EFFECT OF ORDER OF DRUG ADMINISTRATION AND REPEAT PLACEBOS ON THE GALVANIC SKIN RESISTANCE IN HUMAN SUBJECTS

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In a recent study (1), the authors recorded the Galvanic Skin Response (GSR) preceding and following the intramuscular administration of atropine and placebo in ten subjects. The results showed a significant increase in resistance level following atropine as contrasted with the control, thereby indicating the possibility of applying this technic to the study of certain drugs. However, further control studies were indicated because the placebo control preceded the trial in which atropine was administered.

Two additional experiments have been performed to evaluate the effect of order of administration of drugs on the GSR. The first series was designed to consider the influence of order of administration on the previously reported drug effects. This was done by injecting atropine, pilocarpine and placebo in a varied order in 24 subjects. The second series tested the effect of repeat placebos in 10 subjects. The results of both experiments are included in this report.

MATERIALS AND METHODS

There were 24 white male subjects in the first experimental series. In each individual the GSR was recorded on three separate occasions preceding and following the injection of atropine sulfate (gr. $\frac{1}{150}$), pilocarpine hydrochloride (gr. $\frac{1}{16}$) or physiological saline (1 cc). The atropine and pilocarpine dosages were dissolved in 1 cc of physiological saline. All injections were administered intramuscularly into the extensor surface of the upper arm. The subjects were normal college males ranging in age from 20–39. All tests were performed in the morning between the hours of 8 and 12 A.M. in the same room. The time between pairs of tests varied from 2 to 34 days. In 22 subjects the three tests were completed in 21 days while the other two required 38 and 39 days.

All possible arrangements of the two drugs and placebo were administered in the first experimental series as shown in Table I. This consisted of six sequences of administration each of which included four different subjects. Individuals were assigned to each sequence in a varied order. This design resulted in the two independent Patterns (A and B) illustrated in Table I and served the following purposes:

TABLE I

Patterns of drug and placebo administration

Experimental Order	Pattern A, Drug Sequence			Pattern B, Drug Sequence		
	1	2	3	4	5	6
First...............................	X	P	A	X	A	P
Second.............................	A	X	P	P	X	A
Third...............................	P	A	X	A	P	X

A—Atropine; P—Pilocarpine; X—Placebo.

1. The subject effects were minimized by having each individual serve as his own control.

2. The effects arising from particular combinations of drugs and order were counterbalanced.

3. The effects of introducing different sequences of drug administration could be partially evaluated by performing separate statistical analyses for Patterns A and B. This provided independent experimental tests on separate subject populations and furnished additional evidence for generalizing the obtained results.

There were 10 white male subjects in the second experimental series each of whom was tested on three different occasions with a placebo consisting of 1 cc of physiological saline. All injections were administered intramuscularly into the extensor surface of the upper arm. As in the first experiment, these subjects were normal college males and ranged in age from 24–35 years. All tests were made in the morning in the same room. The time between pairs of tests varied from 1 to 67 days. In eight subjects the three tests were completed in 10 days.

In each test performed in both experimental groups, continuous measurement of skin resistance was made using a modified Wheatstone bridge which was connected to a D.C. amplifier and direct writing oscillograph (1). Zinc-zinc sulfate electrodes were attached to the hypothenar eminence of the palm of each hand and the recording started (1). Following a short period, the test substance was injected and the GSR was continuously recorded for 45 minutes. This time interval consisted of 12 sections of record each being $3\frac{3}{4}$ minutes long. Calibrations were made prior to, at the middle and end of each experiment in order to check the stability of the equipment. This also permitted individual readings to be made from the closest of the three calibrations.

<div align="center">RESULTS</div>

Table II shows the statistical analysis of the results obtained in the first experiment which considered the question of order of drug administration. The findings are presented separately for Patterns A and B.

The data used for the analysis were obtained from the eleventh section of record (41 minutes following the injection). This period was selected because sufficient time had elapsed following the injection to permit the drug effects to be manifested (1, 2). Resistance readings were made from the last calibration on

TABLE II

Analysis of variance for pilocarpine-atropine-placebo series

Source of Variation*	Sums of Squares (SS)†		Degrees of Freedom (DF)‡		F-Ratio§		Confidence Level¶
	Pattern A	Pattern B	Pattern A	Pattern B	Pattern A	Pattern B	
1) Between subjects (total)..............	201.61	223.34	11	11			
2) Between subjects drug by order interaction..............	42.50	36.16	2	2	1.20	0.87	$P_{(2,9)}20\% = 1.98$
3) Between subjects 'error'..............	159.10	187.18	9	9			
4) Within subjects (total)..............	328.04	170.82	24	24			
5) Drug effects.........	85.01	88.38	2	2	3.76	9.82	$P_{(2,18)}.2\% = 8.73$ $5\% = 3.55$
6) Order effects........	7.36	0.57	2	2	0.33	0.06	$P_{(2,18)}20\% = 1.76$
7) Within subjects drug by order interaction..............	31.92	0.81	2	2	1.41	0.09	$P_{(2,18)}20\% = 1.76$
8) Within subjects 'error'..............	203.74	81.06	18	18			
9) Total..............	529.64	394.16	35	35			

* Row *1* is the sum of rows *2* and *3* for the 'Sums of Squares' and the 'Degrees of Freedom'. Row *4* is the sum of rows *5, 6, 7*, and *8* for the 'Sums of Squares' and 'Degrees of Freedom'. Row *9* is the sum of rows *1* and *4* for the 'Sums of Squares' and the 'Degrees of Freedom'.

Row *2* indicates the 'between subjects' effects of the drugs which depend on their order of administration.

Row *7* indicates the 'within subjects' effects of the drugs which depend on their order of administration.

† The 'Sums of Squares' divided by the corresponding degrees of freedom indicates the amount of the total variance contributed by each of the factors named in the rows.

‡ The 'Degrees of Freedom' is the number of observations minus the number of algebraically independent linear restrictions placed on them.

§ The F-ratio $= \dfrac{SS_1/DF_1}{SS_{error}/DF_{error}}$

¶ The 'Confidence Level' is expressed as the percent probability $P_{(df_1,df_2)}$ that an F-ratio of the size indicated or larger would be expected to occur by chance.

each record in each experimental session. The high and low resistance readings were converted into $\sqrt{\text{micromhos}}$ using the formula $\sqrt{1/R \cdot 10^6}$: (1, 2) and then averaged. These data were then processed using an analysis of variance design described by Lindquist (3).

His design provides a means of evaluating the statistical significance of some of the variables which are included along with the main effects of drug and order. This is done by means of counterbalancing as shown in Table I, and provides a more appropriate error term against which to evaluate the statistical

significance of the main effects. One shortcoming of the design is the failure to provide an estimate of the variance of possible subject by drug interaction which is independent of order of administration. A subject by drug interaction would occur if different subjects showed different magnitudes of reactivity to the different injections. This can lead to an inflated error term and thereby lower the obtained significance. The sequence effects of the two Patterns are included with other differences between the groups. This prevents an independent evaluation of the statistical significance of any variability introduced by sequence differences.

TABLE III

't' ratios and confidence levels for differences between pairs of drugs

| | 't' Ratios* | | | | Confidence Levels† |
| | Pattern A | | Pattern B | | |
	Pilocarpine	Atropine	Pilocarpine	Atropine	
Atropine.......................	3.51		3.83		$P_{(df\ 20)}$ 1% = 2.85
Placebo.......................	2.93	0.58	1.64	2.19	2% = 2.53
					5% = 2.09
					10% = 1.73
					20% = 1.33

$$* \text{ t-ratio} = \frac{M_1 - M_2}{SE_{12}}$$

† The 'Confidence Level' is expressed as the percent probability $P_{(df)}$ that a t-ratio of the size indicated or larger would be expected to occur by chance.
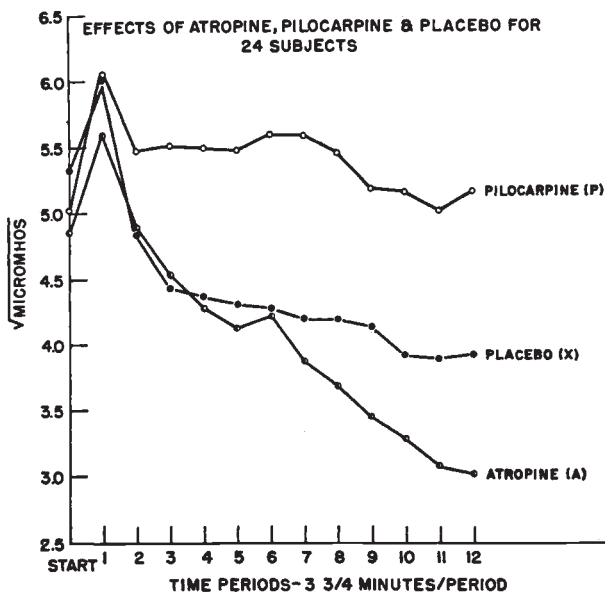

FIG. 1

Table III shows the significance of the differences obtained between each pair of drugs and placebo for Patterns A and B. The "t" ratios were obtained by subtracting the mean of the results for each Pattern for one drug from the mean of the results of the same Pattern for another test substance. This value was divided by the standard error of the difference.

Figure 1 graphically illustrates the results obtained with the three test substances in the entire first series without separating the data into Patterns A and B. Each point on the graph represents the average $\sqrt{\text{micromhos}}$ in a $3\frac{3}{4}$ minute section of record for all of the 24 subjects. This was done for each of the three test substances.

Table IV shows the analysis of the results obtained in the 10 subjects tested with repeat placebos. The data in this series were also obtained from the eleventh section of each record (41 minutes after injection). The calculations were made in the same manner as in the first experimental series.

TABLE IV

Analysis of variance for placebo-placebo-placebo series*

Source of Variation	Sums of Squares	Degres of Freedom	F-Ratio	Confidence Levels
1) Order effects..........................	0.86	2	0.95	$P_{(2,18)}20\% = 1.76$
2) Subject effects........................	24.51	9	6.01	$P_{(2,18)}\ 1\% = 6.01$
3) Order by subject interaction.........	8.15	18		
4) Total..............................	33.52	29		

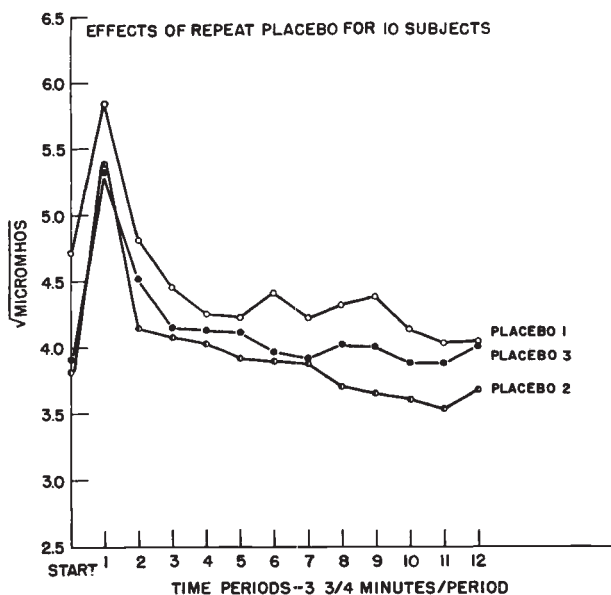* See Table II for explanation of the entries in this table.

FIG. 2

Figure 2 shows the results of the three placebo tests in the second experimental series. Each point on the graph represents the average $\sqrt{\text{micromhos}}$ for a $3\frac{3}{4}$ minute section of record for all of the 10 subjects. This was done for each of the three placebo runs.

<div align="center">DISCUSSION</div>

The general form of results of the drug-placebo series is illustrated in Table II for Patterns A and B. The effect of the drugs is significant for Pattern A at the 5 % level and at the 0.2 % level for Pattern B. The effect of order is not significant at the 20 % level for either Pattern. The variance* which is attributed to error for Pattern A is 11.3 and for Pattern B is 4.5. These values may include some of the effects of order and might, therefore, be somewhat larger than the true value. However, a variance due to order of 3.7 was found in Pattern A and 0.3 in Pattern B. This makes it unlikely that the effect of order would become significant if evaluated against a smaller error.

More important than the possible statistical significance of order in the design, is the relative magnitude of effects attributable to order as compared to the drugs. The variance due to the drug effects for Pattern A is 42.5 and for Pattern B 44.4. The variance attributable to the order effects for Pattern A is 3.7 and 0.3 for Pattern B. These data indicate that the effects of order are considerably less than the drug effects. Therefore, it is possible to conclude that the significance obtained for the drugs is not materially affected by variations introduced by order.

The first experiment was primarily intended to evaluate the effect of drug order; however, it was also possible to analyze individual drug effects. Table III shows the differences between the means of individual drugs and placebo. The difference between atropine and pilocarpine is significant at the 1 % level for Patterns A and B. The difference between atropine and placebo is significant at the 5 % level for Pattern B and not significant at the 20 % level for Pattern A. The difference between pilocarpine and placebo is significant at the 1 % level for Pattern A and not significant at the 10 % level for Pattern B. These results are in the theoretically expected direction although some of the above findings were not statistically significant. However, in other experiments (4), larger dosages of atropine (gr. $\frac{1}{100}$) and pilocarpine (gr. $\frac{1}{12}$) have been found to produce greater and more significant effects.

Figure 1 graphically shows the combined results obtained in the first experimental series. For the first $7\frac{1}{2}$ minutes of record the placebo, pilocarpine and atropine curves follow a similar pattern. The pilocarpine level shows very little change in the rest of the experiment. The placebo and atropine curves are similar for the first 22 minutes. From this point, the placebo and atropine curves separate with the atropine curve showing the more rapid drop. Patterns similar to these have been found in other experiments (1, 2, 5).

The analysis of the results in the second experimental series (Table IV) is

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* The variance is equal to the 'sums of squares' divided by the corresponding 'degrees of freedom' (Table II).

simpler because no drug effects were involved. The effects of order are not significant at the 20 % level. The subject effects are significant at the 1 % level and account for the major part of the obtained variance. Figure 2 graphically shows the similarity between the placebo curves for each of the three tests. This indicates that adaptation does not occur for the conditions under which these experiments were performed. Therefore there is not enough variation between repeat placebo tests in the same individuals to account for the differences found between placebo and drugs in other experiments.

CONCLUSIONS

1. In 24 subjects the GSR levels were recorded following injections of atropine, pilocarpine and placebo in a varied order. The results indicate that the observed drug effects are independent of order of administration.

2. Ten subjects were similarly tested following three placebo injections. The data shows that significant adaptation did not occur between tests.

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