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Confounding Effects of Procaine Hydrochloride and Adequate Baseline on Conditioned Drinking[1,2]

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WAYNER, M. J. AND S. FRALEY. *Confounding effects of procaine hydrochloride and adequate baseline on conditioned drinking.* PHARMAC. BIOCHEM. BEHAV. **1**(5) 551–556, 1973.–Two experiments were carried out to asses the effects of subcutaneous administration of procaine hydrochloride on the acquisition and extinction of so-called conditioned drinking. The dose and general procedures were the same as that in previous studies on this phenomenon in which the procaine had been administered as a local anesthetic with the hypertonic saline to eliminate noxious stimulation associated with the injection. The data were clear and demonstrate that procaine hydrochloride decreases wheel turning behavior and that the injection procedure by itself decreases drinking and eating under these conditions. In addition drinking, and to a less extent, eating continued to increase under these test conditions for at least 50 days and indicate that baseline conditions must be determined more carefully in the future. Results indicate that procaine hydrochloride decreases motor activity and seems to be unrelated to conditioned drinking under these conditions. If increases in drinking occur following the injection phase of the experiment, such increases represent a return to a normal elevated baseline which appears to be enhanced because of the attenuation of drinking due to the injection procedure.

Motor activity Procaine hydrochloride Conditioned drinking Drinking Eating

IN GENERAL, attempts to condition drinking have not been successful and the data can be interpreted in a variety of different ways [1]. Recently, the results of several reports indicate that drinking in the rat which follows the subcutaneous administration of hypertonic saline can be conditioned to complex external stimuli and that the conditioned drinking is relatively resistant to extinction [4,5,6,]. These data seem to have been confounded by the use of procaine hydrochloride with the subcutaneous administration of hypertonic saline to eliminate noxious stimulation associated with the injection [3,4]. In addition, conditioned drinking does not appear to be a specific response and is only one of several competing activities which seem to be elicited under these conditions [2]. These results were interpreted as indicative of a general state of increased motor excitability. The purpose of the present study was to reexamine this phenomenon under similar conditions and without procaine hydrochloride. Results indicate that procaine hydrochloride decreases wheel turning behavior but has no appreciable effect on water imbibition or food consumption. In addition, the data indicate some important considerations concerning the adequacy of baselines in behavioral testing.

METHOD

Animals

Ten male hooded rats 384–486 g in weight, were selected from our colony and were placed in individual cages with free access to food and water for three weeks prior to the beginning of the experiment.

Procedure

Animals ate Purina lab chow blocks and obtained water by licking ball pointed stainless steel spouts attached to graduated plastic cylinders by rubber stoppers. Graduated cylinders were replenished daily with water at room temperature. At the same time each day water tubes were removed from the home cage for one hr before the animal was placed in the test chamber. Weight, home cage food and 23 hr water intakes were recorded daily throughout the experiment.

The baseline period consisted of 7 days. At the end of the one hour deprivation period animals were weighed and carried to the test room in an opaque plastic box. The test room was illuminated by overhead lights and temperature was maintained at 71 ± 5° F. Background white noise was

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[2] The authors are indebted to Dr. Tibor Palfai who suggested that long baselines be utilized.

provided throughout the session. The test chamber consisted of an individual cage attached to a Wahmann Co. activity wheel and fitted with a water tube identical to that in the home cage. Water spouts protruded one cm into the cage. Each animal remained in the test chamber for 45 min with free access to the activity wheel, water and Purina lab chow blocks. At the end of the session animals were returned to the home cage where they had immediate access to food and water. Number of wheel revolutions, water and food intakes in the test cage were recorded. Food was measured to the nearest tenth of a gram.

The injection period consisted of 7 days. Animals were randomly divided into two groups of five each. The experimental group was injected subcutaneously with 2.5% procaine-HCl in distilled water (w/v). Two 1.0 cc injections were administered under the loose skin of the dorsal thoracic region. The dose and procedures are identical to those of a previous experiment [2]. The control group was injected subcutaneously with the same volume of distilled water. Immediately after injection animals were placed in the carrying box where they remained for one min and were then brought to the test cage. All other conditions were the same as during the baseline period.

The post-injection period consisted of 10 days. Animals received the same treatment as during the baseline period.

These are the procedures which were followed in Experiment 1. In Experiment 2, the procedures were identical except that another ten male hooded rats were selected, 385–429 g in weight, and the baseline and post-injection period was extended to 28 and 21 days respectively.

RESULTS

The results of Experiment 1 are summarized in Figs. 1, 2 and 3 where the mean water intake in ml, mean food consumption in g, and mean number of wheel revolutions during the 45 min test session for the two groups of 5 animals each are presented as a function of days during the course of the experiment. The experimental group means are represented by solid circles connected by a solid line and the control group by solid circles connected by a broken line. The days on which injections were administered are indicated by downward vertical arrows. The data are somewhat perplexing. Apparently, five animals are not an adequate random sample of our rat population, as indicated by the baseline data of Figs. 1 and 3, and large differences between experimental and control animals occurred in water intakes and activity. Both groups ate approximately the same amount of food under these conditions, Fig. 2. The differences are most obvious for water intakes and wheel revolutions on the seven days following injections in Figs. 1 and 3. The injections of both distilled water and procaine hydrochloride seem to depress water consumption during the test session with immediate increases in both groups for five days following the injection period. There were no appreciable decreases in food consumption in Fig. 2 which could be attributed clearly to the injections. Although these results clearly indicate that procaine hydrochloride in quantities which we employed in previous experiments did not increase drinking during the test sessions, the injection procedure did seem to prevent additional increases in water consumption as indicated by an increasing baseline and had no clear effects on food intake; whereas, injections with procaine hydrochloride def-

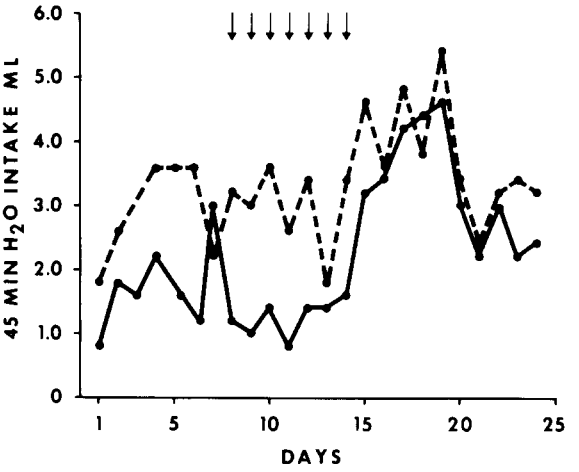

FIG. 1. Mean water intakes in ml for both groups during the 45 min test session plotted as a function of days. Experimental group, solid circles connected by a solid line. Control group, solid circles connected by a broken line. Downward vertical arrows indicate injection days.

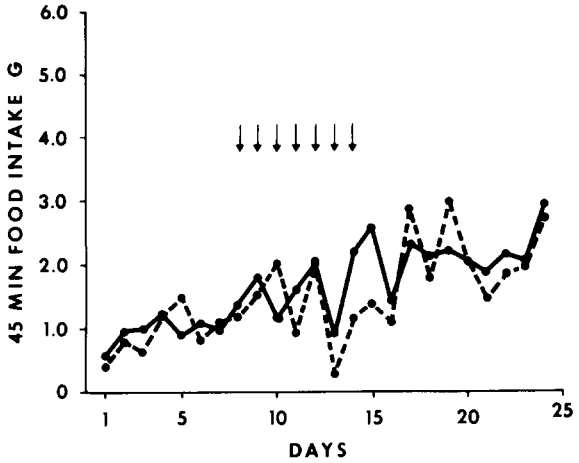

FIG. 2. Same as Fig. 1, except, mean food consumption in g for both groups during the 45 min test session plotted as a function of days.

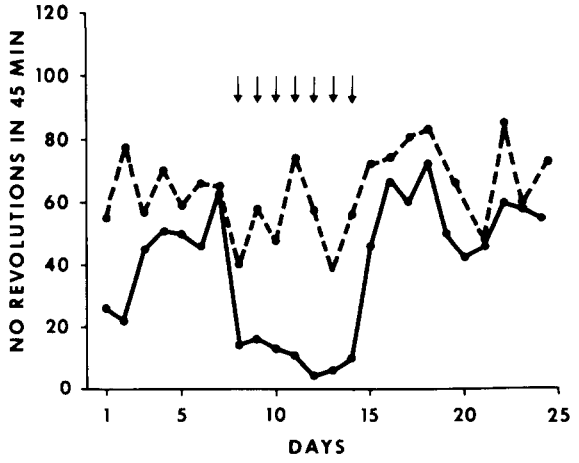

FIG. 3. Same as Fig. 1, except, mean number of revolutions for both groups during the 45 min test session plotted as a function of days.

initely decreased wheel turning behavior. Consequently, the experiment was repeated with the establishment of more adequate baselines for each measure before the injections were administered.

The major results of Experiment 2 are summarized in Figs. 4, 5 and 6 where the mean water intakes in ml, food consumption in g and wheel turns in revolutions for both groups in the 45 min test sessions are presented as a function of days during the course of the experiment. The downward pointing arrows indicate the days on which animals were injected. The experimental group means are indicated by solid circles connected by a solid line and the control group by solid circles connected by a broken line. In this experiment the two groups appeared to be similar in terms of all three variables. The injection procedure produced an obvious decrease in water intakes and a slight but apparent decrease in food consumption. There was an obvious decrease in wheel turning behavior due to the injection procedure which was much greater and more dramatic in the experimental group. These data confirm the results of Experiment 1 and demonstrate that the injection procedure in the control group produces a clear decrease in water imbibition and smaller decreases in food consumption and wheel turning behavior. Procaine hydrochloride has a differential effect and reduces wheel turning behavior drastically under these conditions.

There was no evidence of increased water imbibition due to the procaine hydrochloride. As a matter of fact, there might have been a differential decrease due to the procaine hydrochloride in Experiment 1, Fig. 1, and then a return to an elevated baseline comparable to the control group. The effect is not as great but similar in Experiment 2 as illustrated by the intakes on Days 34, 35, 36 and 37 in Fig. 4.

The increases in baseline during the course of Experi-

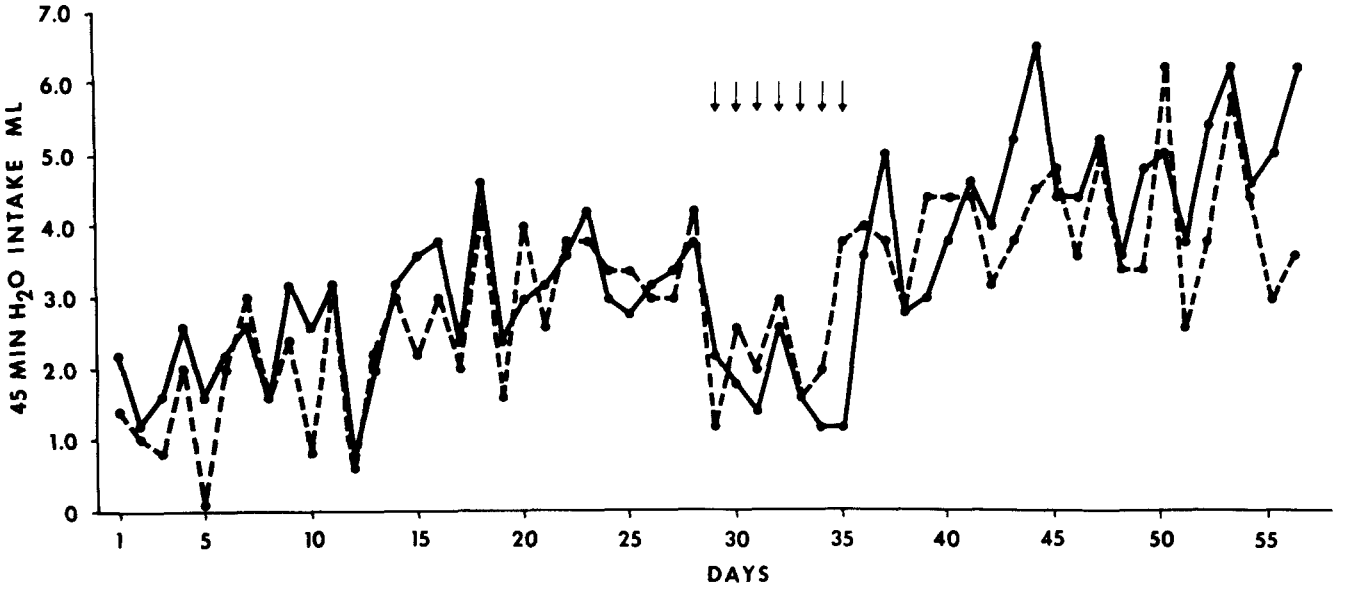

FIG. 4. Same as Fig. 1.

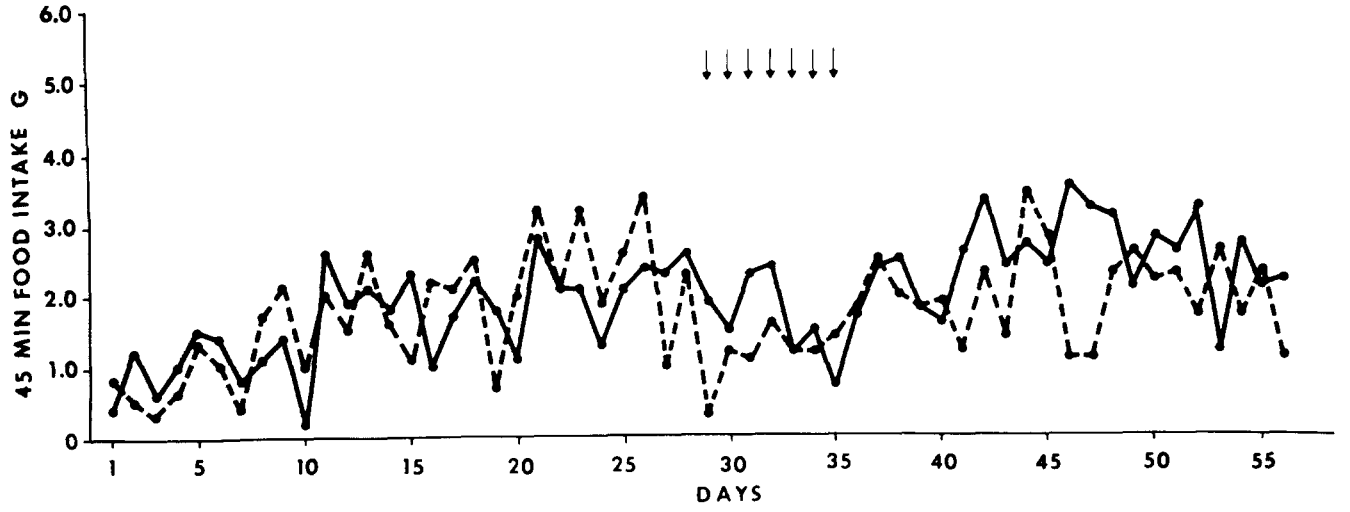

FIG. 5. Same as Fig. 2.

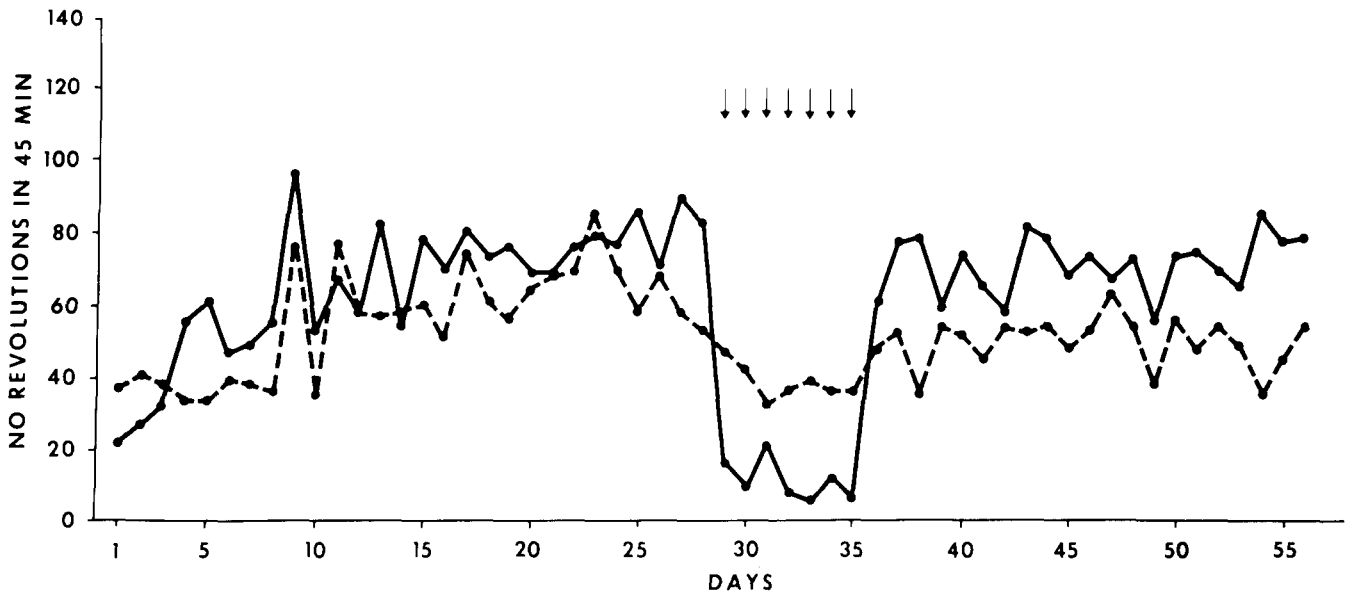

FIG. 6. Same as Fig. 3.

ment 2 can be observed more readily in Figs. 7, 8 and 9 where the data have been rendered less variable by averaging. Moving averages were calculated according to:

$$S(u) = 1/k \sum_{j=u}^{k+u-1} x_j$$

where x_1, x_2 · · · are the daily sequential means and $u = 1, 2, \cdots$ and $k = 8$ and the sequence S is formed on the basis of the sum of k consecutive terms. These sequences are illustrated in Figs. 7, 8 and 9 where the downward vertical arrow indicates the change from pre to post injection. The data of the 7 injection days were not included in the averaging procedure. The increasing trend in water consumption from about 2 ml to 5.5 ml over the 56 day period is clearly evident. A similar trend of increasing food consumption to 3 g can be observed in Fig. 8. An increasing trend in wheel turning behavior occurred only over the first 10 days and initial differences between the two groups were maintained throughout the experiment.

There were no significant changes in 24 hour water intakes or food consumption or body weights which could be attributed to the injections. As the moving average data indicated a plateau effect, the experiment was terminated at the end of 56 days.

DISCUSSION

These results indicate unequivocally that procaine hydrochloride under present experimental conditions, similar to those of a previous study, do not produce conditioned drinking. If anything, the drug plus the injection procedure reduces water intake, food consumption, and a definite decrease in wheel turning behavior occurs. Therefore, these data on procaine hydrochloride provide no special support for any other alternate explanation, including the poisoning hypothesis [3], for our previous results. However, the gradual but significant increases in both water imbibition and food consumption over a relatively long period under these conditions and the decrease due to the injection procedure are factors which must be accounted for in any interpretation of the data. It is now obvious that the baseline of our previous study [2] was not completely adequate and that the increases in water and food intakes following the decreases due to the injection procedures might represent a return to a normally elevated baseline as indicated in Fig. 7.

Because of the decrease in wheel turning behavior due to the procaine hydrochloride, it seems reasonable to assume that the drug decreases motor activity by some general depressant action on neural excitability. Such an interpretation agrees with the previous findings that procaine hydrochloride decreases the salt arousal of drinking [7, 8]. As conditioned drinking and salt arousal of drinking have been related to an increase in motor excitability [2], procaine hydrochloride would be expected to attenuate drinking under both sets of conditions. The procaine injections might suppress the increase in neural excitability which seems to be increasing from day to day in the experimental test situation. In the control group, a similar effect might be produced by inhibition of the same mechanism due to the noxious stimulation associated with the injection.

The so-called conditioned drinking of previous reports which appears to be resistant to extinction might possibly be an artifact due to a normal increase in baseline under these conditions. The drinking and eating might be attributed to an increase in motor activity which results from an increase in the efficiency of the unconditioned environmental stimuli to produce a nonspecific increse in motor excitability. Such an interpretation also explains the nonspecificity of drinking under these conditions [2]. Hypertonic saline when administered not only increases drinking but also the readiness to respond to other types of stimulation. Therefore, it seems unlikely that specific conditioned drinking in a classical sense or as some type of aversion to poisoning has been demonstrated beyond a reasonable doubt.

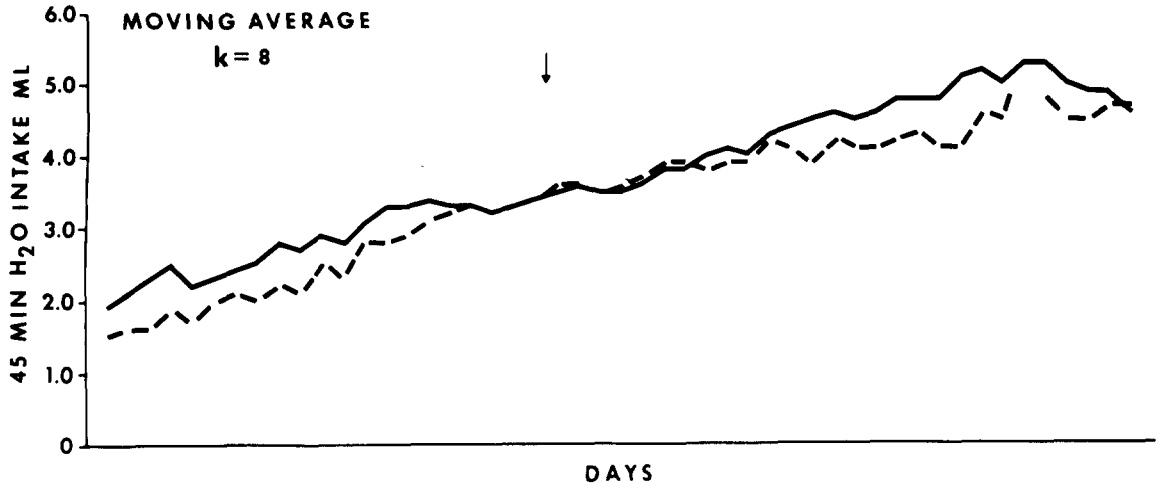

FIG. 7. Moving average sequences, k = 8, calculated for the data in Fig. 4.

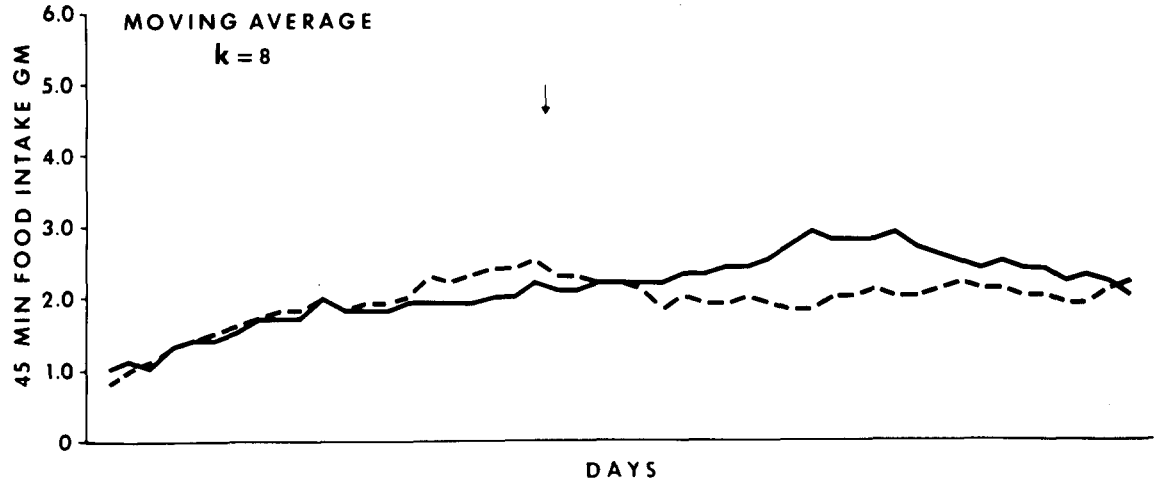

FIG. 8. Moving average sequences, k = 8, calculated for the data in Fig. 5.

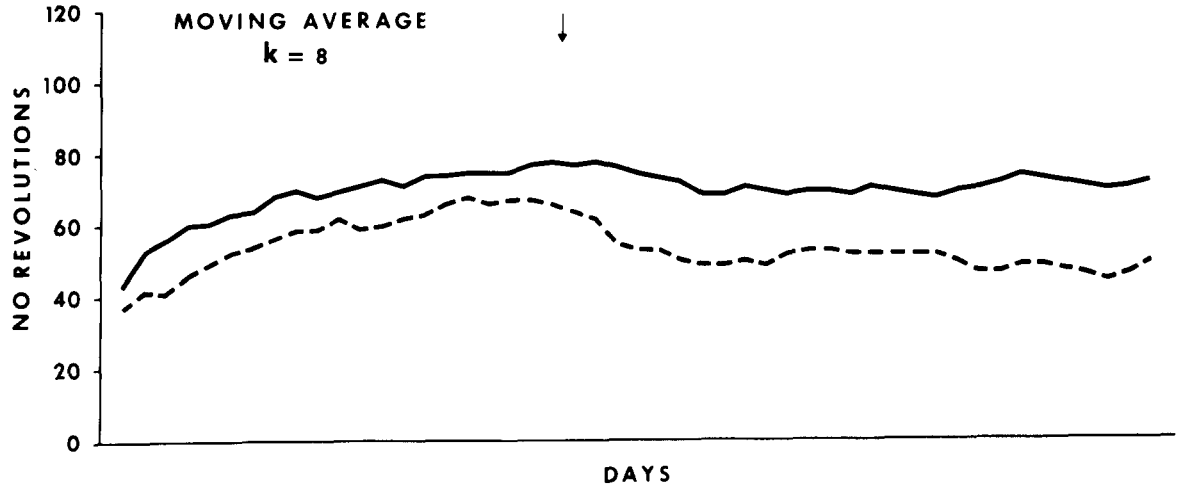

FIG. 9. Moving average sequences, k = 8, calculated for the data in Fig. 6.

REFERENCES

1. Cravens, R. W. and K. E. Renner. Conditioned appetitive drive states: Empirical evidence and theoretical status. *Psychol. Bull.* **73:** 212–220, 1970.
2. Cott, A., M. J. Wayner and J. Millner. Conditioned drinking: A specific or nonspecific response. *Physiol. Behav.* **9:** 219–227, 1972.
3. Mineka, S. M., E. P. Seligman, M. Hetrick and K. Zuelzer. Poisoning and conditioned drinking. *J. comp. physiol. Psychol.* **79:** 377–384, 1972.
4. Seligman, M. E. P., S. Mineka and H. Tillit. Conditioned drinking produced by procaine, NaCl, and angiotensin. *J. comp. physiol. Psychol.* **77:** 110–121, 1971.
5. Seligman, M. E. P., C. E. Ives, H. Ames and S. Mineka. Conditioned drinking and its failure to extinguish: Avoidance, preparedness, or functional autonomy. *J. comp. physiol. Psychol.* **71:** 411–419, 1970.
6. Wayner, M. J. and A. Cott. Conditioned drinking and the effects of saccharin on its recovery after lateral hypothalamic lesions. *Physiol. Behav.* **7:** 201–206, 1971.
7. Wayner, M. J., D. Gawronski and R. Mueller. Effects of procaine-HCl on the salt arousal of drinking. *Physiol. Behav.* **2:** 249–254, 1967.
8. de Wied, D. Effect of autonomic blocking agents and structurally related substances on the salt arousal of drinking. *Physiol. Behav.* **1:** 193–197, 1966.