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EFFECT OF SELECTED DRUGS ON CONTROL OF MORPHINE WITHDRAWAL

HYPOTHERMIA INDUCED BY MORPHINE OR CONDITIONAL STIMULUS

ASSOCIATED WITH MORPHINE

BY

RICHARD B. DRAWBAUGH

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

PHARMACOLOGY AND TOXICOLOGY

UNIVERSITY OF RHODE ISLAND

1974

CONDITIONING OF MORPHINE EFFECTS

MASTER OF SCIENCE THESIS
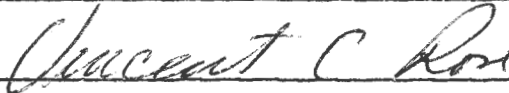
OF

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UNIVERSITY OF RHODE ISLAND

1974

ABSTRACT

The present investigation was undertaken to demonstrate how a conditional stimulus (CS) similar to the action of morphine, can increase rectal temperature during morphine abstinence. Also, the study implicates certain neurotransmiters which are involved in the effect of conditional stimulus and of morphine to affect rectal temperature.

Rats were given two equally spaced injections of morphine sulfate daily, each injection being paired with a bell. The bell was presented for one minute and the injection was given during the last 15 seconds. This procedure was followed for 13-15 days. Twenty-four hours after the last injection the bell was presented alone.

The rats learned to increase their body temperature following the presentation of the bell. This increase was specific only to animals that had the bell paired with morphine prior to challenge treatment. This change in temperature was shown to be approximately equivalent to an injection of 12.5 mg/Kg at 24 hr after the last morphine injection. When naive animals were exposed to a bell, no change in temperature was observed. Those rats which had received a random bell or no bell during addiction demonstrated no change in temperature when presented with the CS 24 hr after the last injection.

Naloxone, a narcotic antagonist, produces hypothermia in normally addicted rats only if given within 12 hr after the last morphine injection. In contrast, when administered to CS-morphine paired animals which received only the CS 24 hr after the last morphine injection, naloxone caused a hypothermia. This data suggest that the CS and morphine are working by either the same or parallel pathways in the brain.

The CS induced increase in temperature was blocked during withdrawal when the animals were pretreated with phenoxybenzamine (2 mg/Kg), mecamylamine (2.5 mg/Kg), haloperidol (0.2 mg/Kg) and benztropine (0.625 mg/Kg) but was not blocked by cyproheptidine (2 mg/Kg). Morphine induced increase in temperature was blocked by mecamylamine, phenoxybenzamine and cyproheptidine but was not blocked by haloperidol or bentropine. Propranolol (2 mg/Kg) had little effect on the increase in temperature due to the CS or morphine when given at 24 hr after the last CS-morphine pairing.

The CS was not able to affect other withdrawal symptoms such as shakes, ptosis, piloerection, loss in body weight, or writhing when presented 24 hr after the last morphine dose.

These data indicate that the increase in temperature elicited by morphine during withdrawal can be classically conditioned. Such a response required a functional autonomic and central nervous system.

ACKNOWLEDGEMENTS

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INTRODUCTION

Roffman et al. (1972) have demonstrated that hyper-
thermia can be conditioned during morphine administration
by pairing a neutral stimulus (bell) with morphine injec-
tions. This conditioning procedure requires approximately
24-30 pairings of the bell and morphine (Roffman et al.,
1972). The resulting data led to the hypothesis that the
conditional stimulus, acting on the brain, may affect the
same receptors that morphine affects. Being able to under-
stand the conditioning associated with morphine administra-
tion may be of great value in treating human addicts. It
should be reasoned then that those behaviors that are
paired with each morphine injection must be extinguished
along with the actual physical process of drug administra-
tion in order to cure addiction.

Conditional responses due to morphine administration
were first seen as a salivary reflex by Collins and Tatum
(1925). Shortly thereafter Dr. Krylov, of the Tashkent
Bacteriological Laboratory in Petrograd, observed that after
repeated morphine injections in dogs they would vomit when
the investigator entered the room, a response seen initially
immediately following the morphine injection. Wikler and
Pescor (1967) demonstrated further, using the classical con-
ditioning paradigm, that the environment associated with

abstinence can act as a conditional stimulus (CS) and can elicit withdrawal symptoms when the rats were placed on that environment many months after the primary abstinence period. In addition, recent evidence indicates that the persistence of abstinence-associated conditioning in post-morphine dependent monkeys reflects a possible mechanism for the relapse to drug taking behavior (Goldberg and Schuster, 1970).

The present investigation sought evidence to establish:

1) Whether the CS acts on pathways that are sensitive to the action of morphine.

2) Whether naloxone, a drug which is a pure narcotic antagonist (Blumberg and Dayton, 1973), can elicit hypothermia following the CS in 24 hr abstinence rats, therefore, supporting the hypothesis that the CS and morphine affect temperature by similar neuronal pathways.

3) The mechanism of action of the conditional stimulus on the temperature regulatory system of the rat and its relationship to the mechanism of action of morphine on the temperature regulatory system of the rat.

LITERATURE SURVEY

Conditioning Associated with Narcotic Addiction

Conditioning associated with narcotic addiction has been demonstrated in a number of experiments.

Utilizing the salivary conditional reflex as a conditional response, Krylov (1927) observed, in the course of certain serological investigations, that upon repeated hypodermic injections of morphine into dogs, certain symptoms that normally follow injections occurred in the dogs as soon as Krylov entered their quarters. It is known that after an initial injection of morphine is given to a dog, nausea and salivation culminated by vomiting will occur. After five or six days of morphine injections Krylov could produce salivation and nausea in the animal by touching him. Two more days passed and his entrance into the room caused the onset of nausea, salivation, and finally vomiting.

Collins and Tatum (1925) serendipitously observed the same phenomenon that Krylov had seen following seven or eight injections of morphine. Kleitman and Crisler (1927) then replicated Collins and Tatum's experiment by using morphine as the unconditional stimulus and thereby systematically conditioning and extinguishing salivary conditional reflexes in dogs.

Utilizing environmental factors associated with morphine abstinence, Wikler and Pescor (1967) demonstrated that a rat undergoing withdrawal in his home cage will, when placed in that cage three months later, show the classical withdrawal signs of wet shakes and writhing. They also demonstrated, along with Thompson and Ostlund (1965), that animals addicted and withdrawn in one environment will self-administer a narcotic drug when placed back in that environment for up to six months after the last day of narcotic ingestion.

Goldberg and Schuster (1967, 1970) utilized nalorphine, a morphine antagonist, to demonstrate conditioned abstinence changes induced by nalorphine in post morphine dependent monkeys. They observed that after pairing a neutral stimulus (light) with nalorphine injections, the neutral stimulus could, when presented alone, elicit conditional responses (emesis, salivation and decreased heart rate). These responses are normally only observed following the nalorphine injection in morphine dependent animals. However, they could not condition the hypothermic effect that follows nalorphine administration. Goldberg et al. (1971) demonstrated that monkeys would self-administer saline, to overcome an antagonistic effect, if they previously had been given nalorphine under the same conditions.

Thompson and Pickens (1969) reviewed the literature of conditioning and drug dependence through 1969. They

concluded that much of drug self-administration can be explained by operant behavior. Antecedent conditions (Kolb, 1962), current stimulus circumstances (Cofer and Appley, 1964), qualitative and quantitative properties of the reinforcing drug, as well as stimuli associated with drug administration (Ausubel, 1964; Weeks and Collins, 1964), all have the ability to act as variables that do affect drug-reinforced response. They concluded, finally, that drug dependence can be analyzed using the operant paradigm and thus provide answers to the underlying mechanisms of drug dependence.

Beach (1957) reported that environmental stimuli acted as a secondary reinforcer in morphine dependent rats. A similar experiment was published by Wikler and Pescor (1967). Beach's experiment was intended to change the environment by giving the rats a choice of either the original environment or a new one instead of placing them into their original environment, as was done by Wikler and Pescor. The animals preferred the environment in which they experienced addiction and withdrawal to the unfamiliar neutral ones. Thus, it was concluded that rats would, when abstinent, show a preference for distinctive environments which had previously been repeatedly associated with relief of withdrawal symptoms (Kumar, 1972). It was further concluded that environmental stimuli can become secondary reinforcers after repeated pairing with the effects of morphine and

that the learning involved may contribute to the maintenance of dependent behavior.

Utilizing a self-administration technique Kumar and Stolerman (1972) showed that animals given morphine in their drinking water would drink large amounts of quinine following cessation of morphine in the water source. They concluded that the bitter taste of quinine alone was the reason for the large intake and they further concluded that taste had become a secondary reinforcer.

Utilizing both classical and operant paradigms Crowder et al. (1972) showed that animals given morphine injections paired with a buzzer will bar press for the buzzer and a saline infusion. They concluded that the buzzer and the saline injection had acquired secondary reinforcing properties. It was further concluded that a stimulus can become a secondary reinforcer without being a discriminative stimulus for an operant.

Utilizing state-dependent learning Hill et al. (1971) and Rosecrans et al. (1973) showed that rats could discriminate drug (morphine) and non-drug (saline) states. Hill's group concluded that when an addict takes an injection he is not only attempting to regain the initial unconditioned effects of the drug, but also to reinstate some of the learned or reinforcing experiences which can only occur in the drug condition.

The Rosecrans group did not attempt to explain their

results in terms of practical importance, but rather they explained their results in biochemical terms which will be discussed below.

Utilizing a classical paradigm, Roffman et al. (1972, 1973) paired a bell with morphine injections. The neutral bell eventually acquired properties of a conditional-stimulus, similar to morphine, which was shown to prevent withdrawal hypothermia during the 72 hr period following the last morphine injection. They concluded that to demonstrate that a conditional stimulus can block one withdrawal symptom would be to parallel the ritual that human addicts follow to postpone the onset of withdrawal. A human addict follows a set pattern when he administers the drug, and if the drug is not available the ritual alone (conditional stimulus) can postpone abstinence (Weidman and Fellner, 1971).

Conditioning associated with morphine ingestion can thus be demonstrated by the use of Pavlov's classical condition techniques. Also, the conditioning can be demonstrated by using an operant conditioning procedure or by combining both classical and operant procedures. Yet another way that conditioning associated with morphine has been observed is by using the state-dependent learning paradigm.

Neurochemical Systems Involved in Temperature Regulation

Feldberg and Myers (1963) postulated that body temperature is regulated by the balance of three monoamines

(5-HT serotonin, DA dopamine, and NE norepinephrine) in the anterior hypothalamus. This hypothesis was based on experiments in which serotonin or norepinephrine was administered intraventricularly and their effect on temperature recorded. Serotonin caused hyperthermia and norepinephrine caused hypothermia in the cat. In the rat similar evidence has been observed using serotonin and norepinephrine (Feldberg and Lotti, 1967; Breese and Howard, 1971). Besides these amines, dopamine (Kruk, 1972) and acetylcholine (Lomax et al., 1969) might also be involved in temperature regulation.

Utilizing the intraventricular injection technique, Jacob and Peindaris (1973) administered injections of serotonin to rabbits and observed, like Feldberg and Myers, an increase in body temperature. However, if the animals were pretreated with cyproheptidine (antiserotonin drug), the increase in temperature due to serotonin was antagonized. Jacob and Peindaris also injected NE intraventricularly and observed an increase in temperature (contrary results to those of Feldberg and Myers). When phenoxybenzamine was given one hr before the norepinephrine, the hyperthermia due to norepinephrine was antagonized. Propranolol (B adrenergic blocker) did not alter NE hyperthermia in rabbits. Chlorpromazine (a phenothiazine, antipsychotic) drug known to antagonize dopamine, caused hypothermia by itself. When norepinephrine and serotonin followed chlorpromazine no change in the norepinephrine hyperthermia was

observed and a very slight increase was noted in the serotonin treated animals.

Recent studies have indicated that cholinergic mechanisms in the hypothalamus may be involved in the central control of body temperature. Although the levels of acetylcholine (ACh) in the hypothalamus are relatively low when compared with the monoamines, the enzymes for ACh's synthesis and degradation are also present, suggesting that acetylcholine could fulfill a neurotransmitter role in this particular brain region (Hall, 1973). However, the role of acetylcholine on temperature regulation in the rat is still questionable (Myers, 1969). Many factors, such as route of administration, amount of substance, and environmental temperature, could account for discrepancies in whether or not acetylcholine directly affects the regulatory system. Nicotine has been shown to cause a rise in temperature, and if mecamylamine is given before nicotine the rise in temperature is blocked (Lomax and Kirkpatrick, 1969). The main conclusion from this study was that nicotine somehow changes the hypothalamic set point. Thus, nicotinic receptors play some role in the hypothalamic cholinergic thermoregulatory system.

Thermoregulatory Behavior

Homeotherms regulate their body temperature by 1) physiological or autonomic responses mediated by way of the sympathetic nervous system, and 2) behaviorial means involv-

ing coordinated and voluntary motor activity. There have been long discussions concerning the terms "autonomic" (physiological) versus "behavioral" thermoregulation since behavior can also be considered physiological. These terms are readily accepted and Cabanac (1972) has suggested that one speak of thermoregulatory behavior and thermoregulatory physiological responses in place of the more ambiguous terminology as behavioral thermoregulation and physiological thermoregulation.

Generally speaking, an animal in his natural environment compensates for fluctuations in temperature simply by moving to a warmer or cooler place (Richards, 1974). This movement of the organism to a more desirable thermal environment can be called by definition, thermoregulatory behavior (Hensel, 1973). The organism controls heat gain and heat loss by changing the physical characteristics of his environment by behavior such as avoidance, huddling, nestling, or putting on clothing such as is the case with man. Only recently has there been an increasing appreciation of the fact that, when given freedom to choose, homeotherms generally rely more on thermoregulatory behavior than on thermoregulatory physiological responses to alter their body temperature (Richards, 1974).

How are these responses motivated? It is safe to assume that organisms are motivated by states of "pleasantness" (comfort) and "unpleasantness" (discomfort). There
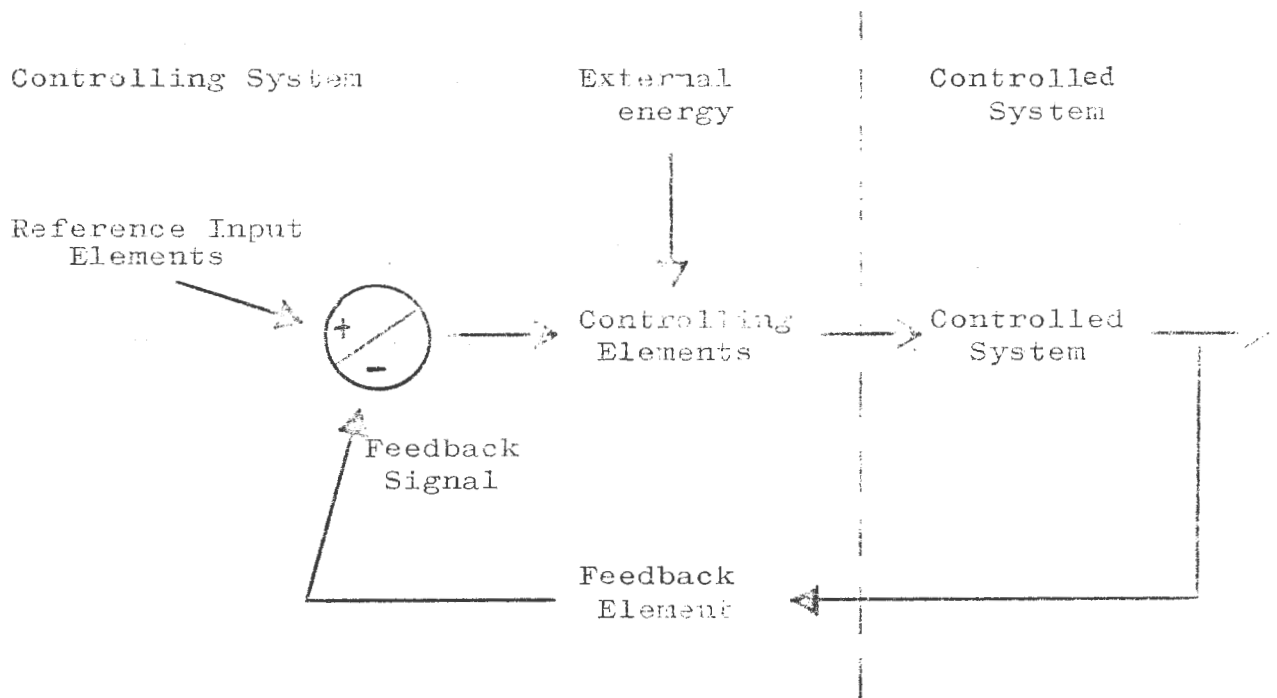
Fig. 1   Block diagram of a behavioral system of temperature regulation modified from Stolwijk and Hardy (1966). The desired outcome of the system is relative constancy of body temperature. The system also may represent autonomic regulation deriving internal energy rather than external as this diagram represents.

is evidence that consciousness plays a big role in determining what state is desirable to be comfortable. Corbit (1969) and Adair, et al. (1970) have shown that the rat and monkey will behaviorally control their environmental temperature when their preoptic-anterior hypothalamic area are thermally stimulated. The regulation is shown schematically in figure 1. This general diagram represents a modified version of Stolwijk and Hardy's (1966) view of the behavioral system. This type of diagram can and is used to explain both Corbit's and Adair's data. This diagram or one very similar has been used by many physiologists working in the area of thermo-regulation. The terms such as reference input elements, controlling elements, feedback elements, etc., may seem rather general but this field has grown so rapidly in recent years that organization of the data can best be explained using these terms in an engineering concept of control systems. Simply, the information has come at one time and no one has been able to synthesize all the ideas and propose a system identifying specific brain areas as to their exact function within the thermoregulatory system.

EXPERIMENTAL

(1)　　Chemicals

Chemicals used were U.S.P. grade or equivalent. Mor-
phine sulfate was obtained from Mallinkrodt Chemical Co.,
New York, New York. Naloxone hydrochloride was obtained
from Endo Laboratories, Inc., Garden City, New York. Benz-
tropine mesylate, cyproheptidine hydrochloride, and mec-
amylamine hydrochloride (Inversine) were obtained from Merck
Sharpe and Dohme Research Labs, Philadelphia, Pennsylvania.
Phenoxybenzamine hydrochloride (Dibenzyline) was obtained
from Smith, Kline and French Labs, Philadelphia, Pennsyl-
vania. Propranolol hydrochloride was obtained from Ayerst
Labs, Inc., New York, New York. The haloperidol (Haldol)
was obtained through the courtesy of McNeil Laboratories,
Fort Washington, Pennsylvania.

All drugs were dissolved in distilled water with
the exception of haloperidol, which was suspended in 0.5
percent carboxymethylcellulose. Doses are presented in
terms of salts. The volume of each injection never ex-
ceeded 0.8 cc, and all saline injections were equal in
volume to their corresponding drug treatment injections.

(2)　　Animals

Male hooded rats of Long-Evans strain, random-bred,
weighing 250-300 grams at the beginning of the experiments,

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were obtained from Rockland Farms, Philadelphia, Pennsylvania and from Charles River Breeding Farms (Canadian Breeding Farm and Laboratories, Inc.), Wilmington, Massachusetts. All animals were experimentally naive for this study. The rats were housed in individual cages in a room maintained at 21-23°C with the lights alternating on a 12-hour dark-light cycle. Food (Wayne Lab Blox) and water were available ad libitum except during the injections and during the physiological measurements.

(3)   Conditioning Procedure

Conditioning consisted of giving an injection of morphine sulfate paired with a bell (Tandy Corporation, Fort Worth, Texas) (78 db 20 kHz SPL measured one meter from the bell) twice daily at 0830 and 2030 for 12 to 14 days (Table 1). The injections of morphine were spaced 12 hr apart beginning with 10 mg/Kg/injection, and were increased by 10 mg/Kg every third injection until 100 mg/Kg/injection or 200 mg/Kg/day was reached (Table 1). The rats were maintained at this dose for 2-4 days and then withdrawn.

The procedure for injection during the morning session was as follows: Each animal was taken out of its home cage (one animal injected at a time), placed in a plastic container and taken to a sound attenuated and temperature controlled room (21°C $\pm$ 0.5) 40 feet from the room where the animals were housed. Immediately after entering the chamber the animal was removed from the plastic container

and placed into a single-pan balance to be weighed and then returned to the plastic container. The bell was turned on and after 45 seconds the animal was picked up and securely held, one hind leg and the head, so as to prevent the animal from movement and the injection was given. Then the animal was again returned to the plastic container, and after a total of 60 seconds had elapsed, the bell was turned off. The rat was then immediately returned to his individual cage. Each day the order of animals going through this procedure was changed.

The identical procedure was followed during the evening session with the exception that the body weight was not taken at that time.

(4)   Testing Procedure

The test procedure for the experiments using mecamylamine, phenoxybenzamine, propranolol, haloperidol, benztropine and cyproheptidine to evaluate their control of morphine withdrawal hypothermia was the following:

1. The same animals were used throughout each experiment.

2. Temperatures of each animal were taken 10 min prior to and 30 min after their last morphine injection.

3. Temperatures of all animals were again taken 24 hr later, prior to test drug administration and a designated period of time following test drug administration.

4. The animals were then divided into two groups, those receiving morphine and those receiving the bell. 30 min after the test treatment the temperatures were taken again.

(5)   Temperature Measurements

All temperature measurements were taken at designated times using a digital thermistor thermometer (Digitec Model 8500-2 by United Systems Corporation, Dayton, Ohio). The rectal probe (Model 402, Yellow Springs Instrument Co., Maryland) was inserted five cm (Myers, 1973) into the rectum for one minute (Lomax, 1970). Each animal had his temperature taken immediately before and 30 minutes after the 0830 injection on two successive days preceding withdrawal. These four insertions of the probe allowed the animals to adjust to the procedure. Also, the animals were handled with great care during both the adjustment trials mentioned above and during the experimental measurements. One hand was placed on the back of the animal about midline, the thumb and first finger holding the tail with a minimal amount of pressure (just enough to keep the animal still). The other hand inserted the probe (coated with mineral oil) the proper distance and held it in place until the required time was reached.

(6)   Measurement of Withdrawal Symptoms

Rats dependent on morphine are removed from their home cages and placed into a novel stainless steel cage

($9\frac{1}{2}$" x 7" x 7") (Wikler and Pescor, 1967) for the purpose of observations. The animals were observed for 30 minutes during which the following symptoms were measured:

Shakes - These are movements of the head and/or body which resemble the behavior an animal exhibits when water is poured over him. The frequency of shakes was tabulated during the 30-minute session.

Ptosis - This condition was present when the animal's eyelids are drooping but not closed tightly and he is capable of movement. The animal moves periodically, and this state was distinguishable from sleep. The amount of time spent in this state was measured by elapsed timers during the 30-minute observation period.

Writhing - This consists of dragging the abdomen on the floor of the observation cage or arching of the back, neither of which is accompanied by yawning. The existence of this symptom was measured during the 30-minute observation period.

Piloerection - This symptom was observed when the rat's fur stands out from the body. The occurrence or absence was measured after the animal had time to groom following placement into the cage. This was done so as not to report raised fur that might have resulted from handling.

Changes in body weight and temperature were measured just prior to placing the animals in the observation cages.

All of the measurements were made at 0, 24, 48, 72 hours following the last morphine injection. These observations were always made in the morning.

(7)   Statistics

The Student's "t" test was used to determine the significance of a difference between two correlated means (i.e., pre-challenge and post-challenge temperatures). The two temperatures, pre and post, were recorded for each individual rat and the column designated "change" was arrived at by subtracting the post-challenge temperature and the pre-challenge temperature of each animal.

TABLE 1

SCHEDULE OF MORPHINE ADMINISTRATION

DAY	DOSE/INJECTION[1]	TOTAL DAILY DOSE
1	10	20
2	20	40
3	30	60
4	40	80
5	50	100
6	60	120
7	70	140
8	80	160
9	90	180
10-14	100	200

[1] Dose in mg/Kg.

RESULTS

A. Specificity and Reproducibility of the Conditional
   Stimuli

In order to determine if the time of day affects rec-
tal temperature changes due to morphine administration, ad-
dicted animals and naive animals' temperatures were taken
before and 30 minutes after a morphine or saline injection,
respectively, at both 0830 and 2030. Data presented in Ta-
ble 2 showed that morphine did not affect rectal tempera-
ture differently in the evening than in the morning. Also,
the pre-injection temperature did not differ. Normal ani-
mals were observed not to have any difference in their tem-
peratures whether taken at 0830 or 2030.

Data presented in Table 3 showed that the bell con-
sistently increased the temperature at 24 hr of withdrawal
in morphine-addicted rats which had the bell paired with
each injection during addiction. Further, the five groups
presented that received morphine and the bell throughout
addiction in five different experiments showed little dif-
ference between experiments. These data were taken from
different experiments conducted during this investigation.

The specificity of the bell's effect on rectal tem-
perature is summarized in Table 4. The bell had no effect
on (1) animals that had never received the drug, (2) on
morphine-addicted animals which received a random bell

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TABLE 2

COMPARISON OF TEMPERATURE CHANGE DUE TO MORPHINE ADMINISTRATION[1] IN ADDICTED AND NAIVE ANIMALS AT 0830 and 2030 HR

HOUR OF DAY	N[2]	Rectal Temperature (°C), Mean $\pm$ S.E.[3]		
		Pre-Injection	Post-Injection[4]	Change
addicted rats				
0830	28	37.38$\pm$0.04	39.17$\pm$0.04	+1.79$\pm$0.05
2030	28	37.50$\pm$0.03	39.21$\pm$0.02	+1.73$\pm$0.03
naive rats				
0830	36	37.86$\pm$0.05	37.88$\pm$0.06	+0.01$\pm$0.04
2030	36	37.78$\pm$0.04	37.83$\pm$0.06	+0.05$\pm$0.05

[1]Morphine given i.p. (100 mg/kg), 12 hr after phine injections in addicted rats.

[2]No. of animals in each condition.

[3]S.E. refers to standard error.

[4]Temperature taken 30 min after the morphine injection.

[5]Received saline injection, i.p.

TABLE 3

EFFECT OF MORPHINE AND THE CONDITIONAL STIMULUS

ON RECTAL TEMPERATURE

TREAT-MENT	N[1]	Rectal Temperature (°C), Mean $\pm$ S.E.[2]		
		Pre-Challenge	Post-Challenge[3]	Change[4]
bell (CS)	6	37.40±0.09	38.06±0.06	+0.67±0.08
	6	37.00±0.08	38.04±0.06	+1.04±0.05
	6	37.39±0.09	38.06±0.04	+0.67±0.07
	18[6]	37.31±0.03	38.05±0.02	+0.75±0.06
morphine[5]	6	37.27±0.10	38.87±0.21	+1.60±0.15
	6	37.21±0.09	38.90±0.11	+1.09±0.11
	12[6]	37.25±0.07	38.89±0.09	+1.62±0.08
morphine[5]	6	37.39±0.07	39.06±0.06	+1.67±0.07
+	12	37.19±0.07	39.28±0.28	+2.09±0.08
	36	37.36±0.03	39.10±0.05	+1.73±0.04
CS	20	37.34±0.05	39.20±0.06	+1.87±0.05
	12	37.41±0.06	39.27±0.06	+2.09±0.06
	86[6]	37.36±0.03	39.17±0.05	+1.82±0.05

[1] No. of animals in each group.

[2] Refer to legend of Table 2.

[3] Rectal temperature taken 30 min after treatment.

[4] Each animal used as his own control (+ denotes increase in rectal temperature and - denotes decrease in rectal temperature).

[5] 100 mg/kg, given i.p.

[6] Combined data from replications.

TABLE 4

COMPARISON OF DIFFERING TREATMENTS DURING ADDICTION AND
THEIR RESPECTIVE BELL EFFECTS ON RECTAL TEMPERATURE
DURING WITHDRAWAL

Treatment During Addiction Phase[1]	N[4]	Bell Effect During Withdrawal[2] (Mean $\pm$ S.E.[6])			
		Before CS	After CS	Change[8]	p[5]
morphine[3] + paired bell	18	37.31$\pm$0.03	38.05$\pm$0.02	+0.75$\pm$0.06	<.001
morphine[3] + random bell	10	37.43$\pm$0.04	37.47$\pm$0.02	+0.03$\pm$0.02	N.S.[7]
morphine[3] + no bell	12	37.31$\pm$0.05	37.40$\pm$0.10	+0.10$\pm$0.08	N.S.[7]
no drug + bell	14	37.81$\pm$0.05	37.98$\pm$0.19	+0.16$\pm$0.15	N.S.[7]

[1]Given twice daily for 13-15 days.

[2]Rectal temperature ($^{\circ}$C), taken during 24 hr of withdrawal.

[3]200 mg/kg/day (terminal dose), given i.p.

[4]No. of animals in each group.

[5]Student's "$\underline{t}$" test.

[6]Refer to Legend 3 of Table 2.

[7]N.S. refers to not significant.

[8]Refer to Legend 4 of Table 3.

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during addiction, and (3) on morphine-addicted animals naive to the bell. The only rats whose rectal temperatures were affected (increase) by the bell alone were the animals who received morphine and bell paired throughout addiction.

Table 5 shows that the bell, when presented 24 hr after the last morphine injection, causes an increase in rectal temperature but this increase is not attenuated by additional presentations at 30 minute intervals after the initial presentation. The three presentations at 24.5, 25 and 25.5 hours were for only 10 seconds; only the first presentation was for one minute.

Different doses of morphine were given 24 hr after the last morphine injection as can be seen in Table 6. As the dose increased, the effect on rectal temperature increased until 25 mg/kg was given. No difference in change of rectal temperature existed between 25 mg/kg and 100 mg/ kg doses. The dose of 12.5 mg/kg was observed to be similar in magnitude to the increase in rectal temperature following the bell when presented to conditioned animals.

Data presented in Table 7 show the effect of one dose (100 mg/kg) of morphine over a period of 48 hr. The temperature reached a maximum at 30 minutes after the intraperitoneal injection. This temperature was still high two hr after the injection. These data were used to determine the appropriate time at which the temperature should be recorded following the morphine injection or the presentation of the bell.

TABLE 5

RECTAL TEMPERATURE AFTER CONDITIONAL STIMULUS GIVEN AT 30 MIN INTERVALS

BEGINNING 24 HR AFTER THE LAST MORPHINE INJECTION

| Hours After Last Injection | Min After Last CS[1] | No. of CS Presented | Temperature, Mean ± S.E.[3] | |
			Rectal Temperature	Change[2]
24	0	0	37.46±0.06	--
24.5	30	1	38.04±0.06	+0.60±0.07
25	30	2	38.05±0.06	+0.62±0.06
25.5	30	3	38.04±0.06	+0.60±0.06

[1] CS presented for 1 min at 24 hr and for 10 sec at 24.5, 25, 25.5 hrs, temperature measured just prior to the presentation (30 min after the last bell).

[2] Compared with temperature prior to initial bell (+ denotes increase in rectal temperature).

[3] Refer to Legend 3 Table 2.

Note: 10 sec was used because it was found that 24 hr after last CS-morphine pairing the bell given for 10 sec caused an increase of 0.79±0.06 (N=12).

TABLE 6

EFFECT OF MORPHINE DOSE ON RECTAL

TEMPERATURE DURING WITHDRAWAL

Dose mg/kg	N^3	Mean $\pm$ S.E.[4]	
		Rectal Temperature (°C)	Change[2]
0	30	37.26 $\pm$ 0.02	--
3.13	6	37.50 $\pm$ 0.13	+ 0.24 $\pm$ 0.10
6.25	6	37.94 $\pm$ 0.12	+ 0.68 $\pm$ 0.08
12.50	6	38.28 $\pm$ 0.07	+ 1.03 $\pm$ 0.05
25.00	6	39.11 $\pm$ 0.12	+ 1.84 $\pm$ 0.11
100.00	12	39.14 $\pm$ 0.03	+ 1.88 $\pm$ 0.03

[1] 24 hrs after last morphine injection.

[2] Compared with temperature prior to drug administration (+ denotes increase in rectal temperature).

[3] No. of animals in each group.

[4] Refer to Legend 3 of Table 2.

TABLE 7

TEMPERATURE IN ADDICTED ANIMALS FOLLOWING

A DOSE OF 100/mg/kg of MORPHINE

Hours After Injection	N[1]	Rectal Temperature Mean ± S.E.[2]
0.00	6	37.32 ± 0.19
0.25	6	38.55 ± 0.15
0.50	6	38.87 ± 0.21
1.00	6	38.85 ± 0.25
2.00	6	38.56 ± 0.20
12.00	20	37.34 ± 0.05
24.00	20	37.29 ± 0.07
48.00	8	37.59 ± 0.17

[1]No. of animals in each group.

[2]Refer to Legend 3 of Table 2

B. Similarity Between Mechanisms by which Morphine and Conditional Stimuli Act

Data summarized in Table 8 showed that neither the bell nor morphine (100 mg/kg) was able to increase the temperature following a "pure" morphine antagonist, naloxone (2 mg/kg). Further, naloxone caused a drop in temperature following an increase in temperature due to the bell, an effect which is normally only seen following an injection of morphine. This experiment was carried out 24 hr after the last morphine injection, when naloxone given alone (2 mg/kg) only caused a slight drop in rectal temperature. This information suggests that the bell and morphine were acting on either a single or parallel pathways which meet at some point eliciting the same effect.

To further substantiate the similarity of physiological mechanisms (bell and morphine) the bell or morphine was given following bell and naloxone (2 mg/kg). Neither the bell nor morphine could reverse the effect of the antagonist.

C. Role of Autonomic Nervous System in the Effect of CS and Morphine

If the autonomic nervous system was required for mediating the effect of morphine related CS and morphine on the thermoregulatory system, interaction with a ganglionic blocker (mecamylamine) would indicate if this system was involved. Propranolol (2 mg/kg), a beta adrenergic blocking agent, should prevent hyperthermia following the

TABLE 8

EFFECT OF NALOXONE, MORPHINE OR CONDITIONAL STIMULUS ADMINISTERED AFTER

AN ABSTINENCE PERIOD OF 24 HR IN MORPHINE ADDICTED RATS

| Sub-Group | Test Treatment | N | Rectal Temperature (°C) Mean $\pm$ S.E.[5] | | | |
			Before	After	Change[3]	p[4]
1	None	30		37.00 ± 0.08		
2	Naloxone[1]	6	37.00 ± 0.08	36.68 ± 0.06	-0.34 ± 0.12	$< .05$
3	Morphine[2]	6	37.27 ± 0.10	38.87 ± 0.21	$+1.60 \pm 0.15$	$< .001$
4	Bell	6	37.00 ± 0.08	38.04 ± 0.06	$+1.04 \pm 0.11$	$< .001$
5	Morphine + Naloxone	6	38.65 ± 0.17	37.37 ± 0.26	-1.28 ± 0.09	$< .001$
6	Bell + Naloxone	6	38.06 ± 0.06	36.17 ± 0.06	-1.91 ± 0.08	$< .001$

[1] 2 mg/kg given i.p.

[2] 100 mg/kg given i.p.

[3] Refer to Legend 4 of Table 3.

[4] Student's "$\underline{t}$" test.

[5] Refer to Legend 3 of Table 2.

TABLE 9

EFFECT OF MORPHINE OR CONDITIONAL STIMULUS ADMINISTERED AFTER NALOXONE

Test Treatment	N[7]	Rectal Temperature (°C) Mean ± S.E.[8]			
		Before	After	Change[5]	p[6]
Saline + Bell	6	37.39±0.09	38.06±0.04	+0.67±0.07	<.001
Bell + Naloxone[1]	6	38.06±0.06	36.20±0.04	+1.86±0.10	<.001
Bell[3]	3	36.21±0.03	36.25±0.09	+0.05±0.03	N.S.[9]
Morphine[2,4]	3	36.18±0.05	36.30±0.11	+0.11±0.05	N.S.[9]

[0] Tests made 24 hrs after the last morphine injection.

[1] 2 mg/kg, i.p.

[2] 100 mg/kg, i.p.

[3,4] Animals for these groups had received Bell + Naloxone before either the bell again or morphine.

[5] Refer to Legend 4 of Table 3.

[6] Student's "t" test.

[7] No. of animals in each test group.

[8] Refer to Legend 3 of Table 2.

[9] Refer to Legend 5 of Table 4.

Note: Saline + Bell and Bell + Naloxone are the same animals. The Bell and Morphine groups were derived from the 6 animals of Bell + Naloxone.

CS or morphine if β receptors are involved in mediating the production of hyperthermia. Furthermore, if ⍺ receptors are involved in mediating either the effect of CS or morphine on rectal temperature, giving phenoxybenzamine (2 mg/kg), an ⍺ adrenergic blocker, would prevent hyperthermia. If both ⍺ and β adrenergic blockers were involved in the hyperthermic response due to morphine or the CS, then both propranolol and phenoxybenzamine would be necessary to prevent the increase in temperature.

Data presented in Table 10 indicate that mecamylamine (2.5 mg/kg) pretreated animals (one hour) do not show any increase in temperature due to either treatment by the bell or a morphine injection. Giving mecamylamine alone does not change the temperature of 24 hr abstinence rats. Morphine alone increased the temperature two degrees (Table 2), and the bell alone at 24 hr of abstinence increased the temperature by almost one degree (Table 2).

Data summarized in Table 11 indicate that animals pretreated (one hour) with propranolol show an increase in temperature following either the CS or morphine. Furthermore, propranolol alone at 2 mg/kg caused no significant change in the withdrawn animals. Thus, blocking of β receptors did not block the increase in temperature due to the CS or morphine, thereby suggesting that B receptors do not play a major role in this phenomenon. However, data summarized in Table 12 indicate that animals pretreated

TABLE 10

EFFECT OF MECAMYLAMINE, MORPHINE OR CONDITIONAL STIMULUS ADMINISTERED AFTER AN
ABSTINENCE PERIOD OF 24 HR ON RECTAL TEMPERATURE IN MORPHINE-ADDICTED RATS

Blocking Drug	Challenge Treatment	N[6]	Rectal Temperature (°C), Mean $\pm$ S.E.[8]			
			Pre-Challenge	Post-Challenge	Change[4]	p[5]
None	CS + Morphine[3]	12	37.19$\pm$0.07	39.28$\pm$0.28	2.09$\pm$0.08	<.001
Mecamylamine[1]	--	12	36.83$\pm$0.04	36.95$\pm$0.07	0.12$\pm$0.04	N.S.[7]
Mecamylamine[1,2]	CS	6	36.87$\pm$0.03	37.06$\pm$0.08	0.19$\pm$0.09	N.S.[7]
Mecamylamine[1,2]	Morphine	6	36.79$\pm$0.09	36.85$\pm$0.09	0.06$\pm$0.04	N.S.[7]

[1] 2.5 mg/kg, given intraperitoneally.

[2] Given 1 hr before challenge treatment.

[3] 100 mg/kg, given intraperitoneally.

[4] Each animal used as his own control.

[5] Student's "t" test.

[6] No. of animals in each test condition.

[7] Refer to Legend 7 of Table 4.

[8] Refer to Legend 3 of Table 2.

TABLE 11

EFFECT OF PROPRANOLOL, MORPHINE OR CONDITIONAL STIMULUS ADMINISTERED AFTER AN
ABSTINENCE PERIOD OF 24 HR ON RECTAL TEMPERATURE IN MORPHINE-ADDICTED RATS

Blocking Drug	Challenge Treatment	N[6]	Rectal Temperature (°C), Mean $\pm$ S.E.[8]			
			Pre-Challenge	Post-Challenge	Change[4]	P[5]
None	CS + Morphine[3]	36	37.36±0.03	39.10±0.05	1.73±0.06	<.001
Propranolol[1]	--	18	37.28±0.08	37.30±0.07	0.02±0.06	N.S.[7]
Propranolol[1,2]	CS	12	37.29±0.07	38.09±0.09	0.81±0.04	<.001
Propranolol[1,2]	Morphine	6	37.31±0.07	38.21±0.10	0.90±0.17	<.001

[1] 2 mg/kg, given intraperitoneally.
[2] Given 1 hr before challenge treatment.
[3] 100 mg/kg, given intraperitoneally.
[4] Each animal used as his own control.
[5] Student's "_t_" test.
[6] No. of animals in each test condition.
[7] Refer to Legend 6 of Table 4.
[8] Refer to Legend 3 of Table 2.

TABLE 12

EFFECT OF PHENOXYBENZAMINE, MORPHINE OR CONDITIONAL STIMULUS ADMINISTERED AFTER AN ABSTINENCE PERIOD

OF 24 HR ON RECTAL TEMPERATURE IN MORPHINE-ADDICTED RATS

Blocking Drug	Challenge Treatment	N[6]	Rectal Temperature (°C), Mean $\pm$ S.E.[7]			
			Pre-Challenge	Post-Challenge	Change[4]	P[5]
None	CS + Morphine[3]	36	37.36±0.03	39.10±0.05	+1.73±0.06	<.001
Phenoxybenzamine[1]	--	18	37.36±0.09	37.35±0.08	0.00±0.04	N.S.[8]
Phenoxybenzamine[1,2]	CS	12	37.38±0.08	37.37±0.07	-0.01±0.02	N.S.[8]
Phenoxybenzamine[1,2]	Morphine[3]	6	37.33±0.08	37.68±0.09	+0.35±0.14	<.05

[1]
 2 mg/kg, given intraperitoneally.
[2]
 Given 1 hr before challenge treatment.
[3]
 100 mg/kg, given intraperitoneally.
[4]
 Each animal used as his own control.
[5]
 Student's "t" test.
[6]
 No. of animals in each test condition.
[7]
 Refer to Legend 3 of Table 2.
[8]
 Refer to Legend 6 of Table 4.

(one hour) with phenoxybenzamine (2 mg/kg) did not show
hyperthermia after the bell, and showed only a slight in-
crease following morphine. Phenoxybenzamine alone at 2 mg/
kg had no effect on the temperature of the 24 hr CS-morphine
deprived animals. Therefore, blocking of the alpha recep-
tors caused a dramatic reduction in the hyperthermic ef-
fects of the CS or morphine.

D. Study of the Physiological Pathways of the Central
    Nervous System

If central catecholamines and/or 5-hydroxytryptamine
are required in mediating the effect of morphine related CS
and morphine on rectal temperature, then drugs blocking
action of these substances should prevent the occurrence of
an increase in temperature. Haloperidol, a dopaminergic
blocking agent (at the receptor), should block the morphine
or CS-induced hyperthermia if the increase in temperature
is dependent on dopaminergic activity. Furthermore, if
cholinergic neurons are involved, administering benztropine
should prevent hyperthermia due to either or both the CS
and morphine. Because of the modulatory effect of ACh as
a possible regulator of dopamine release (Glowinski, et al.,
1973), benztropine and haloperidol might produce the same
effect. Still another compound, cyproheptidine (5-HT
antagonist), should cause a block of hyperthermia due to
either or both CS and morphine if serotonin is involved in
the hyperthermic response. If more than one transmitter

(dopamine, acetylcholine, or serotonin) is involved, then two or more of the compounds might be required to prevent the hyperthemic effect due to the CS or morphine.

Data summarized in Table 13 indicate that halo-peridol (2 mg/kg) pretreatment (2 hr) was able to block the hyperthermia due to the CS, but had no blocking effect after 100 mg/kg of morphine sulfate. Haloperidol alone decreased the temperature by almost one-half of a degree. Therefore, all changes due to the CS or morphine are computed after correcting for the hypothermia caused by haloperidol administration and are compared to the temperature either after the bell or after morphine injection. Thus, the data suggest that the CS is operating by using dopaminergic pathways because haloperidol blocked the increase in temperature that should have followed the bell.

Table 14 summarizes the data obtained after pre-treating (30 min) the withdrawn animals with benztropine (0.625 mg/kg). Following this pretreatment, there was no significant change from the initial temperatures (before benztropine was administered). When morphine or the CS was given to the animals, benztropine acted similarly to haloperidol since it blocked the hyperthermia normally seen after morphine. This similarity of haloperidol and benztropine action on both the CS and morphine appears to support a hypothesis that the CS is working via a cho-linergic-dopaminergic linked system and that morphine is

TABLE 13

EFFECT OF HALOPERIDOL, MORPHINE OR CONDITIONAL STIMULUS ADMINISTERED AFTER AN ABSTINENCE PERIOD OF

24 HR ON RECTAL TEMPERATURE IN MORPHINE-ADDICTED RATS

Blocking Drug	Challenge Treatment	N[6]	Rectal Temperature ($^{\circ}$C), Mean $\pm$ S.E.[7]			
			Pre-Challenge	Post-Challenge	Change[4]	P[5]
None	CS + Morphine[3]	12	37.41+0.06	39.27+0.06	+2.09+0.06	<.001
Haloperidol[1]	--	12	37.46+0.06	37.01+0.07	-0.45+0.06	<.001
Haloperidol[1,2]	CS	6	37.08+0.04	37.11+0.03	+0.03+0.02	N.S.[8]
Haloperidol[1,2]	Morphine	6	37.13+0.09	39.36+0.08	+2.22+0.14	<.001

[1]
0.02 mg/kg given intraperitoneally.

[2]
Given 2 hr before challenge treatment.

[3]
100 mg/kg, given intraperitoneally.

[4]
Each animal used as his own control (+ denotes increase in rectal temperature and - denotes decrease in rectal temperature).

[5]
Student's "t" test.

[6]
No. of animals in each test condition.

[7]
Refer to Legend 3 of Table 2.

[8]
Refer to Legend 6 of Table 4.

TABLE 14

EFFECT OF BENZTROPINE, MORPHINE OR CONDITIONAL STIMULUS ADMINISTERED AFTER AN ABSTINENCE PERIOD OF

24 HR ON RECTAL TEMPERATURE IN MORPHINE-ADDICTED RATS

Blocking Drug	Challenge Treatment	N[6]	Rectal Temperature (^{O}C), Mean $\pm$ S.E.[7]			
			Pre-Challenge	Post-Challenge	Change	P[5]
None	CS + Morphine[3]	20	37.34+0.05	39.20+0.06	+1.87+0.08	<.001
Benztropine[1]	--	20	37.29+0.07	37.36+0.06	+0.07+0.09	N.S.[8]
Benztropine[1,2]	CS	10	37.40+0.08	37.71+0.11	+0.31+0.06	<.05
Benztropine[1,2]	Morphine	10	37.34+0.08	38.65+0.18	+1.32+0.16	<.001

[1]
0.625 mg/kg given intraperitoneally.
[2]
Given 30 min before challenge treatment.
[3]
100 mg/kg, given intraperitoneally.
[4]
Each animal used as his own control.
[5]
Student's "t" test.
[6]
No. of animals in each test condition.
[7]
Refer to Legend 3 of Table 2.
[8]
Refer to Legend 6 of Table 4.

working through a nondopaminergic system.

Data for cyproheptidine (2 mg/kg and 4 mg/kg) pre-
treated animals (45 min) summarized in Table 15 indicate
that following cyproheptidine, the increase in temperature
due to the bell was not blocked to the same extent as the
increase in temperature due to morphine was blocked. The
CS-induced increase in temperature was blocked slightly;
however, morphine induced hyperthermia was blocked signif-
icantly. Administering cyproheptidine by itself at either
dose caused no significant change in temperature in the 24
hr CS-morphine deprived animals. Although not as con-
clusively as in the case of haloperidol or benztropine,
the data seem to suggest that with cyproheptidine, mor-
phine's effect on temperature was blocked while CS-induced
hyperthermia was affected to a lesser degree.

E. Determination of the Conditional Stimulus Effect on
    Other withdrawal Symptoms

In order to find out if the bell had any effect on
other withdrawal symptoms (body weight loss, shakes,
ptosis, piloerection and writhing) the rats were addicted
as previously described getting morphine during the pre-
sentation of the bell. Following addiction they received
either the bell or nothing every 12 hr and their with-
drawal symptoms were measured 24, 48, and 72 hr after the
last morphine-bell pairing. Other animals who received

TABLE 15

EFFECT OF CYPROHEPTIDINE, MORPHINE OR CONDITIONAL STIMULUS ADMINISTERED AFTER AN ABSTINENCE PERIOD OF

24 HR ON RECTAL TEMPERATURE IN MORPHINE-ADDICTED RATS

Blocking Drug	Challenge Treatment	N[6]	Rectal Temperature ($^\circ$C) $\pm$ S.E.[8]			
			Pre-Challenge	Post-Challenge	Change[5]	p[6]
None	CS + Morphine[4]	28	38.34+0.05	39.20+0.06	+1.87+0.09	<.001
Cyproheptidine[1]	--	14	37.44+0.04	37.41+0.04	-0.03+0.03	N.S.[9]
Cyproheptidine[2]	--	14	37.45+0.07	37.43+0.04	-0.01+0.03	N.S.[9]
Cyproheptidine[1,3]	CS	7	37.43+0.05	37.97+0.14	+0.54+0.11	<.01
Cyproheptidine[2,3]	CS	7	37.40+0.03	37.92+0.12	+0.52+0.17	<.01
Cyproheptidine[1,3]	Morphine[4]	7	37.39+0.08	37.72+0.26	+0.34+0.23	N.S.[9]
Cyproheptidine[2,3]	Morphine[4]	7	37.46+0.07	37.65+0.21	+0.19+0.20	N.S.[9]

[1] 2 mg/kg, given intraperitoneally.
[2] 4 mg/kg, given intraperitoneally.
[3] Given 45 min before challenge treatment.
[4] 100 mg/kg, given intraperitoneally.
[5] Refer to Legend 4 of Table 3.
[6] Student's "t" test.
[7] No. of animals in each test condition.
[8] Refer to Legend 3 of Table 2.
[9] Refer to Legend 6 of Table 4.

morphine alone during addiction received either the bell or nothing following the same procedure mentioned above.

Data presented in Table 16 showed that the bell did not significantly affect the withdrawal symptoms, at any of the time periods measured, except shakes at 72 hr. It seems doubtful that this effect at 72 hr has any real meaning because no effect of the CS was observed prior to that time. Further investigation would determine if this was a real effect.

Since at a terminal dose of 200 mg/kg/day the bell did not affect any of the withdrawal symptoms (except the isolated instance of 72 hr shakes and temperature), it was decided to double the terminal dose to determine if this may aid in the ability of the CS to affect withdrawal symptoms other than temperature. Table 17 shows that again the CS did not affect any withdrawal symptom (except temperature) when given every 12 hr during withdrawal. (Note: The dose/day was double the schedule used for 200 mg/kg/day terminal dose, the number of days given morphine was equal.) These data suggest that using the present experimental design, the CS does not affect any withdrawal symptoms that were being measured, regardless of the terminal dose.

TABLE 16

EFFECT OF THE CONDITIONAL STIMULUS ON SELECTED WITHDRAWAL SYMPTOMS DURING THE PRIMARY ABSTINENCE PERIOD FOLLOWING TERMINAL DOSE OF 200 mg/kg/day

Treatment During Addiction[6]	Treatment During Withdrawal	Symptoms,[2] Mean ± S.E.[4]				
		Weight loss(g)[3]	Shakes	Ptosis[1]	Piloerection[8]	Writhing[8]
		24 Hours				
Morphine	None[1]	4.75 ± 1.18	2.71 ± 0.43	37 ± 18	25-28	10-28
Morphine	Bell[1]	4.90 ± 1.58	4.40 ± 1.89	0 ± 0	8-10	4-10
Bell + Morphine	None	2.11 ± 0.65	2.67 ± 0.41	0 ± 0	8-9	0-9
Bell + Morphine	Bell[1]	4.80 ± 0.87	3.30 ± 0.78	0 ± 0	10-10	4-10
		48 Hours				
Morphine	None[4]	25.14 ± 1.89	5.50 ± 0.60	6 ± 6	28-28	13-28
Morphine	Bell[4]	26.80 ± 2.44	4.60 ± 1.58	0 ± 0	10-10	5-10
Bell + Morphine	None[4]	25.56 ± 1.78	5.56 ± 1.16	16 ± 13	9-9	2-9
Bell + Morphine	Bell[4]	27.70 ± 2.17	3.90 ± 0.50	4 ± 4	10-10	7-10
		72 Hours				
Morphine	None[5]	25.50 ± 2.15	5.36 ± 0.52	12 ± 11	28-28	13-28
Morphine	Bell[5]	29.00 ± 4.23	6.70 ± 2.25	30 ± 30	10-10	4-10
Bell + Morphine	None	21.67 ± 2.49	6.11 ± 1.40	0 ± 0	9-9	3-9
Bell + Morphine	Bell[5]	27.10 ± 2.79	3.70 ± 1.03	0 ± 0	10-10	6-10

[1]Bell presented at 12 and $23\frac{1}{2}$ hr.

[2]Symptoms measured for 30 min, except for body weight

[3]Refers to loss from zero time.

[4]Bell presented at 12, $23\frac{1}{2}$, 36, $47\frac{1}{2}$ hrs.

[5]Bell presented at 12, $23\frac{1}{2}$, 36, $47\frac{1}{2}$, 60, and $71\frac{1}{2}$.

[6]Terminal morphine dose, 200 mg/kg/day.

[7]Measured in seconds (duration) during 30 min of observation

[8]Number of animals showing symptom out of total number observed in each group.

[9]Refer to Legend 3 of Table 2.

TABLE 17

EFFECT OF THE CONDITIONAL STIMULUS ON SPECIFIC WITHDRAWAL SYMPTOMS DURING THE
PRIMARY ABSTINENCE PERIOD FOLLOWING TERMINAL DOSE OF 400 mg/kg/day

Treatment During Addiction[2]	Treatment During Withdrawal	Symptom,[1] Mean ± S.E.[10]					
		Temperature Change[3]	Weight Loss[4]	Shakes	Ptosis[8]	Pilo-erection[9]	Writhing[9]
		24 Hours					
Morphine	None	+0.04±0.08	6.60±1.67	4.40±0.84	70±43	10-10	3-10
Bell + Morphine	None	-0.09±0.11	6.00±0.71	1.00±0.71	0±0	4-4	1-4
Bell + Morphine	Bell[5]	+0.76±0.09	9.83±1.66	1.67±0.61	212±96	6-6	1-6
		48 Hours					
Morphine	None	+0.09±0.09	25.60±3.86	3.00±0.52	6±6	10-10	9-10
Bell + Morphine	None	+0.07±0.14	29.50±1.86	4.50±1.44	9±9	4-4	3-4
Bell + Morphine	Bell[6]	+1.09±0.08	31.83±3.01	3.83±1.58	0±0	6-6	3-6
		72 Hours					
Morphine	None	-0.10±0.14	23.20±4.79	2.90±0.57	9±6	10-10	7-10
Bell + Morphine	None	+0.08±0.10	24.00±7.80	5.75±1.25	0±0	4-4	3-4
Bell + Morphine	Bell[7]	+0.99±0.10	30.50±2.60	5.17±1.30	0±0	6-6	5-6

[1] Symptoms measured for 30 min, except for body weight and temperature.

[2] Terminal dose of morphine, 400 mg/kg/day.

[3] Refers to difference from temperature taken 30 min after last morphine injection.

[4] Refers to loss from zero time.

[5] Bell presented at 12 and 23½ hr.

[6] " " " " , 36 and 47½ hr.

[7] " " " " " , 47½, 60, and 71½ hr.

[8] Measured in seconds (duration) during 30 min of observation.

[9] Number of animals showing symptom out of total number observed in each group.

[10] Refer to Legend 3 of Table 2.

DISCUSSION

This study is especially significant in that it demonstrates that the conditional stimulus and morphine affect the body temperature in morphine addicted rats through a similar neurophysiological pathway initiated by different neurosubstances. In this study, rats given a conditional stimulus paired with morphine, when given the conditional stimulus alone during withdrawal, exhibited an increase in temperature analogous to the effect of morphine.

Additional withdrawal symptoms (wet shakes, ptosis, piloerection, body weight loss and writhing) were observed. The conditional stimulus was found not to change any of these symptoms. Therefore, under the present addiction schedule the change in temperature was the only conditional withdrawal symptom that was measured. However, this does not mean that temperature is the only conditionable withdrawal symptom. Rather, this system was most easily conditioned and by possibly varying the conditioning procedures it might be possible to alleviate the severity of other withdrawal symptoms.

The following discussion will cover three areas. The first part will include the evidence establishing the ability of the CS to cause a rise in temperature during withdrawal, similar to the change seen following a morphine in-

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jection. The second part will deal with the physiological mechanisms involved in mediating temperature changes following the CS and morphine. The last part will deal with the significance of these findings.

The addiction schedule was modified from a previous experiment by Roffman et al. (1973), so that instead of four injections per day, only two injections were administered. The terminal dose (200 mg/Kg) was still reached in 10 days as in the experiment by Roffman et al. (1973). The rationale for the reduction of injections each day was the hope that the CS will be more effective when give at 12 hr intervals as the animal will be more greatly motivated to relieve withdrawal symptoms, at each injection, unlike the erratic motivational state of the rat in the other experiment.

Some other withdrawal symptoms, as previously mentioned, were observed at 24 hr after the last morphine or CS-morphine injection. Thirty minutes prior to this measurement the bell was presented to some animals from both groups. As was previously stated the bell affected only the temperature. This may be due to the inadequate number of pairings, as other experimenters (Wikler and Pescor, 1966; Kumar, 1972) had a minimum of 45 pairings in conditioning experiments, while in the present experiment the maximum pairings was 30.

Another possible reason why the bell did not affect the other withdrawal symptoms may be due to the temporal

pairing of the bell and the injection. It might be necessary to present the bell for a longer period of time after the injection or increase the duration of bell presentation. This would insure that the onset of drug action would definitely occur during the presentation of the CS. Also, other stimuli (i.e., visual-strobe light or gustatory-1% saccharine) may be found to be effective in either reducing or eliminating withdrawal symptoms, along with temperature. Another possibility is that more than one stimulus may be needed to control specific symptoms of the withdrawal syndrome. All of these possibilities must be considered in order to realistically evaluate the effect of environmental cues on drug-taking behavior in rats. It is an accepted fact that humans go through many rituals (Wikler, 1971) before and during drug administration and that parts of these rituals become conditional stimuli. Therefore, it seems probable that animals receiving morphine can be conditioned by different cues either separately or simultaneously. It is just a matter of selecting relevant cues to be paired with the drug administration.

Many investigators measure the rectal temperature one hour following morphine administration (Lotti, et al., 1969; Gunne, 1960 and Martin et al., 1963). However, each investigator had his own particular addiction schedule and it was thought that since the present schedule was not similar to any of the above, a time to measure temperature

following morphine administration should be experimentally determined. Therefore, a dose of 100 mg/Kg was administered to addicted rats and maximum hyperthermia occurred 30 minutes following the injection. Thus the time for all temperatures to be taken was 30 minutes after each treatment (CS, morphine or CS-morphine).

The effect on temperature by morphine was observed not to change at 0830 and 2030. This factor is important in that diurnal rhythms may have caused the animal to behaviorally perceive or physiologically react differently to the injection in the morning as compared to the injection at night. This can be reasoned by the fact that the analgesic effects of morphine are different in the morning as compared to the evening (Lutsch and Mans, 1972). Also, it is known that the indoleamine levels change over the course of the day (Bliss, 1973) and since they are postulated to be involved with temperature regulation it is important to determine if the hyperthermic effect of morphine is altered. These factors are important because of their close involvement in the conditioning process. If the physiological and behavioral factors are different at the times the CS-morphine pairing is presented, the animal might be perceiving only half the pairing (self-conditioning) and the other half might be involved in an extinction process. In any case, since the temperature change is the same at the two time periods (0830 and 2030) it is at least safe to assume that

physiologically morphine is affecting the thermoregulatory center in a similar manner. Only by experimentation will the behavioral factors be determined as being no different at the 0830 and 2030 times.

Control of Morphine-Withdrawal Hypothermia by a Conditional Stimulus

The following section contains evidence that a conditional stimulus can, like morphine elicit a rise in temperature during withdrawal in animals addicted to morphine-CS.

1. In the presence of the CS, 24 hr after the last morphine-CS pairing, the rats showed a significant increase in rectal temperature. But if no CS was presented the conditioned animals exhibited no change in temperature at 24 hr of withdrawal. If morphine was administered the typical increase in temperature was observed. Also, if the morphine-CS was given at 24 hr withdrawal the usual increase in temperature was observed.

2. In the presence of the CS, 24 hr after the last morphine injection, the rats showed no change in rectal temperature. Also, presenting the bell to animals who received the bell randomly throughout addiction, produced no effect on rectal temperature. Thus, the bell acted as a CS only when paired with morphine during the addiction phase. The CS did not, however, cause the same change in temperature as morphine (100 mg/kg) when given 24 hr after the last CS-morphine pairing. Instead it was approximately equivalent to 12.5

mg/kg of morphine in its effect on a withdrawal animal's
temperature. Further, the time of the presentation was
found to be only 10 sec in duration to cause the increase
in temperature. And if given at 30 min intervals after the
initial increase in temperature due to the CS, no cumula-
tive or additional changes were observed.

These data suggest the following:

1. The neutral stimulus has acquired conditional properties.

2. The magnitude of the bell with respect to its ability
    to change the temperature is not as strong as the
    terminal dose of morphine it was paired with.

3. The multiple CS presentations did not produce any cumu-
    lative effect when given successively at 24 hr withdrawal.

These conclusions should not be interpreted as claim-
ing that the conditional bell equals 12.5 mg/kg because
statement three clearly shows that not to be true. If it
were equal to 12.5 mg/kg morphine, then continued presenta-
tions should cause an increase in rectal temperature that
would equal the 100 mg/kg dose of morphine. Also, a more
important physiological factor must be considered. Is it
desirable for the organism to increase its temperature to
a hyperthermic state?

First, a consideration must be made as to the status
of the homeostatic mechanisms of the thermoregulatory sys-
tem. That is to say, does chronic morphine change the "set
point" of the thermoregulatory system and therefore change

the temperature at which the organism now calls normal. It has been postulated that such a situation does occur (Lotti et al., 1965) where the "set point" changes. It is difficult to assess which way it might go, but since much of the withdrawn animal's day is spent in a hypothermic state (present experiment) it should be safe to assume that his "set point" may fall. If this is the case then the rise in temperature following the CS may be perceived as being similar in magnitude as the change following 100 mg/kg of morphine. This can be seen in that no matter what the temperature was before the CS, the final temperature following the CS was always the same. This suggests that the animals may have to go only to some point (hyperthermic, compared to new set point) and the change is perceived to be equivalent to the change following the terminal dose of morphine.

Even if the "set point" of the thermoregulatory system does not change, the withdrawn animals may just raise their temperature to a comfortable level. Simply, they possess a range (i.e., 37.7-38.1) at which they find body comfort. It is known that organisms strive to maintain a state of comfort (Hardy et al., 1971). Since the thermoregulatory system is easier to change than other body systems (Richard, 1973), and trying to condition it seems not to be an exception because it is behaviorally regulated, the above reasoning appears logical. This also explains the lack of ability of the CS to cause a cumulative effect

by repeated administrations. The rat has reached a comfort-
able state thus behaviorally he is not motivated to raise
his temperature any more and thus he does not.

Physiological Mechanisms Involved in Mediating Temperature
Changes Following the CS and Morphine

This section contains evidence that the effect of the
CS and that of morphine on temperature is mediated by dif-
ferent transmitters, but that common paths may exist in the
thermoregulatory neural net.

The use of each compound used to analyze the experi-
ment will be discussed separately.

1. In the presence of mecamylamine neither the CS nor mor-
   phine was able to increase the rats' temperature. The
   dose was determined by the criterion that it by itself
   did not affect the temperature. Those data support the
   idea that the autonomic nervous system was involved in
   mediating the temperature changes following the CS or
   morphine. This block by mecamylamine was at efferent
   ganglia, thus preventing any communication between the
   thermoregulatory center and peripheral mechanisms (i.e.,
   adipose tissue, blood vessels) which would create an
   increase in temperature or a pyrogenic effect.

2. In the presence of propranolol or phenoxybenzamine the
   CS and morphine's effect on rectal temperature were un-
   affected by the former and blocked by the latter. These
   data suggest that β receptors are not involved in the

mediating temperature changes that follow the CS or morphine. Centrally β-receptors have been shown to play little if any role in thermoregulation (Rudy and Wolf, 1971). Also, peripherally the role of β receptors within the mechanisms involved in temperature changes are limited to causing a decrease in body temperature and increasing lipolysis to increase heat production (Steiner, 1973). This latter use of β-receptors would not fit because the rise in temperature by the CS and morphine occurs too quickly and since the β receptors have been blocked, a reduction was observed. The dose of propranolol was determined by its inability to change temperature by itself and behaviorally it has been shown not to cause any changes in activity (Weinstock and Speiser, 1974), at the dose used which may indirectly alter the temperature. The pretreatment time of 1 hr was used as the peak tissue levels seen in the rat observed at between 45 min and 75 min (Hayes and Cooper, 1971).

The selection of propranolol as a β-blocker may not have been the best choice. This compound is distributed both centrally and peripherally, therefore its effects cannot be localized as with a compound such as practolol which works exclusively centrally (Wong & Schreiber, 1972). Since few β-receptors, if any, are involved in central thermoregulatory processes this problem is not that critical.

Phenoxybenzamine, the blocker which did block the CS and morphine's effect on temperature was also not the best drug to be used in this kind of study. The use of phenoxybenzamine is widespread, but other more specific α blockers (phentolamine) exist and would allow for easier interpretation of data (Goldstein & Munoz, 1961). In this experiment phenoxybenzamine completely blocked the CS and allowed morphine to raise temperature only slightly. If the dose was slightly raised the complete block probably would have resulted. The dose of phenoxybenzamine used has been previously shown to block electroencephalogen and blood pressure changes that may result from a stimulation of brain receptors (Goldstein and Munoz, 1961). The pretreatment time used has been shown to be the optimal time for blocking NA effects on temperature (Jacob and Peindaris, 1973).

This block of the temperature changes would be expected just by the drug's peripheral effect alone. By blocking vasoconstriction and the ability to activate some of the peripheral thermal receptors as well as a partial block of central α receptors known to be involved in causing an increase in temperature, it can readily be seen why the CS or morphine would not change the temperature after phenoxybenzamine. The control experiment is to separate the central from peripheral

action to determine if one or the other plays a greater role in blocking the CS and morphine's effects on temperature (Carlson, 1973).

3. Haloperidol, which blocks dopamine at the receptor site was able to completely block the rise in temperature following the CS and had no effect on morphine's ability to raise rectal temperature. This dose was selected because it could completely block the bell's effect on temperature and not affect morphine's rise in temperature caused by morphine. It did cause a decrease in temperature by itself, but this can be observed with doses as small as 0.1 mg/kg. The pretreatment time had been used by Niemegeers et al. (1969) in behavioral studies, therefore this time (2 hr) was tried and found to be effective in blocking the temperature change in withdrawn animals previously attributed to the CS.

4. In the presence of benztropine, morphine's hyperthermic effect was not blocked, but the bell's effect on rectal temperature was blocked. Since the drug is a centrally acting anticholingergic, it was deduced that ACh was involved in mediating the CS hyperthermia in the brain. The pretreatment time was determined by Puri et al.(1973) as having optimal biochemical effects. Also, because the dose used produced no effect on temperature, it was decided to use this dose.

5. In the presence of cyproheptidine, morphine's effect on temperature was partially blocked but the bell's effect

was only slightly reduced. The difference was not as clear as the block of CS and/or morphine by the other compounds, but the variability may account for some of the difficulty in interpreting the results of this experiment (CS). The choice of compound was not that good because cyproheptidine has properties other than antiserotonin which prevent it from being specific (antihistaminic). The dose and pretreatment was taken from an experiment by Jacob and Peindaries (1973) who found that 3 mg/kg of cyproheptidine would antagonize an increase in temperature due to .3 mg/kg 5-HT by 90%. They also showed that at 3 mg/kg there were no effects on norepinephrine or dopamine with respect to body temperature. Thus the only problem may be its antihistaminic effect. Histamine given intraperitoneally has been shown to cause hypothermia (Solczanzi and Gabor, 1973), have shown that histamine has little if any direct effect on the thermoregulatory center, but little is really known concerning the role of histamine in thermoregulation, or if a role even exists.

Other laboratories have studied the relationship of serotonin to the thermoregulatory system following morphine. Samanin et al. (1972) have shown that midbrain raphe lesions block acute effects of morphine on temperature. They conclude that serotonin is involved in the acute effects of morphine on temperature. Warwick et

al (1973) support the acute findings of Samanin's group,
but show that 5-HT is not involved in the response to
morphine in tolerant animal hyperthermic. The method
they used to addict the animals was by pellet implant-
ation which may affect temperature systems differently
than i.p. injections of morphine for 10 more days than
the pellet implantation. Warwick's group also conclude
that serotonin was involved in the initial hypothermic
response to morphine and some other transmitters in-
volved in the hyperthermic effect. The basic premise
of their argument is not true because they cite Lotti
et al. (1965) who shows that acute morphine (1 to 10
mg/kg) cause hyperthermia, therefore it is possible for
hyperthermia to occur after every injection. Thus the
role of 5-HT in mediating morphine's effect on temper-
ature needs additional study.

6. Finally, naloxone affected the CS and morphine in the
same way. Naloxone caused a large drop in temperature
following the CS or morphine, 24 hr after the last mor-
phine injection. When naloxone is given alone 24 hr
after the last morphine injection, only a small drop in
temperature is noted. These data suggest that an in-
teraction has occurred between a narcotic antagonist
and the learned conditional effects of morphine
(Drawbaugh and Lal, 1974).

A working explanation of the above data is presented

graphically in Figure 2. The change in temperature observed
following morphine administration could be mediated central-
ly by 5-HT and peripherally by ACh at the ganglia and ACh
and NE at the effector sites. This can be deduced by cypro-
heptidine's ability to block serotonin from working at the
thermoregulatory center. The increase in temperature follow-
the the CS was deduced to be mediated by ACh and DA because
benztropine and haloperidol blocked the change in tempera-
ture normally seen after presentation of the CS. Further,
at some point the pathways of morphine and the CS which af-
fect temperature meet, as can be seen by the fact that
naloxone blocks more than one system, affects the CS and
morphine similarly and that a ganglionic blocker and
adrenergic blocker were able to block temperature increases
following the CS and morphine.

Figure 3 is an outline of what may be happening when
the CS is presented, when morphine is present in the system
and how the system is self-controlling by having at least
one feedback loop. This diagram is intended to give only
approximations and not to be the exact physiological thermo-
regulatory scheme. There is little agreement among physiol-
ogists on how the thermoregulatory system works; however,
they do agree upon the center or controlling system and that
feedback loops exist.

Morphine affects the reference input elements by means
of a transmitter substance. This substance in turn affects
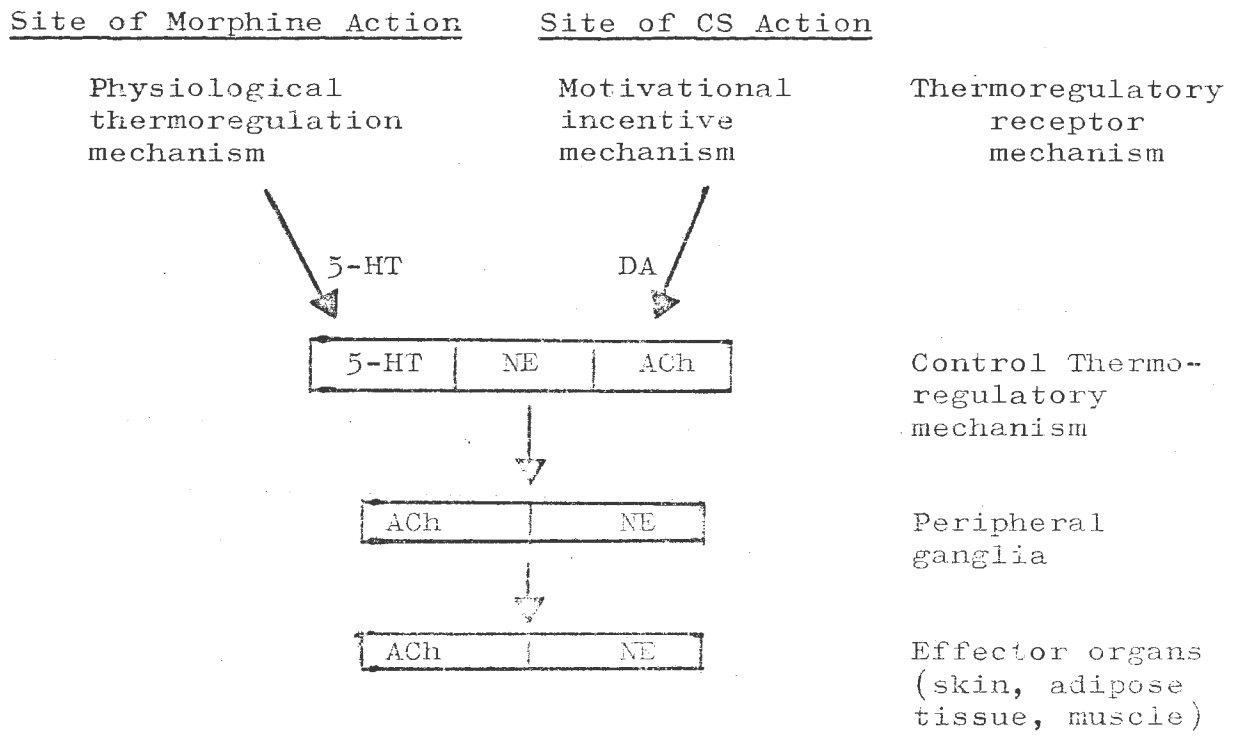a "receptor" which has the ability to be both excited and

Site of Morphine Action   Site of CS Action

Physiological   Motivational   Thermoregulatory
thermoregulation   incentive   receptor
mechanism   mechanism   mechanism

5-HT   DA

| 5-HT | NE | ACh | Control Thermo-
                          regulatory
                          mechanism

| ACh | NE | Peripheral
                          ganglia

| ACh | NE | Effector organs
                          (skin, adipose
                          tissue, muscle)

Fig. 2. Block diagram designed from the data
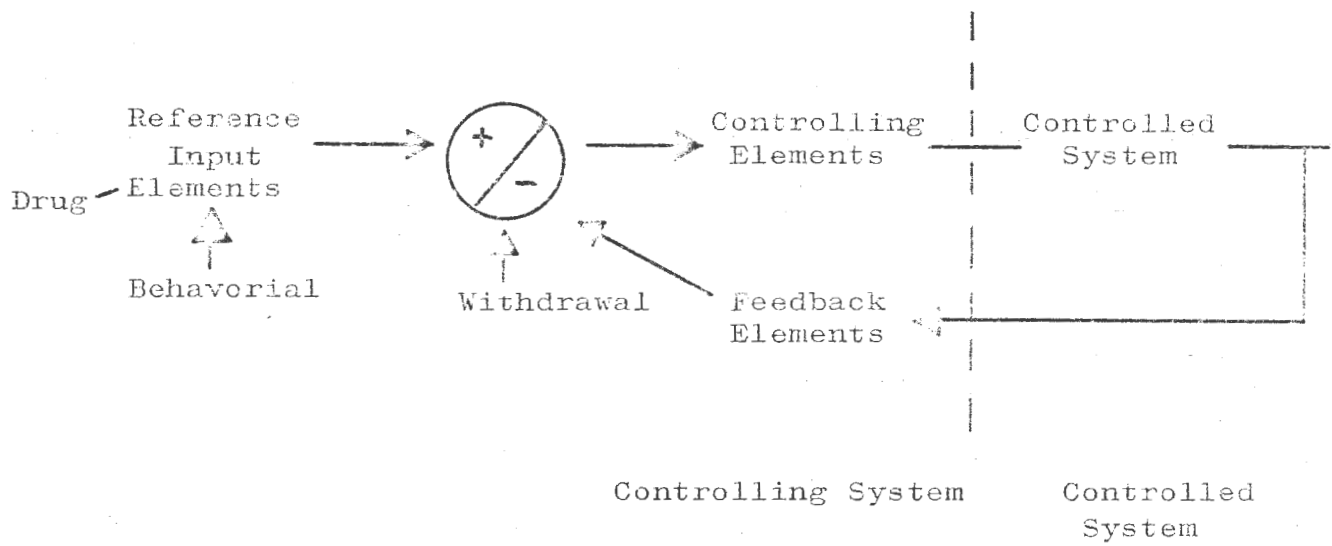   presented in the conditioning exper-
         iment.

Fig. 3 Block diagram of an automatic regulator and
possible inputs related to the present ex-
periment.

inhibited. It is this receptor that has many arms to different elements which are labeled controlling elements. These elements affect vasomotor activity, shivering, sweat and panting, which are located under the heading of controlled system. It is at this point where at least one feedback loop exists which returns to the receptor and inhibits it. This inhibition results in a dropping of temperature in the case of morphine, i.e. as the drug is metabolized the effect on the "receptor" by the reference input drop and the feedback loop begins to affect the receptor and the temperature begins to fall. (Inhibition refers to the ability of the system to compensate for the increase in temperature due to morphine and does not necessarily mean that the receptor is turned off.) Behavioral stimuli work the same way as they are able to affect the reference input elements by specific neurotransmitters. In this study morphine and CS, by different neurotransmitters, affect the reference input elements which in turn cause stimulation of the "receptor." By stimulating the receptor (this does not mean that only one receptor exists), an increase in temperature or hyperthermia exists. The ability of the CS to eventually raise the temperature by itself may be termed thermal motivation (conscious experience) (Corbit, 1973). This increase in temperature causes thermal comfort, but to rise to morphine's hyperthermic level would cause discomfort and not be desirable, thus explaining why the CS causes an

increase which is considerably less than morphine. Also, because this is a behavioral change it is transient allowing the feedback loop to again affect the "receptor" and the temperature falls. This would require a drop in the set point, otherwise the feedback loop would not work because the CS is just above the normal temperature, but then the question arises, what is causing the temperature to fall again? This may be due to peripheral transmitters like ACh which are released and acting on supersensitive receptors (Paton, 1969) during withdrawal. They in turn affect peripheral thermoregulatory effectors causing a drop in temperature.

IV Significance of the study

The ability of naloxone to block the physiological responses evoked by conditional stimuli in the same manner as it blocks the unconditioned morphine effects has both theoretical and practical implications. Of theoretical importance is the suggestion, from this study, that the conditional stimulus may evoke activity in the brain pathways specifically sensitive to the agonist actions of morphine and to morphine dependence. Alternatively, the common belief that naloxone acts only by displacing morphine from its receptor may be questioned. It seems as though oversimplification of naloxone's action has led investigators to believe that this compound has only one action in the organism, i.e. displacing morphine from the receptor. The question then arises of the unlikelihood of a substance at any dose affecting one very specific group of receptors, namely those involved with morphine action. Rather, naloxone may exert an agonistic influence on brain substrates originally insensitive to naloxone but rendered sensitive by the actions of morphine. Other experiments showing the inability of narcotic agonists to reverse actions of narcotic antagonists (Wikler, Fraser & Isbell, 1953) raise similar doubts on the accepted mode of action of narcotic antagonists.

The practical importance of this finding is related to the use of narcotic antagonists in the therapy of narcotic addiction. The current rationale behind the use of narcotic antagonists in the treatment of heroin addicts is

that treatment with these drugs will result in the extinction of heroin consumption because of the blockade of the "high" sought from agonistic effects of illicit heroin. The present data suggest that narcotic antagonists may also be valuable in extinguishing heroin habit associated with the conditional placebo effects of heroin-seeking behavior. These effects have been considered to be major factors in the relapse of the addiction (Wikler, 1971), and it is therefore imperative to investigate the site and mechanism of this conditional behavior to perhaps arrive at some efficacious method of treatment of addiction.

In conjunction with this, physiologists are interested in determining the site and mechanisms of drugs. This interest coincided with the aims of this experiment in the study of the effects of morphine and the CS on temperature in the rat. Temperature changes due to morphine apparently result from a direct action upon thermoregulatory centers within the anterior hypothalamus. Some evidence in support of this view is found in investigations in which rectal temperatures were recorded following microinjections of morphine into various regions of the hypothalamus and surrounding brain areas (Lotti et al., 1965).

The approach taken in this experiment to differentiate the neural pathways used by the CS and morphine is rather unique. There have been few attempts, up to this time, to determine the neural pathways used by morphine to affect temperature.

CONCLUSIONS

1) The conditional stimulus may evoke activity in the brain pathways which are specifically sensitive to the actions of morphine.

2) The conditional stimulus and morphine probably utilize a peripheral mechanism involving ACh and also receptors in the sympathetic nervous system.

3) Centrally the conditional stimulus acts by means of a dopaminergic pathway.

4) Centrally morphine acts by means of a seratinergic pathway in altering body temperature.

5) The CS and morphine have a common path, however, they converge at this pathway by different routes.

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