Reports and Letters

Evaluation of Four Activity Techniques for Monkeys

In a study of whole-body x-irradiation (*1*), it was thought that lightly motivated behavior such as "spontaneous" activity, rather than strongly motivated behavior, might provide the more sensitive measure of radiation damage. The large variety of activity apparatuses previously devised is evidence of the difficulties involved, which are greater with monkeys than with rats.

Few of these apparatuses have been studied in respect to reliability (*2*). Nor, apparently, has anyone attempted to discover the validity of any technique or to compare one technique quantitatively with another. This is surprising in view of the interest in activity measures shown by biologists, psychologists, physiologists, and especially, pharmacologists. The purpose of this study was to find a procedure suitable for the monkey and to determine its reliability and validity. To this end, ratings by an observer, Siegel's electric-eye technique (*3*), and two variations of the suspended-floor technique were compared, and the reliability of each was established.

Nineteen *Macaca mulatta* served as subjects. An expanded metal cage (24 in. by 18 in. by 22 in.) was equipped with a plywood floor that was suspended free of the walls on instrument shock mounts. Two 4-lb shock mounts, mounted face to face and bolted to the cage floor and the corners of the plywood floor, gave adequate vertical movement without horizontal sway. The following four methods of measuring activity were used.

1) A work adder (*4*) was mounted below each corner of the cage. The work adder consists of a large ratchet wheel, with very fine teeth, attached to a pulley, about which was looped a weighted piece of steel fishing leader connected to the cage corners. The ratchet wheel responded to small, as well as large, movements when the cage floor was depressed. When the floor returned to its normal position, a pawl held the wheel, and the wire slid back over the pulley. The work adders and the observer reading them were located inside a cupboard under the cage, hidden from the monkey's view. The total score for all four work adders was used in the statistical analysis.

2) A microswitch that was sensitive to a 0.005 to 0.008-in. excursion, was mounted beneath the center of the floor, just far enough so that movement, but not the animals' weight, would close it. The switch operated an electric oscillator that drove an electric counter at the rate of 10 impulses per second.

3) A beam of visible light, focused on a photo-electric cell, operated a counter through a relay. One observer recorded scores of two counters, which were in separate rooms.

4) An observer noted total activity on a rating sheet bearing five linear rating scales of length 10 cm, that contained five equally spaced reference points. The rater, whose presence was familiar to the monkeys and therefore did not arouse them, sat 10 ft in front of the cage.

The experimental room was isolated but not soundproof, so that slight noises from the colony, laboratory, and counters were audible. An animal was allowed time in the cage to familiarize itself with its surroundings. During this period, all equipment was operating. Upon a light signal given by one observer, all observers recorded readings and ratings simultaneously once each minute.

Reliability coefficients, correlating the odd-minute interval scores with the even-minute scores are presented in Table 1, as are the intercorrelations among methods, based on the total 60-minute score for each animal. These correlation coefficients are all significant at better than the 1-percent level of confidence.

On the basis of the reliability coefficients, there is little choice between these four techniques for measuring the spontaneous activity of a monkey. The intercorrelations vary somewhat more, but all correlation coefficients are statistically significant. Although a test is reliable, it is not necessarily valid—that is, it may or may not be measuring the desired behavior. The high degree of intercorrelation suggests that the three mechanical devices were measuring the same or quantitatively related behavior. The microswitch scores correlated least well with the other measures. This probably reflects the difficulty in obtaining equal sensitivity of the system for all animals. One of the accepted ways of validating a test is to compare scores on it with observer ratings. Both the electric-eye and the work-adder scores correlate highly with activity ratings by an experienced observer. Because of its somewhat higher reliability coefficient and its readier adaptation to multiple-unit, automatic recording, the electric eye was judged best for recording cage activity by a monkey.

Table 1. Reliability coefficients and intercorrelations of four activity measures ($N = 19$).

Item	Rating	Work adders	Electric eye	Oscillation
Rating	.976	.968	.946	.849
Work adders		.932	.928	.838
Electric eye			.993	.789
Oscillation				.993

The electric-eye technique was then used to measure the activity of 16 monkeys during a 44-day period. A reliability coefficient of .948 was obtained by correlating the sums of the odd days' activity scores with those of even days. Such a correlation coefficient is significant at the 1-percent level of confidence.

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References and Notes

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Enhancement of the Central Nervous System Effects of Strychnine and Pentobarbital by Diphenhydramine

The discovery in recent years of new compounds having marked effects on the central nervous system (CNS) has greatly stimulated interest in this aspect of the field of neuropharmacology. One of the earliest of these drugs was diphenhydramine (Benadryl), widely used for its antihistaminic activity but possessing in addition certain so-called "side-effects" involving the central nervous system which are apparently unrelated to its antihistaminic potency (*1*). Among these side-effects of this drug in man are drowsiness, which often follows administration of therapeutic doses (*2*), and convulsions after ingestion of toxic quantities (*3*). Thus the compound seemingly exerts a "depressant" effect at low-dosage levels and a "stimulant" effect after excessive quantities.

Table 1. Effect of diphenhydramine plus pentobarbital on sleeping time of mice (probability < 0.01).

Agent	Avg. sleeping time (min)
Diphenhydramine	0.0
Pentobarbital sodium	35.0
Diphenhydramine plus pentobarbital sodium	52.3

Table 2. Effect of diphenhydramine plus strychnine on mice.

Agent	No. fell (per 25)	No. died (per 25)
Diphenhydramine	0	0
Strychnine	8	4
Diphenhydramine plus strychnine	24	19

Subsequent to the publication of reports concerning these clinical and experimental observations, several investigators described the potentiation of barbiturate sleeping time in mice following prior administration of relatively small quantities of Benadryl (*4*). In studies directed toward the elucidation of a CNS site of action for the compound, it was observed that Benadryl would also cause a reappearance of spontaneous convulsive activity in the cat when it was administered on the decay curve of a strychnine-induced convulsion (*5*).

Recent experiments in this laboratory have shown that pretreatment of mice with a given quantity of Benadryl, which, by itself, effects no apparent alteration in the appearance or activity of the animals, will enhance the gross CNS effects of either pentobarbital or strychnine. The methods employed were as follows.

Groups of ten male white mice, NIH strain, weighing 18 to 22 gm each, were randomly divided into subgroups of five mice each. One subgroup of each group was given 20 mg/kg of Benadryl hydrochloride by subcutaneous injection, while the other functioned as a control group. Twenty-five minutes later the stimulant or depressant drug was administered to all ten animals in each group by intraperitoneal injection.

The mice that received 1.0 mg/kg of strychnine sulfate were then placed on a slanting wire screen, 0.25-in. mesh, and the numbers falling after development of convulsive activity in each group were determined, the end-point being the fall resulting from the loss of ability to cling to the screen. For those animals that received the barbiturate (50 mg/kg pentobarbital sodium), differences in sleeping times were ascertained, the return of the righting reflex being the end-point and the time being taken from the barbiturate injection.

The results are summarized in Tables 1 and 2. The disparity between the two groups that received strychnine in the number of animals dying was undoubtedly influenced to an unknown degree by the effect of the fall itself, so that this difference is probably not as important as the figures would indicate.

These studies demonstrate that diphenhydramine, at a single dosage level that, by itself, exerts no gross effect, can enhance the CNS activity of two compounds long recognized as being pharmacological antagonists. In an attempt to account for this diphenhydramine activity, two modes of action might be considered. The first would involve an inhibition of the biotransformation of the barbiturate and strychnine, similar to the action of β-diethylaminoethyl diphenylpropyl acetate hydrochloride (SKF 525-A) (*6*) to whose structure diphenhydramine bears considerable resemblance. The second possible mode of action would postulate a more direct effect on elements of the CNS, resulting in alterations in levels of neuronal activity that, in turn, could quantitatively affect the response to the second drug.

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Fractionation of Acrasin, a Specific Chemotactic Agent for Slime Mold Aggregation

The aggregation stage during slime mold development is a device whereby the individual myxamebas are brought together in a compact mass preliminary to the formation of the organized fruiting body. Several workers have shown (*1–3*) that the process is chemotactic and that the aggregative center and the cell streams radiating therefrom produce a specific attractive material that was given the generic name *acrasin* by J. T. Bonner (*2*).

Sometime ago, B. M. Shaffer obtained leachings from cells in postaggregative stages that could cause dispersed myxamebas to aggregate in the direction of the point of application (*3*). A small, early aggregate on a thin agar layer was dispersed mechanically. A tiny square plug of agar was cut out and inverted over a glass slide in a moist chamber. By these means, the dispersed cells existed in a film of water between the slide and the agar and adhered to the glass. Very small drops containing leachings from a single pseudoplasmodium were applied at short intervals to the meniscus around the plug and within 5 minutes evoked pronounced elongation and streaming of the test cells toward the point of application. Shaffer found that the attractive material was highly unstable and lost all activity within 15 minutes at room temperature, although, when it was frozen, it remained active for longer periods of time.

We can now report (*4*) the stabilization of acrasin and its fractionation into two components, neither of which attracts cells when alone but only in combination.

Dictyostelium discoideum was grown on glucose peptone agar with *Aerobacter aerogenes* by methods previously described (*5*) and incubated until the cells had aggregated. The entire plate contents were dumped into cold dilute HCl at *p*H 3.5. A clear solution was obtained by decantation and centrifugation. This solution, when neutralized, was shown to possess acrasin activity by the Shaffer procedure. Washings obtained from cells prior to aggregation or a long time thereafter were biologically inactive, as were preparations obtained by leaching at *p*H 7.

The washings at *p*H 3.5 retained activity indefinitely when they were kept in the acid condition but lost activity rapidly upon neutralization. The preparations were concentrated by vacuum distillation at 50°C, the *p*H being held constant at about 3.5 by periodic additions of base. The 200-fold concentrate was put through a loosely packed cellulose powder column with *p*H 3.5 HCl. A fraction of the eluate, distinguished by its fluorescence in the ultraviolet, showed strong biological activity and was stable thereafter at neutrality. The neutralized solution was taken to dryness and provided a fine yellow powder. The yield was 150 mg from 16 growth plates, each containing about 10^9 myxamebas.

Samples containing 6 mg of crude powder were run in ascending bar chromatograms on Whatman No. 1 paper cylinders (15 in. wide) with 80-percent ethanol. Two strongly fluorescent bands appeared with R_f values of 0.3 (fraction A) and 0.1 (fraction B). These were eluted with water and tested separately and in combination by the Shaffer procedure. Table