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Review Article

Techniques Utilized in the Evaluation of Psychotropic Drugs on Animal Activity

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INTRODUCTION

THE EVALUATION of chemical compounds for potential psychotropic activity involves their sequential evaluation in a battery of pharmacologic tests. This screening protocol serves to eliminate toxic or inactive compounds and at the same time allows for the stepwise formation of a profile of pharmacodynamic and toxicological information. The sensitivity of this entire program is heavily dependent upon the initial screening tests involving measurements of animal activity. It is ironic that, of all of the procedures that comprise a screening program, these are the most critical, yet the least standardized, most highly individualized, and most vulnerable to environmental factors. It is therefore, important that careful thought and organization of test criteria precede actual laboratory work. The investigator should determine the optimum set of experimental conditions for his laboratory, since external influences on drug responses will vary from one laboratory to another. It is the purpose of this paper to acquaint the reader with the problems of evaluating animal activity, the advantages and limitations of present-day test apparatus and procedures, the parametric variables which may alter drug response, and the design and statistical techniques which may increase the efficiency of this type of experimentation.

OBSERVATIONAL TECHNIQUES

General Considerations.—The subjective observation of drug-induced changes in the activity of laboratory animals has long been a preliminary procedure in drug testing. This initial procedure can provide information concerning general pharmacological and behavioral drug actions as well as preliminary toxicological data. These total data comprise one facet of the preclinical experimental protocol needed for any attempt on the part of the investigator to predict the clinical activity

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of the drug. Two approaches can be utilized in the evaluation of potential psychotropic agents through the use of gross observational techniques (1). The first of these is the *criterion behavior* approach in which a form of abnormal human behavior is selected and its basic characteristics are determined. The next step is the induction in test animals of a form of behavior which is as nearly as possible similar to this human behavior. The search is then made for new drugs which will modify this behavior at low doses. This might be exemplified by the production of "experimental neuroses" in laboratory animals and the examination of test drugs on this behavior. The second approach is the use of *criterion drugs*. The effects of clinically established drugs are determined in animals using a wide variety of tests, and then the new compounds are studied on the most sensitive of these tests. Most laboratories actually use a composite of these procedures.

The generation of an adequate rating scale for observational procedures requires that the behavior patterns of the experimental animals be carefully examined. As the spontaneous behavior of animals is studied, the events occurring within the animal's behavior can be organized into patterns that are dependent upon the variability of these events (2). Careful examination of these behavior patterns can be made and documented to provide a basis for rating programs (3). Since these observable patterns are under the influence of innumerable internal and external factors, the emphasis on the development of a rating program must be placed upon the use of carefully controlled conditions. There are many variables that must be carefully regulated before the observational test procedure can be used efficiently. The all important variable is the test animal itself. Certainly, all of the attributes of the proper experimental design used in any pharmacologic procedure should be followed in the selection of the test animal. The health of the animals should be a primary concern, and the operations of the animal colony should ensure that a minimal stressing of the animals occurs. If female animals are to be used, the effect of the estrus cycle must be taken into consideration. The development of estrus in the cat periodically alters baseline activity of the animal, and total ovariectomy will eliminate this problem, yet allow for general behavior patterns to remain virtually unchanged (4).

Environment will play a major role in the animal's total behavioral response. As noted by Brady (5), the effects of drugs upon behavior depend upon the environmental conditions affecting the animal when the pharmacologic agent is being evaluated. The initial standardization of the observation area in regard to temperature, humidity, range area of the animal, etc., should not be altered throughout the experiment in order to prevent minute behavioral changes from occurring. Consideration must also be made of the "arousal-provoking" quality of the experimental situation (6). Irwin (7) used a soundproofed or sound attenuated room for the examination of cat behavior, whereas other investigators have used a standard laboratory room that permitted external auditory stimuli to reach the test animals. The question arises here as to whether unexpected external stimuli can be used as an indication of animal awareness. It would appear that they could contribute to a more adequate assessment of the animal's attentiveness.

The observer is an extremely important variable in the test procedure. This person must be a highly trained, observant, and patient individual. He must be fully cognizant of the animal's normal behavior and totally aware of the possible changes in behavior that a drug can produce. He must be able to recognize and record not only those events that are listed in the test program, but also any others that may occur. The training of an individual is based on experience and is very critical; it cannot be achieved by the presentation of written or oral material describing the procedure. Each of the behavioral changes must be shown to the observer through the use of control drugs, and many experimental trials must be performed to ensure adequate reproducibility of the observer's rating.

The mechanics of the rating procedure should be considered in the design of the rating sheet. Most investigators use a single sheet to note any drug effects over the experimental time period. In order to negate subjective bias of the observer, a booklet form can be used for rating (4). Each of the observational time intervals is placed on a separate page so that the observer does not see the previous rating entries. Also, to help ensure that the observer is examining each item on the list, a place can be allowed at each rating point for "no observable change." The design of the sheet or booklet may also be governed by the type of information retrieval program used by the laboratory. If the material is to be placed on punch cards for later printing, the rating sheets should also be designed so that the key punch operator may accurately transfer the information from the rating sheet to the punch card. The choice of items to be placed on any rating scale must be dependent

upon the skill of the supervisor and the observer in the determination of the animal's ongoing behavior patterns in response to the test situation. A rating scale should originally include as many items for rating as possible, based upon the general behavior of the animal and the discrete items that occur within each behavior pattern, as well as upon known changes elicited by the prototype compounds. Following use of the test scale, certain items can be eliminated from the program if the frequency of these events is found to occur at a very low level. As noted by Irwin (8), it is better to have an excess of rating points rather than a deficiency for the test scale.

There are several weaknesses present in multidimensional observation procedures. First, observers vary in their ability; this is only overcome by standardizing the rating scale and quantifying the observed behavior on the basis of an all-or-none rating of events. Second, a tendency toward subjective bias exists as well as a tendency to have an insufficient number of steps in the rating scale. This latter point would possibly cause a loss of information from the behavioral observation. Subjective bias may be overcome by the use of blind procedures for drug administration and observation, and the loss of information can be overcome by adding more rating items. This excessive subdivision does not appear to distort the data (8). Two further problems must be considered: reproducibility of procedures and data between laboratories, and the difficulty of summarizing the information developed from the test procedure (9). The difficulty in transferring the experimental method from one laboratory to another is well known. These procedures are highly individualized and dependent upon the scientist generating the scale. Extremely well-defined profiles are required to overcome this limitation. Their definition must not only be in writing but in some pictorial form as well.

Observational Procedures with Rodents.—Observational test procedures involving rodents vary greatly depending upon the specific need of the particular laboratory. Some investigators (10, 11) use little or no detail in their rodent tests, but these programs are only a minor part of a larger over-all program of drug evaluation. Janssen (12) used a protocol which involved a large number of tests in rodents and dogs; among these were an open field measure, hot and cold plate tests, a toe pinching procedure, a rotarod test, etc. The question which arises from this type of program is whether these multiple tests are necessary for the evaluation of drug effects at the early level of pharmacologic screening. Perhaps early screens should not be overly burdened, but rather allow for fewer, more definitive tests to show potential drug action. A fairly complete rating scale for mice or rats has been clearly defined by Irwin (13). His procedure for the evaluation of the general activity and acute toxicity produced by the test drug is written in a manner that allows for accurate interlaboratory replication. The paper includes a rating chart used in his laboratory, and shows drawings of the animal postures rated for the righting reflex and passivity items on the scale. Norton (2) has developed a rating scale that is more behaviorally oriented than those produced by others. Her profile for the hamster is well defined and includes five main behavior patterns: sociability, contentment, excitement, defensive hostility, and aggressive hostility. Under each of these main categories there are five subheadings which are specified items of behavior. As an example, the items of squawk, pulling, chasing, biting, and rearing are subheadings under the category of aggressive hostility. She compared the results with the hamster to those obtained from monkey and cat studies, and noted that the test animals responded differently to the individual test drugs. For example, chlorpromazine caused an increase in sociability in all animals, but caused an increase in contentment in the monkey and hamster with a decrease in contentment in the cat. It induced an increase in excitement in the cat and monkey, but a decrease in the hamster.

The depressant activity of drugs has been identified rather well for many years, and the utilization of some of these procedures may contribute to the efficiency of rating programs in psychopharmacology. Lim *et al.* (14) used a procedure to evaluate sedation in rats. Animals were housed individually in small cages and pictures taken of their posture and degree of eye closure, with the activity of the animals being measured by a photocell system. The animals were startled periodically with a mild air blast, and the changes in posture and degree of eye closure rated. The illustration of depressed postures of rats and dogs in this paper can aid in the replication of its data. Cohen and Nelson (15) rated depressive activity of rats, noting the effect of chlorpromazine and pentobarbital on the loss of spontaneous motion, response to stimuli, and the degree of ataxia. Each of these were subdivided into four or five rating items. A simple measure of motor activity that has been used to test depressants is the open field test, a procedure that can easily be incorporated into a rating

program. Furthermore, Brimblecombe (16) considered the open field test with the rat to be a valid measure of emotionality. He noted, however, that by itself it may not be accurate, but in a battery of tests it does give some indication of the drug action on the emotional behavior of the animal. In the classical test, the animals are placed at the center of a circular (17) or rectangular (18) open area which has been suitably marked off, and the number of times that an animal moves across the zone lines is recorded. In addition other rating points such as the degree of grooming and defecation can be checked simultaneously (16, 19, 20). This test can utilize individual or paired rats (19), and has been used as a measure of motor deficit, emotionality, and exploration. These behavior patterns can also be analyzed in different ways. Randrup and Munkvad (21) rated the behavior of individual rats by counting the different types of grooming that occurred as well as recording their locomotion within an area through the use of multiexposure photography. They noted that amphetamine produced a stereotyped activity which they could alter through the use of perphenazine. Bindra and Baran (22) evaluated drug-induced changes on sniffing, lying, grooming, and general activity of male hooded rats, which were individually housed in foot square boxes. Many other behavioral signs can be added and scored in this procedure. Cole and Dearnaley (23) utilized a simple rating program in the evaluation of the effects of reserpine and morphine in rats and mice. They illustrated Straub tail reactions, and also noted the effects of the test drugs on such items as the grasp reflex, piloerection, tremor, and posture. Catalepsy and palpebral ptosis have also been used as observational test items (24). Toman and Everett (25) called particular attention to unusual aspects of behavior; for example, hunching and squinting which are characteristic responses to reserpine. Lim (26) included items of morphine-like fixation of posture and pseudohypnosis in his profile for rating drug effects on rats.

One hesitates to talk about emotionality in rodents, but there are certain aspects of behavior that investigators use for its evaluation. Raitt *et al.* (27) and King (28) used a six-item program with each component rated on a 5- or 6-point scale. Rats were evaluated on their reaction to the presentation of a probe to their snout, response to a light rap on the back with a probe, resistance to capturing, resistance to handling, vocalization during stages of capture and handling, and urination and defecation during these stages. This procedure certainly allows for the assessment of drug effects on the stress induced by the observer's interaction with the test animal, but one wonders if this type of emotional rating can be correlated with clinical effects.

Sulser *et al.* (29) have utilized a different test situation in their rating of drug effects on behavior in the rat. The animals were placed on top of a box about 1 ft. high. Control animals would move about and explore their surroundings for a few minutes, then cluster together, and groom themselves, but would not leave the top of the box. Test drugs altered this response; for example, rats given a benzoquinolizine compound (RO-41284) remained motionless and isolated from each other for about 3 hr. Rats treated with amphetamine would dash about helter-skelter, while rats given RO-41284 plus desmethylimipramine would move around the edge of the box and fall off or leave the box. Votava *et al.* (30) examined the effect of experimental drugs on the central nervous system with the aid of a test involving orientation activity in the rat. The animal was placed in a small chamber and after a short interval was allowed to move into a second and larger chamber when a barrier was removed. The animal was then rated on various motor responses, such as the number of times it moved through the door, the amount of grooming, etc. Welker (31) has examined sniffing as an aspect of exploratory behavior in the albino rat. In a rather detailed paper, he noted the various aspects of this behavior and how it was altered by various stimulants and depressants.

Most rodent rating scales utilize individual animals, but the social interaction of these animals should not be overlooked. If social interaction is to be included in a rating scale, some excellent discussions (32, 33) of this behavior in rats and mice can be consulted. Silverman (34) has recently discussed the use of ethology as a means of observing animal behavior with increasing precision. He has developed a well-defined and integrated rating profile for social interaction, which includes the categories of exploration, investigating, mating, aggression, and other signs. Rats were isolated for several days and then introduced into the observation cages in pairs; only one of the test animals was treated with placebo or chlorpromazine, and a counterbalanced order of presentation was introduced into the experimental design. Chance and Silverman (35) evaluated chlorpromazine, amphetamine, and amobarbital on the latter test. It is possible that within groups of rats, different degrees of social interaction will occur. Irwin (36) reported that there was a significant negative

correlation between individual treadwheel counts and the time devoted to interactional behavior of rats; *i.e.*, animals which showed a high degree of spontaneous activity in the treadwheel did not score high in social interaction.

Certain aspects of social behavior lend themselves well to the testing of psychotropic drugs. The aggressive tendency of rodents in certain situations has been used in an attempt to duplicate clinical situations. When mice are isolated for a period of time and then placed together, an aggressive behavior, which can be rated (37–39), is produced between the two mice. An instinctive behavior that is seen in some rats is the mouse-killing reaction. Certain rats will almost immediately kill a mouse when it is introduced into the rat's cage. While many psychotropic drugs are not specific antagonists of this response, it has been noted that antidepressants will block it (40).

If animals are to be re-used in these test procedures, prior experience must be taken into consideration. Marriott and Spencer (41) examined the exploratory behavior of rats under the influence of various psychotropic drugs. Their studies, based on that of Steinberg *et al.* (42), used a Y-shaped box and noted the number of complete entries that the rat made with all four feet into one of the arms of the box. They found that chlorpromazine reduced exploratory behavior, but that meprobamate and chlordiazepoxide increased it. However, the effect of chlordiazepoxide on exploration was completely inhibited by a single previous exposure to the Y box. This fact should be taken into consideration if open field tests or other forms of exploratory behavior measurements are to be used.

The addition of many of the aforementioned behavioral signs or procedures to the standard pharmacological screening test used in observational studies may aid in the identification of potential psychotropic agents. These rating scales in their final form should be as quantitative as possible. It is relatively easy to quantify certain physiological signs within the test structure; these include such items as pupil size, cardiac and respiratory rates, and body temperature. One caution that must be noted is that the desire for exacting quantification may yield a rating scale that, through the time and effort required for its usage, drastically reduces the efficiency of the total rating program.

Observational Procedures with Cats.—A requisite for gross observational studies is that the test animal must possess a fairly stable personality for the prolonged periods of time required for laboratory study; in addition, the animals must show a sufficient range of spontaneous behavioral patterns to allow accurate studies to be made. Cats seem to fit these qualifications (43). Irwin also favors the cat as a test animal for its value in predicting drug effects in man. He noted that its sensitivity to various behavioral drug effects appears to be more like that of man than any other laboratory animal, with the ratio between doses producing behavioral and side effects in the cat being closely approximate to that of man. A disadvantage in the use of the cat is that it shows atypical effects to certain drugs; for example, it is stimulated by certain narcotic analgesics and antihistamines (44); also chlordiazepoxide produces an effect in the cat that lasts for several days. The cat, along with the dog and monkey, allows a wider range of behavior and drug personality interactions to be observed than one would see with the rat or the mouse (45).

The cat observational technique is not truly a preliminary test in that the rodent screen should be initially utilized to eliminate a large number of inactive and toxic compounds from the test series. This is necessary since the procedures involving cats require the use of colonies that become invaluable because of the time involved in establishing behavioral baselines. Drugs that are to be tested on these animals must also have some toxicological data available for the estimation of initial drug dosage. The drugs should be given orally to allow a better clinical correlation of data, although other routes have been considered (8). Drugs can be administered in capsule form to the cats who are restrained either in a box or in the arms of an assistant. The capsules are placed on the back of the tongue using a long curved forceps or hemostat, and the animal's mouth is then held shut. However, the oral route is not without its disadvantages; for example, chlorpromazine often produces vomiting in the cat (46), and thus, the animal will not receive the full dose of the drug.

As with the other species, the environment and method of observation can influence the behavior of the cat. Individual animals can be observed within (43, 47, 48) or when removed from their home cage (49). Multiple animals have been tested either as pairs of unrestrained, free-roaming cats (4) or as four cats restrained by leashes to allow only minimal overlap of animal test areas (6). The rating of individual animals allows for the notation of behavioral and physiological signs influenced by the interaction with the observer or with other aspects of the test environment, but does not allow for the evaluation of social interaction between test animals. This disadvantage

can readily be overcome by using multiple animals in the test sequence. As noted before, Irwin *et al.* (6) chose to rate multiple animals within a sound attenuated room to minimize external stimuli, while others (4) rated the animals in a regular laboratory room which allowed for the animals to react to random sounds from the external environment.

The rating scale should be developed with an intimate knowledge of normal behavior of the cat in the surroundings in which the test is to be conducted. It would appear that the test procedure and environment should generate as much behavior as possible in the test animals. The behavior patterns to be observed should be reliable and consistent within the test sequence. As an example, the authors, during the preliminary development of a cat rating program, presented to the cats a mouse within a plastic chamber. All of the cats did not respond with the typical mouse-kill pattern of behavior, even when the lid was removed from the plastic cage. This type of test then could not be used as a part of the behavioral scheme unless the cats were screened for this specific reaction. This type of selection, involving the elimination of various cats because of the lack of reactivity to certain test sequences, reduces the heterogeneity of the test sample. It would appear to be a better course to randomly select a series of healthy cats and build the test around these animals rather than to find the animals to suit the test.

Various types of rating scales have been developed for cats. Norton and deBeer (43) examined behavior patterns of individual cats and selected four main rating categories (sociability, contentment, excitement, and hostility). Sociability and hostility were categories selected to represent opposite reactions of the animals directed toward the observer, while contentment and excitement were selected to represent opposite patterns reflecting the emotional attitude of the cat in his accustomed surroundings. Each of these main headings had five subheadings of behavioral signs, and the scoring system was based on the frequency of occurrence of these signs. Using this procedure, drugs were administered orally to cats; and it was noted that chlorpromazine, among the compounds tested, reduced sociability to the least degree, but produced the greatest reduction in hostility. Sharma *et al.* (47), using the same type of rating profile, also showed a decrease in sociability with chlorpromazine and reserpine. Irwin (6, 13) used a much more comprehensive scoring system for the evaluation of drug effects. Cats were restrained on leashes so that they had approximately 1 ft. of interanimal overlap to allow some play or aggressive activity. The observer conducted the experiment for 5 hr. following drug administration. During this time the animals were rated on major changes of behavior, changes in interaction with the observer, and changes in interaction between the animals. Items rated included: time to sleep, lying down, alertness, curiosity, reactivity, locomotion, restlessness, stereotypy, grooming, vocalization, effect (playful, placid, fearful, aggressive), staggering gait, pupil size, heart rate, respiratory rate, limb weakness, relaxation of the nictitating membrane, and deep sleep. Chlorpromazine produced a diminution of play, grooming activity, fearfulness, or aggressiveness, especially in low doses. Hostile behavior was almost always suppressed, but some animals did show an increased fear or aggression after drug administration (45). Kinnard *et al.* (4) used a 32-item rating scale which included measures of social interaction, interaction with the observer, and general behavioral or physiological changes of the individual animal. Activity of the pairs of free-roaming cats was stimulated through the use of play objects and periodic presentations of catnip to the animals. Using this method, chlorpromazine or perphenazine, imipramine, pentobarbital, and *d*-amphetamine could be readily differentiated from each other. Data output from this and other multi-item rating programs tends to become voluminous, and a need for data reduction is apparent. Through the use of a computer program, adapted from one used for the clinical rating of patient symptoms, the 32 variables in the latter test were reduced to nine factors of behavior. In the past, investigators have preselected behavioral patterns and then subdivided these patterns through the use of specific animal actions, such as the subdivisions of yowling, hissing, and piloerection for aggression. Through the use of the computer program, the results of a test can be grouped into major factors based upon the intercorrelation of the responses of the animals to the experimental drugs. This type of analysis of the data may lead to a more efficient definition of the drug activity.

Rice and McColl (50) rated cat behavior using a small profile that included autonomic signs, somatomotor effects, and behavioral effects. The behavioral signs rated were howling, habit change, and hostility. Cole and Glees (51) rated the ability of cats to obtain food from a horizontal glass tube, their ability to walk along a ladder to obtain this food, and their performance in a placing reflex test involving the hind legs.

The procedures previously described have used

the normal behavior of laboratory cats as their baseline. However, it is quite conceivable that the effect of the drug on this type of behavior might not correlate with the drug's clinical activity. Investigators have attempted to induce abnormal behavior in cats and then test drugs upon this altered activity. Masserman (52) trained cats to open a box to receive a pellet of food following a light signal; after learning this event, the cat was trained to turn a switch to initiate the program. The animals were then subjected to an air blast or a mild shock which induced a motivational conflict behavior between conditioned hunger and fear. Under these conditions the animals developed startle and phobic reactions to sound and light stimuli as well as other neurotic behavioral patterns (53). Jacobsen and Skaarup (54, 55) have used this technique to study psychotropic drugs. They noted that chlorpromazine, in total doses of 0.1 to 2.0 mg. s.c., did not alter the neurotic reaction in cats, although benactyzine did. This type of response might be compared to the conditioned emotional responses (CER) used in rat behavioral techniques. Mixed results have been obtained from this procedure, and it was reported that chlorpromazine and reserpine did not alter the emotional response of the rat in this test (56). It is possible that this specific type of conflict behavior in the cat may not be a definitive test for all types of psychotropic drugs. Sacra *et al.* (57) produced a conflict behavior in cats by administering shock through the tail of a mouse whenever the cat attacked the mouse. The cat thus received a shock when it went to pick up the mouse. Following several presentations of this response, a conflict behavior pattern developed. Chlorpromazine and meprobamate were found to be effective in protecting against this type of response.

The environment and observer also play a major role in the final drug effects observed in the conflict studies. Masserman (58) noted that sedative and tranquilizing drug effects were greater when the test animals (cats or dogs in conflict behavior) were in the accustomed security of their home cages than when in a state of alert anticipation during transportation to the laboratory; the new environment, on the other hand, enhanced the stimulant effects of the test drugs. He also noted that the drug effects were dependent upon the difference in handling of each animal by different experimenters no matter how constant the research protocol was. It has been pointed out that observer reliability and consistency is extremely important and that this should be rated within the test program (59). The age of the animal used is also important. Pechtel *et al.* (60) showed that kittens, compared to older cats, adapted less well to laboratory routine and learned tasks of lever pressing and audiovisual discrimination. The young animals readily developed neurotic patterns under the stress of adaptive conflict.

Observational procedures have been used in experiments that involved different routes of drug administration or types of pretreatment. Feldberg and Sherwood (61) have analyzed the behavior of cats following the injection of test drugs into the lateral ventricle of an unanesthetized animal. Behavioral changes were not rated, but were noted in a general manner. Haley and Dasgupta (62) observed the changes caused by an intracerebral injection of LSD in conscious dogs and cats, but again no rating scale was used. Elder and Dille (63) administered LSD to singly caged cats and rated autonomic responses, spontaneous behavior, and response to types of stimuli (auditory, visual, and tactile). Various pharmacologic agents were then used in an attempt to antidote the LSD response. Sturvesant and Drill (64) analyzed the effect of mescaline on the behavior of cats. Rowe *et al.* (65) noted the behavior of reserpinized cats following monoamine oxidase inhibitors, and included photographs of the cat's behavior. Burdock *et al.* (66) analyzed the behavior of laboratory animals before and after the production of hypothalamic and midbrain lesions in the animals. Behavior of the animals can also be observed and rated in conjunction with neurophysiological recordings of brain activity (67).

Observational Procedures with Other Species.—The dog has been used by many investigators as a test animal for observational techniques, but apparently it is secondary to the cat in these procedures. Lang and Gershon (68) have used the dog in a procedure involving the intravenous administration of yohimbine to the animal. The induced behavior before and after the use of potential antagonists was then rated. The rating program presented was extremely well defined with 17 main divisions of behavior, each with a specified 3-point scale.

The monkey would appear to be the ideal test animal to use in observational techniques, especially if one considers its place on the phylogenetic scale. However, the use of the monkey, possibly because of costs and other reasons, is not so widespread as it should be in this type of pharmacologic program. Many investigators (69–75) have tested potential psychotropic drugs on monkey behavior. The viciousness of monkeys, such as the rhesus and the cynomolgus,

can be used as a tool for the evaluation of potential psychotropic agents. The animals, as in the rodent or cat studies, can be tested singly or in a procedure which involves chained pairs of monkeys to allow interaction (74). Knapp *et al.* (70) tested chlorpromazine and piperacetazine in dogs and squirrel monkeys, and noted that the drug effects may have been more discernible in the dog than in the monkey. In spite of this, the monkey should still be considered a prime test animal for observational studies since its behavioral patterns, in comparison to other laboratory animals, more closely resemble those of the human.

INSTRUMENTAL TECHNIQUES

Spontaneous Locomotor Activity.—Methods for measuring small animal activity have been used since the turn of the century. The devices were, for the most part, mechanically operated and used by psychologists to study the normal behavioral patterns of laboratory animals (76, 77). However, the search for psychoactive agents gave impetus to the need for instruments which would accurately assess the ability of these compounds to alter the normal spontaneous locomotor activity of small animals.

Activity recording units can be classified into four main groups involving four different types of activity cages: those which are immobile and record activity independently of cage movement (photocell activity cage); those which rotate about a central axis as the animal runs (treadwheel); those cages which are vertically or horizontally displaced as a result of animal movement (jiggle cage); and those which tilt on a fulcrum (tilt cage). The first type (fixed) is considered a direct recording apparatus because animal movement itself is recorded, whereas the latter three instruments are the indirect type because they basically record cage movement rather than animal movement. Since Riley and Spinks (78) reviewed the early prototypes of these instruments in 1958, this phase of the paper will emphasize the types of activity recording units developed since then.

An ideal device for measuring spontaneous activity would be one that is (*a*) sensitive to all types of motor movements, (*b*) sensitive to minor changes in animal activity, (*c*) free of positive feedback of stimuli, (*d*) free of carry-over momentum, (*e*) independent of animal weight variation, (*f*) capable of delineating between the activity of animals receiving placebo and animals treated with small doses of psychotropic compounds, (*g*) capable of recording a stable baseline of activity over short trials, (*h*) capable of simple operation and adjustment, (*i*) capable of being used with a simple direct-reading digital recorder. Needless to say, an instrument possessing all of these characteristics has yet to be developed.

The photocell activity cage has been used extensively as a drug screening device to determine the effects of psychotropic compounds on the spontaneous activity of small animals. It operates on the photocell system, in which light beam interruptions due to animals in motion are converted into electrical impulses which are transmitted to digital counters. Activity of the animal is thus reflected as a summation of light beam interruptions due to lateral movements. Many modifications of the photocell activity cage have been described in the literature and reflect the attempts of investigators to maximize its sensitivity. Dews (79) utilized a rectangular single beam unit to study psychomotor stimulants; Winter and Flataker (10) also used a single beam but reflected it twice off the sides of the cage; Kinnard and Carr (80) used a circular single beam unit to determine the activity patterns of central nervous system depressants, *e.g.*, secobarbital and meprobamate. A circular two-beam unit was utilized to study the ataractic properties of chlorpromazine and sodium pentobarbital (81). Most investigators employ horizontal beams; however, an apparatus with a single vertical light source has been used satisfactorily (82). The beam is directed from the ceiling of the unit downward onto several photocells placed below a Lucite floor. The use of a single light source precludes the possibility of variations in light intensity among the beams as in units with multiple light sources. The disadvantage is that fecal boluses may block off the operation of the vertically directed beams. Woodard (83) designed an apparatus with six photoelectric cells equally spaced around the perimeter of a circular raceway. A single light source was housed in a circular compartment in the center of the cage. This design takes advantage of the natural instinct of the rodent to confine its activity to the periphery of any confined environment; however, it places limitations on the spontaneous activity which satisfies the animal's strong instinct for exploratory behavior.

Tedeschi *et al.* (84) used another approach to the evaluation of psychoactive compounds on the motor activity of rats. They devised a photocell unit which was only large enough for a rat to rear up but precluded him from moving laterally. Rats placed in such a test situation at first elicited exaggerated behavior which progressed to complete inactivity toward the end of their

confinement. The authors suggested that drugs which reduce motor activity can be tested during the initial period of increased activity and, conversely, drugs which increase motor activity may be tested during the latter part of confinement when activity is markedly reduced. The effects of established psychoactive agents were measured in both the confined motor activity (CMA) test and the conventional photocell activity cage. Caffeine, in an oral dose of 5 mg./Kg., produced a 200% increase in activity in the CMA test; whereas, 5 to 350 mg./Kg., orally, did not increase the activity level in the conventional photocell apparatus. Similarly, tranylcypromine (5 mg./Kg. orally), a clinical antidepressant, produced a 200% increase in activity in the confined motor activity test; whereas, the chronic administration of tranylcypromine (5 mg./Kg., orally, twice a day) failed to produce any consistent or significant effects on motor activity in the conventional apparatus (85). In the CMA test, the effect of *d*-amphetamine was greatly magnified in that 0.24 mg./Kg., orally, increased activity 200%; whereas, in the conventional motor activity test, an oral dose of 5 mg./Kg. produced only a 40% increase in activity. A significant limitation of the conventional photocell activity cage was noted (86) when an oral dose of 16 mg./Kg. of *d*-amphetamine was administered to mice. This dose produced a decrease in lateral movement and induced tremors of the head and limbs analogous to the well-known clinical side effects of restlessness, irritability, and emotional disturbance. This type of activity resulted in a decrease in beam interruptions in the conventional unit, but under the CMA tests, may have been accurately registered as an increase in activity. Thus, by monitoring only one component (rearing) of spontaneous activity, Tedeschi has been able to correlate the preclinical and clinical effects of well-established stimulants and antidepressants. The CMA test was also effective for quantifying the effects of drug which decreased spontaneous activity, but the sensitivity was not significantly greater than that of the conventional photocell unit. The data suggest that it may not be necessary to measure total activity but only selected components of activity known to be affected by certain classifications of compounds. The problem is that psychotropic agents have a diffuse action on the nervous system and psychopharmacological techniques in existence today cannot, with certain exceptions, delineate the component effects of drug action.

Another novel approach to the recording of animal activity was the use of electroconductive slats placed across the floor of the test cage (87). Each slat was made of varnished Masonite and painted with silver-based conductive paint. As each animal made contact with the slats, an electronic relay activated an externally powered counter. This appears to be a satisfactory method of recording locomotor activity of small animals. It is less bulky than the photobeam units and can readily be installed to activate timers and measure areas of the test environment explored. Mitchell (88) devised another type of unit in which the lateral motion of an animal on a galvanized iron plate caused it to move slightly, inducing an electric current in a coil with a bar magnet core. The currents thus produced were then amplified electronically and recorded as activity.

More sophisticated methods of monitoring small animal activity have been developed, such as the transmission of vibrations produced by animal motion, using a piezo-electric head (89), a 30-gauge galvanized steel diaphragm (90), or a crystal phonograph cartridge (91). In most of these procedures, activity is recorded by means of a stylus recorder, resulting in records which are difficult to quantify. Alvarez-O'Bourke (91) solved the problem of quantifying such graphs by eluting each record with acetone and quantitatively evaluating it with a spectrophotometer on the principle that the amount of ink laid down by the pen is proportional to the frequency and intensity of the movements of the animal. Otis (92) designed a chamber which operated on a floor displacement principle. Normal floor vibrations which occurred during locomotion were transduced into modulated electrical signals that operated from 1 to 3 electromagnetic counters depending on the intensity of floor movements. Only downward motion was recorded, and the counter circuits were preset so that one counter will fire at the slightest movement of the animal, a second counter only if the animal's activity is moderate, and a third, only if violent activity occurs. The value of this apparatus is that it detects both the occurrence and intensity of movement, which the photocell cage is not capable of. Otis attached this device to a treadwheel so that the experimental animals would have access to either unit. Therefore, he was able to compare the effects of psychoactive compounds on activity with both devices separately and combined. The data of his studies indicated that the treadwheel failed to discriminate between intraperitoneal injections of placebo and 2.5 mg./Kg. of amphetamine, 30 mg./Kg. of imipramine, and moderate doses of other psychostimulating agents. In contrast, the floor displacement apparatus did delineate between

placebo treatment and equivalent doses of the same stimulating drugs. The over-all conclusion of this study was that the combined chamber–treadwheel combination was the most sensitive method for detecting drug effects. Knoll (93) developed an electronic device in which an animal moved over four aluminum plates, and counters registered every crossing from one plate to another. The advantage of this method is that as many as 24 mice can be tested at one time. Shillito (94) measured mouse locomotor movements by a capacitor system including six brass probes. When an animal moved past a probe, its capacitance was altered, resulting in an imbalance in the electrical circuit, which caused a dot to be recorded on a moving kymograph. The data were analyzed by merely counting the dots, and as many as four mice were tested simultaneously without causing an overlapping of marks. This device is similar to the "antenna cage" (95) in which a rat moves around a radio antenna placed in the middle of the cage; his movements change the capacity between the cage and the antenna. The latest electronic activity device[1] generates a soundwave of a frequency and level that cannot be detected by most animals. Any motion within an experimental cage into which this sound wave is directed produces disturbances in the received portion of the wave, causing the receiver to produce electrical impulses which can activate many types of recording and counting devices.

Melander (96, 97) used a photographic technique similar to Rothlin and Cerletti (98). The experimental mice were painted with a dye which emits visible light when activated by ultraviolet light. The animals were then exposed for 5 min. to panchromatic film. The resulting films afforded the investigator information on the types of movement, but they were not quantifiable.

Several modifications of the tilt-type activity cage have been described in the literature. Basically, the cage pivots on a central fulcrum, tilting in response to the weight of the animal as he moves from one part of the cage to the other. Sensing contacts (microswitches), below the platform, register the gross movement of the animal on digital counters. The apparatus is sensitive only to movement in line with the sensing contact on the platform below. Minor movements such as rearing, grooming, etc., which are not large enough to tilt the cage are not monitored. Tilt-type units have been described by Campbell (99) and Kissel (100), and the latter reported a lack of habituation to the unit probably due to the positive feedback typical of all moving-type devices. The limitation of these units is that only running activity is recorded to the exclusion of important minor movements (tremors, etc.) which may be drug induced. This limitation was overcome by Caviezel *et al.* (101), who added a closed air-Marey tambour system which recorded activity by means of a work adder. Thus, while the microswitches below the tilt platform recorded running movements, the downward displacement of the ball-bearing fulcrum set in motion the tambour membrane, and this recorded minor activity. Bastian (102) and Bourgault *et al.* (103) utilized a rectangular cage which tilted on a wide metal fulcrum, perpendicular to its axis; thus, an animal must run along the long axis of the cage in order to register a count; movement perpendicular to the long axis of the cage was not recorded. Also, minor movements were not monitored, and various types of activity were not differentiated as is true of other instruments of this class.

The jiggle cage has been used extensively to evaluate the effects of drugs on small animal activity. Earlier designs were usually of the spring-suspended type in which animal movement vertically displaced the freely swinging cage (104). Several interesting modifications of the jiggle cage have been described. Cho (105) used a 500-Gm. Toledo scale for the rat and a 500-Gm. dietetic scale for the mouse; movement of the animal vertically displaced the platform of the scale causing upward and downward deflections of a pen writing on a moving kymograph. Schallek (106) and Sandberg (107) used a unit (Williamson Development Co., West Concord, Mass.) in which the cage was suspended on a resilient cantilever beam which permits slight sideways motion of the cage in response to the animal's activity. The sideways motion is proportional to the acceleration imparted by the animal. Such motion caused a switch to close momentarily whenever the integrated accelerations reached a predetermined level. A similar apparatus was described by Chappel (108); the cage was suspended by a ball and socket joint from a spring steel or spring bronze cantilever beam. Movements of the animals were recorded by electrical counters activated by contact of the cantilever with a stationary screw. The sensitivity of the apparatus can be adjusted for body weight differences by turning the screw regulating the gap between the stationary contact and the cantilever beam. The advantage of this unit is that small movements such as

[1] Ultra-Sonic Motion Detector, Alton Electronics Co., Gainesville, Fla.

grooming, biting, gross respiration, head bobbing and swaying can be recorded, although the movement of the cage, itself, is relatively slight. A jiggle platform (Lehigh Valley Electronics, Fogelsville, Pa.) has been developed which oscillates horizontally on a set of ball bearings. Sudden movements, *e.g.*, turning, running, jumping, etc., cause the platform to move slightly, making and breaking an electrical contact. A wide range of sensitivity is accomplished by the raising or lowering of a conical pendulum into or out of a metal ring. However, this apparatus is only suitable for measuring the activity of rats or hamsters and cannot monitor mouse activity. In general, the suspended jiggle cage has several limitations. First, most of the units lack a dampening effect so that carry-over momentum generally exaggerates the amount of activity; second, it is a moving or mobile unit which may generate a small amount of positive feedback stimuli; third, the animals satiate rapidly so that accurate estimation of peak drug effect is required or else the effect will be missed; finally, the units are difficult to calibrate; attempts to maximize the sensitivity of such cages in order to pick up minor movements result in excessive residual excursions after animal activity has stopped; on the other hand, decreasing the sensitivity precludes the monitoring of minor movements. An advantage is that various types of behavioral patterns can be monitored on a pen recorder; however, such records are difficult to quantitate and are only useful for *visual* comparison of the changes in behavior elicited by various drugs.

The revolving treadwheel turns as the animals run or walk, and revolutions in either direction are recorded on counters. For a review of the early units (prior to 1958) the reader is referred to Riley and Spinks (78). Skinner (109) also reviewed the parameters of the exercise wheel in 1933. Because of the large positive feedback generated by animal movement, habituation to this unit does not occur so that it is possibly the only unit in which cross-over studies can be carried out (110). This apparatus is capable of detecting the stimulating effects of monoamine oxidase inhibitors (110) which cannot be monitored with immobile units. Irwin (110) indicated that the treadwheel can also detect doses of drugs which disorganize behavior. Because of the mobility of the apparatus, animals generally sustain their behavior over a long time interval and, therefore, it appears that the unit is of more value in the study of depressants where it is desirable to obtain stable, sustained levels of activity as a baseline. In order to study stimulants in this type of unit, Wiedemeijer *et al.* (111) limited the mice to a section of the turning wheel by fixing a partition within it causing the animals to produce a smaller baseline of spontaneous activity. Since the apparatus only measures running activity in revolutions, Royer *et al.* (112) increased the information output by attaching a tachometer–generator and recorded the output on an Esterline–Angus pen recorder.

Investigators have used both graphic and numerical recording techniques to illustrate drug-induced changes in spontaneous activity. Graphic recording illustrates types of activity (rearing, jumping, grooming, etc.), and, therefore, can indicate specific qualitative alterations on behavior; digital counters, on the other hand, reflect only quantitative changes. The tendency today is to record only quantitative changes, because it is relatively simpler and less expensive to attach some type of digital counter to the activity unit. However, this must be done with guarded caution, for each method of recording gives important information about drug effects. For instance, Tonini (113) has indicated that sedative, subhypnotic doses of barbiturates did not decrease numerical values in his actograph unit, but did alter the activity graphs (short, intense, and more frequent bursts of activity with long periods of rest between); whereas, tranquilizers, in therapeutic doses, decreased numerical values but did not alter the graphic record. Thus, both procedures should be used in the preliminary evaluation of psychoactive compounds.

Forced Motor Activity.—The neurotoxic effects of psychoactive compounds have been evaluated by "fall-time" tests which measure motor coordination of experimental animals. There are two types of "fall-time" methods: those using inclined planes, and those using rotating rods and cylinders. A discussion of the fixed incline plane procedure can be found in the paper by Riley and Spinks (78). The data are usually expressed as the average time that a group of animals can stay on or as the percentage of animals falling off within an arbitrary period of time. The first of the horizontal rod-type of instrument was the hollow screen cylinder devised by Young and Lewis (114) for testing insulin. Mice which could not hold on to the rotating cylinder fell into metal trays. It was later used in the assay of curare (115) and the measurement of sedation (116). The horizontal rod method appears to be the more popular device today because of the simplicity of construction and objectivity of measurement. It was used by Dunham and Miya (117) for detecting neurological deficits of psychotropic agents in rats and

mice; Herr (118) compared the effects of antidepressants and tranquilizers on the rotarod performance of mice; and Plotnikoff *et al.* (119), studying the effect of stimulants, was able to distinguish between amphetamine which enhanced rotarod performance and caffeine which was inactive; Kinnard and Carr (80) studied several types of depressants and suggested the combined use of the rotarod and the photocell activity cage to characterize and differentiate between various types of depressants. The objectivity of the apparatus was increased recently by an electronic circuit which automatically stops a timer when the animal falls to the compartmentalized platform beneath the rod (120).

Otis (121) assessed motor coordination by forcing animals to perform on a drum 18 in. in diameter. When an animal could not maintain his performance, he slipped to the back wall plate of his compartment, tripping a microswitch which transmitted an impulse to a digital counter. Both the number of times and the total time the wall plate was depressed were recorded.

An important factor which has been neglected in forced motor studies is the "free ride" animals will take periodically throughout the trial. A "free ride" is defined as one revolution in which the animal holds on without walking. This could result in the failure to detect minor depressive actions of drugs and neurotoxicity, because a drug might decrease motor coordination without impairing the ability of the animals to wrap themselves around the rod and ride. In one study (86) the average per cent rides for the training trials ranged from 0.2–11.1%, and the range among the placebo-treated animals was 0.0–10.5%. While 0.2% riding may be insignificant, especially at the higher speeds and longer time trials, the higher value of 11.1% may mask drug effects. The results may be affected to a greater degree when large doses of a depressant drug are administered, because the animals may have enough strength to hold on, even though the drug has affected their coordination. Per cent rides as high as 57.6% were recorded with 16 mg./Kg. of chlorpromazine, administered orally to mice. It is suggested that investigators observe animal performance carefully and take into consideration the possibility that erroneous conclusions concerning drug effects may be drawn from such data.

Parametric Influences on Drug Response.—Although the literature is filled with many papers dealing with the effect of psychotropic drugs on motor activity, very few investigators have been concerned with the various parameters which might significantly influence drug response as measured in the various devices described above. Such parametric effects may differ not only among the various classifications of compounds but from drug to drug within a single classification. Thus, by experimental manipulation of environmental factors, a drug may be made to have a stimulant effect, a depressant effect, or no effect on motor activity (122). It is, therefore, of extreme importance that investigators take into consideration the parameters which have been shown to influence drug response and utilize the combination of variables which will produce the optimum results. For instance, test and housing aggregation size, sex, illumination of the test environment, availability of food prior to the test, and route of administration can be powerful determinants of the magnitude of drug effect. Wright *et al.* (83) reported that promazine produced an immediate equal decrease in the activity of individual mice and groups of mice; whereas, pentobarbital in low doses produced a greater increase in the activity of animals tested singly than those tested in groups. Brown (123) found no differential effect of chlorpromazine on the activity of single and grouped mice, but Watzman *et al.* (124) reported that chlorpromazine had a greater depressant effect on activity of mice tested in aggregations of five than on those tested singly. This drug–aggregation interaction may indicate that chlorpromazine potentiates the tendency for grouped animals to clump together and thus register relatively low activity. When dose-response curves for test aggregations of one, five, and nine animals per test unit were compared (125), the slopes indicated that chlorpromazine produced a greater depressant effect on the larger test aggregations than on animals tested singly; furthermore, the slopes for both multiple animal groups were approximately the same so that there appeared to be no advantage in using as many as nine (126) animals per test unit. It has also been reported (125) that chlorpromazine produced a significantly greater effect on animals housed and tested under the same aggregation conditions than those housed and tested under different conditions. Since amphetamine has been shown to stimulate aggregated animals more intensely than isolated animals (127), the group situation gives evidence of accentuating both depressant and stimulating drug effects.

An experimental condition producing a powerful effect on drug response is the illumination of the test environment (125). It is well documented that the spontaneous activities of rodents (128) and rabbits (129, 130) are affected by illumination conditions. Chlorpromazine had a greater effect

on spontaneous motor activity of mice tested in the dark than in the light during the latter half, but not the first half, of a 2-hr. test (125). Under the placebo condition the decrement in activity of mice in the last hour of the trial was much steeper and decreased to a much lower level in the animals tested in the illuminated condition. Testing in the dark produced no increase in activity in the initial exploratory phase but did greatly decrease adaption during the latter part of the session. Apparently, the high curiosity and exploratory behavior in the initial part of the test was powerful enough to overcome the effects of illumination.

It has also been reported that the activity of females was consistently higher than males in the photocell activity cage, and that chlorpromazine had a significantly greater depressant effect on the spontaneous activity of females than males, during the first 0.5 hr. of the trial (125). It was thus concluded that conditions which elevate normal activity (aggregation, darkness, female sex, same housing, and test groupings) enhance the depressant effect of chlorpromazine on spontaneous activity. On the other hand, when the normal activity level is low, such as at the end of a test session in an illuminated environment, the effect of chlorpromazine is slight. The influence of these factors appears to be class-specific. For instance, while the activity response to perphenazine and chlorpromazine (phenothiazines) is influenced by the same environmental factors and in the same direction, the response to pentobarbital is not (83, 126). The response to pentobarbital was not differentially affected by test illumination, as was the response to phenothiazines; however, it did produce a consistently greater effect on fasted than satiated animals, while responses to the phenothiazine compounds were not differentially affected by the feeding condition of the animals. The authors recommended (125) that the most sensitive measure of chlorpromazine effect in the photocell activity cage could be obtained by testing an aggregation of five female mice for a period of 0.5 hr. beginning 30 min. after i.p. administration of the drug (86).

Another parameter, often neglected, which may alter response to a drug is the difference in sensitivity among the measuring units. If more than one instrument is being used, then the investigator must demonstrate equality of sensitivity (131). Failure to do so may lead to inaccurate interpretations of drug effects. Since there is a difference in sensitivity among photocell units, even from the same manufacturer (125), it is imperative that the various levels of all experimental variables be exposed to each unit in a factorial design or else all animals under all conditions should be tested in a single unit.

The location of the photocell beams is also an important determinant of drug effect on motor activity. The use of two right-angle (crisscross) beams yielded a better delineation of low doses of chlorpromazine than three parallel beams (132). However, there was a greater drug effect with the two peripheral beams than middle beam in the parallel arrangement, with no significant drug–beam interaction in the crisscross arrangement. The data suggested that the most sensitive measure of chlorpromazine effect would be with an arrangement of two pairs of peripheral beams at right angles to each other.

The importance of evaluating drugs at certain test intervals is borne out by Dews (79) in measuring the influence of certain drugs on the locomotor activity of mice with the photocell activity cage. He found that the initial part of the trial (first 15 min.) produced more reliable data than any other test interval. This is the period of exploratory hypermotility which occurs when animals are first placed into a new environment. The high reproducibility of the data during this period is probably due to the fact that the animals search to the same degree to satisfy their curiosity and, therefore, the variability of movement among the animals is small. Borsy *et al.* (133) studied several classical tranquilizers by their ability to inhibit this orientational hypermotility. This appears to be a sound approach to the evaluation of depressants because the test compounds were made to challenge a natural, unlearned reaction similar to the clinical situation for which these compounds are used. Bonta (134) reinforced this exploratory behavior by moving mice from a large rectangular cage in a dark room to a small round test cage in an illuminated room and measured spontaneous activity for the first hour only. Reserpine, azacyclonol, benactyzine, and chlorpromazine abolished this hypermotility, but meprobamate was successful only in ataxic doses. These tests are in contrast to the studies in which drug-induced hyperactivity is used as the baseline of performance (135, 136).

Otis (92) illustrated the importance of evaluating drug effects over long, as well as short, periods. He reported that deanol, phenelzine, and imipramine produced quantitative differences in spontaneous activity relative to placebo controls, depending on whether they were tested for 3 or 16 hr. The data suggested that different drugs may require different testing periods in order to achieve significance.

Irwin (137) has reported on the influence of

internal and external factors on spontaneous activity in the treadwheel; female rats were more responsive to drug effects than male animals; hyperactive animals were found to be significantly more responsive to both stimulant and depressant drugs than hypoactive animals. In the female rat, peak running activity occurred every fourth day in correlation with the estrus cycle (138). Older rats performed more intensely but with shorter spurts of activity and maximum treadwheel activity was observed in animals between 87 and 120 days (139), 175 days (140), and 300 days old (141). Jones *et al.* (142) reported that running activity in the wheel varies inversely with age and directly with experience although these relationships are not linear. Desroches (143) also found that activity in the treadwheel declined with age but reported that prior experience in the unit did not influence this decrease. The discrepancy in the literature is probably due to differences in environmental conditions, such as temperature, illumination, and sound levels of the experimental room. Ström has described extensive studies on the suitability of the treadwheel for the evaluation of substances having potential central depressant action (144).

The literature is sparse with regard to the influence of experimental conditions on rotarod performance. However, there is some evidence that manipulation of speed and rod diameter alters the magnitude of drug response. Plotnikoff *et al.* (119) studied the effect of amphetamine and other stimulants at three different speeds (11.44, 18.30, and 29.28 r.p.m.) and reported that the effect of amphetamine was smallest at the lowest speed. Watzman *et al.* (86) found that chlorpromazine produced a greater depressant effect on mice when they performed on a 2-in. diameter rod rather than on a 1 or 1.5-in. rod.

It is apparent from the foregoing discussion that environmental and experimental factors must be carefully controlled; for, as mentioned before, the effects of drug on behavior are largely a function of the situation in which they are studied.

Repeated Tests of the Same Animals.—The advisability of re-using animals in experimental situations has been controversial and was briefly discussed earlier. It is highly desirable, if possible, to give repeated tests to the same animals when evaluating the effects of psychotropic agents on behavior. Not only is it an economic advantage, but a more sensitive test is obtained because the consistency of individual performances usually found in repeated tests means a smaller variation in scores against which the effects of drug can be measured. On the other hand, the disadvantage of repeated tests on the same animals is that both drug and behavioral carry-over effects may occur from one test to the next, especially when there is a short time interval between sessions. Adler (145), utilizing the photocell activity cage, found a greater depressant effect of tetrabenazine on the motor activity of rats in the second of two tests with a 1-week intertrial period. Rushton *et al.* (146) have shown that even a single brief experience in a Y-shaped maze, lasting only 3 min., markedly modified subsequent reactions to an amphetamine–amobarbital mixture. Ross and Schnitzer (147) reported an elevation of activity level of mice 2 weeks after the animals had been tested for locomotion while under the influence of a single dose of *d*-amphetamine sulfate. The same authors later showed in a separate experiment, that the drug was not directly involved in the later elevation of activity and theorized that the animals learned to be more active due to their prior treatment of the stimulating drug. Watzman *et al.* (124) tested mice twice in the photocell activity cage, 1, 3, 7, and 14 days apart. The scores in the second test were generally lower than those of the first test, and this behavioral carry-over effect was greater for groups given a second test at intervals of 1 or 3, rather than 7 or 14 days after the first test. Even at the 7 and 14-day intervals the recovery of the original activity level was not complete. A more important finding in this experiment was that chlorpromazine produced a greater depressant effect on activity in the first 0.5 hr. of the second session than of the first session. This effect was clearly not due to accumulation of the drug from the first session because the greater drug effect was found in the second session after the 14-day interval as well as after the 1-day interval. The authors concluded that investigators must be careful when using the same animals more than once in tests of drug effects. They recommended that the sequence be counterbalanced giving placebo first to half of the animals, and the drug first to the other half. The high over-all correlation in activity between the first and second sessions showed that the use of repeated tests on the same animals can increase the sensitivity of the test of drug even though there is a difference in performance and a different magnitude of drug effect between the two sessions. Thus, the effects of the drug can be tested adequately with a greatly reduced number of animals by the use of a repeated test design.

Statistical Treatment of Activity Data.—Parametric tests of statistical significance, such as the analysis of variance and *t* tests, assume

that the frequency distribution of scores is nonskewed and that the variance among the experimental groups is approximately equal. Usually, because of the extreme variations in activity performance among small animals, it is necessary to transform the data and thus normalize the frequency distribution. If this is not done, the non-normality is likely to be accompanied by a loss of power in the F and t tests and a corresponding loss of efficiency in estimation of treatment effects (148). Furthermore, extremely high raw scores are likely to lead to a misinterpretation of relative magnitudes if they are farther from the mean than the lowest scores. Logarithmic or square root transformation of data may normalize the distribution of scores by reducing the magnitude of the high scores more than the low ones. In a recent study of the photocell activity cage (124), a comparison of the raw, square root, and logarithmic forms of experimental data indicated that the raw scores were skewed in a positive direction with extreme high scores being much farther from the mean than were the extreme low scores. The logarithmic scores were skewed in the opposite, negative direction, whereas the square root scores were skewed to the least degree (positive direction). Also, spontaneous activity appeared to be most stable when the scores were in the square root form. Irwin (110) has used square root transformation data on locomotor activity in the treadwheel, and Kissel (100) found it necessary to transform tilt cage data into logarithmic form. The skewness of a frequency distribution can be roughly estimated by comparing the relative distance of the highest score and the lowest score from the mean or median value. A more refined procedure (149) requires the estimation of the mean (M), median (Mdn) or mode, and standard deviation (S.D.). Either of three equivalent equations may be used as follows:

$$\frac{\text{M} - \text{mode}}{\text{S.D.}} = \frac{\text{M} - [\text{M} - 3(\text{M} - \text{Mdn})]}{\text{S.D.}} = \frac{3(\text{M} - \text{Mdn})}{\text{S.D.}}$$

The value of a normal frequency distribution is 0, and therefore data which are distributed in a manner approaching normality would have a value close to 0.

Because reliability and reproducibility of data are of extreme importance to scientific investigators, it is desirable to use the most sensitive measure of reliability. One statistical procedure which is extremely useful is the split-half method (150), in which stability of performance for each animal within the same session can be readily computed. For example, if the spontaneous motor activity of an animal is measured over a 2-hr. period in 15-min. segments, the performance of the even time intervals (2nd, 4th, 6th, and 8th) is correlated with the scores recorded in the odd time segments (1st, 3rd, 5th, and 7th). The resulting correlation coefficients can then be tested for statistical significance of differences by the method described by Edwards (151).

Rotarod performance of rodents treated with central nervous system depressants has been recorded as the per cent decrease in performance times (81) or the per cent of animals (118, 152, 153), falling off at a predetermined time (all-or-none method). A more consistent dose response effect was obtained for chlorpromazine when the "performance time" method of recording was used (87). The all-or-none method, in principle, does not appear to be satisfactory. If an animal scores 179 sec. under a low dose and 20 sec. under a higher dose in a 3-min. trial, a satisfactory dose response curve would be achieved under the time-response procedure; whereas, under the all-or-none method, 100% depression would be reported for both doses. There is even some question as to the suitability of the "performance time" method of recording the data. Because all trials are terminated at some point of time, the data are usually skewed with the majority of trained animals scoring at the "ceiling" or maximum time limit of the experiments with some animals scattered throughout the middle and lower part of the frequency distribution. Therefore, parametric tests of significance, such as the analysis of variance and t tests, cannot be properly applied for the reasons given above.

Since the drug effect may vary with environmental factors, it is desirable in activity experiments to study the effect of a drug over a range of conditions. This can be done efficiently by the use of the factorial design which permits broader generalizations to be drawn than a group of individual studies which are limited to a single set of conditions (154). The statistical treatment of activity data of this type can be efficiently analyzed by the analysis of variance test. It is a flexible statistical procedure which is capable of treating several levels of experimental variables simultaneously. By providing estimates of interactions between doses and the other variables of the experiment, it gives important information on parameters which govern or influence the appearance of a particular drug effect.

SUMMARY

It would appear that a total pharmacological evaluation of psychotropic drugs must include the use of at least two different animal species in an

observational program. If time and funds are available, a third species can be added. The rodent (mouse or rat) and the cat would seem to be the favored animals for this procedure with the monkey being the third species. The rodent screen should provide not only behavioral or pharmacodynamic data, but also toxicological information for the protection of animals used in subsequent studies. The rodent observational scale should include the signs characteristic of autonomic and central nervous system changes, and certain behavioral measurements obtained through the use of open field tests and the rating of signs, such as grooming, etc. More important, an additional sequence should involve the measurement of social interaction of rodents. This latter point is too often omitted from the typical observational scale. The cat can be used as a test animal once sufficient toxicological data has been obtained. It provides the stable baseline of behavior that may give a better correlation of drug effects than the data obtained from the rodent studies. The cat procedure should allow the generation of a high degree of behavior, and the rating scale should include a sufficient number of rating points so that information is not overlooked. The use of paired, free-roaming cats may be advantageous over other techniques in that their activity may be increased over caged or restrained animals. Data reduction is extremely important in these tests, and the use of certain computer programs may serve to fulfill this need. The clinical predictiveness of observational data is undoubtedly more reliable when the number of species involved in the tests is increased. The addition of the monkey to the rating procedure increases this reliability and provides some natural behavior patterns (aggression) that are not seen routinely in other test animals. Even with the additional species, preclinical predictiveness is at best difficult. To overcome this and other inadequacies, better models of behavior must be developed for the test animals. It would appear that the "experimental neuroses" developed in test animals should be re-evaluated and a new set of baselines formed. The present methods of developing conflict behavior by using adverse stimuli may not be the suitable method for attempting the differentiation of drug effects, but this would appear to be a major area for future work in observational research.

The fixed type of activity unit such as the photocell cage appears to be the preferable apparatus for monitoring small animal activity. It measures lateral motion directly and does not introduce any movement artifacts typical of the indirect type of activity cage. The indirect measuring units, such as the jiggle cage, are difficult to calibrate and the data, too often, represent frequency of cage movements rather than animal movements. The sensitivity of the direct type of activity apparatus has been vastly increased by investigators with the use of intricate electronic circuitry. Further efforts to increase sensitivity with still more complex and sophisticated instrumental design does not appear to be necessary. Present concepts of drug evaluation are based on the assumption that *total* movement must be measured in order to monitor drug effects sensitively. However, investigators (84) have accurately simulated the clinical effects of stimulants by recording only one component of behavior, while the recording of total locomotor activity failed to do so. This is not surprising in view of the fact that the primary effects of amphetamines in humans are nonlocomotor in nature (restlessness, irritability, anxiety). Similarly, depressant compounds may alter selectively only a few component behavioral patterns of total activity. Therefore, research should turn its attention toward delineating, if possible, the specific behavioral patterns affected by particular compounds or classification of compounds. If successful, the use of complex, expensive apparatus capable of recording every type and all degrees of movement would not be justified. Papers have been reviewed which indicate that the qualitative and quantitative effects of a drug are a function of the environment in which it is tested. Because such parametric influences vary from laboratory to laboratory, it is incumbent upon each investigator to determine the optimum set of experimental conditions for his laboratory.

Because of the great variability in activity between animals, proper statistical treatment of data is extremely important to the behavioral scientist. He depends to a great extent on the power of tests of statistical differences to give meaning to his data. Since parametric tests (analysis of variance, t tests) assume that frequency distributions are normal, it is imperative that investigators consider the transformation (log, square root) of raw data before applying these statistical tests.

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Research Articles

Synthesis of ^{14}C-Labeled Isomers of Dichlorodiphenyldichloroethanes (DDD)

By R. E. COUNSELL and ROBERT E. WILLETTE

1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl-^{14}C)ethane (*o,p'*-DDD-^{14}C), 1,1-dichloro-2-(*m*-chlorophenyl)-2-(*p*-chlorophenyl-^{14}C)ethane (*m,p'*-DDD-^{14}C), and 1,1-dichloro-2,2-bis-(*p*-chlorophenyl-^{14}C)ethane (*p, p'*-DDD-^{14}C) were synthesized by acid catalyzed condensation of chlorobenzene-^{14}C with excess 2,2-dichloro-1-(*o*-, *m*-, and *p*-chlorophenyl)ethanols. The carbinols were prepared in good yields by reverse addition of the chlorophenyl Grignard reagent to dichloroacetaldehyde. Purity was determined by thin-layer and gas chromatography. The I.R., U.V., and NMR spectra of these compounds are discussed.

INTEREST in the development of radiopharmaceuticals suitable for adrenal photoscanning prompted the present study. For this purpose, an agent that selectively concentrated in the adrenal and could be labeled with a γ-emitting radionuclide was necessary. This paper describes the synthesis of ^{14}C labeled isomers of 1,1-dichloro-2,2-bis-(chlorophenyl)ethane (DDD) to be utilized in tissue distribution studies. The synthesis of other DDD isomers and ^{125}I and ^{131}I isomers will be reported elsewhere.

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