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BRIEF COMMUNICATION

Crossover Effects in Experimental Investigations with Human Subjects

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LANDAUER, A. A. *Crossover effects in experimental investigations with human subjects.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1141–1145, 1975. – Simple crossover studies with drugs may introduce artifactual results. Volunteers are frequently less apprehensive when tested on the second occasion and experimental medication may result in different performance measures. Empirical evidence is given to demonstrate this point and a suggestion is made as to how it can be overcome.

Crossover effects Experimental design Human drug research Method Transfer effects

THE use of crossover designs in pharmacological research seems to have gained a quite unwarranted status. For instance, in a recent review of the effects of antianxiety drugs on human performance McNair [2] analyzed the methods used in the numerous investigations which he reports and found that in 68 percent of the studies a crossover design was used. He also reports that 64 percent of the investigations were performed under double-blind conditions, that the vast majority of experiments were made with normal males (88 percent), and that active medication consisted of a single dose (70 percent).

Thus, the majority of pharmacological experiments with antianxiety drugs which test behavioral effects and which find their way into the scientific literature employ healthy male volunteers who receive a single dose of the active medication and a single dose of placebo (or of a comparison drug) under double-blind conditions.

The simplest type of crossover design in drug research with human subjects is to give Drug A and Drug B consecutively and determine the order of administration by chance. In place of one of the drugs, placebo can be given. Therefore, in this type of design there are two groups of subjects: one group which starts on Drug A and receives Drug B afterwards; and one group which is given Drug B first, followed by Drug A. It is better to have an equal number of subjects in each group, though this is rarely achieved in practice owing to extra-experimental causes. However, there is nothing wrong with having an unequal number of subjects in the two groups, provided the appropriate statistics are applied to the data.

It has been known for a long time that special care should be used in crossover studies in which a transfer of effects may take place. Poulton and Freeman [4] examined a large number of crossover studies in which transfer effects had been found. Though most of the experiments they review are taken from the field of human performance, the paradigm they advance holds equally well to other domains. According to these investigators, transfer can be symmetrical and asymmetrical. By starting conditions at random in a fully-balanced Latin Square design, symmetrical transfer effects are adequately controlled.

A typical asymmetrical transfer effect occurs when performance is lowered under stress conditions during the first test, and only marginally improves when the second test is given under normal conditions; while subjects who are first tested under non-stress conditions perform at a higher level, which is only slightly debased in the subsequent test under stress conditions.

If the experimenter suspects that there may be some asymmetrical transfer effects which occur in a crossover design with drugs, he can compare the effects of Drug A administered to Group 1 (Drug A first group), with the effects of Drug B administered to Group 2 (Drug B first group). For the time being he disregards the effect of the second drug which was administered to each group. Unfortunately this method is not very sensitive to small differences between treatment conditions, in particular if individual differences are large. Such a design becomes a two independent groups model with all the shortcomings the investigator was trying to avoid when he decided to use a crossover design.

One factor which may account for asymmetrical transfer effects with normal volunteers in crossover experiments with drugs may be the following. Most subjects are somewhat apprehensive and anxious when they arrive for the first time at a laboratory or hospital to take part in a drug experiment. This is particularly marked in young men who have no medical or paramedical background and who

TABLE 1

MEAN PERFORMANCE, POOLED OVER TESTING DAYS

		Effect Due to Alcohol	
	Overall Effect	Without Alcohol	With Alcohol
Overall Effect	–	284	231
Effect Due to Drug			
Oxprenolol	280	321	238
Placebo	235	246	224

TABLE 2

ANALYSIS OF VARIANCE, POOLED RESULTS

Source of Variance	SS	MS	*df*	F-Ratio
Between Subjects	308104		19	
Within Subjects	897500		60	
Drug Effect	39650	39650	1	1.266
Error (Drug Effect)	594912	31311	19	
Alcohol Effect	55073	55073	1	22.469*
Error (Alcohol Effect)	46569	2451	19	
Drug X Alcohol Effect	18514	18514	1	2.464
Error (Interaction)	142782	7515	19	
Grand Total	1205604		79	

*$p<0.001$

have not participated in previous investigations. These subjects represent the type most sought after in drug experiments.

Subsequent to their first session the volunteers become aware that psychological and physiological tests are not unpleasant and that even blood-letting by experienced staff is only mildly discomforting. Consequently, when subjects return for their second trial, they are no longer as anxious as they were on the first testing day. Therefore, if an antianxiety drug such as a tranquillizer is being evaluated, the drug will tend to affect performance of subjects on the first testing day only.

Some confirmation for this point of view comes from a recent crossover study in which the Profile of Mood States questionnaire [3] was administered to 20 young men. No significant difference was found between the active drug (a beta-adrenergic blocking agent) and placebo. However, when the data were analyzed according to testing days, it was found that there was a significant difference on two dimensions which this questionnaire measures. On the first testing day subjects were significantly more anxious (t = 3.053, df = 19, $p<0.01$) and more depressed (t = 2.647, df = 19, $p<0.02$) than on the second testing day. The results denote that different stages of anxiety and depression existed when the active drug was administered to subjects. The effect appears to be symmetrical and by starting an equal number of subjects in each condition, probably is controlled adequately.

As far as performance measures are concerned, similar effects have been obtained. In a recent study which investigated the behavioural effects of oxprenolol and alcohol on skills similar to those used in car driving [1] a

TABLE 3

MEAN PERFORMANCE ON EACH TESTING SESSION, DATA OF GROUP 2 IN *ITALICS*. THE MEAN OF THE TWO ENTRIES APPEARS IN TABLE 1. GROUP 1: DRUG–PLACEBO. GROUP 2: PLACEBO–DRUG.

		Effect Due to Alcohol	
	Overall Effect	Without Alcohol	With Alcohol
Overall Effect	–	249	213
	–	*319*	*250*
Effect Due to Drug			
Oxprenolol	178	178	177
	382	*464*	*300*
Placebo	284	319	248
	187	*174*	*200*

simple crossover design was used. On each of the two testing days (once with medication and once with placebo) subjects were tested twice: first without and later with 1 ml/kg of ethanol. This confounding order effect has always been used in our drug-alcohol research. In one treatment of the data performance over testing days was pooled. This could be done since half the subjects started on placebo and half on the active drug. The results of one test, the driving score on the Martin Simulator, are shown in Table 1. A summary of the analysis of variance is given in Table 2. As can be seen the only difference which was significant was due to alcohol consumption.

Another way of examing the results of this experiment is to consider that two groups of subjects were used: Group 1, which was given oxprenolol on the first experimental day and placebo on the second day, and Group 2, which received placebo on the first day and oxprenolol on the second. Table 3 shows the mean for each day under the same conditions as Table 1: each of the two means in Table 3 has a single mean in Table 1.

A new analysis of variance was made of these data. The model used was the Three-factor Experiment with Repeated Measures (Case 1) model which has been fully described by Winer [5]. It has one between subjects variable (Group 1 compared with Group 2) and two within subjects variables, which were identical to those used in the other analysis. In other words, the effects of drug and alcohol were again examined after any effect due to treatment order had been isolated. The summary of the analysis of variance is presented in Table 4. The drug and the alcohol conditions each had a significant main effect. Furthermore, the interactions of drug with group, drug with alcohol, and the triple interaction of drug, alcohol and group were all significant.

This second-order interaction is plotted in Fig. 1. It can be seen that on the first testing session performance both with and without oxprenolol was very similar. Moreover, on

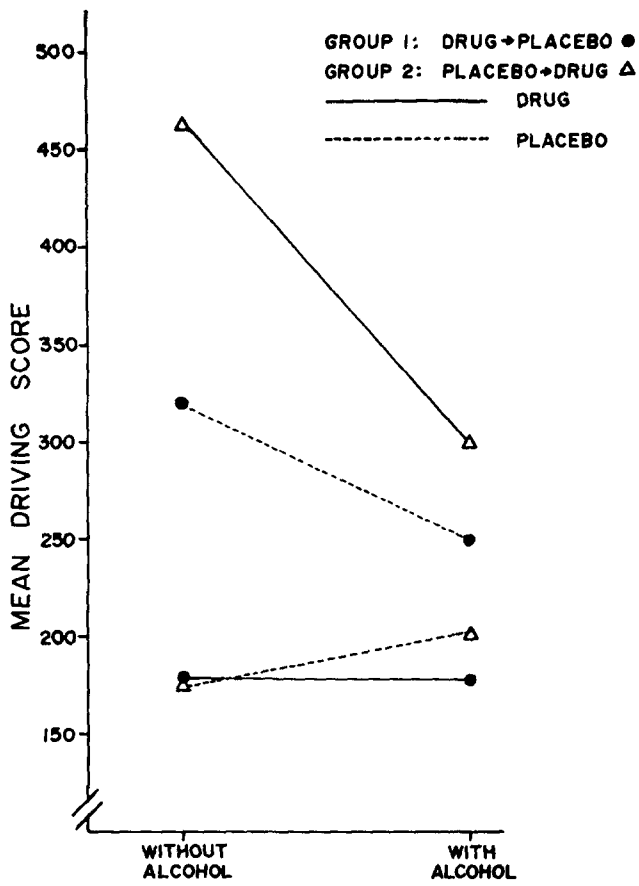

FIG. 1. Mean driving score. Each point is based on 10 observations. The higher the score, the better the performance.

TABLE 4

ANALYSIS OF VARIANCE, SEPARATE GROUPS

Source of Variance	SS	MS	*df*	F-Ratio
Between Subjects				
Between Groups	57943	57943	1	4.169
Error (Between Groups)	250161	13898	18	
Total Between Subjects	308104		19	
Within Subjects				
Drug Effect	39650	39650	1	5.045*
Drug × Group Effect	453457	453457	1	57.702†
Error (Drug Effect)	141455	7859	18	
Alcohol Effect	55073	55073	1	24.115†
Alcohol × Group Effect	5462	5462	1	2.391
Error (Alcohol Effect)	41107	2284	18	
Drug × Alcohol Effect	18514	18514	1	5.711*
Drug × Alcohol × Group Effect	84435	84435	1	26.048†
Error (Drug × Alcohol Effect)	58347	3242	18	
Total Within Subjects	897500		60	
Grand Total	1205604		79	

*$p<0.05$ †$p<0.001$

this occasion the well-documented detrimental effects of alcohol intake were apparently attenuated by the effect of practice in both the drug and the placebo group. On the second testing day, when subjects were more familiar with equipment and procedure, there was a general improvement in performance. However, the subjects who received oxprenolol on this occasion achieved a better score than those who received placebo. With subsequent alcohol consumption the performance decline was quite marked in both groups of subjects. This probably accounts for the significant drug-group, drug-alcohol and drug-alcohol-group interactions.

It may be prudent to abstain from crossover designs in pharmacological research with human subjects. This applies in particular to investigations with drugs which have an antianxiety effect and where behavioral measures are used. In this case it can reasonably be predicted that a smaller antianxiety effect should occur with normal subjects during the second testing session, since most test anxiety should have dissipated.

One way to overcome some possible problems caused by such asymmetrical transfer effects would be to test subjects on 3 occasions. The first session would be on placebo only (single-blind) and the subsequent sessions would be the usual crossover double-blind conditions. This procedure, which is being used by some investigators, would tend to reduce any artefactual effects which are due to the test anxiety of normal, naive volunteers.

REFERENCES

1. Landauer, A. A., D. A. Pocock and F. W. Prott. The effect of oxprenolol and alcohol on skills similar to those used in car driving. Paper presented at the 2nd International Congress of C.I.A.N.S., Prague, 1975.
2. McNair, D. M. Antianxiety drugs and human performance. *Archs gen. Psychiat. Chicago,* **29:** 611–617, 1971.
3. McNair, D. M., M. Lorr and L. F. Droppleman. *Profile of Mood States Manual.* San Diego: Educational and Industrial Testing Service, 1971.
4. Poulton, B. C. and P. R. Freeman. Unwanted asymmetrical transfer effects with balanced experimental designs. *Psychol. Bull.* **66:** 1–8, 1966.
5. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1962.