METHOD & DESIGNS

The use of strain rank orders

in assessing equivalence of techniques

GERALD E. McCLEARN,¹ *Institute for Behavioral Genetics, University of Colorado, Boulder, Colo.*

In situations where effects of previous testing make it impossible to compare putatively equivalent forms of a behavioral test, inbred strains may be useful. To a considerable extent, animals from an inbred strain represent replicate individuals. Different naive samples from the same strain may be tested in different forms of a test. There is presumptive evidence that the two forms are assessing similar functions if samples from different strains that differ on the behavior give consistent ordinal rankings. An illustration is given of the use of this approach in assessing methods of measuring alcohol preference in mice.

In animal behavioral research it is frequently of interest to ascertain the equivalence or nonequivalence of two or more procedures, methods, or pieces of apparatus. One operational approach to the problem is to determine if manipulation of the same independent variable gives similar results with the two procedures. Often, however, the question arises in the first instance because some operation, in fact, has not yielded similar results. A case in point would be a failure to replicate the distribution of practice effect, using the same intertrial intervals, but in different mazes.

Another approach would be to apply reliability considerations appropriate to alternative forms of psychometric tests. If the same animals are tested by technique A and technique B, and a high correlation between scores is found, the technique may be regarded to be measuring the same thing, at least in large part. One difficulty of this approach is that practice and order effects may obscure the results. Another problem is that the animals typically employed in behavioral research (particularly rats) are partly but not intensively inbred, so that there will be a restriction of range effect without the compensating virtue of stability of genotype over time.

The relative stability of the genotypes of rigorously inbred animals offers a solution that circumvents the problems associated with the necessity for repeated measurements on the same individual. Because each animal of an inbred strain is a genetic replicate of every other (or at least, very nearly so; see McClearn, 1967, for discussion) one can, in effect, measure the same animal with several techniques, with repeated measures on each, but with the animal naive at each measurement. In practice, this approach involves the examination of the constancy of ordinal relationships of strain differences across techniques.

An example may be taken from work on alcohol preference in mice. Rodgers and I (McClearn & Rodgers, 1959, 1961; Rodgers, 1966) showed highly significant differences in alcohol preference of several inbred strains using the following method:

The animals were housed for testing in individual cages with a standard laboratory ration freely available. Water and a 10% ethanol solution were freely available through drinking tips extending through the cage tops. The drinking bottles were 25-ml graduated cylinders, supported at approximately 45 deg angles by wooden racks above cages. For 14 successive days, at approximately the same time daily, the quantities of water and of ethanol solution consumed during the previous 24 h were recorded. In order to counter position effects, the positions of the cylinders in each cage were reversed after the sixth and tenth days in the first experiment and after every third day in several later experiments. For each animal, a daily preference ratio was obtained by determining the percentage of the total liquid consumption which was from the ethanol solution bottle—i.e.,

$$\frac{\text{ethanol solution consumed}}{\text{water consumed} + \text{ethanol solution consumed}}$$

Thus high values indicate alcohol preference and low values indicate water preference. The mean of the daily preference ratio over the 14-day test period was then taken to provide a mean preference ratio for each animal.

The results of these studies showed C57BL to be high preference animals, RIII and C3H/2 animals to have intermediate preference, and BALB/c, A, and DBA/2 to have low preference ratios.

Comparability of standard and short version alcohol preference tests. The standard 14-day alcohol preference test was initially adopted arbitrarily. It is a long observation period, yet it seemed necessary because of the clear tendency of the C57BL animals to increase their ethanol intake from relatively modest quantities to high consumption over at least a two-week period. There would be an obvious advantage if an alternate procedure could be found which would permit shorter term observations, but which would yield the same relative strain rank orderings as the longer 14-day test. To this end the following experiment was undertaken. Male animals from each of six strains (C57BL, C3H/2, BALB/c, RIII, A, and DBA/2) were assigned to one of two groups. One group was tested in the standard manner for 14 days, with bottle position changes being made every third day, on a choice between 10% ethanol and water. The other group was tested for only three days. For 11 preceding days the animals of this group had been housed in pairs with both water and a 10% ethanol solution available. The positions of the bottles were interchanged in the same fashion as for the first groups, but no daily consumption readings were taken. Results of the study, shown in Fig. 1, indicate the essential comparability of strain rank orders under the two conditions.

Comparability of single and double cylinder testing situations. Although this short test would appear adequate for most purposes, it was felt desirable to try other versions of alcohol preference tests before a change in methodology was settled upon.

One other approach which has been used for assessing preference for beverages in experimental animals has been to present the alternate solutions singly on alternate days. To determine if single cylinder testing would be useful in alcohol preference studies with inbred strains of mice, six male animals from each of the six strains were tested on both the standard double cylinder and on the single cylinder situation. (Two animals of the RIII strain were terminated because of illness.) Each animal was tested for 24 days with two six-day periods on one condition alternating with two six-day periods on the other condition. Half of the animals of each strain were tested in the

order double-single-double-single, and the other half in the order single-double-single-double. Expressing results from both procedures as the ratio of the ethanol solution consumed during the period to the total amount of ethanol solution and water consumed during the same period permits direct comparison of the two methods. The results are shown in Fig. 2, and it is apparent that the single cylinder situation does not permit the degree of differentiation among strains that the double cylinder situation allows.

Alcohol acceptance under thirst motivation. It is apparent that in the usual testing circumstances, strain differentiation depends at least in part upon the increase in C57BL intake as a function of time of exposure to the choice situation. It seemed possible that the same effect might be obtainable by increase of thirst motivation. Six male animals from each of the standard strains were tested. On Day 1, the animals were weighed, placed in

individual cages, and provided with a single cylinder containing tap water. Daily readings were taken of water consumption until Day 3, when the cylinder was removed. Twenty-four hours later, animals were given cylinders containing 10% ethanol; 24 h later, the amount consumed from the ethanol solution under the conditions of thirst motivation was recorded. Figure 3 shows the results. It is apparent that the strain rank ordering obtained by this method is essentially the same as that obtained in the longer 14-day standard test, or in the short version described above.

These results show that some procedures are essentially interchangeable for purposes of assessing alcohol preference, and that another procedure is not. Apart from the practical implications for research on alcohol consumption in animals, these results indicate the usefulness of strain differences in assessing the comparability of different procedures.

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On assessing verbal stimulus hierarchies¹

CECIL A. ROGERS, JR., DEPARTMENT OF PSYCHOLOGY,
UNIVERSITY OF ARIZONA, Tucson, Arizona 85721

Typical word-association procedures result in verbal response hierarchies for a specific stimulus word. The problem of assessing corresponding stimulus hierarchies is discussed. Three approaches are entertained: obtaining associations to all of the words in the dictionary, clerical cross-indexing of existing response-hierarchy data, and obtaining backward associations which are then used in forward association. A comparison of data obtainable by the last two methods is presented.

Deese (1962) states that the associative meaning of a word may be defined in terms of (a) the distribution of responses (response hierarchy) to that word when it is used as a stimulus in free association; and (b) the associative distribution (stimulus hierarchy) or collection of stimuli to which the word occurs as a response. Applications of word-association norms for response hierarchies were stimulated by publication of the Russell-Jenkins (1954) norms. Further impetus was provided by the shift in emphasis of interference theories of forgetting from retroactive to proactive agents, as signaled by Underwood (1957) and Underwood & Postman (1960). Norms on verbal-association

response distributions have been well-exploited, including studies on paired-associate learning (Coleman, 1963), mediation (Jenkins, 1963), controlled recall (Bilodeau, Fox, & Blick, 1963), sentence recall (Rosenberg, 1966), interference generation (Blick, 1965), semantic generalization (Mink, 1957), and organizational characteristics of free recall (Cofer, 1965).

On the other hand, norms on stimulus distributions have not been generally available for several reasons. First, there is a methodological problem as to how one might assess the stimulus distribution for a given word. While, in assessing response distributions, it is simple enough to ask S to say or write the first word that a given item makes him think of, it is quite another problem to get him to produce a stimulus for some word. Next, even if we could contrive to get Ss to produce a list of stimulus words, the obtained stimulus distribution for a word might not differ from its response distribution. It is well known that many verbal associative networks are tightly knit (e.g., *cold* often elicits *hot* as its primary and vice-versa). Third, while almost every word, when used as a stimulus, produces some response distribution which is fairly reliable and accurate, we might be hard-pressed to think of a procedure which would ensure us that we have sampled all or even many high frequency producers of a given response. Fourth, if somehow we do collect items which Ss say