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Effects of a Fixed Time Schedule and Body Weight on Ethanol Self-Administration

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OEI, T. P. S. AND G. SINGER, *Effects of a fixed time schedule and body weight on ethanol self-administration.* PHARMAC, BIOCHEM. BEHAV. **10**(5)767–770, 1979.—Two experiments are reported. The first study showed that the patterns of ethanol acquisition using the schedule induced self-injection paradigm, are more similar to those of heroin than methadone or nicotine but dissimilar to that of d-amphetamine. The second study showed that ethanol intake once established was not maintained by removal of the schedule. The results also showed that schedule induced behavior may be stressful as indexed by a significant increase of plasma corticosterone levels.

Ethanol Schedule induced Self-administration Corticosterone

MOST studies of ethanol self-administration have used an oral route employing drinking bottles. In a recent review, Meisch [4] showed the difficulties encountered using this procedure. Recently, a new method for inducing voluntary self-administration of drugs using a schedule induced self-injection (SISI) paradigm, which overcomes the difficulties reported, has been developed for rats [3, 6, 7, 8]. Employing the SISI paradigm in rats, different rates and patterns of acquisition for nicotine, methadone, heroin and d-amphetamine were reported. The patterns of acquisition of d-amphetamine, methadone and nicotine at 100% body weight condition without a fixed time 1 minute (FT1) food delivery schedule were very low and similar for all these drugs. At 80% body weight without a fixed time 1 minute (FT1) food delivery schedule, rats self-injected more heroin than methadone and nicotine. On the other hand, rats in this condition self-injected more d-amphetamine than saline controls or any of the other drugs studied. However, at 80% body weight and a FT1 food delivery schedule condition rats further increased their rate of self-injection for heroin, methadone and nicotine; but the rate of acquisition of heroin and methadone was similar and the rate for amphetamine was lower than without the schedule.

These findings lead to the conclusion that an interaction of environmental, nutritional and pharmacological factors is important for voluntary self-administration of high doses of nicotine and methadone, whereas for heroin, the pharmacological properties of the drug alone are sufficient to induce high rates of self-administration. However, in the case of d-amphetamine, an interaction of nutritional and pharmacological properties of the drug is the critical variable. These findings thus show that different drugs interact differently with environmental and nutritional factors to produce a differential pattern of rate of acquisition of drugs. Understanding the pattern of the rate of acquisition of drug can be used to reclassify drugs and can also provide an alternative approach to the treatment of human addiction.

The present experiments were designed to study the pattern of acquisition and maintenance of ethanol using the SISI paradigm.

Falk *et al.* [1] reported that schedule-induced polydipsic rats developed chronic hypertension. The general findings of Falk *et al.* [1] suggest that 'intermittent-feeding' schedule may be stressful. In order to test this hypothesis, the plasma corticosterone levels were also measured.

EXPERIMENT 1

In this experiment, the rate of self-injection of ethanol at 100%, 80% body weight, and 80% body weight with a food delivery schedule was compared to that of saline controls under similar conditions.

METHOD

Animals

Forty-three naive male Wistar albino rats weighing approximately 350 g were used. All animals were housed individually in a temperature controlled room with a 12 hr light/dark cycle. Food and water were available ad lib. In experiments requiring rats at 80% of their body weight, these were reduced prior to surgery and then maintained at that weight, with water available ad lib.

Apparatus

The experimental chamber was a modified Skinner box (35×32×32 cm) with a bar and food pellet dispensing unit attached to one side of the walls. The bar was situated 5 cm and the pellet dispensing unit 3 cm from the grid floor. The bar operated a syringe infusion pump (Sage instruments, model 341) which delivered 0.07 ml of ethanol solution or saline when triggered. A timing device set for a fixed interval of 5 sec was incorporated into the drug delivery system so that any further bar presses by the animals during the 5 sec infusions were not rewarded with drug or saline injections. Cumulative records were used to record the number of bar

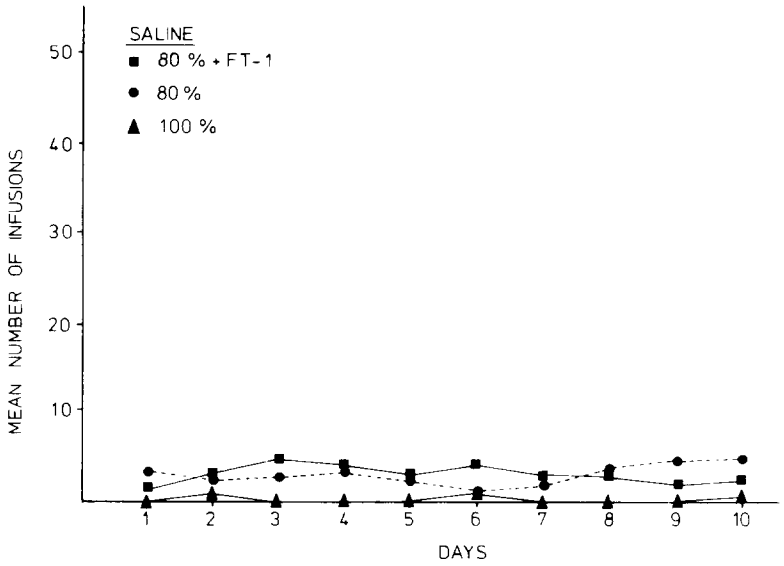

FIG. 1. Mean number of saline infusions for the three treatment groups over 10 days.

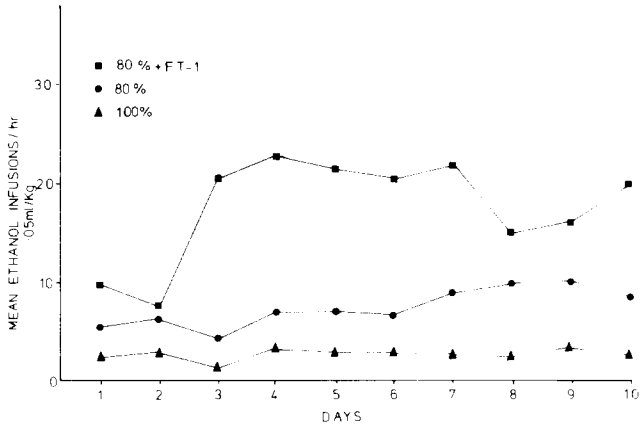

FIG. 2. Mean number of ethanol infusions (0.05 ml/kg/infusion) for the three treatment groups over 10 days.

presses and infusions during test sessions. Noyes food pellets (45 mg) were delivered regularly, one each minute, to the animal when the fixed-time 1 min (FT1) schedule was operating.

Drugs

99.5% ethyl ethanol (CSR Chemical Co.) was prepared for intravenous administration prior to each test session by mixing it in 0.9% sterile saline at a dose of 0.05 ml/kg/infusion. The concentration of ethanol was 19.9%. The anaesthetic used for the surgery consisted of a combination of pentobarbital sodium and chloral hydrate. The solution was injected intraperitoneally.

Procedure

Animals were weighed, anaesthetized and cannulae of sp. 28 polythene tubing were implanted into the jugular vein. The cannulae were maintained in position by leather jackets worn by each animal. The cannulae were connected to a flexible swivel system allowing each animal relatively free movement at all times. Following recovery from surgery (3 days), the animals were placed in the Skinner box for 1 hr/day testing sessions for 10 consecutive days at the same time each day. Each session began by priming the animal with an initial dose of ethanol or saline.

Biochemical Assay

Immediately after the last testing session, each animal was decapitated. Blood from its cervical wound was collected for determination of plasma corticosterone (11-OHCS) levels. Corticosterones were assayed in the 50 μl aliquot of plasma by competitive protein binding using a modified version of Murphy's [5] assay procedure.

RESULTS

The overall mean infusion/hr/day for the three groups self-injecting saline and ethanol are plotted in Fig. 1 and Fig. 2 respectively.

A two-way ANOVA with repeated measures over 10 days was first applied to the ethanol data to test for the effect of schedules on the rate of acquisition of ethanol. The results of ANOVA showed significant main effects of schedule conditions (SC), $F(2,21)=47.22$, $p<0.001$, days (D), $F(9,189)=1.74$, $p<0.05$ and for interaction of schedule and days, $F(18,189)=1.71$, $p<0.01$. These results suggest that rats under different schedule conditions self-injected significantly different amounts of ethanol and at significantly different rates. Post hoc Scheffe analysis showed that the mean of ethanol intake through self-injection over the 10 day period for rats under the 80% body weight and FT1 condition was higher than the mean of ethanol intake of rats under the 80% body weight alone ($p<0.05$) which in turn was higher than that for rats at 100% body weight ($p<0.05$). This pattern can be clearly seen in Fig. 2.

In order to compare the data of ethanol and saline, a two-way ANOVA was applied to the combined data. The statistical results revealed significant main effects for drugs,

TABLE 1

MEAN PLASMA 11-OHCS LEVELS (μG/100 ML) AT THE END OF THE TENTH TESTING SESSION FOR ETHANOL AND SALINE UNDER THE EXPERIMENTAL CONDITIONS

	100% Body Weight			80% Body Weight			80% Body Weight + FT1		
		Mean	SD		Mean	SD		Mean	SD
Saline	(n=6)	12.67	10.2	(n=5)	9.80	8.8	(n=6)	14.10	5.9
Ethanol	(n=8)	22.0	10.6	(n=8)	57.25	21.5	(n=7)	58.14	17.5

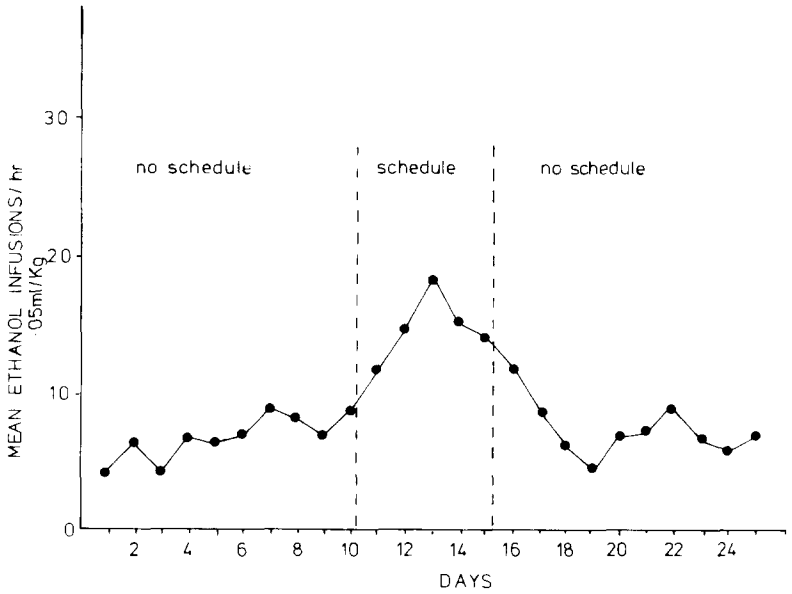

FIG. 3. Mean number of ethanol infusions (0.05 ml/kg/infusion) for the 80% body weight animals over 25 days under a FT1 schedule or no schedule conditions.

$F(1,37)=66.52$, $p<0.001$, for schedule conditions, $F(2,37)=34.04$, $p<0.01$, and for interaction of drugs and schedule conditions, $F(2,37)=19.15$, $p<0.01$, suggesting that overall rats self-injected more ethanol than saline and that the rate of ethanol self-infusion was dependent on the schedule conditions. Scheffe post hoc analysis revealed that rats at 80% body weight conditions with or without a FT1 schedule showed significantly higher rates of self-injection when ethanol rather than saline was available.

The mean plasma 11-OHCS levels (μg/100 ml) for the saline and ethanol groups over the three schedule conditions are shown in Table 1. Three samples of the blood had to be excluded from the statistical analysis because they were contaminated during the biochemical analysis. A two-way ANOVA revealed main effects for drugs, $F(1,34)=77.3$, $p<0.01$, for schedule conditions, $F(2,34)=4.05$, $p<0.05$, and for the interaction between Drugs and Schedule Conditions, $F(2,34)=4.45$, $p<0.05$. This suggests that plasma corticosterone level was dependent on the Drugs and Schedule Conditions. Post hoc Scheffe revealed that only rats in the 80% and 80%+FT1 Conditions showed an increase in plasma 11-OHCS levels (see Table 1).

DISCUSSION

The findings that the rate of acquisition of ethanol for the 100% body weight condition was not significantly different from the three saline groups suggest that ethanol under the 100% body weight condition is not reinforcing. However, at 80% body weight condition the rate of acquisition was increased significantly suggesting the nutritional factor interacts with ethanol to affect the sensitivity of the pharmacologic of ethanol. When a FT1 food delivery schedule was introduced, the rate of acquisition of ethanol was increased even higher suggesting a FT1 schedule is an important enviromental variable in ethanol self-administration. This very high rate of acquisition of ethanol is due to the interaction of schedule, pharmacological properties of ethanol and nutritional factors. This conclusion is consistent with the results of previous findings using this paradigm for nicotine, methadone, and heroin [7].

Plasma 11-OHCS results showed that only rats in the high self-intake groups (i.e. 80% and 80%+FT1) had significant increase of plasma 11-OHCS. These findings thus show that schedule induced self-injection is stressful only if combined with ethanol intake. This interpretation is consistent with the findings of Falk [1] on schedule induced chronic hypertension.

EXPERIMENT 2

Results from Experiment 1 showed that at 80% body

weight condition, a FT1 food delivery schedule is an important variable for the high rate of acquisition of ethanol self-administration. The question whether a FT1 food delivery schedule is important in the maintenance of ethanol self-administration is unclear. The aim of this study was to test the effect of the removal of the schedule on the maintenance of ethanol self-administration using a within subjects ABA design.

METHOD

Animals

Seven animals were used. They were reduced to 80% body weight prior to surgery and then maintained at that weight throughout the experiment. The holding conditions were the same as in Experiment 1.

Procedure

The apparatus and the procedures were similar to that used in Experiment 1 except that in this experiment animals were tested for 25 days consecutively, i.e. they were tested for 10 days without a FT1 food delivery schedule, 5 days with the schedule and then another 10 days without the schedule. The dose level for the ethanol was the same as in Experiment 1.

RESULTS

The present analysis was based on data of 6 rats. The mean number infusions for the animals over the 25 days period are plotted in Fig. 3. The statistical analysis used was planned contrasts on repeated measures [2]. Two planned contrasts were used. The first contrast compared the data from the first 10 days with the subsequent 5 days. This was to test for the effect of introduction of a schedule on the rate of acquisition of ethanol self-administration. The second contrast compared the data from the 5 days with a FT schedule and the last 10 days without the schedule. This was to test for the effect of removal of the schedule on the maintenance of ethanol self-administration.

The results of the planned contrasts showed that the first contrast was significant, $F(1,15)=21.89$, $p<0.01$, suggesting that introduction of a FT1 food delivery schedule significantly increased ethanol intake. This can be clearly seen in Fig. 3. Similarly, the results of second contrast were also significant, $F(1,15)=18.00$, $p<0.01$, indicating that removal of the FT1 schedule significantly decreased ethanol self-administration.

DISCUSSION

The results of the present experiment showing that the introduction of a FT1 food delivery schedule increased the rate of ethanol acquisition is consistent with the results of Experiment 1. The removal of the schedule caused a corresponding decrease in the rate of ethanol self-administration. This suggests that not only is the schedule important in the acquisition phase of ethanol intake through self-administration but also in the maintenance phase of ethanol intake. It is interesting to note that during the acquisition phases, rats showed little individual differences in the rates of self-administration of ethanol. However, there were considerable individual differences during the maintenance phase. In this phase, the rates of ethanol self-infusion of all rats except one only returned to the 'initial' levels when schedule was not operating. However, the rate of ethanol self-infusion of one rat increased with days. At the 25th day of the experiment, the rat was self-injecting at the rate of 276 infusions/hr whereas the average rate of self-injection for the other 6 rats was 8 infusions/hr.

When the data from this rat was included in the analysis no significant difference was observed between the conditions where a FT1 schedule was and was not operating. This was due to an increase in error variance from 6.72 to 548.1. With this increase in error variance, it is not surprising that no significant differences were observed.

GENERAL DISCUSSION

The patterns of the self-infusion of ethanol using the SISI paradigm shows a step-wise increased pattern of ethanol intake with 80% body weight condition and 80% body weight+FT1 condition. This pattern of ethanol acquisition is similar to that reported for heroin [6,7]. The intake pattern of rats in the 80%+FT1 condition for ethanol, however, is similar to that of methadone but dissimilar to that of d-amphetamine [8].

The present findings show that the ethanol intake pattern, once established, is not maintained when the schedule is removed. This environmental factor may not be a critical variable in the maintenance of ethanol intake. Nutritional or biological factors may be critical. This is consistent with clinical observation in humans, where chronic alcohol intake is generally associated with nutritional deficit. Individual differences during the maintenance phase which showed that one rat became a 'chronic alcoholic' by steadily increasing ethanol intake suggest individual differences in the predisposition to ethanol intake.

We have shown that a FT1 food delivery schedule in combination with ethanol at 80% body weight may be stressful for the animal as indexed by increases in plasma 11-OHCS levels.

REFERENCES

1. Falk, J. L., M. Tang and S. Forman. Schedule-induced chronic hypertension. *Psychosom. Med.* **39**: 252–263, 1977.
2. Grant, D. A. Analysis of variance tests in the analysis and comparison of curves. *Psychol. Bull.* **53**: 141–154, 1956.
3. Lang, W. J., A. A. Latiff, A. McQueen and G. Singer. Self-administration of nicotine with and without a food delivery schedule. *Pharmac. Biochem. Behav.* **7**: 65–70, 1977.
4. Meisch, R. A. Ethanol self-administration: infrahuman studies. *Adv. Behav. Pharmac.* **1**: 35–84, 1977.
5. Murphy, B. E. P. Some studies of protein-binding steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. *J. Clin. Endocr. Metab.* **27**: 973–990, 1967.
6. Oei, T. P. S., G. Singer, D. Jefferys, W. Lang and A. Latiff. Schedule induced self-injection of nicotine, heroin and methadone by naive animals. In: *Stimulus Properties of Drugs: Ten Years of Progress*, edited by F. Colpaert and J. Rosecrans, Elsevier: North Holland, 1978.
7. Oei, T. P. S., G. Singer and D. Jefferys. The interaction of a fixed time schedule and body weight on self-administration of drugs. *Psychopharmacologia*, 1979 (in press).
8. Takahashi, R., G. Singer and T. P. S. Oei. Schedule induced self-injection of D-amphetamine by naive animals. *Pharmac. Biochem. Behav.* **9**: 857–861, 1978.