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# ULTRASONIC MEASUREMENT AND AUTOMATIC ANALYSIS OF GENERAL ACTIVITY IN THE RAT[1]

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Problems in measurement of general activity have prevented its use as an index of drive. An ultrasonic device for measuring general activity appears to remedy many of the faults of other methods. A system utilizing the ultrasonic technique in conjunction with automatic data collection is presented, together with results obtained during conditions of 12-hr. light-dark cycles or continuous illumination, with ad-lib access to food and water. Automatic data collection permits fine temporal resolution of activity measurements, use of autocorrelational methods to detect periodicities in the data, and observation of general activity over extended periods of time.

The general activity of organisms has long been considered a relatively direct manifestation of their drive state. Despite more than 50 years of study, however, the precise relationship between general activity and drive state remains unclear (Cofer & Appley, 1964, pp. 269–277). The inability to give operational meaning to the concept of general activity is in part dependent upon problems in the measurement of activity. Various devices have been used to measure activity (Hall, 1961; Mitchell, 1959; Munn, 1950; Reed, 1947), but each has revealed certain limitations. Stabilimeters and activity wheels provide proprioceptive feedback to the animal; photocell techniques, open fields, and the like are not capable of detecting certain kinds of bodily motion which contribute to general activity.

The ideal general activity measuring device should not influence the activity of the organism being studied and should be able to register a representative sample of the organism's total behavior, if not all bodily motion. The ultrasonic device (Peacock & Williams, 1962) offers an approach to these requirements, in that its acoustic signal of 40 kc. is not heard by the rat at the power levels used and its sensitivity is sufficiently great so that minor bodily movements can be recorded. A three-dimensional pattern of standing waves is set up in the space containing the animal. Movement by *S* through the nodes and antinodes of the standing wave pattern causes a change in the reflected signal which is detected by a ceramic microphone and amplified electronically. The number of pulses obtained from the apparatus is proportional to the linear extent of the movement; calibrations with mechanical objects moving through distances of 2.6–6.5 cm. at various velocities approximating rodent movements reveal that the device is primarily responsive to the extent of movement, although a slight effect of movement velocity is seen. The ultrasonic device can be regarded as an extension of the principle used in the photocell technique, with the difference that a very large number of acoustic standing wave nodes are used as motion detectors in place of a few light beams.

As the first step in a systematic program of activity research, the present investigators have examined the general activity patterns of laboratory rats under ad-lib food and water conditions using the ultrasonic device. The controlled variables during the experiments were ambient temperature, noise level, light-dark ratio, genetic stock, sex (male), and age of *S*s at the onset of the experiment. In short, an attempt was made to obtain a sample of

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general activity under well-defined conditions in order to assess the capability of the ultrasonic device.

There is considerable evidence (Bruce, 1960; Cloudsley-Thompson, 1962; Halberg, 1960; Pittendrigh, 1960) that a variety of biological systems seems to wax and wane regularly within specified time periods. The most usual period is circadian (about 24 hr.). Although psychologists have long known about the diurnal activity rhythm of rats (Munn, 1950; Reed, 1947), apparently no attempt has been made to examine systematically the activity records at shorter time intervals. The major reasons for analyzing the periodicity of activity rhythms are: (*a*) it is possible that standard motivational variables, e.g., deprivation, sex of organism, have differential effects upon activity at different points in a cycle which would probably not be detected by conventional methods of measurement and analysis, and (*b*) periodicity analysis can detect effects which are hidden or obscured by the large variability of the individual *S*'s responses. Thus, the present investigators have supplemented conventional methods of analysis with routine evaluations of the periodicity of activity data by means of autocorrelation and related techniques (Bendat, 1958, 1962; Mercer, 1960) in order to determine whether such analyses are fruitful with ultrasonic activity measurements.

The present paper reports the results obtained during two replications of a baseline study, describes the system used to collect the data, and compares the general activity of rats under conditions of continuous light and a 12-hr. light-dark cycle.

## METHOD

### *Subjects and Environmental Conditions*

The *S*s for the baseline studies, 24 male albino rats of the Dublin DR strain, 90 days old at the start of the experiment, were housed in a sound-shielded room with fluorescent lights, operated by a timer, which were turned on at 6 A.M. and off at 6 P.M. During the dark hours, the room was dimly illuminated by a pilot light on a power supply and by the glow of vacuum tube filaments in the electronic apparatus in the room. The room was entered only once each day, between 2:50 and 3 P.M., to change food and water supplies and to exchange litter pans. The *S*s lived in individual wire cages suspended within open-front boxes lined with acoustic absorbing material which minimized reflection of the ultrasonic signal. The electronic apparatus within the room produced no sounds, and a white noise (80-db. SPL) was introduced to mask extraneous sound.

Twelve additional animals of the same sex and strain were kept under the same conditions, except that during exposure to continuous light the fluorescent lights were left on 24 hr. each day.

### *Apparatus*

A circuit diagram of the ultrasonic device has been previously published (Peacock & Williams, 1962). For the purpose of the present experiment, the basic circuit was modified slightly. Each of the 12 cages was supplied with its own amplifier, power supply, receiver, and transmitter. A single master oscillator, crystal controlled, provided the 40-kc. signal for all 12 channels. The use of a single oscillator was necessary in order to prevent interaction of the 12 channels resulting from small differences in oscillator frequencies. The output of each ultrasonic amplifier, which consisted of an irregular waveform, was led by a coaxial shielded cable to an integrator located in an adjoining room. The integrator amplified the signal in one vacuum tube stage, rectified the signal, and stored it as a charge on a low-leakage capacitor. When the charge on the capacitor reached a preset voltage, it activated a relay which simultaneously discharged the capacitor and sent a pulse to a counter. The counters were constructed of stepping switches and had a capacity of 999. A digital clock operated a keypunch control unit every 5 min., causing it to start a punch cycle on an IBM 024 keypunch. The punch sequence included a 4-digit number representing the time of day, a channel identification code for each channel, and 12 sets of 3 digits representing the number of counts stored in each channel. As the data were serially read from the counters onto the punched card, each counter was returned to 0. The punch cycle took approximately 5 sec. to complete, but each channel was inoperative only a fraction of a second every 5 min. Thus, 288 cards, each containing the summated activity of 12 *S*s properly coded, were produced during a 24-hr. period.

### *Procedure*

When *S*s arrived from the supplier they were placed in the individual experimental cages for a habituation period of 3 days with all experimental conditions maintained. For the next 40 days their activity was recorded at 5-min. intervals. Power failure during the first study resulted in the loss of 2 separate days' data, one 6 days and one 16 days after the onset of the experiment. During the replication of the baseline study, the data collection period was shortened to 38 days in order to match the first study. Owing to failures in the

data acquisition system, information from two channels in each study was incomplete. The results are based on data from at least 10 *Ss* in each replication.

The procedure during the comparison of continuous light with 12-hr. light-dark cycle conditions was essentially the same. After the habituation period, data were collected for 12 days with the room lights coming on at 6 A.M. and going off at 6 P.M. Beginning with Day 13, the lights were left on continuously for 9 days.

## Results

The diurnal rhythm of the albino rat is clearly shown in Figure 1, in which the mean hourly activity of 20 *Ss* over a period of 38 days is plotted as a percentage of their total daily activity. During the light period, each hour's activity represented less than 2% of the total daily activity; during the dark period, each hour's activity represented 6–8% of the total daily activity. Thus, the 12-hr. dark period contained more than 75% of *S*'s entire activity. The onset of the dark period was accompanied by an abrupt increase in the amount of activity which was followed by a slight decline until about 10 P.M. A fairly steady amount of activity occurred during the remainder of the dark period, with perhaps an increase during the last hours of the night. The sudden onset of the lights at 6 A.M. was followed by a sharp decrease, with *Ss* remaining quiescent during most of the day. The caretaker's entry into the experimental room at 1450 hr. occasioned a slight increase of activity coincident with light offset. It is probable that the caretaker's entry would have had a greater impact on activity level if *Ss* were not provided with ad-lib food and water. The data point for 1500 hr. is artificially depressed because two 5-min. periods are omitted from the calculations just prior to 1500 hr.

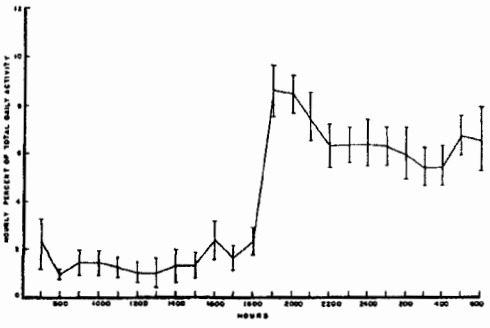

Fig. 1. Mean hourly activity of 20 rats over a period of 38 days, expressed as a percentage of total daily activity. (Vertical bars indicate ±1 *SD*.)

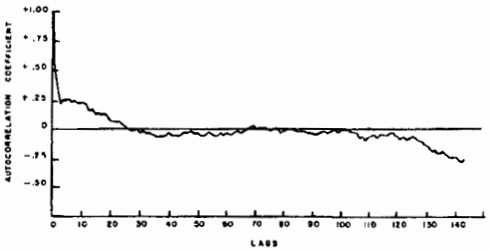

Fig. 2. Mean autocorrelation function of 10 *Ss* on an arbitrarily selected experimental day.

The two replications of the baseline study revealed no significant differences attributable to the different experiments. Only two of the 24 data points showed no overlapping of the standard deviations, and the general pattern of activity was the same in both studies.

An autocorrelation analysis was performed on the data of each *S* on each of the 38 days. The mean autocorrelation function on a typical day (Figure 2), calculated by using Fisher's *z* transformation, showed a sharp exponential decay from $r = 1.00$ to $r = 0.00$ during the first 20–25 lags. A lag of 5 min. was used in the analysis. This result indicates that the total activity in successive 5-min. periods up to approximately 1 hr. was highly correlated; i.e., the total activity of *Ss* in any given 1-hr. period was very similar to the total activity in the preceding 1-hr. period. With higher lags, the mean daily autocorrelation function remained essentially at zero until the maximum lag of 144 was reached. At this point, the autocorrelation coefficients became significantly negative ($p < .05$), confirming the 12-hr. periodicity shown in Figure 1.

The mean autocorrelation function combined the data of all *Ss* on each day of the experiment, but the individual *Ss* may show periodicities which are obscured by averaging. Representative individual records of significantly positive and negative autocorrelation coefficients are offered in Figure

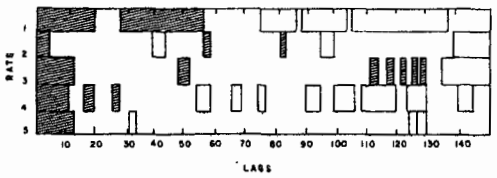

FIG. 3. Positive (crosshatched rectangles, > +.163) and negative (open rectangles, > −.163) autocorrelation coefficients, for the first 5 *Ss* on an arbitrarily selected day, which reached significance ($p < .05$).

3. Each individual *S* on a given day showed numerous significant periodicities; when all individual records were averaged, however, the random array of significant lags resulted in near zero values for all but very short and very long lags.

The light-dark cycle is a major determinant of the pattern of activity of *Ss* maintained with ad-lib supplies of food and water. In Figure 4, the hourly mean activity in actual counts is plotted for 12 *Ss* who were kept on a 12-hr. light-dark cycle for 12 days, followed by a 9-day period of continuous light. The diurnal rhythm produced by the 12-hr. light-dark cycle is apparent in Figure 4. When the *Ss* were abruptly changed to continuous light, the diurnal rhythm disappeared and was replaced by an even distribution of activity across the 24 hr.

The similarity between the results presented in Figure 1 (expressed as mean hourly percentage of total daily activity) and those shown for the 12-hr. light-dark cycle in Figure 4 (actual counts) suggests that either mode of data presentation is suitable for future studies.

Although the averaged data revealed only the diurnal variation in activity during the light-dark cycle condition, each individual *S* again showed intermittent periods of activity and rest during both illumination conditions. During cyclical illumination, the individual *Ss* alternated rest and activity irregularly and fairly rapidly; under continuous illumination, the frequency of these alternations decreased.

The mean autocorrelation function for the continuous illumination period showed no significant periodicities beyond the first

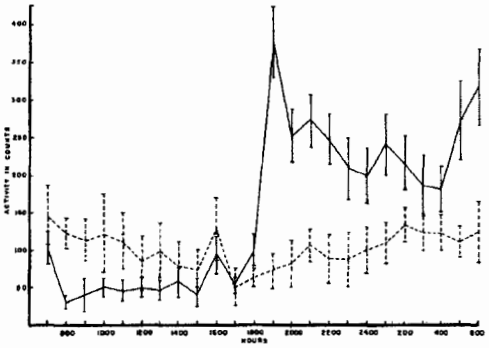

FIG. 4. Hourly mean activity of 12 rats on a 12-hr. light-dark cycle (solid line) followed by continuous illumination (dashed line). (Vertical bars represent ±1 *SD*.)

four lags. During the cyclical illumination period, lags of up to 10 were significantly positive, as were the longer lags representing the diurnal rhythm. An individual record of significant positive and negative lags is presented in Figure 5, where it can be seen that continuous illumination increased the number of positively significant coefficients at longer lags and thereby distributed the significant lags more uniformly across the experimental period. Furthermore, the preponderance of negatively significant long lags seen in cyclical illumination was reduced by continuous light.

## DISCUSSION

The ultrasonic device and its associated data acquisition system appear to be useful tools for the investigation of the effects of

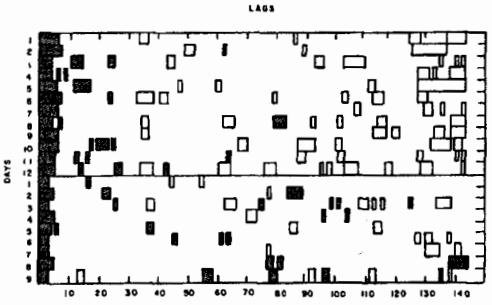

FIG. 5. Significantly positive (crosshatched rectangles) and significantly negative (open rectangles) autocorrelation coefficients for a single *S* during 12 days of a 12-hr. light-dark cycle followed by 9 days of continuous illumination.

various variables on the general activity of the rat. The data of the present studies accord well with information obtained through the use of other measuring devices (Hall, 1961; Munn, 1950), but, in addition, the automatic data collection system permits the recording and analysis of activity data with a higher degree of temporal resolution than heretofore possible. No previous investigation has quantified for extended periods of time the exact distribution of activity during a 24-hr. period.

The present results indicate that the technique of autocorrelation is a useful method of assessing the presence of periodicities in both group and individual activity data. Although the diurnal rhythm of the rat has been known for some time (Hunt & Schlosberg, 1939a, 1939b), the autocorrelational analyses provide an estimate of the rhythm's magnitude in terms of the familiar correlation measurement scale. Inspection of Figure 2 also suggests the rhythm is not an all-or-none phenomenon, but develops gradually from the eleventh (c. Lag 132) to the twelfth (Lag 144) hour. Apparently cyclical light, as an external *Zeitgeber*, is a very powerful synchronizer of the general activity of rats. Under continuous illumination, the periodicities in the activity of individual *Ss* are more evenly distributed over the experimental session, with little evidence of synchronization among *Ss* (Figure 5).

Consistent with the *Zeitgeber* interpretation are the numerous significant positive and negative coefficients obtained in the autocorrelational analyses of individual *Ss* given cyclical illumination. The occurrence of the significant coefficients seems to be random except at the very short and very long lags (Figure 3). Apparently, there is no *Zeitgeber* to organize these periodicities.

A surprising result of the present experiments was the failure to substantiate Richter's (1927) claim that rats showed increased activity approximately every 3–4 hr. under ad-lib conditions. Neither the conventional nor autocorrelational analyses of the present data revealed such a periodicity. A possible explanation of this result is that the ultrasonic technique does not permit a discrimination between food getting and other forms of general activity, a discrimination made in Richter's apparatus.

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