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A SEQUENTIAL RESPONSE METHOD OF STUDYING COMPLEX BEHAVIOR IN ANIMALS AND ITS APPLICATION TO THE MEASUREMENT OF DRUG EFFECTS[1]

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A behavioral testing method was demonstrated to be applicable to the study of detrimental drug effects on complex behavior in rats. The method required the subject (*S*) to respond in a certain sequence to four identical response sites spaced 90° apart within a cylindrical test compartment. After each *S* was shaped to perform its one particular sequence within this quadrilaterally symmetrical environment, and its performance was brought to a stable level, drug effects were then studied on a battery of such *S*s whose sequential response habits were representative of a continuum of sequence complexity. Experiments with a drug which induces hallucinatory and confusional states in man showed that the method yields in the rat quantitative measures of detrimental behavioral effects in terms of dose-response and dose-time relationships in addition to providing an estimate of the interactive effect between drug dose and behavioral complexity. It was found that for a given magnitude of behavioral detriment, drug dose and behavioral complexity of sequence were inversely related. That is, a *S* required to perform a simple sequence needed a larger drug dose to interfere with its habit a unit amount than did a *S* with a more complex one; or, expressed another way, a given drug dose had a greater behavioral effect the more complex the sequence. Finally, an empirical ranking of response sequences along a functional behavioral complexity dimension was presented.

This report describes a behavioral testing method developed in an attempt to study in animals the effects of hallucinogenic and other drugs having detrimental effects on complex behavior in man. However, since the method may also be applicable to the systematic interspecies study of the detrimental behavioral effects of other classes of experimental treatments (*e.g.*, CNS lesions or ablations, acute dietary deficiencies), the method will be described in considerable detail, and results obtained in experiments in this one application, drug effects, will be discussed.

METHOD

The method is predicated on the proposition that one of the most complex, yet variably complex, response patterns common to a wide range of species is a chain of responses performed sequentially in space as well as time. It was reasoned that since these response chains are so liable to interference from external influences, they would similarly be sensitive to other disruptive effects including endogenous ones. The idea is certainly not new, for mazes of one type and another have existed for many years. Conventional mazes, however, are characterized by a multiplicity of cues for solution. The sequential response method to be described here is designed to approach an ideal cue-depleted environment so that the cues utilized in the performance of sequential response patterns are probably endogenous rather than discriminated from the environment.

The apparatus consisted of two parts; the animal's compartment, and the electronic control network containing the necessary components to program, interpret and record the events of interest. The animal's compartment can be described as a cylinder placed on end. The animal on the floor is faced with four response sites spaced 90° apart around the cylindrical walls, labeled clockwise A, B, C and D. *S* secures rewards by making responses

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to these sites in a designated sequence. Because the four response sites appear identical and the entire environment is also quadrilaterally symmetrical, the possibility for confusion in space should thus be maximized. It was argued that sequential behavior required of an animal in such an ambiguous environment would be susceptible to minute detrimental effects, *i.e.*, could be disrupted to some degree.

In practice, a response sequence may require *S* to respond to any of the sites more than once or even to omit some of them. Any possible combination of sites can be required in any possible sequence for a "simple sequence", or pairs of simple sequences can be required in alternation for a "sequence alternation". At the end of each sequence *S* is rewarded at the reward site (D) where sequences invariably end, and *S* thus cyclically performs sequences and collects rewards at a self-paced rate. Every response performed in the appropriate sequence is recorded as correct, all others as errors. If an error is made at any point, *S* is required to begin the entire sequence once again. After each *S* is shaped to perform its one particular response sequence and its performance brought to a stable level, experimental effects are then studied on a battery of such *S*s whose sequential response habits are representative of a continuum of sequence complexity.

By choosing appropriately complex sequences, engineering the test compartment to match the animal, and requiring responses and providing rewards compatible with it, the method should thus be directly adaptable to the comparative study of the same class of complex behavior in a relatively wide range of species, including mammals, aquatic animals, and perhaps crawling insects as well. Moreover, a systematic investigation of several dimensions of complex behavior in animals is facilitated by the ability to manipulate sequence complexities both within and between species.

The method is thus similar in certain respects to Hunter's (1928) use of the temporal maze, and to the method developed by Jenkins (1927) to study the learning capacities of the kitten (Shuey, 1931), white rat and guinea pig (Riess, 1934), rhesus monkey (Fjeld, 1934), and cebus monkey (Koch, 1935). The Jenkins apparatus also was cylindrical, but it was constructed of open wire mesh and had only three response sites on the floor at 3 o'clock, 12 o'clock, and 9 o'clock (called 1, 2, and 3, respectively). *S* responded to these in sequence in order to be given access into a smaller cylindrical reward cage at the center of the apparatus. Sequences were always of the basic form, 1-2-3-2-1-2-3-2-1, *etc.* *S* was tested by rewarding it when the particular sequence in force was performed, running to criterion performance on that sequence, then moving to the next one. Testing was begun with sequence 1-reward, then after criterion was met, successively to 1-2-reward, 1-2-3-reward, 1-2-3-2-reward, 1-2-3-2-1-reward, 1-2-3-2-1-2-reward, *etc.* Each species was tested in this manner and the length of sequence attained was reported as a measure of that species' learning ability.

The present method is also in one sense similar to an operant technique employed by Mechner (1958) and others. Mechner required *S* to respond to one bar at least N times and then respond to a second bar once in order to secure a reward. Thus the number of responses executed on the first bar served as the cue for a response to the second bar, which Mechner termed a process of "response-produced cues". It can be seen that previous responses may serve as cues to subsequent responses in the sequential response method also, especially in partially repetitive sequences such as A-B-C-B-C-*D*, or A-A-*D*.

In the sequential response experiments reported here, the experimental animal was the rat, and the test compartment designed for it (Fig. 1) was a cylinder 22 in. in diameter, illuminated by a 7.5w frosted bulb suspended above the center of the cylinder 18 in. from the floor. Each response site consisted of a circular metal disc on the wall 1 in. from the floor, a "knowledge of results" light positioned directly over the disc, and on the floor, a flush pedal which closed a micro-switch when depressed. Thus the rat made responses to the sites merely by walking over the floor pedal, with correctness being indicated by the light going "on" as each correct response in the sequence was made and going "off" immediately as *S* left that site. When responses were made to the appropriate sites in the appropriate sequence, *S* could then complete the sequence at the fourth site (D) where the metal disc on the wall was in this one case

actually a door hinged at the top. By inserting its head into the hole made by pushing open the door, the rat was given access to a fountain (Polidora and Meyer, 1961) containing a small volume of liquid delivered after the last correct (D) response. After the animal removed its head, the door closed of its own weight and once again appeared identical to the other three sites.[3]

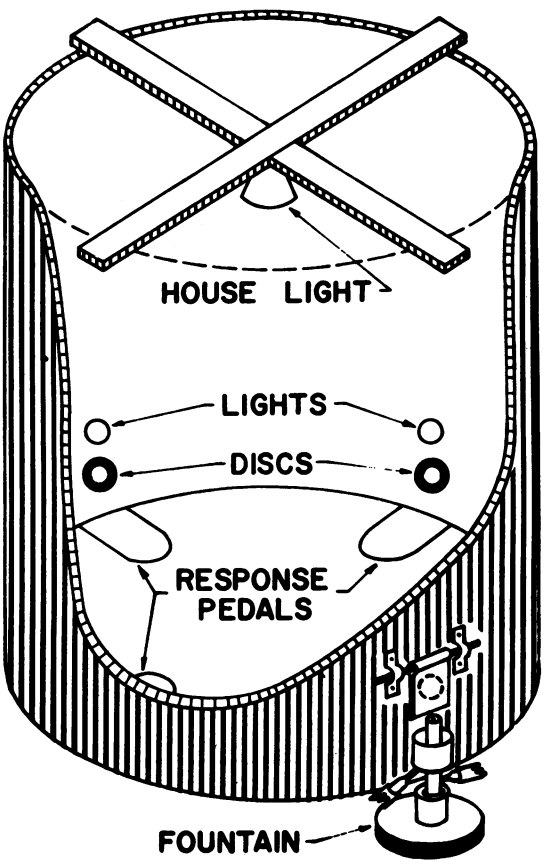

Fig. 1. A cut-away drawing of the sequential response test compartment for the rat. The enclosure around the fountain which prevented *S* from escaping, the liquid reward reservoir, tubing, solenoid valve, light sockets, and electrical wiring were omitted from the drawing to facilitate clarity.

Male Long-Evans strain hooded rats were shaped to their respective sequential response patterns and their performance brought to a stable level by daily 1-hr testing (approx. 22-hr water deprivation, 0.04 ml water rewards, 1 hr *ad lib* water a variable interval after each test session). Shaping itself required from 5 to 18 1-hr sessions (differences being due primarily to sequence length and complexity), and stable performance was attained in a maximum of 15 additional sessions, five to eight being more common. Since improvement leading to stable performance was relatively rapid once begun, the attainment of stability, judged over a block of three successive sessions, was clear in most cases. Of 51 rats tested to date, 43 (84%) were shaped to perform acceptably.

When stable performance was attained, daily I.P. saline injections were administered 10 min after the beginning of a session as each *S* usually had attained its characteristic response and reward rate by that time. Drug injections were similarly administered. Except during injections and the initial test sessions with a new drug or a new dose, the experimenter remained in the control room, while the test compartment containing *S* was in a darkened, sound-attenuated room. For each *S* a drug session was preceded by a saline session the day before and followed by a non-tested day, and successive drug sessions were separated by at least three, but more usually by seven days.

In order to demonstrate the applicability of the sequential response method to the measurement of detrimental behavioral drug effects, the data from one compound has been collected for presentation here. JB 329, N-ethyl-3-piperidylcyclopentyl phenylglycolate hydrochloride, "Ditran", (Abood, *et al.*, 1959; PSC Bulletin, March 1962, p 35) was chosen as a representative hallucinogenic agent having confusion-inducing effects in the human (Meduna and Abood, 1959) and measurable behavioral effects in the rat (Abood, *et al.*, 1959; Biel, *et al.*, 1961). Because of a progressive tolerance exhibited to this compound, however, it was administered no more than three times to a given *S*.

The experiments were conducted in two laboratories (see footnote 1) with two identically designed but different test units and with three different batteries of *S*s. Since no systematic differences have been detected between these data, they are presented without designation.

[3]A cylindrical test compartment substantially different in scale and design, but identical in concept, has also been developed for the monkey. With the use of the single control network, the method is currently being evaluated in this species as well.

RESULTS

Figure 2 illustrates the saline control performance of two *Ss*, one having the most, the other the least stable performance of the 43 *Ss* tested. The three test sessions depicted were run on the same three successive days for each *S*. It may be seen in the graphs, as well as in the summary histograms, that the rate at which responses were made, the proportion of them which were correct (*i.e.*, within the sequence as designated), and hence the rate at which rewards were dispensed were relatively stable for these 1-hr sessions.

Rates of individual *Ss* differed considerably even within a particular sequence, but intrasubject rates typically remained stable and characteristic for up to six months from the time adaptation, deprivation, and initial shaping was begun, usually at 60 days of age.

Figure 3 illustrates the effect of three doses of JB 329 (0.25, 0.5, and 1.0 mg/kg) on two rats with sequences of unequal complexity, one with the "simple sequence" A-C-*D* (*D* indicating the rewarded D response), the other with the "alternating sequence" A-*D*/C-*D*, in which first only A-*D* is effective, then after a reward, only C-*D*, and so on. Several points are illustrated in this family of curves; the relative stability of saline performance of these two *Ss*, the dose-time dynamics of each drug dose represented within each session, the dose-response relationship represented across the three curves for each *S*, and finally the dose-sequence relationship illustrated by a comparison between *Ss* at each dose. These final two relationships are further illustrated by comparing the saline and drug median reward rate histograms in each graph.

It can be seen that drug effects are reflected primarily in the percent correct responses (error rate), not in response rate. In other words under the effect of the drug *S* continued to respond at a relatively stable rate, but fewer of the responses made were linked together into the relevant sequence. As a rule, when the drug effect began to wane and *S* began to link responses into sequences once again, it

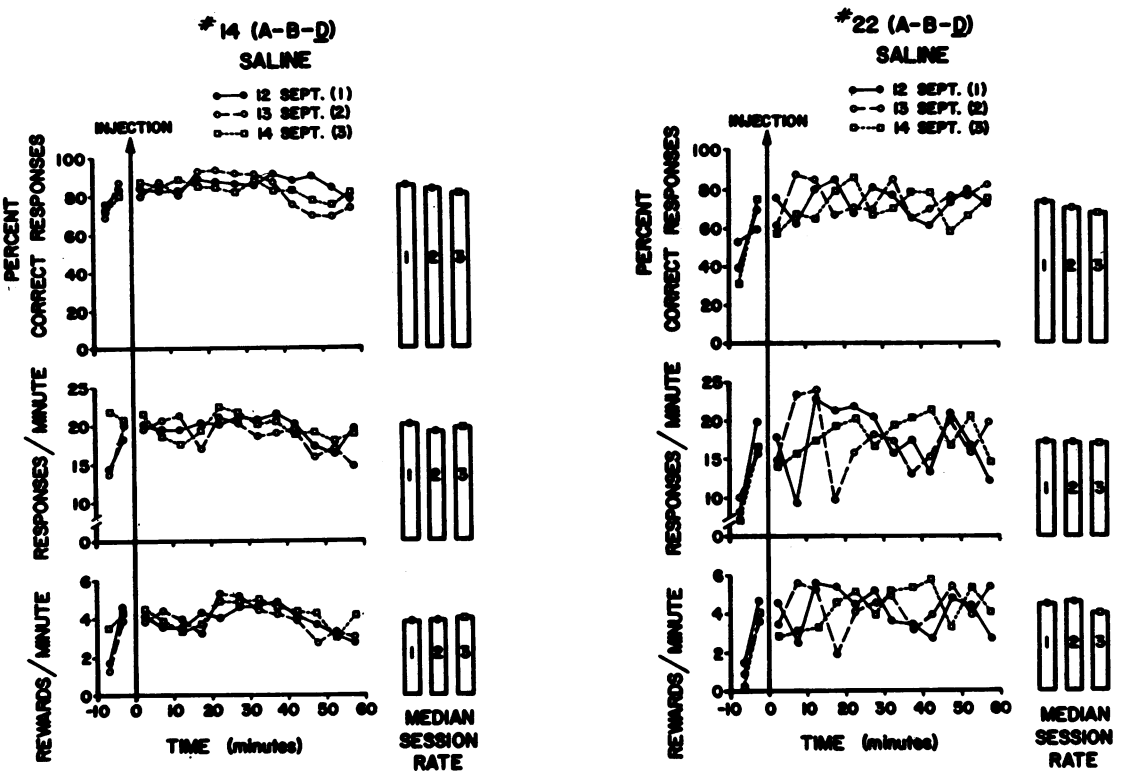

Fig. 2. Sequential response performance of the two rats having the most and the least stable performance of the total population of 43 *Ss*. As in Fig. 3 below, the median session rates to the right of each graph were computed between the time of injection and the end of the session.

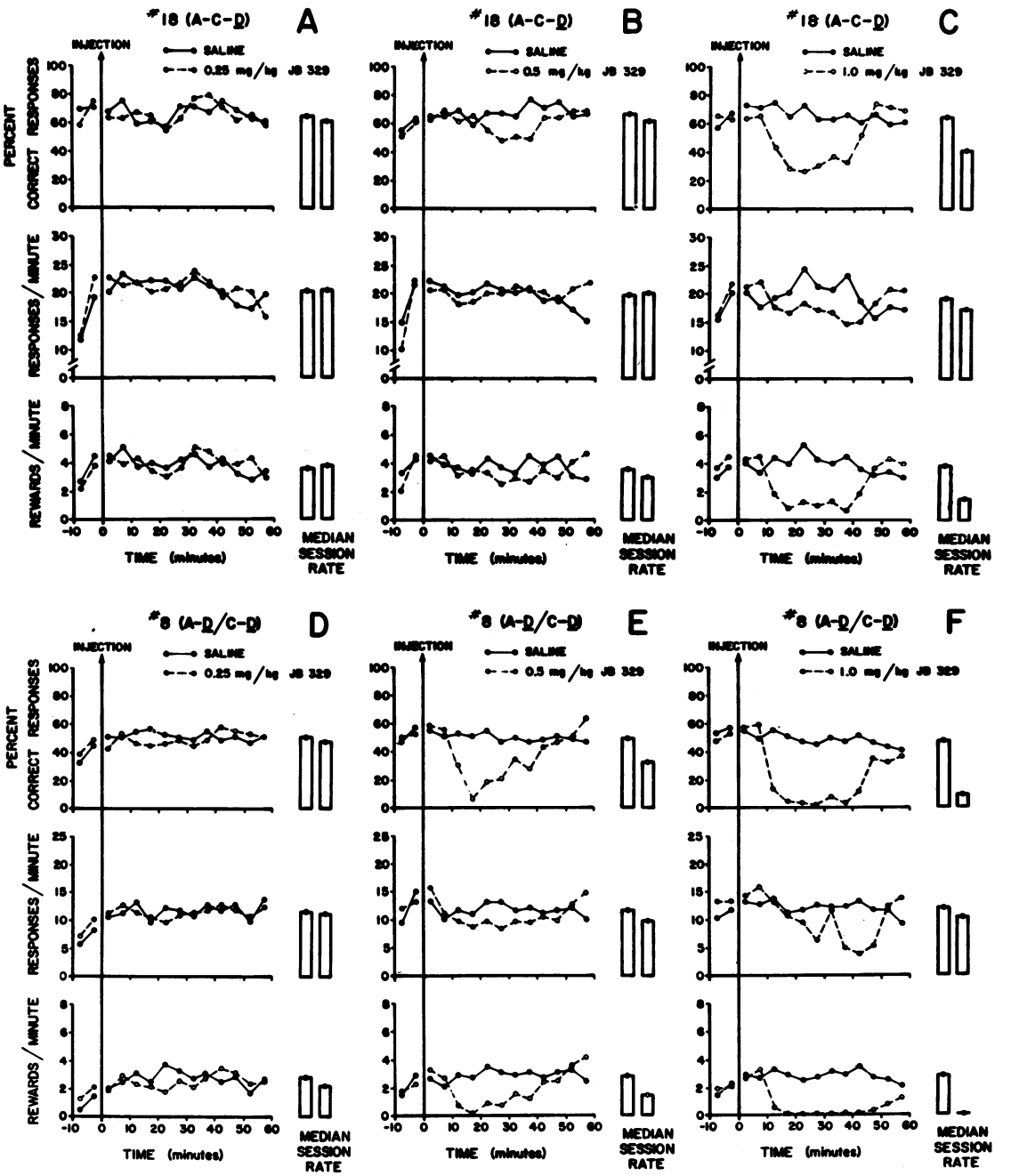

Fig. 3. Detrimental effect of three doses of JB 329 on the sequential response performance of two rats with sequences of unequal complexity.

was evidenced first by an increased correct response rate followed by an increased response rate, seldom the reverse (*cf.* Fig. 3C, E and F). When the effect was severe, as in Fig. 3F, the response rate generally decreased gradually after several minutes of nonrewarded performance suggesting that extinction and drug effects were confounded in this interval. Finally, the duration of effect was typically positively correlated with its severity.

In order to illustrate the systematic dose-response and dose-sequence effects of this

detrimental treatment on sequential behavior in general, every drug session median reward rate for every *S* tested was compared with that *S*'s saline session median reward rate the day before, and a Decrement Ratio, expressed as [(drug minus saline)/saline], was computed. These data are found in Fig. 4 where Decrement Ratio is plotted for each sequence studied along five JB 329 dose parameters. The six arrowed points correspond to the sessions illustrated in Fig. 3. In want of a better prediction, sequences were spaced equidistant on the abscissa and were positioned in increasing order of severity of behavioral effect (Decrement Ratio), and lines representing the five drug doses were fitted by inspection. Plotted in this way, the inverse relationship between sequence and behavioral effect for a given drug dose (slanted dosage lines), the direct relationship between dose and severity of effect (ranked dosage lines), and the sensitivity of the method to this treatment (separated dosage lines) are evident. It should be noted that a Decrement Ratio of 1.0 represents a maximum, and since a ratio of 1.0 was necessarily considered off-scale and its true magnitude indeterminate but probably greater than 1.0, dosage lines were drawn accordingly.

Several control experiments were necessary to characterize more closely the nature of these

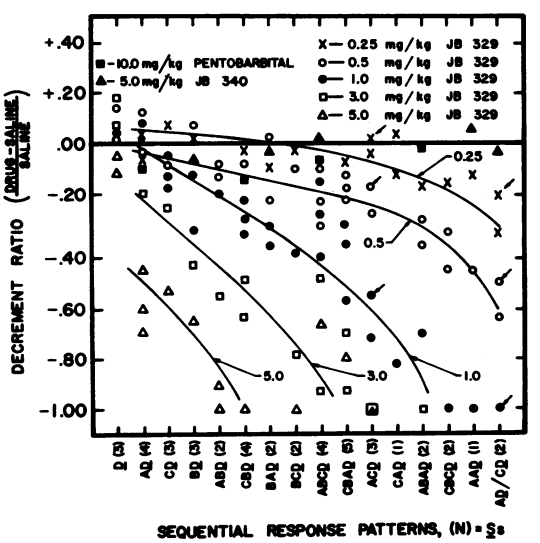

Fig. 4. Detrimental effect of five doses of JB 329 on the sequential response performance of each of the 43 *Ss* tested, together with control data with JB 340 and pentobarbital sodium.

behavioral drug effects and the manner in which they were measured. The leftmost sequence in Fig. 4 is *D*, indicating that these three *Ss* were required to step on the D pedal, collect a reward, back off in order to "open" the D micro-switch, step on the pedal once again, collect another reward, and so on. These *Ss*, with, in effect, no spatial sequence, serve to illustrate the effect of this drug on the instrumental acts, consummatory responses, and incentive value associated with rewards in this situation. It may be seen that detrimental effects are not evidenced even at high doses. Two additional types of control experiments are depicted in Fig. 4, one with a barbituate anesthetic, pentobarbital sodium, the other with JB 340, a congener of JB 329 which is similar in structure and in anticholinergic and antihistiminic potency, but which does not induce hallucinatory or confusional states in man (Abood, *et al.*, 1959). It may be seen that in neither of the three 10 mg/kg pentobarbital sodium experiments, nor the five 5 mg/kg JB 340 ones, were consistant or large effects on sequential response performance found.

Finally, several *Ss* shaped to water deprivation-water reward conditions were tested with 15% sucrose-water rewards after water satiation in order to test the possibility of artifactual results due to higher tissue drug concentrations in the dehydrated rat. In general, similar drug effects were noted, but since both saline and drug session response rates were greatly depressed, the sensitivity of the test was correspondingly decreased, making comparable quantification questionable and leaving the issue unresolved.

DISCUSSION

The data of the control experiments represented in Fig. 4 support several interpretations regarding the detrimental behavioral effects observed in this situation. Not only did the JB 340 experiments indicate that the JB 329 effects were not attributable to its autonomic properties, but it was also shown in the *D*-sequence experiments that JB 329 had no detectable effect on thirst or reward retrieving behavior. Furthermore, the pentobarbital experiments, indicating that even a moderate degree of ataxia and incoordination minimally affects proficient sequential response be-

havior, suggest that the observed effects were not related to possible motor side-effects of the drug.

In additional support of these interpretations is the fact that the severity of behavioral effect was not a direct function of drug dose alone, but rather was an interactive function of dose and sequence. Therefore since the performance of simpler sequences was less affected by a given dose, and since the pharmacological effects of the drug were operative during every session, simpler sequences may be considered also as control conditions indicating that the drug itself had little direct affect on the individual response elements of sequential response patterns. Incorporating these interpretations with the observation that under the drug's effect *S* continued to make responses but failed to link them into relevant sequences, it may be concluded that the drug exerted its action primarily on the inter-response linkages of sequential response patterns.

An examination of the ranking of sequences in Fig. 4 reveals no readily evident correspondence between this ranking and what might have been defined *a priori* to be sequence complexity, indicating that the empirical ranking presented is more meaningful at least for the elucidation of these lawful drug-behavior relationships. It is proposed that this empirical ranking of sequences constitutes a functional dimension of "behavioral complexity".

Within this framework then, certain functional principles of behavioral complexity for this set of conditions are suggested by the order of sequences in Fig. 4: (1) in general, short sequences are less complex than longer ones; (2) sequences requiring *S* to traverse the middle of the cylinder are more complex than comparable sequences not requiring center crossing (*e.g.*, B-*D* *vs* A-*D* or C-*D*); (3) sequences which require a return to a response site are more complex than comparable ones (*e.g.*, A-B-A-*D* or C-B-C-*D* *vs* A-B-C-*D* or C-B-A-*D*); (4) "counting" sequences (*e.g.*, A-A-*D*) are more complex than all others studied with the sole exception of the "alternating sequence" A-*D*/C-*D*; (5) sequences A-B-C-*D* and C-B-A-*D* are inordinately simple considering their length, a principle [like (2) above] which undoubtedly is influenced by the wall-seeking tendency native to this species; and finally (6) the terminally curved dosage lines fitted to the data in Fig. 4 suggest that the linear spacing of sequences did not apply so well for the highly complex sequences as for the simpler ones.

Thus these data lend support to the original assumptions and rationale of the sequential response method by demonstrating that the method provides a means of studying a continuum of complex behavior, as well as providing a means of sensitively measuring experimental effects on this behavior in the rat.

REFERENCES

Abood, L. G., Ostfeld, A., and Biel, J. H. Structure-activity relationships of 3-piperidyl benzilates with psychotogenic properties. *Arch. int. pharmacodyn.*, 1959, **120**, 186-200.

Biel, J. H., Abood, L. G., Hoya, W. K., Leiser, H. A., Nuhfer, P. A., and Kluchesky, E. F. Central stimulants: II Cholinergic blocking agents. *J. Org. Chem.*, 1961, **26**, 4096-4103.

Fjeld, H. A. The limits of learning ability in rhesus monkeys. *Genet. Psychol. Monog.*, 1934, **15**, 369-535.

Hunter, W. S. The behavior of racoons in a double alternation temporal maze. *J. Genet. Psychol.*, 1928, **35**, 374-388.

Jenkins, T. N. A standard problem box of multiple complexity for use in comparative studies. *J. Comp. Psychol.*, 1927, **7**, 129-145.

Koch, A. M. The limits of learning ability in cebus monkeys. *Genet. Psychol. Monog.*, 1935, **17**, 163-234.

Mechner, F. Probability relations within response sequences maintained under ratio reinforcement. *J. exp. Anal. Behav.*, 1958, **1**, 109-121.

Meduna, L. J., and Abood, L. G. Studies of a new drug (Ditran) in depressive states. *J. Neuropsychiatry*, 1959, **1**, 1-3.

Polidora, V. J., and Meyer, P. M. Simple devices for liquid reward delivery. *J. exp. Anal. Behav.*, 1961, **4**, 382.

Riess, B. F. Limits of learning ability in the white rat and the guinea pig. *Genet. Psychol. Monog.*, 1934, **15**, 303-368.

A selective list of drugs used in Psychiatry. *Psychopharm. Service Center Bull.*, March, 1962.

Shuey, A. M. The limits of learning ability in kittens. *Genet. Psychol. Monog.*, 1931, **10**, 287-378.

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