not happen, we assume that if a master oscillator is present it receives feedback from its followers and thus population interactions occur. A master oscillator does not seem to be present, since slightly below the critical level a circadian remnant is still observed, as in Fig. 4, but it is not observed in the minimal eye (0.02 of the population). If the eye is a population of circadian oscillators, the minimum population should show circadian oscillations at least in the first free-running period because each oscillator's phase should still be influenced by the previous light-dark cycle and a circadian rhythm should be observed. Minimal (0.02) eyes express only ultradian frequencies, so this model does not seem to fit. The last alternative of a population of noncircadian oscillators is the most likely because the circadian range and period shorten in proportion to the remaining population until the critical level is reached; below that level a circadian remnant is expressed, and finally only shorter ultradian frequencies appear. The higher order interactions that produce the circadian rhythm are lacking when the population is reduced below the critical level.

Pavlidis (*3*) has suggested that systems of coupled oscillators may be responsible for circadian periodicities at biochemical levels in single cells as well as in populations of interacting cells. The eye of *Aplysia* is an example of a population of interacting neuronal oscillators that produces circadian periodicities.

JON W. JACKLET
JEFFREY GERONIMO
Department of Biological Sciences, State University of New York at Albany, Albany 12203

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Anomalous Retinal Pathways in the Siamese Cat: An Inadequate Substrate for Normal Binocular Vision

Abstract. *All major retinal pathways in the Siamese cat are abnormal, with almost total crossing of the projections to the pretectum and superior colliculus. These projections represent a marked disruption in the customary neural substrate for binocular vision, which implies a consequent impairment in stereoscopic depth perception. Crossed eyes, commonly seen in the Siamese cat, may therefore arise from a neuroanatomical defect in the primary visual pathways.*

Crossed eyes and squint have been associated with improper control of the eye muscles and have therefore been considered to be a deficit in the oculomotor system (*1*). If the ability to utilize binocular information about distance is lost, then the vergence mechanisms coordinating symmetrical eye movements would be disrupted, for there would be inadequate sensory input to guide the oculomotor system (*2*). Because of the high incidence of crossed eyes in the Siamese cat, we investigated the primary visual pathways in this animal. Guillery found abnormalities in one of the three pathways from the retina (*3*). We show that all of the major retinal pathways of the Siamese cat are abnormal.

In the normal, ordinary cat, the retinal ganglion cells send their axons bilaterally to three major visual centers: the dorsal lateral geniculate nucleus of the thalamus, the pretectum, and the superior colliculus (*4–6*). Axons from the nasal retina of each eye cross to the opposite side of the brain and make their connections contralaterally, whereas axons from the temporal retina remain almost exclusively uncrossed and terminate ipsilaterally, for the most part (*6–8*). As a result of this partial crossing of optic nerve fibers, each subcortical visual center receives binocular information about the contralateral visual field, because the nasal retina of one eye views the same visual field as the temporal retina of the other. It is the precise organization of the visual pathways that most probably forms the substrate for normal binocular vision. If a portion of the input from one eye were disarranged or missing, binocular integration in the visual pathway would be disrupted. The retinal projections of the Siamese cat form an anatomical system which is disorganized, and which is consequently an inadequate substrate for normal binocular integration.

Three adult (unrelated) Siamese cats and one 6-week-old kitten were used in these experiments. In each animal the right eye was enucleated aseptically under deep anesthesia. Five to eight days after the operation, the animals were again anesthetized and perfused through the heart with 0.9 percent saline followed by 10 percent formaline-saline. Frozen sections of the brain were cut coronally at 30 μm; every fifth section was stained for degenerating axons and their terminals