INSTRUMENTATION & TECHNIQUES

Automated analyses of circadian rhythm activity of small arthropods

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An automated apparatus is described which measures circadian activity rhythms of small invertebrates in darkness or in the presence of monochromatic light. Back-and-forth activity in tilt cages is measured without physical contact by magnet-activated reed switches. Four such cages are grouped around a mercury pen lamp, whose spectra lines can be selectively filtered, and all are placed in a large enclosure with light-baffling for complete control of intensity and color, delivered to each animal. Activity, measured as reed-switch closures, is counted digitally and punched out periodically onto paper tape. An interactive computer program reads, stores, plots, and further analyzes the data.

Studying behavior of animals with comparatively limited behavior repertoires requires, first, the isolation of some measurable behavior, and second, a way of measuring it. The behavior of invertebrates, which can be classed as limited, is of interest because the invertebrate visual system has provided much of the available information about basic visual processes. This information, obtained by electrophysiological measurement, has revealed the light properties to which the animals' visual system is responsive.

Many invertebrates have visual cells with peaks of sensitivity (electrophysiologically determined) in two or more parts of the spectrum, in marked contrast to vertebrates and other invertebrates with single peaks of sensitivity in one spectral region (Wasserman, 1973). One such invertebrate is the nocturnal wolf spider, *Lycosa*. The visual cells of its principal eyes are sensitive in both the UV and blue-green regions of the spectrum (DeVoe, 1972; DeVoe, Small, & Zvargulis, 1969). Although this animal appears to rely most heavily on touch, taste, and vibration reception

(Gertsch, 1949), its visual sense is second in keenness only to that of the jumping spider (Homann, 1928, 1931; Land, 1969). Attempts at behavioral assessment of the wolf spider's color discrimination (Frings, 1941; Peckham & Peckham, 1887, 1894; Schlegteendal, 1934; Young & Wanless, 1967) have so far yielded no unequivocal results. Wolf spiders tend to freeze or flee when exposed to optomotor stimuli (Schlegteendal, 1934). Our observations have shown that they are insensitive to electric shock in an avoidance conditioning situation. On the other hand, spiders have circadian activity rhythms that are modifiable by light (Cloudsley-Thompson, 1957; Tongiorgi, 1959). Frank and Zimmerman (1969) have shown that the spectral sensitivities of the circadian emergence rhythms of *Drosophila* can be measured by such modifications: intensities of different wavelengths of light were found which evoked equal resettings of the "dark" rhythms. It seemed, therefore, that a similar method, applied to the wolf spider's activity, would allow us to investigate the animal's behavioral discrimination of color.

Activity rhythms of small arthropods have been recorded by using running wheels (Roberts, 1960; Zwicky, 1970), piezoelectric vibration transducers (Tongiorgi, 1959), capacitance-change detectors ("Entomex," LKB-Farad, Sweden), and tilt cages (Cloudsley-Thompson, 1957). The development of our instrumentation was governed by the small size and weight of our animals, for which tilt cages appeared

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most suited.¹ In such a cage, the animal's back-and-forth movements cause the cage to tilt at either end. The need for illuminating as many animals as possible with monochromatic light was solved by grouping tilt cages around a mercury pen lamp whose spectral lines are selectively filtered. Activity is logged by using simple digital electronics to punch activity data onto paper tape for later, off-line computer analysis.

ENCLOSURE CHAMBERS

To entrain initial circadian activity rhythms to a 12:12 light-dark cycle, animals are maintained in plastic cages (TV Terrarium "Fernco," Montgomery Ward) in a living chamber. For measurements of circadian activity under other, experimental, light regimens, subjects are transferred to a nearly identical experimental chamber containing the tilt cages and the mercury pen lamp. The chambers are modified monkey experimental chambers (Silencer Model 385453-01, Koppers Co., Baltimore, Md.), 132 cm high x 71 cm wide x 89 cm deep, painted flat black inside and provided with intake and exhaust fans to minimize heat increases when light sources are turned on. The air intakes and exhausts are blackened vacuum-cleaner hoses to eliminate light leaks. To reduce vibrations, the chambers are corner-mounted on combination vibration isolators (KPG-40-6 Korpads and Korgrips combined with Elastomer mountings, Series F, No. FBB 260, from Korfund Dynamics, Westbury, New York). In the experimental chamber, where vibration elimination is critical, the tilt cages stand on a 32-mm-thick x 61 cm x 50 cm stone slab, which rests on 70 kg of sand in a metal tray. Especially quiet fans (No. 745 Venturi "Whisper Fan," Rotron, Inc., Woodstock, New York) are connected to the ends of the intake and exhaust hoses, rather than on the chamber itself. Complete lightproofing during experiments is obtained by an opaque black oilcloth curtain covering the experimental chamber. The chamber's inside temperature is monitored with a remote-reading thermometer (Weather Hawk Model 2354, Charts No. 32, Taylor Instruments, Arden, North Carolina) and is maintained between 22° and 24°C.

EXPERIMENTAL LIGHT SOURCE AND LAMP HOUSING

The experimental light source is a Pen-Ray Argon-Mercury lamp with a Model SCT 1, 117-V, 4-A power supply (Ultra-Violet Products, Inc., San Gabriel, California) run from a constant-voltage 117-V transformer (Raytheon No. RVA 250). We chose the lamp for its small size and 360-deg radiation pattern, and because it imposes minimal heat load on the experimental chamber and the lines at 365, 436, and 546 nm for monochromatic stimuli can be

Table 1
Filters for Isolating Mercury Spectral Lines and Intensities Measured at Two Cage Positions

Spectral Line (nm)	Filter	Average Intensities in quanta/sec/cm ²	
		Front of Tilt Cage	Back of Tilt Cage
365	UG-1 Schott	4.28×10^{12}	1.34×10^{12}
436	No. 50 Wratten	1.49×10^{12}	4.15×10^{11}
546	No. 74 Wratten	1.53×10^{12}	4.78×10^{11}

isolated with filters. The lamp bulb hangs down in a housing, 37 cm high and 7.5 cm on a side, constructed of 3-in.-sq aluminum tubing and covered on both top and bottom. From the top cover, a 25-mm-diam tube, 50 cm long, projects through a fitted opening in the chamber roof and allows the lamp cord and warm air from the lamp to exit (see Figure 3). The lamp itself is centered inside the housing (by three screws) in the middle of four 5-cm-sq windows cut in the four sides of the tubing and covered with plates of Pyrex No. 7740 borosilicate glass (ESCO Optics, Oak Ridge, New Jersey) to prevent ozone leakage and convective heat movements. Over each window, a 40-mm-sq, three-slot filter holder is fitted on a threaded metal frame. A 75-mm-diam cover (for light isolation) with a 25-mm hole for the emerging light beam is screwed over the filter holder. The filter holder holds 30-mm-sq filters. All glass, gelatin neutral density, and gelatin color filters (see below) were cut down to this size from the standard 50-mm-sq size. The gelatin filters were mounted into slide mounts designed for Kodachrome and Kodak Ektachrome-X 110 transparencies.

For intensity attenuation, we use Kodak Wratten 96 neutral density filters (Eastman Kodak Co., Rochester, New York). The color filters used to isolate the mercury spectral lines at 365, 436, and 546 nm are shown in Table 1. The UG-1 is a Schott glass filter (Schott Glass, Duryea, Pennsylvania) similar to Wratten 18A; the remainder are Wratten filters described in the Wratten Filter Handbook (Eastman Kodak Co.). The light intensities with each color filter were measured radiometrically, using a calibrated PIN-10 UV photodiode (United Detector Technology, Santa Monica, California). The photodiode surface was placed at the positions corresponding to the fronts and backs of the tilt cages in order to calibrate the maximum and minimum light intensities incident upon the animals. Values for the measured intensities, in quanta/sec/cm², are tabulated in Table 1.

EXPERIMENTAL STATIONS

Four identical tilt cages were each constructed from rectangular metal cans, 75 mm long x 50 mm square cross section (No. C12-k1.02, Vector Electronic Co.,

Figure 1. Tilt cage shown in profile (left) and from bottom (right). (1) Metal can, (2) filter frame for cover glass, (3) fulcrum block, (4) light blocking washer, (5) U-shaped wire for magnet, (6) magnet, (7) hypodermic tube supports for U-shaped wire, (8) hole for water tube.

Sylmar, California). They were painted black on the inside and capped on the open end by epoxying on a Kodak 50-mm-sq filter frame. The frame holds a 48 x 50 x 0.17 mm cover glass (cut down from 48 x 60 mm cover glasses, A. H. Thomas Co., Philadelphia, Pennsylvania), which serves to keep the animal in but is too thin to attenuate even the UV wavelengths appreciably. At the bottom of each case, a 10-mm-diam hole was cut to admit a 6-mm-diam, wick-filled glass tube from a rubber-stoppered water bottle (see Figure 2). The wick, 25-mm-wide cotton twill tape, does not harden and darken at its end after long use, as do wicks for alcohol lamps, and serves as a water source for the animal throughout the experimental period. (Wolf spiders can remain without food for periods longer than 15 days, but must have continuous access to water.) A 40-mm-diam black metal disk slipped over the glass tube and held in place by a rubber grommet, shields the underside of the cage from possible light entry through the hole and prevents the animal's escape through this hole. Centered with a clamp, the water tube does not touch any part of the cage and so does not affect its movement.

A small square brass block was screwed to each of two sides of each cage. At the bottom of each block, a small indentation was made with a machinist's centering punch. These indentations rest on the points of 25-mm-long sewing machine needles (Size 18), which thus provide the pivots for the cages' tilting. The blocks were made to slide in slots cut into the cage sides to provide the balancing of each cage; thin metal washers block light entry through these slots. For each cage, a 13-mm-long x 1-mm-sq permanent magnet (Hamlin No. H-31-604; Hamlin, Inc., Lake Mills, Wisconsin) is cantilevered from the rear of the cage over a reed switch (Hamlin Mini-2-115). This scheme provides contactless opening and closing of the reed switches upon cage tilts. It also provides a "snap-action" effects, as with all reed switches: Relatively large (1-2 mm)

movements of the magnet are required to open and close the reed switch, due to magnetic hysteresis. As a result, only full cage movements, which are limited by stops, are detected, while small rocking motions and bounce at the stops are not. To set the lever arms of each cantilevered magnet, it was epoxied to a U-shaped piano wire that slid into short (12-mm-long) pieces of hypodermic tubing epoxied in parallel to the underside of the cage. This permits the magnets to be slid onto each cage before an experiment and slid out at the end before cage cleaning. Figure 1 shows a tilt cage in profile and from below with the magnet in place.

The adjustable stops under each end of the tilt cage are screws capped with rubber plungers from plastic tuberculin syringes. These stops are adjusted so that movement against them is slightly more than sufficient to open or close the reed switch. When properly balanced with the cover glass and magnet in place, but no animal inside, a force of no more than 100 mg at one end of the cage is sufficient to tilt it to that end from the other. This minimum weight is less than that of our animals, which range from 0.85 to 2.00 g.

Each tilt cage is mounted via the pivot needles on a 15-cm-sq plastic platform, which also holds the clamp for centering the water tube and a miniature phone jack soldered to the reed switch. The platform is adjustable in height on four long bolts anchored in a 15-cm-sq stone slab base. A sketch of the platform with tilt cage is shown in Figure 2.

With animals in place, each tilt cage (on its platform) is positioned around the lamp housing with the light exit path from each filter holder aligned with the cover glass at the front of each tilt cage. Baffles, made from 1-gal circular ice cream canisters, 25.5 cm high x 23 cm in diam and sprayed black inside and out, are placed over the tilt-cage assemblies to isolate the light incident on one animal from that on any other. A hole cut in the baffle fits over the cover of the lamp housing filter holder, so that when the baffle is

Figure 2. Experimental station with mounted tilt cage. (1-7) Same as in Figure 1, (9) water tube, (10) light blocking disk, (11) sewing machine needle, (12) clamp for water tube, (13) water bottle, (14) tuberculin syringe plunger acting as a stop, (15) reed switch soldered to (16) miniature phone jack, (17) plastic platform, (18) support bolts for platform, (19) stone base, (20) water wick.

Figure 3. Experimental chamber interior. (1, 11, 13, 17-19) As in Figures 1 and 2, (21) miniature phone plug and cable to digital electronics, (22) lamp housing, (23) heat and lampcord outlet tube, (24) screw-on cap over filter holder, (25) light baffle in place over an experimental station.

Figure 4. Schematic drawing of paper-tape-punch format. One half of a dual 24-h timer turns stimulating light on and off. Light-on is indicated by a punch in Bit 0 of Word 1; nothing is punched when light is off. The other half of the dual timer closes a switch every 24 h, indicated by a punch in Bit 5 of Word 1. Otherwise, Bit 4 of Word 1 is punched. Animal activity, detected by opening and closing of the reed switches, filtered by contact bounce filters, and inverted by buffer amplifiers, is counted by 4-bit binary counters and punched in Bits 0 through 4 of Words 2 through 5, one word for each of the four tilt cages. Animal position at back of cage (final position = 1) leads to inverter output being high and Bit 0 being punched. Counters increment when animals move back to front, i.e., when inverters go from high to low. Activity words are identified by Bit 6 punches in Word 1. Bit 7 is punched during manual FEED; Bits 6 and 7 are both punched in the first cycle after power return from a power-off. In all words, arrows indicate that bits were punched when these lines were high (electrically, +5 V).

in place, it completely encloses the experimental station together with its light beam. Figure 3 shows a view of the lamp housing with one baffled and one unbaffled experimental station.

RECORDING ELECTRONICS

The virtues of the reed switch movements as indicators of animal activity are that they may be electronically registered in one way or another. In order to put activity counts in a form in which they can easily be used in later computations, we punch the activity data on paper tape for off-line computer analysis. At regular intervals, light on- and offset, time data, and counts of animal movements are punched in a five-word-byte cycle, as schematically illustrated in Figure 4. Word 1 contains the time and light on/off data. Words 2-5 contain the activity in Cages 1-4, respectively, as counted by 4-bit binary counters, as well as the final front or back position of each animal. For the purpose of synchronizing the subsequent tape-reading program, Word 1 lacks a punch in Bit 6 but is never blank (to prevent some computers from simply skipping it) since either Bit 4 or Bit 5 is always punched, depending on the time markings. Manual feeding of tape at the start or end of an experiment causes Bit 7 to be punched,

including the first punch cycle after manual feed. If power fails during a multiday experiment, this information is conveyed by a combined Bit 6 and Bit 7 punch occurring in the first punch cycle after power returns. Corrections for the duration of this interval can then be made at tape-reading time. To avoid confusion between power-offs and beginnings of experiments, Bit 6 is not punched at all during the initial punch cycle of an experiment. Data in this punch cycle or in the first one after a power-off have to be ignored anyway as such a cycle does not necessarily represent a full sampling period.

Details of the digital circuitry are given schematically in Figure 5, except for the multiplexer of AND/OR gates, which follows conventional design. We used "K" series logic cards (Digital Equipment Corp., Maynard, Massachusetts) because of their excellent interfacing capabilities. The tape punch is a Teletype High Speed BRPE (Teletype Corp., Chicago, Illinois). It was converted to slow-speed intermittent operation by means of a Dayton 25-rpm brake-motor (Dayton Electric Mfg. Co., Chicago, Illinois).

The circuitry consists of three parts. The first is concerned with activity counting, the second with sequencing/multiplexing, and the third with external control. For activity counting, reed switching due to cage movements is filtered by K581, C to F contact

Figure 5. Schematic diagram of the digital circuitry used to count animal activity and punch-out paper tape. Logic was built up of Digital Equipment Corp. "K" series cards; only a few actual pin connections are shown. Operation descriptions are as follows: K003 OR-wired gate expander; K012, OR gate expander; K123, noninverting gate; K124, noninverting AND/OR gate; K138, inverter; K161, octal decoder; K202, flip-flop; K210, binary counter, incremented by negative-going input; K333, pulser, triggered by positive level; K581, self-powered contact bounce filter, high output for open contacts; K683, relay driver.

bounce filters (which go high at opening). It is buffered for increased fanout capability by K138, C to F inverters, and it is counted at each high-to-low transition of the inverter (back-to-front movement in the cage) by K210, A, B, C, D 4-bit binary counters. Both inverter outputs (front or back position of the animal in the cage) and the four outputs of each counter are applied to the multiplexer. As the counters hold only half the number of total back-and-forth movements, their contents plus position information from the inverters permit up to 31 back-and-forth movements to be reconstructed at tape reading (see below), obviating larger counters.

Light on/off's are controlled by a Sealectro 24-h LCT dual timer (Sealectro Corp., Mamaroneck, New York). One half switches the light source on and off and, after K581, A, filtering, is led to the multiplexer. This light timing can be disabled by a manual switch (ON/DISABLE), as, for example, during measurements of activity rhythms in the dark. For delivery of short, single flashes of light, as for resetting dark activity, the onset of this Sealectro timer triggers a Graylab Universal timer (up to 60 min timing; Dimco-Gray Co., Dayton, Ohio) for more precise control of flash durations. The second half of the Sealectro timer provides a switch closing every 24 h. This is filtered by K581, B and is applied, along with its negation from inverter K138, B, to the multiplexer.

A punch cycle is initiated by the switch closing of the sampling interval timer, a Sealectro LCT 60-min timer, capable of timing intervals of 1 to 60 min; we use 5-min intervals. Timer switching, filtered by K581, G, triggers a negative pulse from pulser K333, A which in turn clocks the motor control flip-flop K202 to SET and the K210, E sequencing counter to "1." The flip-flop SET output, led via a K124, C AND/OR gate and a K683 relay driver, starts the punch motor and tape feed. A cam switch on the punch motor, which opens every revolution (i.e., after a word has been punched), is filtered by K581, H and triggers a negative pulse from K333, B which clocks the sequence counter by one more count. The next clock pulse from K333, B after the sequence counter has reached a count of "5," resets the counter to "0" via the K161 decoder and the K003 OR gate. The counter outputs of "1" to "5," as decoded by the K161 decoder, enable Words 1 to 5, respectively, in the multiplexer for punching by the Teletype via the K683 relay drivers. When the sequence counter is reset to "0" after the punching of the fifth word, the K161 decoder output of "0" triggers a negative pulse from pulser K333, C which both clears the K210, A, B, C, D activity counters and clocks the K202 flip-flop to RESET, turning off the punch motor.

A three-position, four-section switch, RUN/OFF/FEED, provides external control. In the OFF mode, loss of power to a time-delay relay (Magnacraft

505Q1CX-1) grounds the CLEAR inputs of the K202 flip-flop and the K210, E sequence counter, thereby preventing them from being triggered by the sampling interval timer. In the FEED mode, the K124 B and C AND/OR gates are enabled, applying power to the punch motor and tape-feed relays and causing Bit 7 to be punched via a K683 relay driver. In both OFF and FEED modes, a red-light-emitting diode (LED) is turned on as warning and a 5-V reed relay (Magnacraft W118DIP-5) latches itself on. The LED (combined with a switch) is Dialco 913-1553-1637-525.

The LED turns off upon initiation of the RUN mode and the 5-V reed relay stays latched. The delay relay turns on after a delay during which the SET input of the K124, E AND/OR gate, wired as a flip-flop, turns this flip-flop on. After the delay, the SET input is grounded and inhibited. At the next punch cycle, the SET output of the K124, E flip-flop is ANDed with the SET output of the K202 motor-control flip-flop in K124, B, causing Bit 7 to be punched during this punch cycle. At the same time, the output of the K124, E flip-flop is negated by inverter K138, A and prevents Bit 6 from being punched during Words 2 to 5. At the end of this first punch cycle, flip-flop K124, E is cleared by the negative pulse from K333, C, Bit 7 is no longer punched, and Bit 6 is no longer inhibited. Thus, in subsequent punch cycles, outputs "2" to "5" are ORed by the K012-K123 OR gate and via K124, A AND/OR gate and a K683 relay driver, and cause Bit 6 to be punched in Words 2 to 5. An experiment is terminated by switching briefly back to FEED to punch Bit 7 before ending at OFF.

Should power go off during an untended experiment (when the control switch is in the RUN mode), the 5-V reed relay unlatches and does not relatch to "on" when power is restored, while the K124, E flip-flop is SET via the time delay relay, as at the initial switching to RUN, above. During the next punch cycle, Bit 7 is punched as described via the enabled output of K124, B and the +5 V at the reed relay are then ANDed at the K124 A, AND/OR gate to cause Bit 6 to be punched also. At the end of the first punch cycle after power-return, the K124, E flip-flop is cleared by the negative pulse from K333, C and Bit 7 is not punched again; Bit 6 is punched in Words 2 to 5 only. To indicate that a power interruption has occurred, the LED is turned on by the unlatched 5-V reed relay. Pushing the RESET pushbutton manually relatches the 5-V reed relay and turns off the LED.

As the backup to the digital logic and to aid in determining number and duration of power-offs, information concerning cage positions (from the K138, C to F inverters), light on/off times, and power on/off (direct connection to the +5-V logic

Table 2
Tape Reading Program: Simplified Flow Diagram

power supply) is led by another group of K683 relay drivers (not shown in Figure 5) to a Simpson No. 2755 10-channel event recorder (Simpson Electric Co., Chicago, Illinois). The duration of a power-off can be estimated from the error between correct time and the time indicated on the event recorder chart paper, which is printed on a 24-h time basis. Similarly, the number of power-offs can be seen by the off-deflections of the channel driven by the +5 V logic power supply. In our experience, power-offs have been infrequent (but not absent) and sufficiently spaced in time so that their correct number could be read from the event recorder tape. The other principal uses of the event recorder output are for checking the tape-reading programs during debugging, and for providing an immediate estimate of animal activity at any time in the course of the experiment. Peak activity times over several days can thus be monitored, and it is possible to make stimulus delivery time and duration contingent upon such observed peak periods.

DATA ANALYSIS

We use an interactive FORTRAN program entitled SPYDERHYTHM, to read the paper tapes and store the interpreted data on magnetic (LINC) tapes. A simplified flow diagram of the tape-reading program is given in Table 2. Further details are available from the authors. The decoding of the number of back-and-forth movements of the animals during the sampling interval is based on their final POSition at

punch time (punched in Bit 0) and the number of *back-to-front* movements, which alone are counted in the K210, A, B, C, and D counters and punched in Bits 1 to 4. The number of total back-and-forth movements is not exactly twice the number of back-to-front movements recorded in these counters. For example, had an animal moved only from the front to the back during a sampling interval, there would have been no record of this in the counters at all. Therefore, it is necessary to compute the total number of back-and-forth movements with the schema illustrated in Table 3. Final POSitions are defined as "1" if Bit 0 is punched, which occurs when inverters K138, C to F are high and signifies that the animals are at the back of the cages. Otherwise, final POSitions are "0."

To store the reconstructed number of back-and-forth movements on tape along with the final POSition itself (so as to have some measure of where the animal stayed during light-on, for instance), the number is negated if the final POSition was 1, otherwise not. However, for zero movements but a final POSition of 1, 0 would result, yet in the 2s complement logic of many computers this is the same as +0. Therefore, what is stored on the digital tape is the reconstructed number of back-and-forth movements +1, negated if the final POSition was 1, otherwise not.²

Finally, due to the fact that activity counters are not cleared directly after their contents are punched but are all cleared together at the end of the punch cycle (by pulser K33, C), activity counted between

Table 3
Activity Decoding Schema

1. Retrieve final POSitions (values of Bits 0 in Words 2-5) of animals in previous punch cycle
2. If the final POS in the previous punch cycle was 1 (animal at back of cage)
and present POS is 1, then change of POS = 0
and present POS is 0, then change of POS = 1
If the final POS in the previous punch cycle was 0 (animal at front of cage)
and present POS is 1, then change of POS = 1
and present POS is 0, then change of POS = 0
3. The total number of back-and-forth movements (position changes) since the last punch cycle is as follows:
If present POS is 1, then number of back-and-forth movements has been $2 \times (\text{counter contents}) + \text{change of POS}$
If present POS is 0, then number of back-and-forth movements has been $2 \times (\text{counter contents}) - \text{change of POS}$
4. Examples of punched data and decoded activity:

Bits Punched					Present POS	Pre-vious POS	Change of POS	Counter Con-tents	Number of Back-and-Forth Move-ments
<u>4</u>	<u>3</u>	<u>2</u>	<u>1</u>	<u>0</u>					
0	1	0	1	1	Back	Back	0	5	10
0	1	0	1	0	Front	Back	1	5	9
0	1	0	1	1	Back	Front	1	5	11
0	1	0	1	0	Front	Front	0	5	10

Note—Doubly underlined bits contain counter contents.

punching and clearing can be lost. Punching the activity words takes 10 sec out of the 300-sec (5 min) sampling intervals. Thus, a maximum of 3% of the activity counts can be lost during this time. Loss of odd numbers of counts can be detected at tape-reading, since previous final POSition + activity will not yield present final POSition. When this happens (infrequently), a "lost activity count" of 1 is added to that measured.

The ultimate purpose of computer reading and analysis is to extract phase shift information from the activity records. To that end, an additional SPYDERHYTHM routine provides for direct plotting of the stored data. The plotter (Calcomp, California) draws in rows, one day below the other, the back-and-forth movements of each animal and the light on/off sequences and labels time axes. Activity periods become visible at a glance, and rapid comparison within and between animals can be made. A more rigorous analysis of phase shift differences is obtained by digitally filtering the stored data. We are presently adapting a digital filter similar to those developed by Binkley, Adler, and Taylor (1973) and Enright (1965). Rhythm and phase shifts can thus be analyzed in relation to particular intensities of differing monochromatic stimuli in terms of their

effect on animal activity. From such data, we hope to learn whether the animals make use of the spectral sensitivities already measured in the receptor cells themselves (DeVoe, 1972).

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NOTES

1. Originally, we tried to measure all activity with a piezoelectric transducer, as did Tongiorgi (1959). For this, a 78-rpm crystal phonograph cartridge (No. 18 with N6-2 Astatic needle, Astatic

Corp., Conneaut, Ohio) rested against a metal tube (as cage) suspended by two elastic bands from horizontal cross bars. In principle, movements above any arbitrary size might be measured by using a voltage comparator to detect suprathreshold outputs of the piezoelectric crystal. In practice, residual floor vibrations, underdamping of the cage suspension, and irreproducibility of transducer positioning combined to give unreliable counts of activity when used with digital counters.

2. We are aware that reconstruction of the back-and-forth

movements could have been handled in hardware such that each movement of an animal in either direction would be counted. Since this would have entailed larger counters and additional logic, reconstruction of activity counts with software seemed to us the easier route.

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ERRATUM

ELIAS, M. F., & ELEFThERIOU, B. E. A behavior-genetic investigation of induction and eduction times for halothane anesthesia. *Behavior Research Methods & Instrumentation*, 1975, 7, 7-10. On page 9 of the article, there is a mistake in the table. The line connecting nonsignificant differences for eduction scores at 2% halothane should indicate *no* differences between the G (16 sec) and J groups (15 sec). In other words, the the solid line should extend to cover the G and J groups instead of stopping at the C group.