Pharmacology Biochemistry & Behavior, Vol. 6, pp. 235–236.

BRIEF COMMUNICATION

Differences in Placebo Effects[1]

W. L. ISAAC AND W. ISAAC

Department of Psychology, University of Georgia, Athens, GA 30602

(Received 12 August 1976)

ISAAC, W. L. AND W. ISAAC. *Differences in placebo effects.* PHARMAC. BIOCHEM. BEHAV. 6(2) 235–236, 1977. – Constant volume injections of water and saline were evaluated in terms of their effects upon locomotor activity in the rat. Both solutions produced an overall decrease in activity and were found to be nonequivalent in their effect.

Placebo Saline Water Activity

INTRAPERITONEAL injection is probably the most common route of administration of drugs to the rat and the most commonly used solutions in which drugs are dissolved for such injections are water and saline [2]. These solutions seem to be used interchangeably by different investigators, apparently assuming that these vehicles produce no change in behavior by themselves. Data to support such an assumption seem to be lacking.

The present study compared the effect upon locomotor activity of constant volume injections of bacteriostatic water and bacteriostatic normal saline. Thus, the consequence of injection could be evaluated and the differential effects of the two solutions could be compared.

METHOD

Animals

Twelve male albino rats, Sprague-Dawley derived (Southern Animal Farms) were used. They were 20 days of age upon arrival in the laboratory. Food and water were available in the home cage at all times. A 12 hr light-dark cycle was maintained in the colony room.

Apparatus

Each of the subjects was housed in a clear plastic mouse-breeding cage 45 cm long, 24 cm wide, and 15 cm high, with a hardware cloth cover, during activity measurements. Each plastic container was placed in a separate sound treated cubicle that was 83 cm deep, 56 cm wide and 56 cm high and open at one end. Each cubical was lighted with a 20 W fluorescent bulb mounted 29 cm above the floor of the activity chamber, providing approximately 160 ft-c (lux) illumination at the floor of the activity chamber. The twelve cubicles were housed in a sound treated room.

A single infra-red light beam bisected that length of each plastic container about 2 cm above the floor. An infra-red filter covered the photocell making it insensitive to ambient illumination. When the animals broke the beam in their chambers counts were recorded on individual counters in a separate room.

Procedure

Upon arrival in the laboratory each of the 12 animals was placed in an individual cage and given constant access to food and water. Five days were allowed for adaptation to the laboratory and daily handling. They were then placed in the individual activity measurement apparati for two days to allow adaptation to the plastic container and the handling and transport procedures. On the eighth day in the laboratory experimental procedures began.

The three experimental conditions, intraperitoneal injection of 1 ml/kg of bacteriostatic water (Abbott), intraperitoneal injection of 1 ml/kg of bacteriostatic sodium chloride (0.9% Elkins-Sinn), or no injection, were given in five independent sequences. Thus, over a 15 day period five measures of activity were obtained under each experimental condition. All animals were subjected to the same experimental condition on any given day. The animals were weighed every three days and the doses adjusted accordingly.

On the appropriate days the animals were injected before being transported to the activity room in plastic containers. Approximately 10 min elapsed between the injection of the first animal and the beginning of activity measurement. The animals were injected in the same order each day. After being placed in the cubicles, activity measures were obtained consecutively for a 5 min period and two consecutive 30 min periods.

All activity counts were transformed by a square-root transformation ($\sqrt{x + 1}$) as recommended by Edwards [1].

[1] This work was supported by a grant (MH 25164) from the National Institute of Mental Health.

Such a transformation has been previously found to be useful in measuring dose related changes in locomotor activity. Analysis of variance was used to evaluate the effects of the experimental treatment on activity for the first 5 min, the next 30 min period, and the total 60 min period.

RESULTS AND DISCUSSION

Analysis of the data obtained from the first 5 min the animals were in the apparatus, using the interaction between the source of variance of interest and the animals as the error term, yielded only an interaction between the treatments and the replications statistically significant, $F(8,88) = 5.14$, $p<0.01$. As may be seen in Fig. 1, this was due to an elevation of the scores for the two injection conditions on the first day only of each condition. On the basis of previous observations the rapid adaptation to the injection and transport procedures was not unexpected.

During the next 30 min a significant treatment effect was obtained, $F(2,22) = 7.03$, $p<0.01$. When these differences were further evaluated by the Duncan's New Multiple Range Test [1], with alpha set at the 1% level, it was found that the noninjection group differed from both injection groups. The two injection groups also differed significantly from each other. A significant replication effect was obtained, $F(4,44) = 7.14$, $p<0.01$, but the interaction between treatments and replications was not significant, $F(8,88) = 0.72$, $p>0.05$.

For the total 60 min following the first 5 min analysis of variance yielded findings similar to those obtained for the first 30 min. Treatment effects were significant, $F(2,22) = 5.86$, $p<0.01$, as were replication effects, $F(4,44) = 5.65$, $p<0.01$. The interaction between treatments and replications was again nonsignificant, $F(8,88) = 1.16$, $p>0.05$. However, Duncan's New Multiple Range Test, alpha set at 0.01, failed to detect a significant difference between the noninjection condition and the saline injection condition. These two groups did differ significantly from the water injection condition.

It is apparent that intraperitoneal injection of either vehicle, water or saline, produces a change in locomotor activity. Further, the changes are not equivalent, indicating that the solutions produce a change in locomotor activity in addition to the simple effect of injection. Our initial expectation had been that if any change resulted from injection it would be an increase in activity. Such an increase is seen only during the initial 5 min adaptation period of the first replication. The observation that a reduction in activity would result from injection of the solutions was not expected. The finding that water produced a greater decrease in activity than the saline solution was also unexpected.

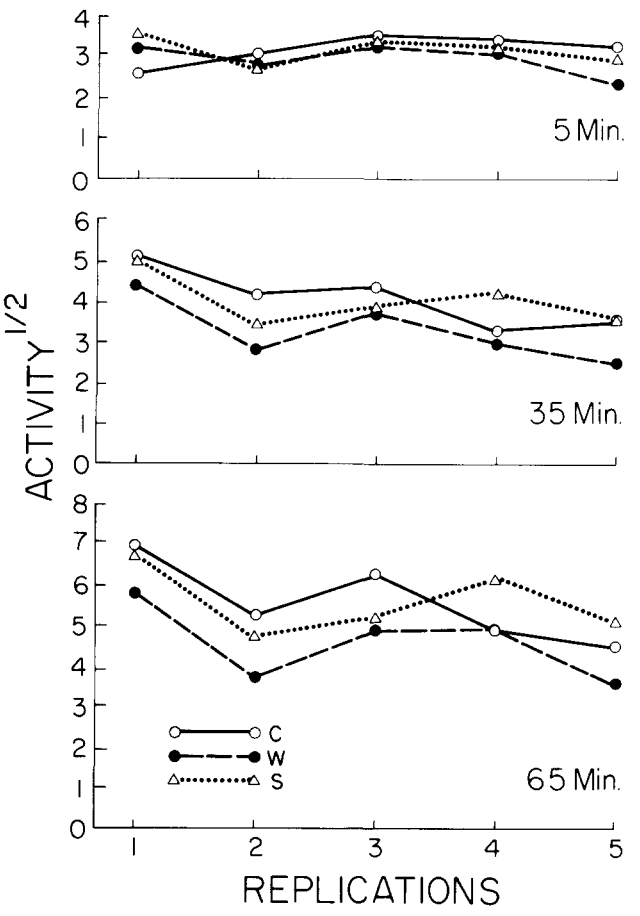

FIG. 1. The effects upon activity of water and saline injections compared with no injection over three time periods.

The present study would serve to emphasize the necessity for placebo injections of the vehicle used in preparing drug solutions under study. It also demonstrates the necessity of adaptation to the injection procedure with the vehicle to be used if such effects are to be eliminated from the outcome of intraperitoneal injections of a drug.

REFERENCES

1. Edwards, A. L. *Experimental Design in Psychological Research*. New York, N.Y.: Holt, Rinehart and Winston, Inc., 1972.

2. Thompson, T. and C. R. Schuster. *Behavioral Pharmacology*. Englewood, New Jersey: Prentice-Hall, Inc., 1968, p. 20.