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NO EVIDENCE FOR A CONDITIONED TASTE PREFERENCE IN RATS USING RELIEF FROM MORPHINE WITHDRAWAL AS THE UNCONDITIONED STIMULUS

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ABSTRACT

Studies dealing with morphine's reinforcing properties have revealed an apparent paradox that the drug is a rewarding stimulus in self-administration paradigms, but a punishing agent in the conditioned taste-aversion situation. The present study attempts to demonstrate morphine's rewarding property in the form of a conditioned taste-preference by pairing a novel flavour with injections relieving withdrawal stress in drug-dependent rats. Rats were made morphine dependent by 15 days of twice daily injections at doses escalating from 25 to 150 mg/kg each. High-dose morphine maintenance injections of 150 mg/kg were then paired with a novel coffee-flavoured solution at 24 or 48 hour intervals over a 10 day training period. Subsequent one and two-bottle preference tests showed a resulting learned coffee aversion which was not seen in control animals receiving the same drug injections and coffee exposure without contiguous pairing of the two. Of great interest was a group of dependent animals which were given coffee-morphine pairings while morphine-sated rather than while morphine-deprived and subsequently did not learn a coffee aversion. Thus, morphine injections during withdrawal stress did not confer positive reinforcing properties on associated taste stimuli, but rather produced learned taste aversion, whereas morphine administered in the absence of withdrawal stress was neutral. These data support the view that in either morphine-naive or morphine-dependent animals the drug is not a rewarding stimulus in the context of the conditioned taste-aversion paradigm.

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INTRODUCTION

By definition, a primary reinforcer is a stimulus event which an organism can associate with certain neutral stimuli resulting in the acquisition of secondary reinforcing properties by these previously neutral stimuli. The secondary reinforcers can subsequently either increase or decrease the probability of the response upon which they are contingent, depending upon the nature of the primary reinforcer (either rewarding or punishing). However, morphine along with a few other psychoactive drugs has been shown to act both as a positive reinforcer and as a punishing stimulus, depending upon the nature of the particular paradigm used to evaluate the drug's reinforcing properties (Cappell, LeBlanc and Endrenyi, 1973). Whereas certain paradigms conclude that morphine is a powerful positive reinforcer (Weeks and Collins, 1964), other experimental situations allow for the opposite conclusion (Cappell et al, 1973).

For example, morphine has been shown to act as a positive primary reinforcer for place learning in rats (Beach, 1957). In his study, rats showed a preference for the goal box in which they were habitually injected. This habit persisted long after morphine injections had ceased, illustrating that certain visual stimuli in the Y-maze had acquired secondary reinforcing properties. Beach's (1957) basic experiment has been successfully

replicated by other researchers such as Schwartz and Marchok (1974) and Kumar (1972).

Numerous studies have also illustrated that animals will learn to press a lever for an injection of morphine, whether it is administered intraperitoneally (Nichols, 1965) or intravenously (Weeks, 1964). If an animal will learn to self-administer a drug, this can be taken as evidence that the drug has positively reinforcing pharmacological consequences. When saline is temporarily substituted for the drug, the bar-pressing response continues to be elicited by the conditioned secondary reinforcers (the self-administration equipment). For example, Goldberg et al (1969) have shown that after a number of response-contingent pairings of light and morphine injections, the light by itself acquired the property necessary to elicit bar-pressing.

A widely studied means of drug self-administration is through a paradigm involving the oral consumption of a morphine solution (Stollerman and Kumar, 1970). The bitter taste of the morphine solution results in its initial rejection. This initial aversion to the bitter taste can be counteracted by first habituating the animals to satisfying their normal thirst during a limited time daily, and then substituting a morphine solution for the water normally presented (Kumar et at, 1968). Subsequently, when both morphine and water are

available in a choice situation, the animals demonstrate a distinct preference for the bitter morphine solution, or for an equally bitter quinine solution (Kumar et al, 1968). Since the bitterness of the drinking solution evidently acquires the property of a secondary reinforcer, the postingestional consequence of drinking the morphine is presumably the primary positive reinforcer in this paradigm.

Morphine was discussed above as a positive reinforcer in three different behavioural contexts: (a) choosing one arm of a Y-maze for morphine reinforcement, (b) lever-pressing for the drug, (c) oral consumption for morphine's pharmacological effect. The Kumar et al (1968) drinking paradigm clearly demonstrates that rats possess the ability to learn an association between taste and the presumably rewarding pharmacological effects of morphine. And yet, when taste and the pharmacological action of morphine is paired in the <u>conditioned taste-aversion paradigm</u>, morphine turns out to be a potent punishing stimulus (Cappell et al, 1973). In the conditioned taste-aversion situation, a drug-naive animal is presented with a novel taste followed by the morphine injection. The animal subsequently avoids the novel taste even though the drug dosage used in the experiment is known to be intravenously self-administered in the lever-pressing paradigm (Cappell et al, 1973). Morphine thus appears to be both rewarding and punishing, depending on the paradigm used to duplicate its properties.

That morphine is a potent aversive agent has long been established by Pavlov (1927). He showed that in dogs an injection of morphine produces a variety of direct physiological effects such as nausea, hypothermia, salivation and changes in heart rate; effects which can be easily conditionable to light, the sight of the syringe or the sight of the experimentor. The light by itself eventually comes to elicit vomiting in the animal. It has been suggested by Amit et al (1975) that certain drugs such as morphine, seem to be aversive only to morphine-naive animals and that the punishing pharmacological effects which accompany the initial doses of the drug are reduced or eliminated with subsequent doses. This implies that, in the conditioned taste-aversion paradigm, a learned taste-aversion should only be observed when morphine-naive animals are used. But this hypothesis does not necessarily imply that it is specifically drug-naivety which obscures morphine's known rewarding properties in the conditioned taste aversion paradigm. It is possible that the conditioned taste-aversion situation, by its very nature of pairing novel taste with subsequent physiological consequences may not be suitable for demonstrating morphine's reinforcing action in the form of a conditioned taste-preference. As its name implies, the conditioned taste-aversion paradigm may, in fact, be useful only in demonstrating a learned taste-aversion.

It is, in fact, worthwhile to note that conditioned-taste preferences are quite difficult to demonstrate and are thus a rarity in the literature whereas the ease with which conditioned taste-aversions are formed is well known (Revusky, 1970). Up to this point in time, only two relevant examples of a conditioned taste-preference in rats have been found. Garcia et al (1967) conditioned increases in preference for a novel flavour in thiamine-deficient rats by pairing thiamine injections with the ingestion of a novel taste. Green and Garcia (1971) showed that recuperation from apomorphine-induced illness served as the positive reinforcer to produce a preference for either of two novel tastes. In both experiments the offset or recovery from illness was the primary reinforcer. The conditioned taste-preference paradigm is thus the opposite analogue of the conditioned taste-aversion situation in which the onset of illness serves as the primary reinforcer.

It is well known that if morphine is chronically administered in sufficiently large amounts, a need state for the drug is developed which is known as physical dependence. Once physical dependence is established, withdrawal of morphine results in physical distress called the "withdrawal syndrome". Rapid recovery from the illness of withdrawal results upon the administration of more drug. Such recovery from the distress of morphine withdrawal is thus analogous to recovery from the apomorphine-induced or thiamine-deficient malaise used successfully as the

positive reinforcer in previous taste conditioned paradigms. Thus, morphine-dependent animals may be used in the conditioned taste-aversion paradigm to attempt to demonstrate a taste-preference by pairing a novel flavour with recovery from morphine withdrawal.

It should be pointed out that rats, whether initially morphine dependent or non-dependent, possess the ability to Learn an association between taste and the pharmacological effects of morphine (Stolerman and Kumar, 1970). The Stolerman and Kumar (1970) oral self-administration paradigm differs from the taste-conditioning situation in many ways, nonetheless it is clear that in both cases the animals learn about the consequences of ingesting a novel-tasting fluid. The conditioned taste-aversion paradigm can be set up so that a novel flavour is paired with a morphine "medicinal" or "maintenance" injection in dependent animals. Thus, one could hope to observe a conditioned taste-preference in such a paradigm for several reasons: (a) rats can learn the association between flavour and positive "medicinal" consequences (Garcia et al, 1967; Green and Garcia, 1971), (b) dependent rats are able to associate taste with morphine's pharmacological effects (Stolerman and Kumar, 1970), (c) morphine is well known as a positive reinforcer in several other paradigms in which drug-dependent animals are used (Beach, 1956; Weeks, 1965; Nichols, 1964), (d) the initial aversive properties of

morphine are presumably attenuated when morphine-experienced or dependent animals are used (Bolotow and Amit, 1975; LeBlanc and Cappell, 1974).

Jacquet (1973) was the first to use morphine "medicinal" injections as the primary reinforcer in an attempt to produce a conditioned taste-preference. She implemented a schedule of conditioning in which morphine-naive animals slowly became drug-dependent during the course of repeated taste-drug pairings. She hoped that following the development of drug-dependence, the emergence of a taste-preference would overcome the expected initial taste-aversion. However, Jacquet found that the initial learned taste-aversion remained throughout the course of training. Consequently, it is unclear whether her study was really a failure to reverse an initial learned taste-aversion, rather than a failure to produce a learned taste-preference.

The obvious way to eliminate the problem of drug-naive animals in the initial conditioning period is to establish morphine-dependence before training begins. In this way, the very first taste-drug pairing is one in which a novel flavour is followed by a medicinal morphine injection. The first study to use such morphine premedication in the conditioned taste-aversion paradigm was Parker et al (1973). Consequently, they reported the demonstration of a learned taste-preference, but in fact what their study seemed to illustrate was the reversal of an initial taste-aversion which was attenuated during the training procedure.

Specifically, Parker et al (1973) premedicated his experimental animals and subsequently measured their level of preference for a novel tasting fluid both before and after the conditioning regimen. Unfortunately, each test of preference used in their study was administered over a four-day period during which the rats were presumably experiencing withdrawal stress. As a result, the stress of withdrawal may have interfered with the normal level of preference for the novel solution over the 96 hour test period. This notion is supported by an untrained control group which received no premedication but exhibited a much higher level of preference for the novel flavour during the 96 hour pretraining preference test. Thus, it appears that just as in Jacquet's (1973) study, an initial aversion to the novel taste was established before the taste-medicine pairings were administered during training.

On the post-training preference test, Parker et al (1973) found that the premedicated animals exhibited a higher level of preference for the flavoured solution than in the pre-training preference test. From this observation they concluded that during conditioning the morphine-dependent animals were able to associate the beneficial consequences of a morphine medicinal injection with the contiguous presentation of a novel flavour. However, their study lacks the one control group essential in proving that an observed increase in preference for a flavoured

solution following exposure is really a conditioned taste-preference and not just the normal increase in preference expected due to repeated exposure to the novel flavour. It is worthwhile to note that the level of preference which they claimed to be as a result of learning never exceeded the level of preference exhibited by his drug-naive, untrained animals. It would appear that what Parker et al (1973) really achieved was the reversal of an inadvertantly produced taste-aversion back to the level of drug-naive, untrained controls. It is clear that the simple elimination of a taste-aversion cannot be described as learning of any kind, unless the appropriate controls are implemented into the design of the experiment.

A lesson to be learned from the Jacquet (1973) and Parker et al (1973) studies is that one must follow two simple rules if an initial taste-aversion is to be avoided: (a) before training, the animals must be premedicated to induce dependence on the drug, (b) the novel flavoured solution must not be paired with morphine withdrawal over long periods of time. In a study published after the completion of thepresent experiments, LeBlanc and Cappell (1974) followed these guidelines and yet still did not demonstrate a learned taste-preference. However, in accordance with the Parker et al (1973) study, LeBlanc and Cappell found that the aversive properties of morphine injections were

attenuated when the injections were given to morphine-dependent rats. No evidence of a learned taste-preference was seen. This attenuation may be accounted for by the relatively low-dose morphine "maintenance" injections administered during training. Perhaps the dose was of an insufficient magnitude to produce a learned taste-preference. On the other hand, as a result of morphine exposure during premedication, the dose may have been insufficient to produce a learned taste-aversion. Thus, a higher dosage of the "medicinal" injection during training may either produce the desired learned taste-preference or its opposite analogue, a learned taste-aversion.

The present study assessed the effects of taste-morphine pairings in morphine dependent animals using fairly high doses of the "medicinal" injection during training. The flaws of the Jacquet (1973) and Parker (1973) studies were avoided. The purpose of the present study was to attempt to demonstrate morphine's well-known rewarding properties using a conditioned taste-aversion paradigm. In this study, the use of morphine-dependent animals served to: (a) prepare the animals for subsequent morphine "medicinal" injections during training, and (b) reduce the aversiveness of morphine administration experienced by drug-naive animals, thus reducing the possibility that such aversiveness may interfere with the learning of a taste-preference. It is well known that rats are able to associate recovery from illness with novel flavours (Green and

Garcia, 1971). Furthermore, it is known from oral self-administration studies that rats can learn about the beneficial pharmacological consequences of ingesting a bitter morphine solution (Kumar et al 1968). Thus, rats presumably have the potential to learn a taste-preference using, as the positive reinforcer, morphine injections into "morphine-needy" animals. The present study examines this potential by attempting to condition a taste-preference in drug-dependent animals.

It is important to point out that such a demonstration of morphine's reinforcing "medicinal" property in the CTA paradigm would have to be regarded as just a "special case" of morphine's well-known rewarding properties since drug-dependent animals were used. Other paradigms clearly have shown that rats initiate and maintain self-administration at doses which do not necessarily produce signs of physical dependence (Woods and Shuster, 1968; Shuster, 1970; Deneau, Yanegita and Seevers, 1969). On the other hand, a demonstration of morphine's punishing property in this study would support the notion that, whether dependent or non-dependent, animals are <u>not</u> able to associate flavour with morphine reward in the conditioned taste-aversion paradigm.

One final note concerning the administration of morphine into drug-dependent animals is that such injections can be executed either during withdrawal stress, or shortly after a

maintenance injection when the animal is not, physiologically, in need of the drug. Thus, the present study also examined the reinforcing properties of morphine administration in drug-satiated, dependent rats. This extra group of dependent animals allowed for a direct comparison between the reinforcing properties of a morphine "medicinal" injection and a presumably "unneeded" morphine injection.

METHOD

The study was divided into three parts although all of the animals in the study were run at the same time. Experiment 1 consisted of three control groups and one experimental group. It was designed to determine whether a morphine maintenance injection in 48-hour drug-deprived, dependent animals could be paired with a novel coffee flavour to produce a conditioned-taste preference. Experiment 2 was identical in purpose to Experiment 1, but the morphine injections were paired with coffee every 24 hours and only one control group was used. Experiment 3 was also designed to determine the effects of pairing a morphine injection with the coffee flavour, but in this case, drug-sated, dependent animals were tested.

Subjects: The subjects were 56 male Wistar rats weighing between 240 and 260 gm at the start of the experiment.

Premedication Schedule (days 1-15): Of the 56 animals, 40 were randomly selected to be given premedication. These rats were put on a morphine injection schedule to induce physical dependence over a period of 15 days. The remaining 16 animals were administered control injections of saline and therefore remained drug-naive.

During the course of premedication, morphine sulfate was administered intraperitoneally twice a day, at 9 a.m. and 6 p.m. The first day's injections were at a dose of 25 mg/kg each and injection doses were increased by 25 mg/kg/day to a final dose of 150 mg/kg each twice a day. The 150 mg/kg (twice per day) dose was then continued from day 6 to day 15. This dose regimen was judged to be sufficient to establish a reliable level of physical dependence to morphine on the basis of studies such as Schwartz and Marchok (1974), Jacquet (1973), Hine et al (1975). Dependence was to have been confirmed by test with naloxone (Way et al, 1969), but the drug was not available, and the test could not be made.

Water was available at all times on days 1-10. On the last 5 days of the premedication regimen, the animals were forced to drink all their water during a 20 minute period once a day, 2 hours before their 6 p.m. injection. Food was available freely throughout the experiment. All fluid given during the course of the experiment was available from 100 ml Richter tubes.

On day 16, the 40 drug-dependent animals were divided into five equal groups; the groups were matched on the basis of water intake over days 14 and 15. Similarly, the drug-naive animals were divided into two equal groups with matched water intake.

Experiment 1

Two of the drug-dependent groups and both drug-naive groups were used in the first experiment.

The Conditioning Trials (days 16-25): Following the second morphine injection on day 15, the animals in the two drug-dependent groups received one 150 mg/kg morphine maintenance injection once every 48 hours over a period of 10 days. Five such medicinal injections were therefore administered in each group. With each morphine injection, a 1.5% coffee solution (decaffeinated Sanka) was made available either 2 hours after or immediately preceding the morphine injection. Specifically, the experimental Group 1 animals received their maintenance injections immediately after each presentation of the coffee flavour. Thus, in Group 1, morphine maintenance was paired with flavour every 48 hours. In Group 2, each morphine maintenance injection was administered two hours before the coffee was made available. Thus, the presumed effects of morphine and novel taste were unpaired, and this group was a backwards-conditioning control group. Each coffee presentation lasted 20 minutes and the coffee solution was the only fluid available on the morphine injection days. Water was made available for 20 minutes on the alternate days at the same hour during the ten-day conditioning schedule. The 20 minute coffee and water intakes were measured to the nearest 0.5 ml.

The drug-naive animals in Group 3 and 4 were treated in a similar fashion to Group 1 during the conditioning trials, in that a morphine or saline injection immediately followed (was paired[1]) with each 20 minute coffee presentation. Group 3 received morphine injections at a 25 mg/kg dose while Group 4 was administered control saline injections immediately following the 20 minute availability of coffee.

To summarize (see Table 1), Group 1 was the experimental group of morphine-dependent animals given the novel taste of coffee followed by a morphine-maintenance injection. In these animals, coffee taste was paired with an injection which relieved the stress of abstinence once every 48 hours. Group 2 received its medicinal injection 2 hours before the novel flavour and was therefore an unpaired, or backward-conditioning control group; coffee and relief from abstinence should not have been associated by this group. Group 4 was a drug-naive control which received saline injections during conditioning. This group was used to reflect the normal or baseline level of coffee intake over the course of developing coffee

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[1] Note: The first time that coffee and a morphine or saline injection was paired in Groups 1, 3 and 4 (day 17), the drug was administered just 30 seconds after the novel-tasting coffee solution was made available. After the injection, the animals were given access to the coffee for the remainder of the 20 minute period. The purpose of this procedure during the first taste-drug pairing was to allow for more control over the amount of initial exposure to the novel taste. On all subsequent pairings, the injections were given immediately after the 20 minute taste presentation.

familiarity. Group 3 was a non-dependent group used to determine whether the changes in coffee preference observed in Group 1 were a result of morphine premedication. Group 3 was expected to develop a strong conditioned taste-aversion.

The Post-conditioning Preference Test: 48 hours following the final taste-drug pairing, all four groups were given 20 minutes of simultaneous access to water and coffee. This two-bottle preference test lasted 48 hours. Water and coffee intakes were recorded after the first 20 minutes, and subsequently for two consecutive 24-hour periods.

Experiment 2

Two dependent groups of eight animals each were used in this part of the study.

The Conditioning Trials: The essential difference during conditioning between Groups 1 and 2 of Experiment 1 and the two analogous groups in Experiment 2 was that the latter two were administered coffee-morphine pairings once every 24 hours for 10 consecutive days instead of once every 48 hours. In all other respects the treatments were identical. Thus Group 5 was analogous to Group 1 in that morphine maintenance immediately followed coffee; Group 6 was an analog of Group 2. Since Groups 5 and 6 received morphine injections every day, the availability of water on alternate days had to be eliminated.

The animals in this experiment thus had the coffee solution as the only fluid available for ten consecutive days.

The rationale for Experiment 2, in which rats were given a morphine maintenance injection paired with flavour every 24 hours, is that the stress of withdrawal increases in intensity for up to 96 hours after the latest injection (Wikler et al, 1953). Therefore, the degree of relief from abstinence or need-reduction may vary and different levels of taste-aversion of preference may result for taste-morphine associations made under different levels of abstinence. Moreover, the lack of alternative sources of fluid could force the Group 5 animals to consume larger quantities of coffee in the event of an initially strong aversion to coffee, and this could alter the strength of morphine-coffee association.

The Post-conditioning Preference Tests: The two-bottle taste preference test was identical to that of Experiment 1 except that the two-bottle test was initiated 24 hours after the final taste-drug pairing on day 25.

Experiment 3

The remaining dependent group of rats, Group 7, was used in this part of the study.

The Conditioning Trials: The rats in this experiment were treated in the same way as Group 1 (in which a medicinal injection

immediately followed coffee exposure) with one exception: a normal medicinal injection was administered two hours befor each coffee presentation and an additional 150 mg/kg injection was given following the coffee. In this way, the morphine injection which followed the novel flavour was not of a medicinal nature. It represented, rather, an administration of morphine to drug-sated, drug-dependent animals.

The Post-conditioning Tests: The two-bottle taste preference test was identical to Experiments 1 and 2.

RESULTS

Table 2 shows the mean coffee intake of each group during the initial 20 minute exposure to the coffee solution. This initial pairing-day data is useful in preparing Figures 1 and 3, which illustrate the subsequent mean coffee consumption of each group after correcting for baseline intake.

An analysis of variance of the initial pairing-day data revealed a significant between-groups effect ($F = 44$; $df = 4,35$; $p < .001$). Subsequent Scheffé tests discovered that: Group 1 was significantly different from Groups 2 and 4 ($p < .01$); Group 5 differed from Group 6 ($p < .01$); and Group 7 did not differ significantly from either Group 2, 3 or 4. These differences between groups in initial coffee intake justify the correction of subsequent coffee intakes with the baseline data given in Table 2.

Experiment 1

Figure 1 illustrates mean coffee consumption relative to each group's initial coffee intake on day 17. It is clear from Fig. 1 that the experimental paradigm was not successful in producing a conditioned taste-preference for the coffee solution. Using the difference from baseline data, an analysis of variance for repeated measures between groups revealed a significant overall between groups effect ($F = 34.6$; $df = 4,35$; $p < .01$). However, the other effects and interactions were found to be

statistically insignificant. Since experiment 1 is primarily interested in Group 1, in which animals were administered morphine maintenance injections immediately following access to the novel coffee flavour, appropriate statistical comparisons among means were done in order to further evaluate the statistical relationship of Group 1 to its three control groups.

Thus, Group 1 consumed less coffee on every trial than did Group 2, which received its maintenance injection two hours before the coffee ($p<.01$). In fact, as Figure 1 illustrates, the mean amounts of coffee drank in Group 1 were less than one-tenth of the mean intakes of Group 2 from day 19 to day 25. In contrast, Group 1 was similar in its low level of coffee consumption to Group 3, the drug-naive group in which novel morphine injections were paired with coffee. The animals in Group 3 acquired a powerful conditioned taste-aversion, as expected, and the levels of coffee intake demonstrated by Group 3 were comparable statistically to Group 1 for three of the four test days, ($p>.05$ on days 19, 23, 25). On day 21, however, Group 1 consumed significantly more coffee than did Group 3 ($p<.05$), thus indicating an attenuation of the learned taste-aversion as a result of premedication.

The taste-aversion evident in Groups 1 and 3 was so potent that all of the animals in Group 3 and one in Group 1 demonstrated a total rejection of the coffee solution at the end of the conditioning period. Consequently, all of the animals in Group 3

consumed all of their fluid on the alternate water days (days 20, 22, 24, 26). Thus, Figure 2 compares the amount of coffee consumed on a particular day to the amount of water intake on the subsequent non-coffee day. This comparison is expressed as the ratio of the coffee intake to the sum of coffee plus water consumed over the two days. The ratios in Figure 2 evidently support the differences illustrated in Figure 1 between Groups 1, 2, 3 and 4. Thus, despite the differences in mean daily coffee intake between the four groups, the mean amount of water consumed on the alternate water days was virtually identical in each group.

While Group 1 demonstrated a steady, low level of coffee intake throughout the conditioning period, the drug-naive saline control (Group 4) steadily increased its mean consumption of coffee so that by day 25 the animals in Group 4 were drinking as much coffee as water during the 20 minute periods on alternate days (Fig. 1 and 2). In order for Group 1 to demonstrate a true preference for the coffee flavour, its mean intake of coffee would have to surpass that of Group 4. But Group 1 consumed significantly less coffee than Group 4 on every trial ($p < .01$), once again providing no evidence for a conditioned taste preference but rather for a learned taste-aversion.

Experiment 2

The results of Experiment 2 are treated separately in statistical and graphical analysis since coffee was the only source of fluid to these animals for 10 days and since coffee and morphine were administered once every 24 hours thus eliminating the alternate water days. An analysis of variance for repeated trials between groups revealed a significant between groups effect but no other effect ($F = 54.6$; $df = 1,14$; $p < .01$).

Group 5, which received morphine maintenance injections just after coffee once every 24 hours nevertheless maintained a very low level of intake of the coffee solution, its only source of fluid throughout the 10 day conditioning period (Figure 3). Despite the fact that Group 5 had no other source of fluid, the average 20 minute coffee intake was only 2.5 ml over the ten days of training. Control Group 6, which was administered the medicinal injections two hours before each coffee presentation demonstrated a level of intake 3 to 4 times higher than Group 5 ($p < .001$ on each trial).

That the low level of drinking observed in Group 5 was not due to the effect of 24 hours of morphine deprivation was demonstrated on day 26 when Group 5 was given a choice of water or coffee. On day 26, the total volume of fluid consumed rose significantly ($p < .01$) due to high water intake. The decreased fluid intake observed in Group 6 on day 26 was probably due to the situational novelty of having access to fluid before first receiving the usual morphine

maintenance injection.

All of the animals in Group 5, which had morphine maintenance paired with taste lost a great deal of body weight (Figure 4) over the 10 day conditioning period indicating severe dehydration and at the same time demonstrating the potency of the taste-aversion in these animals. In contrast, the animals in control Group 6 did not lose any weight over the course of the experiment. Thus the animals in Group 5 had acquired an aversion to the coffee taste to the point of severe weight loss and dehydration. Wet mash had to be supplied to four of the deteriorating animals towards the end of the study (days 21-26) to prevent death from starvation and dehydration.

Experiment 3

The results of Experiment 3 were treated in the same statistical and graphical analysis as were the results of Experiment 1 because of identical methodological considerations. An analysis of variance for repeated trials between groups revealed a significant between groups effect ($F = 34.6$; $df = 4,35$; $p < .01$) but no other effect. The results of this part of the study suggest that drug-satiety in morphine-dependent rats precluded the acquisition of the conditioned taste-aversion illustrated in Group 1 of Experiment 1. Group 7, which was administered its morphine maintenance injections two hours before the usual coffee-morphine pairing did not exhibit the low

level of coffee intake observed in Groups 1 and 3 of Experiment 1 ($p<.01$ for all trials). The procedural difference between Groups 1 and 7 was that in Group 1 coffee was followed by a presumably medicinal drug injection, whereas in Group 7 the injection following the coffee was into a drug-sated, dependent animal. In Group 3, the morphine injection was not physiologically necessary since the animals in Group 3 were not morphine-dependent. As Figure 1 illustrates, both Groups 1 and 3 acquired taste-aversions, but not Group 7.

Moreover, Group 7 did not differ statistically from Group 2 of Experiment 1, a group which did not acquire a conditioned taste-aversion since the maintenance injection and coffee flavour were not paired ($p>.05$ for all trials). Thus it is clear that Group 7 had not acquired the taste-aversion.

The two-bottle preference test on day 27 (Figure 2) demonstrated the typically low level of coffee intake in a choice situation.

DISCUSSION

Under the present experimental paradigm a conditioned taste-preference was not acquired following repeated pairings of novel flavour with morphine "medicinal" injections. Specifically, in Group 1 the premedication procedure did not offset (but did attenuate to a degree) the conditioned taste-aversion acquired by animals which were drug-naive for the initial taste-drug pairing. In support of these findings, Jacquet (1973), and Parker (1973) also demonstrated that morphine dependent animals in a conditioned taste-aversion paradigm did not associate morphine's "medicinal" effect of relieving withdrawal stress with a novel flavour. The recent study by LeBlanc and Cappell (1974), which was published following the completion of the present work, closely resembles the procedure used in this study in attempting to demonstrate the positive properties of morphine in the conditioned taste aversion paradigm. Direct comparisons between the results of the two studies can therefore be made in a meaningful fashion.

Experiment 1 of the present work resembles LeBlanc and Cappell's (1974) study in several respects: (a) the experimental animals were premedicated with morphine to establish physical dependence on the drug (b) the animals in their study were premedicated up to 200 mg/kg daily for 23 days versus 150 mg/kg twice daily for 15 days, (c) the period of drug-

deprivation between each pairing of flavour and withdrawal relief was 42 hours versus 48 hours, (d) in both studies preference levels were monitored at each taste-drug pairing, (e) a one-bottle preference test was used, (f) water was available between pairings. Despite these basic similarities, LeBlanc and Cappell's results do not demonstrate the high degree of conditioned taste-aversion evident in this study. Their results, nevertheless, do support the findings of Experiments 1 and 2 from the point of view that a taste-preference was clearly not acquired by the animals when a morphine medicinal injection was used as the unconditioned stimulus during training. The discrepancy between results is therefore only one of a difference in the degree of taste-aversion found in each study. For example, they found that in dependent animals, the conditioned taste-aversion was significantly attenuated in comparison to their morphine-naive controls. Similarly, the present study demonstrated an attenuation in the degree of taste aversion as illustrated by a comparison of experimental Group 1 and control Group 3 (Figs. 1 and 2). This attenuation was significant, however, only during a part of the conditioning period.

Since the discrepancy between the results of the two studies is quantitative and not qualitative it is worthwhile to examine the procedural differences in an effort to establish the contributing factors to this discrepancy. One possible source

of variance between the studies is the different novel flavour used: saccharin versus coffee. Since the initial level of preference for saccharin is much higher than that for a bitter 1.5% coffee solution, it is plausible that this factor can influence the ease with which a taste-aversion is established or attenuated. For instance, attenuation of a conditioned taste-aversion to a usually preferred saccharin solution may have been easier to offset than a corresponding aversion to the bitter flavour of coffee. In all probability, this factor was not totally responsible for the differences in the extent of taste-aversion found in each study.

A more significant reason for the discrepancy may be the difference between the dosages of the morphine maintenance injections administered in the two experiments. The LeBlanc and Cappell study used just a 20 mg/kg medicinal injection following a premedication regimen of morphine injections at a 200 mg/kg daily level. The present study used a significantly larger 150 mg/kg medicinal injection after a 300 mg/kg daily pretreatment schedule to induce dependence. It is known that with repeated administration, morphine's various effects at a particular dose gradually diminish in strength (Wikler, 1968). This is known as tolerance. Since tolerance to morphine is a concomittant of the acquisition of morphine dependence, it is not surprising that, in the LeBlanc and Cappell study, a 20 mg/kg

injection produced a lower level of saccharin-aversion in a drug-dependent animal than the same dose in a drug-naive one. Such drug tolerance in the conditioned taste aversion paradigm has been previously demonstrated with ethanol by Berman and Cannon (1974). The relatively greater potency of the 150 mg/kg morphine injection used in the present study during taste-conditioning is a reasonable explanation for the greater degree of taste-aversion observed in this experiment. In perspective, the two studies together offer a strong argument against the notion that morephine-dépendent rats are able to associate the beneficial effects of a morphine maintenance injection with a novel flavour in a conditioned taste-aversion paradigm. In either study, no indication of a learned taste-preference was observed after pairing relief from morphine withdrawal with a novel flavour. Thus, the result of pairing a medicinal morphine injection with a novel taste in the conditioned taste-aversion paradigm results in either a learned taste-aversion or, an attenuated taste-aversion if lower drug doses are used.

That the high-dose morphine "medicinal" injection was aversive to the dependent animals in the present study is dramatically illustrated by Experiment 2. The rats in the Experimental Group 5 were administered a morphine-maintenance injection once every 24 hours (versus 48 hours for Experiment 1) so that coffee was their only source of fluid for 20 minutes per

day for 10 days. The conditioned taste-aversion was of such magnitude that most of the animals in the group would only take a few token licks at the coffee solution before refusing it. Consequently, all of the animals were severely dehydrated and under-weight at the end of the ten day conditioning period.

While a morphine medicinal injection of 150 mg/kg in Experiments 1 and 2 was aversive, an injection of identical dosage into the dependent but drug-sated animals in Group 7 (Experiment 3) was not aversive since a taste-aversion was not observed. A conditioned taste-preference was not observed either however, making an interpretation of the results in Experiment 3 difficult but interesting. Perhaps the injection of morphine into drug-sated animals was not punishing due to the already high physiological level of the drug in the animal's system. An overdose of a drug may therefore be of little significance in the conditioned taste-aversion paradigm. This possibility can be readily investigated with drug-naive rats by administering morphine 2 hours before and then immediately after the presentation of a novel taste. A significant attenuation of the expected taste-aversion in this group would support the notion that if an animal is "drugged" before a novel flavour is paired with a second drug injection, the animal does not acquire a taste aversion. Thus, the results of Experiment 3 are interesting from the point of view that a conditioned

taste-aversion was so easily blocked by a pre-injection with the drug.

A valid question to raise at this point is whether or not a morphine "medicinal" injection was really the unconditioned stimulus in Groups 1 and 5 of the present study. It is possible that the observed taste aversion in these groups was a result of the coincidental pairing of coffee and withdrawal stress rather than as a result of the pairing of coffee and the morphine "medicinal" injection. Unfortunately, this study lacks the control group to determine whether dependent rats are able to learn an association between a novel coffee taste and the stress of withdrawal. Pavlov (1927) demonstrated that if the morphine abstinence syndrome is precipitated by a morphine antagonist, then this withdrawal state can be used as an aversive unconditioned stimulus. For example, the light formerly paired with nalorphine (a morphine antagonist) came to elicit vomiting and excessive salivation in morphine-dependent monkeys. It must be noted, however, that in Pavlov's experiments, the light (CS) was paired with the "onset" of the abstinence syndrome precipitated rapidly by nalorphine. In the present paradigm, the novel coffee solution was presented <u>during</u> withdrawal, while the gradual onset of the abstinence syndrome occurred presumably several hours before a taste-drug pairing. Thus, in the present paradigm it is unlikely that the morphine ab-

stinence syndrome served as an unconditioned stimulus to produce the observed taste aversion.

In support of the above conclusion, the LeBlanc and Cappell (1974) study did include a control group of dependent rats which were briefly presented with a novel taste after 42 hours of morphine abstinence. After several such taste presentations, no evidence of a conditioned taste-aversion was observed. LeBlanc and Cappell (1974) suggest that withdrawal may have a direct rather than a learned suppression on drinking, possibly in the form of an unconditioned taste neophobia associated with the withdrawal state. In view of their findings, it is probable that the morphine "medicinal" injection and not withdrawal was, in effect, the aversive stimulus in the present study.

A tentative conclusion to be made from the results of the present study, LeBlanc and Cappell (1974), Jacquet (1973) and Parker et al (1973) is that, in the context of the taste-aversion paradigm, a morphine "medicinal" injection is not rewarding to morphine-dependent animals. In fact, depending on the dosage, such a medicinal injection is likely to be somewhat aversive. In other words, rats appear to be unable to associate the beneficial consequences of relieving the stress of withdrawal with a preceding novel-flavoured solution. This result is not altogether surprising considering the scarcity of learned taste-preferences in the literature. By contrast, the ease with which animals learn to associate the onset of punishing drug effects

with novel flavours is well-known (Cappell et al 1973). In this study, both the drug-naive and drug-dependent animals demonstrated a significant learned taste-aversion after just one taste-drug pairing. Thus, in the context of the conditioned taste-aversion paradigm, rats are able to associate taste with the drug's aversiveness, but not taste with the drug's known rewarding properties, regardless of whether the animals are drug-dependent or drug-naive.

Paradoxically, as Cappell et al (1973) point out, the same drug doses which are found aversive in the conditioned taste-aversion situation are readily self-administered in other paradigms. That being the case, a plausible resolution of this paradox is the possibility that drugs such as morphine can act simultaneously, both as a positive reinforcer and as an aversive agent in the same animal. Thus, Wise, Yokel and deWit (1975) have reported that the same rats which learned to self-administer the drug apomorphine were able to learn simultaneously a conditioned taste-aversion to a novel flavour. The Wise et al (1975) study appears to support the view that a drug injection may be rewarding, as measured by self-administration studies; but at the same time aversive, as measured by conditioned taste-aversion studies.

But how does a paradigm which reflects morphine's rewarding properties differ from a paradigm (the taste-aversion paradigm) which invariably demonstrates the opposite. Wise et al (1975)

suggest that it is the nature or modality of the drug-related stimulus which determines the emergence of morphine's reinforcing property from the learning paradigm. It is well known, for example, that the ease with which certain associations are learned greatly differs (Seligman, 1970). Thus, rats easily learn the association between taste and the onset of illness, but have difficulty in learning the association between taste and the onset of recovery or the onset of an electric shock (Garcia and Ervin, 1968). In other words, the rewarding properties of a drug may be easily associated with visual or auditory stimuli, while the simultaneously present punishing properties of that drug may be readily associated with a novel flavour.

The objection to the above hypothesis is that, first of all, rats are certainly able to associate taste with morphine's rewarding properties (Kumar et al, 1968), although obviously not as readily as they are able to associate a novel taste with illness. Secondly, morphine's unconditioned physiological effects can easily be conditioned to previously neutral visual stimuli such as light (Pavlov, 1927). Although these two points weaken the hypothesis (the associability of drug-related stimuli), Wise's (1975) finding supporting the notion that drugs can serve as compound stimuli is nonetheless of great importance.

Accepting the possibility that morphine can act as a com-

pound stimulus, an alternate hypothesis to explain the selectivity of paradigms in reflecting the drug's reinforcing property may be related to the nature of the paradigm itself. One possibility is that self-administration paradigms are set up to allow the animal to determine, according to its needs, the onset, dosage, intertrial interval and frequency of drug administration. On the other hand, conditioned taste-aversion paradigms are rigidly designed by the experimentor so that the animal has no control over the drug's administration. From intracranial self-stimulation studies it is known that the same stimulus event which is rewarding when precipitated by the animal's contingent response may not be rewarding when administered by the experimentor (Steiner et al, 1969; Cantor, 1971). Whether such data obtained from intracranial self-stimulation studies can be generalised to drug self-administration paradigms is a significant question. Wise et al (1975) would argue against such a generalisation since, in their study, the very same apomorphine injections which the animals self-administered evidently served simultaneously as a punishing agent in producing a learned taste-aversion to saccharine. Thus, at the present time, there appears to be no foolproof explanation of the paradox which suggests that morphine is a rewarding stimulus in self-administration paradigms but a punishing agent in conditioned taste-aversion paradigms.

The present study compiled evidence against the possibility that morphine-dependent animals in the conditioned taste-aversion paradigm are able to associate morphine's beneficial "medicinal" consequences with a novel flavour. It now appears that neither drug-naive nor drug-dependent animals can learn about morphine's rewarding properties in the context of the conditioned taste-aversion paradigm. One remaining possibility is the use of "morphine-experienced" animals. The "morphine-experienced" animals differ from morphine-dependent animals in that the schedule of injections is insufficient to produce physical dependence, nevertheless the animal is not drug-naive at the start of taste-drug pairing. The likelihood of observing a learned taste-preference using morphine-experienced animals in the present paradigm is low, since LeBlanc and Cappell (1974) used such animals with essentially negative results.

It should be pointed out that the present study along with Parker et al (1973), LeBlanc and Cappell (1974) and Jacquet (1973) study simply assumed that the schedule of morphine injections administered in their respective paradigms was adequate to produce, in the rat, a state of physical discomfort or withdrawal during taste-drug pairing. Since no empirical evidence was gathered by either experimenter on this important prerequisite in the animal's physiological state, the possibility exists that gross misinterpretations may have been made on the associability, in rats, of novel tastes with morphine "medicinal"

injections. Without empirical proof of the animal's physiological state during the actual taste-drug pairings it is possible to misinterpret the results in at least two ways: (a) Pairing a novel flavour with morphine in a "dependent" animal that was wrongly assumed to be in withdrawal just before the injection. Such a mistaken assumption leads to the incorrect conclusion that the animals did not associate the beneficial consequences of the drug with a novel taste. The desired associability may have been observed had the timing of the taste-drug pairing been different. (b) An animal that is wrongly assumed to be dependent obviously cannot be in withdrawal at any time. Therefore, the experimenter is pairing taste and drug instead of taste and medicine and the interpretation will be erroneous. Thus, in the present experiment, there may have been several of each type of animal in a group; rats which were not morphine dependent, and rats which were dependent but were not in withdrawal during taste-drug pairing. Unquestionably, verification of dependence and of withdrawal during the pairing of flavour and morphine is fundamental to this study and the LeBlanc and Cappell (1974) study.

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TABLE 1

SUMMARY OF GROUPS

GROUP	TYPE OF GROUP	PREMEDICATION	DAILY INJECTION: 2 HRS BEFORE OR JUST AFTER COFFEE PRESENTATION
	Expt. 1 - Effects of Morphine Medicinal Injections after 48 hrs of Drug Deprivation		
1	experimental	yes	after
2	backward conditioning control	yes	before
3	aversion control	no	after
4	saline control	no	after
	Expt. 2 - Effects of Morphine Medicinal Injections after 24 hrs of Drug Deprivation		
5	experimental	yes	after
6	backward conditioning control	yes	before
	Expt. 3 - Effects of Morphine in Drug-sated Animals		
7	experimental	yes	before & after

TABLE 2

Experiment	1				2		3
Group	1	2	3	4	5	6	7
Mean Initial Coffee Intake (ml)	.4	1.7	1.5	2.8	0.1	1.7	2.5

FIGURE 1

Relative mean intake in 20 minutes of a 1.5% solution of decaffeinated coffee (w/v) in Groups 1, 2, 3, and 4 of Experiment 1 and Group 7 of Experiment 3. Intakes are expressed as (absolute minus baseline plus 3) ml. Baseline values are taken from day 17. Coffee intake was measured every 48 hours in a one-choice test from day 19 to 25.

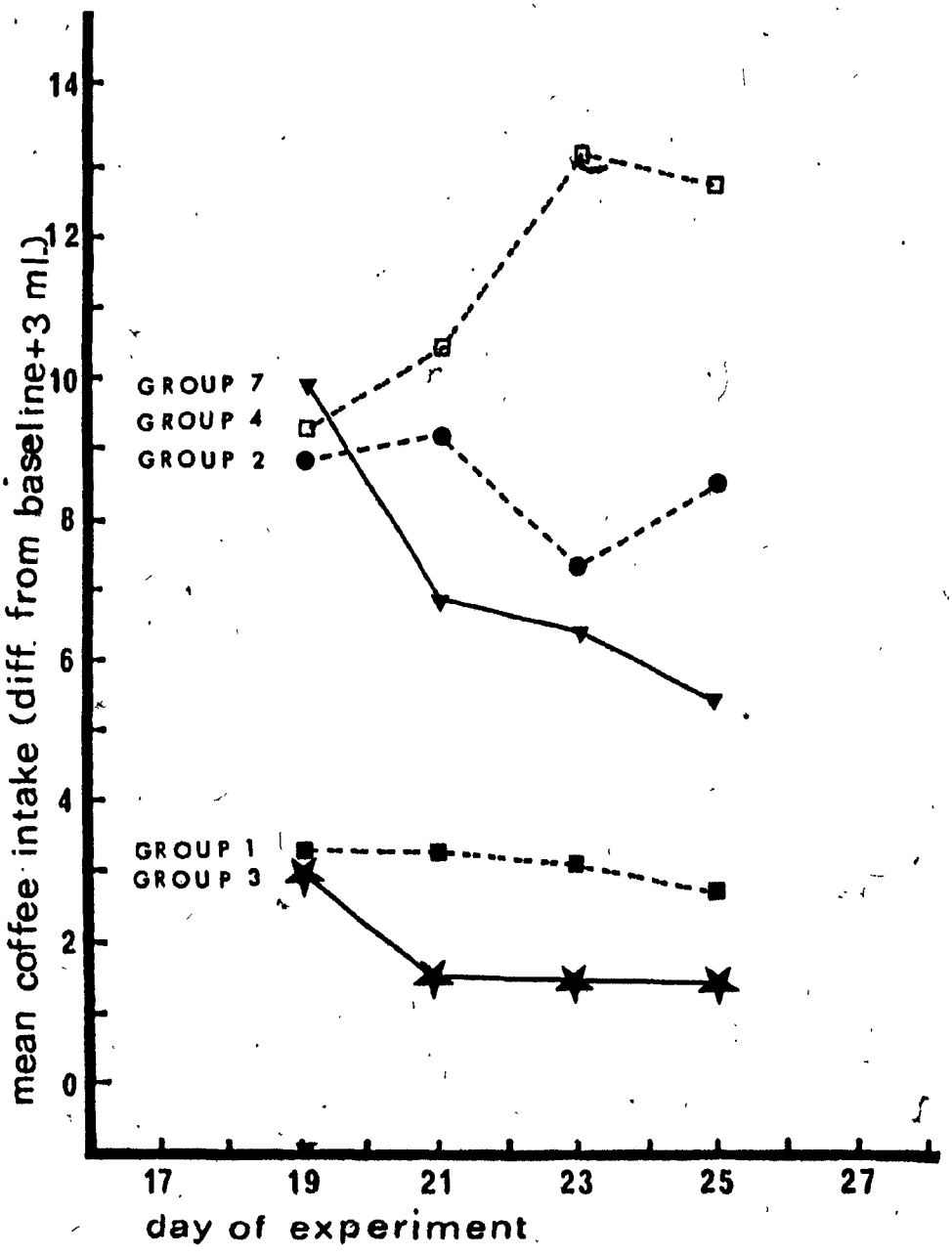

mean coffee intake (diff. from baseline+3 ml)
14
12
10
8
6
4
2
0
GROUP 7
GROUP 4
GROUP 2
GROUP 1
GROUP 3
17
19
21
23
25
27
day of experiment

FIGURE 2

Mean relative intake of the 1.5% (w/v) coffee solution in comparison to the amount of water consumed on the subsequent day. This relative intake is expressed as the ratio of coffee/(coffee plus water). On days 19 to 25, either coffee or water is available for 20 minutes on alternate days. On day 27, a 20 minute choice between coffee and water is available. Day 29 is a 24-hour choice between coffee and water. Groups 1, 2, 3, 4, and 7 are illustrated.

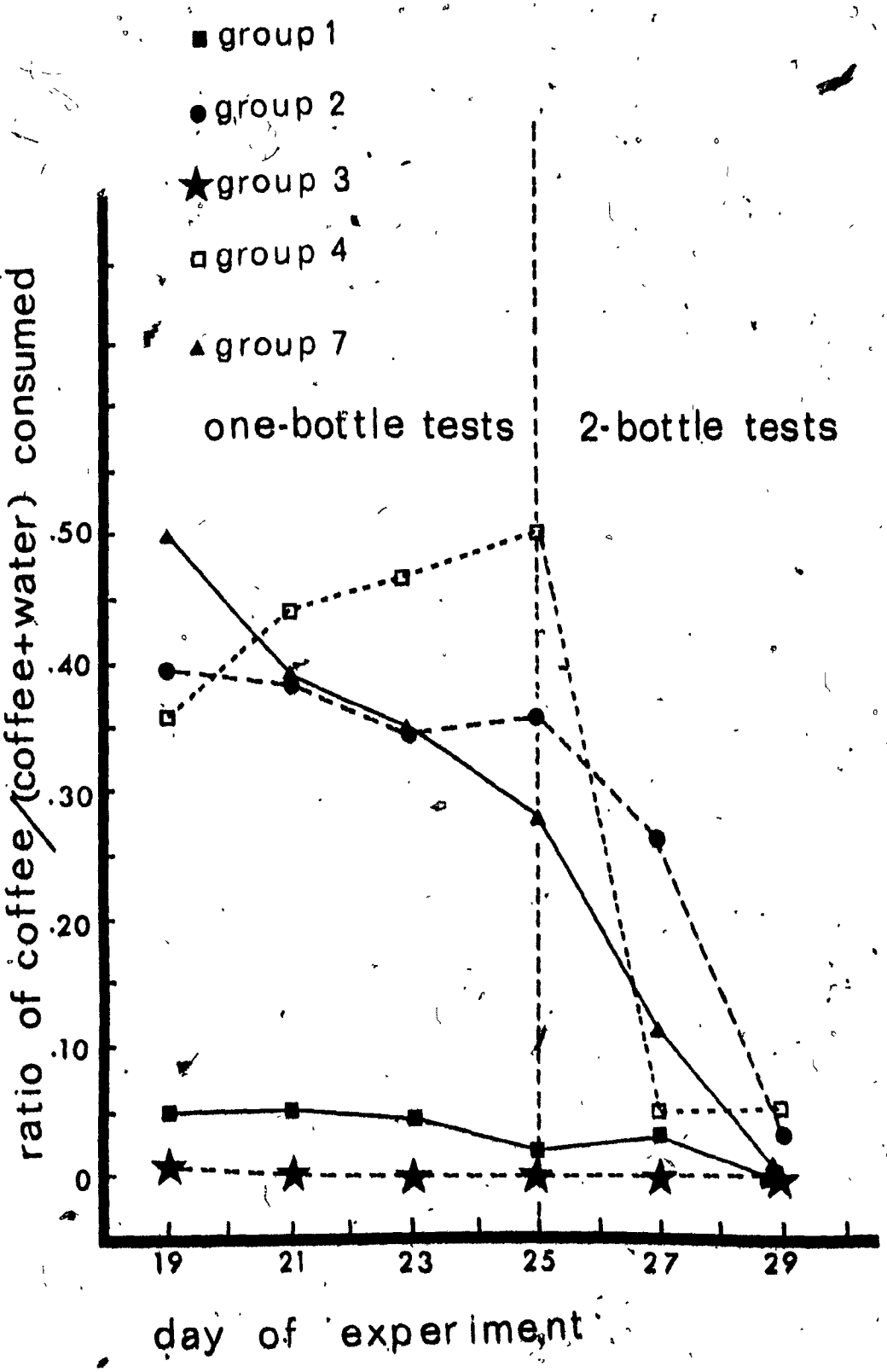

group 1
group 2
group 3
group 4
group 7
one-bottle tests
2-bottle tests
ratio of coffee/(coffee+water) consumed
.50
.40
.30
.20
.10
0
19
21
23
25
27
29
day of experiment

FIGURE 3

Mean intake in 20 minutes of a 1.5% (w/v) solution of coffee in Groups 5 and 6 of Experiment 2. Intakes are expressed as (absolute minus baseline plus 3) ml. Baseline values are taken from day 16. Coffee intake was measured every 24 hours in a one-choice test from day 17 to 25. On day 26 water was simultaneously available and its mean intake expressed.

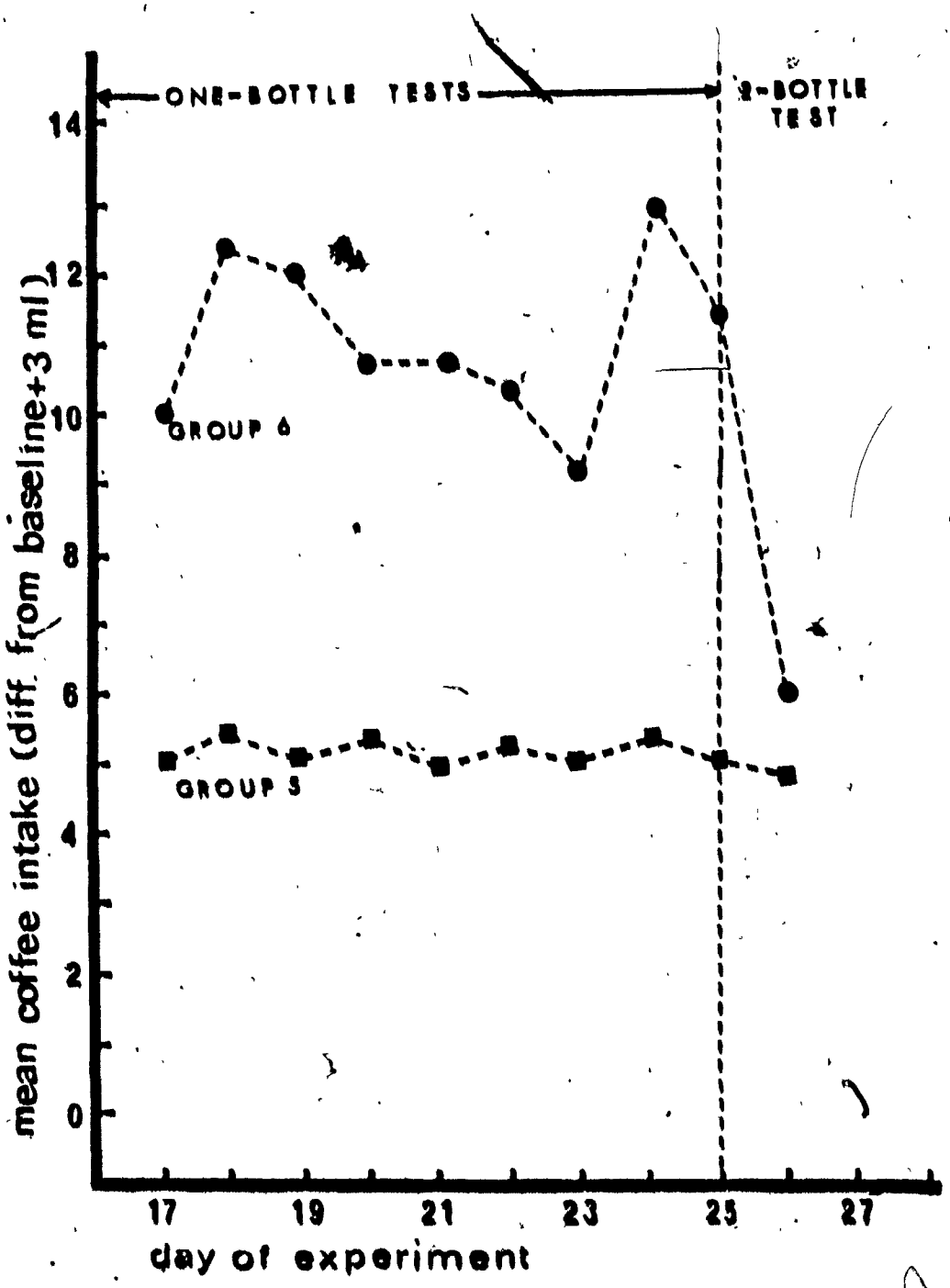
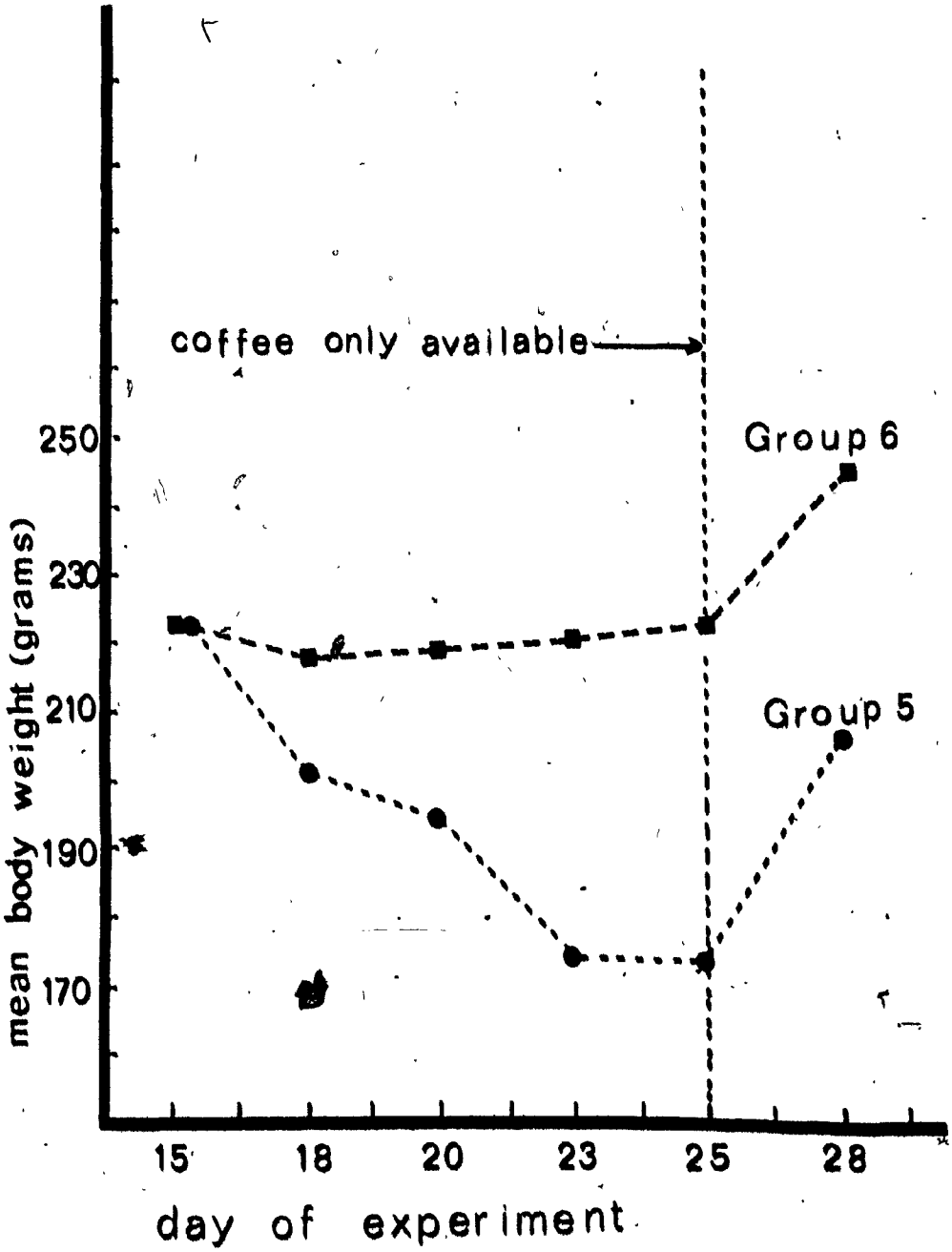

ONE-BOTTLE TESTS
2-BOTTLE TEST
14
12
10
8
6
4
2
0
mean coffee intake (diff. from baseline+3 ml)
GROUP 6
GROUP 5
17
19
21
23
25
27
day of experiment

FIGURE 4

Mean body weights of Groups 5 and 6 of Experiment 2.

Only a coffee solution was available from day 16 to 25.

Unlimited access to water was available from day 26.


coffee only available
Group 6
Group 5
250
230
210
190
170
mean body weight (grams)
15
18
20
23
25
28
day of experiment

APPENDIX A

Analysis of Variance of Baseline Data

SOURCE	df	MS	F
Between	4	6.6	44*
Within	35	.15	

*$p < .001$

APPENDIX B

Analysis of Variance of Experiment 1 & 3
(48 hours between taste-drug pairing)

SOURCE	df	MS	F
Group (G)	4	521.8	34.6*
Error Between	35	15.1	-
Trials (T)	3	16.9	1.2
T x G	12	13.7	1.16
Error Within	105	11.8	-

* $p < .001$

APPENDIX C

Analysis of Variance of Experiment 2
(24 hours between taste-drug pairing)

SOURCE	df	MS	F
Group (G)	1	345.6	54.6*
Error Between	14	6.3	-
Trials (T)	8	10.3	1.21
T x G	8	13.4	1.58
Error Within	112	8.5	-

* $p < .001$