*Pharmacology Biochemistry & Behavior*, Vol. 25, pp. 623–627, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

# Intravenous Self-Administration of Phentermine in Food-Deprived Rats: Effects of Abrupt Refeeding and Saline Substitution

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PAPASAVA, M., G. SINGER AND C. L. PAPASAVA. *Intravenous self-administration of phentermine in food-deprived rats: Effects of abrupt refeeding and saline substitution.* PHARMACOL BIOCHEM BEHAV **25**(3) 623–627, 1986.—The objectives in these experiments were to determine the effects on intravenous phentermine self-administration of abrupt refeeding and of saline substitution in food-deprived rats. In Experiment 1, 32 naive rats, reduced to 80% free-feeding weight (FFW), were assigned randomly to four equal-sized groups. Two groups self-administered phentermine, the other two saline over two consecutive six-day phases. In phase 1, all animals were maintained at 80% FFW, while in phase 2, one phentermine- and one saline-reinforced group was abruptly food satiated. Results showed that phentermine self-administration was suppressed. In Experiment 2, another 32 naive rats were assigned to four equal-sized groups and tested over two consecutive six-day phases. Two groups were FF, the other two were reduced to 80% FFW. In phase 1, a group of FF and 80% FFW animals self-administered phentermine, the other two saline, while in phase 2, treatments were reversed: previously phentermine-reinforced animals self-administered saline and vice versa. Findings showed that 80% FFW rats rapidly discriminate the introduction or removal of phentermine and alter responding accordingly, while FF animals respond at low rates throughout.

Drug dependence Anorectics Food deprivation Abrupt refeeding Saline substitution Rats Extinction

FOOD deprivation has been shown to potentiate intravenous self-administration of psychomotor stimulants in animals [2, 7–9, 20]. More recently, we have shown that intravenous self-administration of phentermine (Duromine), a clinically used anorectic related to amphetamine in structure [21] and having a similar mechanism of action [5, 6, 22], is also potentiated by food deprivation [11–13]. The findings show that when the degree of food deprivation is held constant and graded doses of phentermine are made available in an ascending order, the rate of phentermine self-administration is an inverted-U shaped function of dose [12]. When dose is held constant and animals are subjected to increasing degrees of food deprivation and consequently body weight loss, the rate of self-administration is an inverse function of body weight levels [11]. These findings, it is argued, constitute support for the proposition that the reinforcing efficacy of phentermine, and probably all anorectics having a mechanism of action similar to amphetamine, is amplified following a synergistic interaction between physiological variables arising as a consequence of food deprivation, and the pharmacological properties of the drug [8–13].

If this proposition is correct and the reinforcing efficacy of phentermine is indeed modifiable, then it should also be possible to demonstrate that animals which self-inject phentermine at high rates when food-deprived (FD) and at reduced body weight, will decrease responding when they are food-satiated. The present experiment was a test of this hypothesis. In this experiment, the objective was to determine the relative contribution of deprivational state on the maintenance of phentermine self-administration. Similar investigations have shown that while abrupt refeeding rapidly suppresses drug-reinforced responding [1,8], gradual refeeding results in the persistence of the behaviour [1].

## EXPERIMENT 1: PHENTERMINE SELF-ADMINISTRATION IN REDUCED AND RECOVERING BODY WEIGHT ANIMALS

### METHOD

*Animals*

Thirty-two experimentally naive male Wistar albino rats (Monash University, Clayton, Vic., Australia) weighing between 380 and 400 g were used. The animals were housed individually in a temperature controlled room (21±1°C) with a 12-hr light/dark cycle (lights on 0600–1800 hr). Water was available ad lib in the home cages but not during testing. Feeding conditions are described in the Procedure section.

The experimental chamber was a modified operant box (35×32×32 cm) with a bar and pellet dispensing unit at-

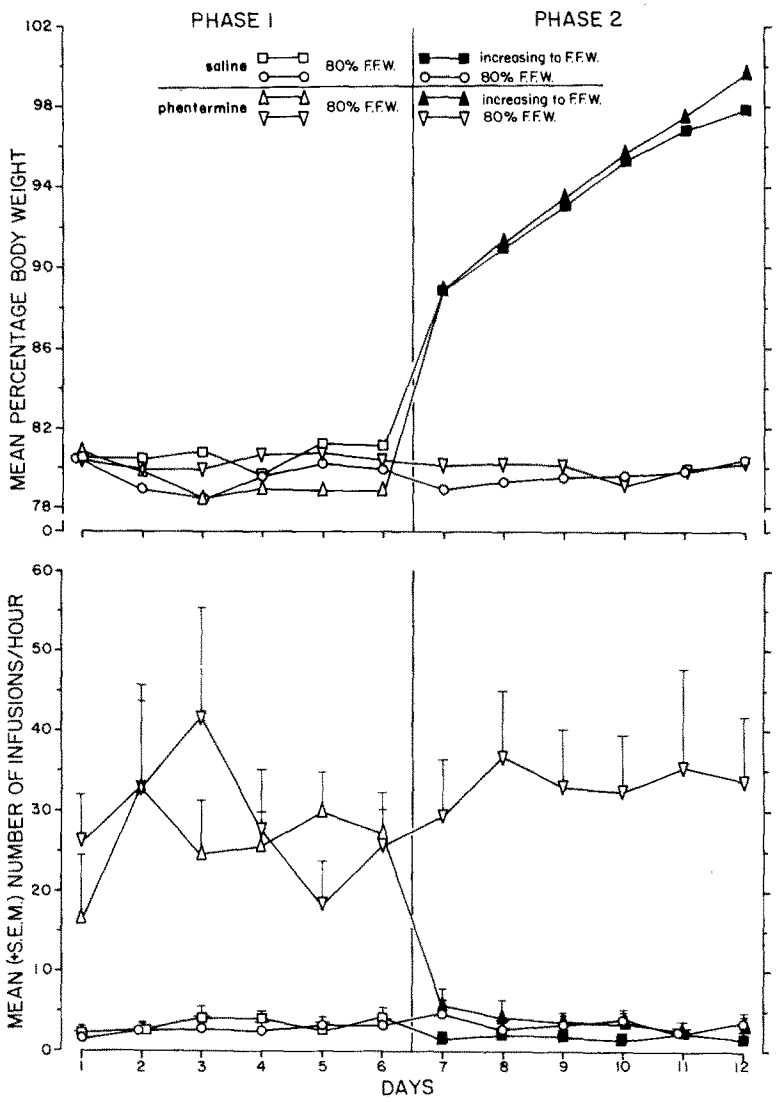


FIG. 1. Mean (+S.E.M.) number of infusions (lower panel) and corresponding percentage body weights (upper panel) over consecutive 1-hr/day test sessions for the two saline- and two phentermine-reinforced groups of animals.

tached 5 and 3 cm respectively to one wall above a grid floor. The bar, when triggered, activated a syringe infusion pump (Sage Instruments, Model 341) which delivered 70 $\mu$l phentermine solution or saline, depending upon the experimental condition. A timing device set for a fixed interval of 5 sec was incorporated into the drug delivery system such that any bar presses by the animal during this period were recorded but produced no programed consequences. Although all bar-presses were recorded, only bar-presses which actually produced an injection of either drug or saline (i.e., infusions) were subjected to statistical analysis and presented in the figures. The number of infusions and bar-presses during the experimental session was recorded on a cumulative recorder.

*Procedure*

All animals were reduced to 80% of their FFW, anaesthetized, and cannulae of SP28 polythene tubing were implanted into the jugular vein, and maintained in position by light weight leather jackets fitted to each animal. The cannulae were connected to a flexible swivel system which allowed relatively unrestricted movement. Following a three-day recovery period from surgery, animals were assigned randomly to four equal-sized groups. Two groups of animals self-administered phentermine, the other two saline, over two phases of six consecutive 1 hr/day testing sessions at the same time each day. In phase 1, all animals were maintained at 80% FFW, while in phase 2 one phentermine- and one saline-reinforced group of animals were abruptly returned to ad lib feeding. The other two groups were maintained at 80% FFW.

*Drugs*

Phentermine hydrochloride (Riker Laboratories Pty. Ltd., Australia) was prepared daily for IV administration in sterile physiological saline at a dose of 0.25 mg/kg/infusion (doses refer to the salt). Drug concentrations were calculated

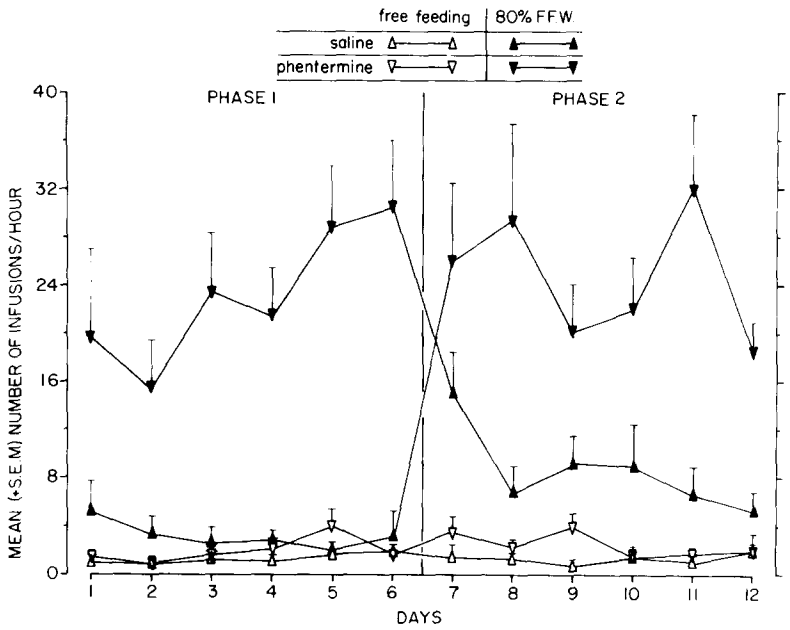


FIG. 2. Mean (+S.E.M.) number of infusions over consecutive 1-hr/day test sessions for the two saline/phentermine- and two phentermine/saline-reinforced groups of animals under free-feeding and 80% free-feeding weight conditions.

daily in phase 2 in order to maintain the dose specified as the animals' weights increased. The anaesthetic used for surgery was pentobarbitone sodium (Sagital, May and Baker Pty. Ltd., Australia) and was injected IP at a dose of 60 mg/kg (60 mg/ml:1 ml/kg).

### RESULTS AND DISCUSSION

The mean number of infusions and percentage body weights over the twelve consecutive test sessions for the two saline- and two phentermine-reinforced groups of animals are presented in Fig. 1.

A four-way ANOVA with two repeated measures (phases and days) was applied to the infusion data in order to determine main effects for drug conditions (DC), feeding conditions (FC), phases (PH) and days (DS), as well as interactions between them.

Significant main effects were found for DC, $F(1,28)=32.457$, and $p<0.05$, and for FC, $F(1,28)=5.406$, $p<0.05$. The significant interaction between DC and FC, $F(1,28)=4.825$, $p<0.05$, suggests that overall, phentermine-reinforced animals maintained at reduced body weight across both phases had the highest infusion rate (see Fig. 1).

There was a significant main effect for PH, $F(1,28)=8.850$, $p<0.05$, and there were significant interactions between DC and PH, $F(1,28)=7.609$, $p<0.05$, and between FC and PH, $F(1,28)=22.72$, $p<0.05$. The significant three-way interaction between DC, FC and PH, $F(1,28)=16.292$, $p<0.05$, suggests that when phentermine-reinforced animals were returned to ad lib feeding in phase 2, responding was attenuated significantly. There were no other significant effects.

In summary, when 80% FFW animals were reinforced with infusions of phentermine, their response rates were significantly higher than were those of saline-reinforced animals. When one group of phentermine-reinforced animals was returned to ad lib feeding, however, responding was attenuated significantly (see Fig. 1).

## EXPERIMENT 2: CROSS SELF-ADMINISTRATION OF PHENTERMINE AND SALINE IN FREE-FEEDING AND FOOD-DEPRIVED ANIMALS

In the previous experiment, it was shown that phentermine self-administration is suppressed by food satiation, even after animals self-administer high levels of phentermine for six days. The present experiment was undertaken to determine the extinction pattern of phentermine self-administration. Thus far, two basic approaches have been utilised to investigate the extinction of stimulant self-administration in animals. In the first approach, the effect upon previously drug-reinforced responding is determined either by substituting saline for the drug, or by simply discontinuing drug delivery [14–17]. In the second approach, the effects of pretreating subjects with drugs which are thought to block or prevent reinforcement are determined [3, 4, 18, 19, 23, 24]. Findings from both lines of investigation have shown that extinction is characterized by a brief initial burst of increased responding, followed by a cessation of responding. The objective in this experiment was to determine the effect on responding of saline substitution in both FF and FD animals previously reinforced with phentermine.

### METHOD

*Animals*

Thirty-two experimentally naive rats were each surgically implanted with a chronic in-dwelling jugular vein cannula according to procedures described previously.

*Procedure*

Animals were assigned randomly to four equal-sized groups. Two groups of animals were reduced to 80% FFW initially, the other two remained FF. Following recovery from surgery, animals were tested over two phases of six consecutive 1 hr/day testing sessions at the same time each day. In phase 1, one of the FF and one of the 80% FFW groups of animals self-administered phentermine, the other two groups self-administered saline. In phase 2, animals previously reinforced with phentermine were transferred to saline, while the two previously saline-reinforced groups were transferred to phentermine. All other procedural details were similar to those of the previous experiment.

*Drugs*

Phentermine hydrochloride was prepared daily at a dose of 0.25 mg/kg/infusion.

RESULTS AND DISCUSSION

The mean number of infusions over the twelve consecutive test sessions for the two saline/phentermine and two phentermine/saline groups of animals appear in Fig. 2.

A four-way ANOVA with two repeated measures (phases and days) was applied to the data in order to determine main effects for feeding conditions (FC), order of treatment presentations (OP), phases (PH) and days (DS), as well as interactions between them.

A significant main effect was found for FC, $F(1,28)=52.982$, $p<0.05$, indicating that, overall, food-deprived animals had significantly higher rates of self-administration than did free-feeding animals.

There were also significant interactions between OP and PH, $F(1,28)=89.75$, $p<0.05$, and between FC, OP and PH, $F(1,28)=79.964$, $p<0.05$, which indicates that while food-deprived phentermine-reinforced animals reduced responding significantly when transferred to saline, and food-deprived saline-reinforced animals increased responding significantly when transferred to phentermine, the order in which they received the treatment influenced significantly the rate of responding. Inspection of Fig. 2 suggests that saline-reinforced responding was significantly higher in phentermine-experienced animals, than was saline-reinforced responding in drug naive animals. The significant PH × DS interaction, $F(5,140)=2.367$, $p<0.05$, indicates that changes in responding over days differed between phases. This significant interaction most probably pertains to the marked increases and decreases in responding that occurred when the treatments were reversed. There were no other significant effects.

In summary, FD animals reinforced with infusions of phentermine decreased responding significantly when saline was substituted for phentermine, while FD animals reinforced with infusions of saline increased responding significantly when phentermine was substituted for saline. It may be noted, however, that the rate of saline-reinforced responding was significantly lower in drug naive animals than was saline-reinforced responding in previously phentermine-reinforced animals.

## GENERAL DISCUSSION

The results from the first experiment show that while animals self-administer phentermine at high rates when food-deprived and reduced to 80% FFW, responding is rapidly and markedly attenuated following abrupt refeeding: an effect shown previously in rats reinforced with intravenous infusions of cocaine [8] and orally self-administered etonitazine [1]. From Fig. 1, it can be seen that following ad lib feeding for 24 hours, animals regained approximately 10% FFW and reduced phentermine-reinforced responding to 22% of baseline values (baseline is defined as mean response rates in phase 1). By the second day of ad lib feeding, responding was decreased to 16% of baseline values and was not reduced appreciably thereafter, despite successive increments of about 2% FFW/day until animals reached predeprivation weight levels on day 12. Thus, while abrupt refeeding attenuates responding, this attenuation is not characterized by successive decrements in drug intake synchronous with progressive increments in body weight. Rather, responding is attenuated very rapidly and after the second day of ad lib feeding (day 8) is independent of amount of weight regained.

That the suppression of phentermine-reinforced responding is in fact a function of abrupt refeeding and not some other variable(s), is attested to by the data from phentermine-reinforced FD animals maintained at 80% FFW across both phases of this study. These data show that FD animals maintained high rates of responding throughout, and in phase 2, their response rates were significantly greater than both the abruptly re-fed phentermine-reinforced animals, and the appropriate saline-reinforced control group of animals (see Fig. 1).

The present findings, therefore, establish that phentermine self-administration in FD and body weight reduced animals is rapidly and significantly suppressed when these animals are abruptly food-satiated. The failure of animals to maintain responding in the absence of food deprivation indicates that the reinforcing efficacy of phentermine is modifiable by the deprivational state of the animal, and provides further support for the contention that physiological variables arising as consequence of food deprivation interact synergistically with the pharmacological properties of phentermine [11–13].

The findings from Experiment 2 show that FD animals rapidly discriminate between the introduction and the removal of phentermine, and alter responding accordingly. Unlike the findings from previous investigations (e.g., [14–19, 22, 24]), however, extinction of responding is not characterized either by a brief initial increase or a cessation of responding, but rather, is attenuated immediately and maintained at a significantly reduced rate.

From the data presented in Fig. 2, it can be seen that following the substitution of saline for phentermine, responding was decreased to 65% of baseline values in the first test session (day 7) and 29% in the second. Sporadic increases in responding occurred in the subsequent two test sessions (days 9 and 10) but overall, responding was decreased to 37% of baseline values. Although the rate of responding following saline substitution was attenuated significantly, it was nevertheless significantly greater (approximately 2.7 times) than that of drug naive saline-reinforced animals, indicating that in FD animals, responding is difficult to extinguish after exposure to phentermine. This finding is in marked contrast to that from the previous experiment which showed that phentermine-reinforced responding was both more rapidly and more completely suppressed following abrupt refeeding. A comparison of these data reveals that relative to saline sub-

stitution, decreases in responding following abrupt refeeding were: 43% greater in the first test session, 13% greater in the second, and 22% greater overall (see Figs. 1 and 2). Thus, it would appear that the deprivational state of the animal is a more salient variable in the maintenance of self-administration.

The data from this experiment also show that when FD animals previously reinforced with saline were reinforced with phentermine, responding was increased significantly, with virtually immediate establishment of terminal performance. In fact, on the first day of access to phentermine, responding was increased by 805% and overall, was increased by 763% relative to baseline values.

Although the present findings show that animals decrease responding significantly when saline is substituted for phentermine and conversely, increase responding significantly when phentermine is substituted for saline, this effect occurs in FD animals only; FF animals respond at low rates throughout.

In conclusion, the findings from the two experiments reported here, show that phentermine self-administration is potentiated by food deprivation and suggest that a synergistic interaction occurs between physiological variables arising as a consequence of food deprivation and the pharmacological properties of the drug. If either the physiological or the pharmacological component is removed—by food satiation or by saline substitution respectively—then responding is attenuated significantly. However, the attenuation of responding caused by saline substitution is neither as rapid nor as complete as that brought about by food satiation, indicating that the rate of self-administration is most sensitive to changes in the physiological state of the animal.

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