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EFFECT OF CONDITIONAL STIMULUS ON MORPHINE

WITHDRAWAL SIGNS IN THE RAT

BY

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CONDITIONAL STIMULUS EFFECTS ON WITHDRAWAL SIGNS

DOCTOR OF PHILOSOPHY THESIS

OF

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to MY PARENTS

ABSTRACT

The present investigation was undertaken to demonstrate the comparisons and contrasts of the conditional stimuli's (CS's) actions with those of morphine in morphine withdrawn rats. The study shows that specific stimuli altered behavioral and physiological withdrawal signs such as: hypothermia, shakes, ptosis, piloerection, writhing and aggression. Also, one stimulus was able to effect two biochemical measures (blood glucose and striatal homovanillic acid) similar to the action of morphine.

Rats were given two equally spaced injections of morphine sulfate paired with different stimuli (bell, drug, oil of anise, saccharin). The stimuli were paired with an injection for 15-25 days. Twenty-four hours after the last morphine injection the appropriate stimulus was presented.

The rats learned to increase their body temperature, reduce wet shakes, increase ptosis, reduce writhing and reduce aggressive responses following the presentation of oil of anise. The bell stimulus only increased temperature. The gustatory stimulus increased temperature and the drug stimuli had no effect on withdrawal signs. The changes observed were specific only to animals that had the respective stimuli paired with morphine prior to challenge treatment. The duration of the CS in the oil of anise study was important, the onset required a time period of greater than 2 min

but no more than 30 min for the maximal effect. When naive animals were exposed to the stimuli, no changes were observed either behaviorally or physiologically. Those animals receiving random CS or no CS during addiction demonstrated no change in observed withdrawal signs when presented the CS 24 hr or 48 hr after the last morphine injection.

In the presence of naloxone, a pure narcotic antagonist, oil of anise-morphine paired animals receiving the CS, 24 hr after the last morphine-CS pairing exhibited no change in withdrawal signs which previously had been changed by the CS. This data implies, indirectly, the release of a morphine-like substance in the production of the CS effects.

In addition to the behavioral and physiological measure brought under stimulus control, two biochemical measures (blood glucose and striatal homovanillic acid) were specifically increased by the CS (oil of anise) in a manner similar to that seen by morphine alone, therefore showing that the conditional responses of morphine addiction are not just behavioral but also involve biochemical systems.

These data indicate that the changes resulting from morphine presentation during withdrawal can be classically conditioned. The responses are of three types - behavioral, biochemical and physiological.

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INTRODUCTION

Roffman et al. (1973) and Drawbaugh and Lal (1974) have
demonstrated that hyperthermia can be conditioned during mor-
phine abstinence by pairing a neutral stimulus (bell) with mor-
phine administration. The procedures used in these two studies
were very similar, requiring between 24-40 pairings of the bell
and morphine. The study by Drawbaugh and Lal (1974) showed
that naloxone could block the conditional phenomenon and thus
concluded that the conditional stimulus, acting on the brain,
may affect the same receptors that morphine affects. Under-
standing the conditioning factors that are present with mor-
phine administration may be of great importance in treatment
of human addicts, at both a behavorial and biochemical level.
It should be understood that treating just the physical as-
pects of drug addiction is not enough, but treatment of the
behavioral aspects is also very important in order to increase
the cure rate of narcotic addicts.

Conditional responses due to morphine administration
were seen as early as 1900 by Faust in dogs injected for 3-4
weeks. Collins and Tatum (1925) reported a conditioned sali-
vary response in dogs after repeated morphine administration.
One year later Pavlov reported that an investigator in his
lab observed dogs vomiting shortly after the investigator en-
tered the room, a response usually seen following morphine
administration. Wikler and Pescor (1970) demonstrated, using
the classical conditioning paradigm, that the environment

associated with abstinence can act as a conditional stimulus and can elicit relapse behavior when the animal is placed in that environment for up to a year following the last morphine injection. In addition, recent evidence indicates that rats can self-administer morphine orally, in their drinking water, and show preference for the conditional stimulus (saccharin) when saccharin is given by itself (Parker et al., 1973). Kumar (1972) paired morphine drinking with an environment and observed that the rats preferred the environment where they received morphine in their drinking water.

The present investigation sought evidence to establish:

1) What is the best schedule of addiction using the bell as a conditional stimulus

2) What is the best kind of conditional stimulus in a rat analog of drug addiction (auditory, gustatory, interceptive or olfactory).

3) Whether naloxone, a drug which is a pure narcotic antagonist (Blumberg and Dayton, 1973), can block other conditional stimuli as it was able to block the auditory CS, therefore, giving further support to the hypothesis that the CS and morphine work by similar mechanisms in the brain.

4) Whether a CS can control not only behavior elicited by morphine, but also biochemical changes usually seen following morphine administration.

5) Whether the conditional phenomenon can be considered as indirect evidence for the stimulus causing its effects by inducing the release of an endogenous morphine-like substance.

LITERATURE SURVEY

Conditioning Associated with Narcotic Addiction

A. Behavioral

It has been established by many investigators that some drug reactions can be elicited by environmental stimuli that have been paired a number of times with the drug administration. That is to say that certain drug reactions can and are indeed conditioned. It is well known that one of the first effects of injection of morphine in dogs is to produce nausea and salivation followed by vomiting. Krylov observed in the course of certain serological experiments that following repeated hypodermic injections of morphine into dogs, the symptoms that normally follow injection, nausea, salivation and vomiting, were seen as soon as the experimenter entered the animal quarters. Initially, Krylov observed, after only 5-6 days of morphine injection, that touching the animal could elicit the drug response. In a few additional days, he noted that his entrance into the room could produce the onset of nausea, salivation and vomiting. Thus the greater the number of previous injections of morphine, the fewer the number of stimuli were required to evoke a reaction that simulated that produced by the drug (Pavlov, 1927).

This report, however, was not the first published report of conditioning narcotic effects even though chronologically the work was completed about 1890. Faust (1940) reported that after 3 to 4 weeks of repeated morphine injections, dogs

were observed to anticipate the injection "as if they felt
the need of a new injection....indeed, in one case the dog
would greet me with lively expressions of joy, when I entered
the cage with syringe in hand and let the injection be made
while he is standing, without being bound in any way." (45,
p. 12.)

Collins and Tatum (1925) also observed serendipitously
the same phenomenon that Krylov and Faust had seen, follow-
ing only 6-8 injections of morphine. Kleitman and Crisler
(1927) replicated the Collins and Tatum experiment by using
morphine specifically as a UCS and thereby systematically con-
ditioning and extinguishing the salivary reflex observed in
dogs.

Crisler (1928) and Kleitman (1929) separately repli-
cated the salivary response observed in dogs which was ini-
tially associated with s.c. injection of morphine and later
elicited by the needle alone. However, this time both investi-
gators reported the exact number of injections to acquisition
of the response and used a specific interval for CS-UCS pair-
ing. Not until 1940 was an experiment (Spragg) carried out
which not only looked at number of trials to acquisition, but
gave variable doses, observed behavior other than salivary re-
sponse and used a specific CS (special room).

Not until Wikler published an article on conditioning
of drug effects in 1948 did a dramatic change in the art of
classically conditioning of narcotic effects occur. The con-
ceptual framework used in this study and subsequent studies

at Lexington is important because it greatly influenced meth-
odologies used in the past two decades. Wikler's studies
used techniques similar to Pavlov but was concerned specifi-
cally with the role of the central nervous system. He was
not able to show a conditional salivary response in decorti-
cate dogs but it could be seen in spinal dogs.

After Wikler's report another period of almost 10 years
lapsed before another conditioning experiment was published
(Bykov, 1957), and the concern shifted from problems involv-
ing classically conditioning morphine effects to questions
about the classical conditioning of morphine withdrawal and re-
lapse (Goldberg & Schuster, 1970; Goldberg et al. 1971;
Parker et al. 1973; Trost, 1973; Wikler & Pescor, 1967). Util-
izing environmental factors that were associated with morphine
abstinence, Wikler and Pescor demonstrated that rats under-
going withdrawal in their home cage, when placed in that cage
3-6 months later, will show withdrawal signs (wet shakes,
writhing). In the same experiment they also demonstrated that
animals addicted and withdrawn in their environment will self-
administer a narcotic drug when placed back in that environ-
ment for up to 6 months after the last day of narcotic injec-
tion. Goldberg and Schuster (1970) utilized nalorphine, a
narcotic antagonist, to demonstrate conditioned nalorphine
induced abstinence changes in morphine dependent monkeys. They
observed that after pairing a neutral stimulus (light) with
injections, the neutral stimulus alone could elicit a condi-

tional response (emesis, salivation). These responses were normally seen only following nalorphine injection of morphine dependent animals. Goldberg et al. (1971) demonstrated that monkeys self-administer saline to overcome nalorphine's antagonistic effects if they had previously been given nalorphine and had the opportunity to self-administer morphine to overcome its effects.

Trost (1973) utilized a differential classical conditioning paradigm on morphine dependent rats and showed that the rats which had a specific environmental stimulus paired with each injection did indeed show a completely different kind of behavior when put into that situation where the stimulus or the environmental conditioner previously paired with morphine was again present. He concluded from this differential training, that stimuli paired with morphine withdrawal distress play a more important role in readdiction liability than stimuli associated with withdrawal reduction. In other words the original physiological need for morphine is not present but the associated behaviors may be energized by the expectancy of an oncoming need to a greater extent than by the expectancy of an oncoming relief. Jones and Prada (1973) showed that dogs also relapse to morphine use after 1-6 months of addiction followed by 1-6 months of withdrawal. Following this enforced abstinence, the dogs recommenced self-administration of morphine when it again was made available. The results seem to indicate that the post-dependent dogs

had maintained their dependence on morphine by self-adminis-
tration. Effects other than those experienced by the initial
exposure (such as nausea and vomiting), were responsible for
this relapse.

Another group of experiments which bring us up to the
present use either rats or primates pretreated with doses of
morphine (to 200 mg/Kg/day) aimed at rendering the animal
physically dependent, and subsequently testing the condition-
ability of withdrawal signs precipitated by either narcotic
antagonists or sudden cessation of morphine injections. This
period began a change in philosophy from conditioning of drug
effects to concern of drug dependency as a whole (Lynch et
al. 1976).

Beach (1957) began this period of experimentation by
reporting that environmental stimuli were able to act as
secondary reinforcers for morphine dependent rats. This was
later substantiated by Wikler & Pescor (1967). In Beach's
experiment, the rats were given a choice of either the original
environment in which they were addicted or a new one whereas
in the Wikler& Pescor experiment all were returned to their
original environment. The animals preferred the environment
in which they had experienced addiction and withdrawal, rather
than the neutral one. Thus it was concluded that rats, when
abstinent, show a preference for a distinctive environment
previously associated repeatedly with withdrawal symptoms
(Kumar, 1972; Thompson & Ostlund, 1965). It can further be

concluded that environmental stimuli can become secondary re-
inforcers after repeated pairings with the effects of morphine.
There are also antecdotal reports that individual learning
may contribute to the maintenance of dependent behavior in
humans as well. Another experiment showing development of
secondary reinforcers were that of Kumar (1972) utilizing a
self-administration technique. They showed that animals given
morphine in their drinking water would drink large amounts of
quinine if morphine was withdrawn from the water source.
They concluded that the bitter taste of quinine was the rea-
son for the large intake. They further concluded that the
taste had become a secondary reinforcer. Yet another example
of a secondary reinforcer was done by Crowder et al. (1972).
Using both classical and operant paradigms, they showed that
animals given morphine injections paired with a buzzer would
bar-press for the buzzer and a saline infusion. They con-
cluded that the buzzer and a saline infusion 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. Roffman et
al. (1973) conducted an experiment using a paradigm somewhat
like Crowder et al. (1972) except that a bell was paired
with morphine withdrawal and the body temperature of the
animal was monitored. They showed that the bell was able
to raise the temperature to normal or slightly above normal,
suggesting that the animals were able to overcome the hypo-
thermic effect of morphine withdrawal by a preparatory re-

sponse to the conditional stimulus. They concluded that they were able to condition the effect of morphine; namely, the ability to alter body temperature. This experiment was later replicated by Drawbaugh and Lal (1974). In the latter study, not only were the temperature effects of morphine conditioned but the conditioned response was also blocked by naloxone. This suggested that morphine and the conditional stimulus may be working through the same pathway(s). These experiments could also be considered to suggest that the CS might be working by means of a morphine-like substance (endorphin).

Few systematic studies have been done with humans dealing with conditioning of narcotic effects or withdrawal signs. Levine (1974) reported that two individuals whom he treated at NIMH Clinical Treatment Center, Lexington were true "needle freaks" and found pleasure in "sticking" themselves without heroin. He explained the "needle freak" phenomenon as a form of operant conditioning. The idea that self-injection is operant behavior that leads to the pleasurable experience of intoxication and is therefore positively reinforced. Continued and increased self-injection in the absence of continuous reinforcement can be considered an instance of secondary reinforcement conditioning. Thus there is a behavior (self-injection) which is directed toward a stimulus with no intrinsic utility that was previously paired with a biologically significant stimulus. Shannon et al. (1976) did a study to see if this "needle freak" phenomenon generalizes to medicinal use of hypodermic needles. They found that no generalization

was observed and concluded that a chain of behavior is in-
volved with injection of heroin which is not present in a
medicinal situation.

O'Brien et al. (1975) attempted to classical condition
withdrawal signs in man as Goldberg & Schuster (1967) had
earlier done in monkeys. They used naloxone as the UCS, au-
ditory tone and oil of peppermint as the CS with each sub-
ject receiving the CS-UCS pairing three-five times per week.
On test days a saline injection was given instead of naloxone
with the tone and smell. Five of eight patients showed evi-
dence of conditiong of abstinence signs.

Thompson and Pickens (1969) reviewed the conditioning
literature and concluded that much of the drug self-adminis-
tration can be explained by means of operant behavior. They
analyzed drug dependence in terms of factors known to con-
trol acquisition, maintenance and elimination of the operant
responses beliving that these same factors should also con-
trol acquisition maintenance and elimination of drug rein-
forced responses. They felt that looking into these specific
areas would lead to a better understanding of the initial
development of drug dependence and perhaps contribute to
work with the problem of drug addiction. They felt that
there are a number of different variables which will effect
drug reinforced responses. These variables include ante-
cedent conditions (Kolb, 1962), current stimulus circumstances
(Cofer & Appley, 1964) qualitative and quantitative properties

of the reinforcing drug as well as a stimuli associated with
drug administration (Ausubel, 1963; Weeks & Collins, 1964).
They concluded their review of the literature by stating that
drug dependence can at least be partially analyzed by using
an operant paradigm and that this approach helps to provide
answers to the underlying mechanisms of drug dependence. They
at no time concern themselves with classical conditioning.
This lack of reference to classical experimentation may be
a problem of semantics or a sincere feeling that drug addic-
tion can best be explained and treated as an overt behavior-
al disorder.

B. Biochemical

Perez-Cruet (1974, 1976) and Lal et al. (1976) are thus
far the only labs which have been able to condition bio-
chemical changes due to morphine. Perez-Cruet utilizing a
paradigm used in another lab (Roffman, et al. 1973; Drawbaugh
& Lal, 1974) paired a buzzer with a narcotic (methadone) and
was able to condition morphine's effects on homovanillic acid
(HVA), a dopamine metabolite. Lal and co-workers using oil
of anise, paired with morphine, were able to also condition
morphine's effects on HVA.

In addition to the use of the gustatory stimulus to
control a brain metabolite, Lal and co-workers were able to
also condition blood glucose levels. This same lab earlier
(Roffman and Lal, 1973) was able to condition biochemical
effects produced by pentobarbital, suggesting that not only
overt behavior due to a drug are able to be conditioned,
but biochemical effects also.

Conditioning Changes of Blood Glucose

The amount of glucose in the blood of animals is regulated by a feedback system (homeostatic). The importance of this system can be seen in the fact that small deviations of glucose levels are suggestive of serious clinical diseases. An increase of fasting glucose was a sign of diabetes mellitus, and a decrease of fasting glucose is a sign of hypoglycemia. The level of blood glucose is especially important because the brain, unlike the majority of other tissue, is not able to obtain energy from lipids and proteins and thus relies heavily on glucose. Therefore, if glucose levels get too low, the brain cannot function normally and if this continues for a long enough time, coma and death may ensue. A minimal level of glucose in the brain is imperative.

The level of blood glucose has been associated with certain levels of behavior. It is well-documented that the central nervous system is very sensitive to alterations in glucose levels. Changes of glucose levels at specific brain sites causes changes of electrical activity at both single unit and gross potential levels (Anand et al. 1964; Brown, 1969; Oomura et al. 1964; Oomura et al. 1969). Also, such changes cause reflex homeostatic alterations of peripheral glucose levels (i.e. adrenals, liver and pancreas) (Muller et al. 1973; Sakata et al. 1963; Szabo & Szabo, 1972), along with changes in feeding behavior (Balagura and Kanner, 1971; Miselis & Epstein, 1970). These types of changes have been

utilized as reinforcers in learning experimentation (Chambers 1956a,1956b).There are other behaviors which glucose levels can be correlated with such as: eating, stress, exercise and arousal. Simply, the amount of glucose in the blood co- varies with many different behaviors and has clinical import- ance. The survey to follow summarizes the experiments in which blood glucose is brought under stimulus control.

The majority of reports on conditioned glycemic re- sponse use insulin as the unconditioned stimulus (UCS). Typi- cally, the administration of insulin is repeatedly paired with a neutral stimulus which does not affect blood glucose levels. After a number of days of pairings, the stimulus alone has the ability to elicit a change in blood glucose. If the change is an increase of glucose, it is called hyperglycemia; if a decrease, hypoglycemia.

A number of investigators in the Soviet Union performed research on human subjects (schizophrenics and diabetics) and observed conditional responses following neutral stimulus pairing with insulin (Lichko, 1959; Zakharov, 1960; Lettes & Pavlov, 1954). The neutral stimulus was able to change (de- crease) blood glucose levels from 12 to 50 mg% depending upon the laboratory.

Dogs have been the next most frequent choice as sub- jects for conditioned glucose response experiments. In these experiments, the presentation of insulin was paired with an auditory stimulus, the stimulus was later able to decrease blood glucose levels with or without a saline injection

(Savchenko, 1940; Leites and Pavlov, 1954; Alvarez-Buylla
and Carrasco-Zanini, 1960).

The remainder of experiments employing insulin as the
unconditioned stimulus used rats as subjects. Woods, Makous
and Hutton (1968) reported that rats who had repeated pair-
ing of stimulus (auditory) with insulin, when given auditory
stimulus + saline ,   a 12 mg% change was observed. The same
laboratory under the direction of Stephen Woods completed a
number of additional experiments (Hutton et al., 1970, Woods
et al. 1969; Woods and Shogren, 1972) showing an average
conditional change in blood gluocse of 25 mg%. It must be
noted that in all the experiments demonstrating conditioned
hypoglycemia, the conditioned response was smaller than or
equal to the unconditioned response.

The duration of the conditioned reponse was from 15 to
30 min and it declines rapidly thereafter (Alvarez-Buyalla
et al. 1960; Woods et al., 1969). Leites and Pavlov (1954)
observed a biphasic response to conditioning procedures. For
about the first 30 minutes a hypoglycemic response occurred
followed by hyperglycemia. This fact is extremely important
and should be noted by investigators unable to obtain condi-
tional changes.

The conditioned response is a function of the number
of conditioning trials. The response is able to develop over
a minimal number of trials, most experimenters reporting from
two to five trials are necessary (Leites & Pavlov, 1954,
Woods et al, 1969). It has also been shown that too many

trials cause the magnitude of the response to lessen and may even invert and get a conditioning of hyperglycemia instead of hypoglycemia (Leites and Pavlov, 1954).

Conditioning of hypoglycemia extinguishes rapidly, two to five trials (Leites & Pavlov, 1954; Woods et al., 1969). Spontaneous recovery has been noted in rat experiments, but the stimulus effect elicited is very small and only after one week since the last test trial.

The proposed mechanism to explain the above-mentioned experiments is as follows: When insulin is administered to an animal paired with a particular stimulus or set of stimuli, the response that follows of that animal to the same stimulus but without insulin administration is a decrease of blood glucose called conditioned hypoglycemia. The conditioned response follows properties of a classically conditioned response, and the mechanism that causes this response appears to be CNS-mediated release of pancreatic insulin.

Morphine Effect on Blood Glucose and Homovanillic Acid Levels

Morphine injected into dog (Ross, 1918; Pierce and Plant, 1928), cat (Dey et al., 1975; Feldberg and Shaligman, 1972; Borison, et al., 1962), rabbit (Araki, 1891; Sable-Amplis, 1972), mouse (Sable-Amplis, 1972) and rat (Sable-Amplis, 1972) caused hyperglycemia, the intensity of which varies according to the species and the dose. Except for Pierce and Plant (1928) and Sable-Amplis (1972) the above studies were limited to the acute effects of morphine on blood glucose levels.

Pierce and Plant addicted dogs from 50-280 days with a terminal dose ranging from 40-220 mg/Kg. However, even with these large variations in dose and duration of addiction the results for all animals were similar. Acute dose of morphine caused a pronounced hyperglycemia, however, within 10 days of injections the effect disappeared (tolerance developed). Upon withdrawal some time between the 2nd and 5th day, a transient period of hyperglycemia was observed.

Sable-Amplis gave mice, rats and rabbits doses of morphine ranging from 10-40 mg/Kg for a period of up to 15 days. In all cases tolerance developed within 7 days, 3 days for the mouse. Gradual increases of 10 mg/Kg injection was not enough to cause the increased blood glucose levels. Thus, suggesting that a rapid tolerance develops to glucose changes following morphine on all species mentioned.

If an extract (brain) of tolerant mice is injected into naive mice the naive animals when injected with morphine will already possess altered metabolic effects due to the extract and show no change due to the morphine (Sable-Amplis, 1972). In rabbits, altered metabolism of glycogens exists for 3 weeks after withdrawal from morphine, thus the effects of morphine on glucose in vivo is dramatic and long-lasting in many types of animals.

Homovanillic acid (HVA) levels have not been studied to any great extent following morphine administration. Therefore, the number of studies correlating HVA with morphine's effect on behavior are even fewer.

Morphine has been shown to increase HVA content of striatum in several animal species (Ahtee and Kaariainen, 1973; Fukui and Takagi, 1972; Kuschinsky and Hornykiewicz, 1972; Sasame et al. 1972; Perez-Cruet, 1976). However, only Kaariainen and Ahtee (1976) have attempted to correlate the change in HVA levels with behavior (analgesia) utilizing the hot plate technique.

They found some degree of correlation of morphine and methadone increases in HVA with peak analgesic action. They concluded that morphine though it does not act primarily on dopaminergic neuronal systems, it does affect striatal dopamine areas. This suggests that at least one and possibly other alterations in behavior due to morphine may be associated with changes in HVA levels.

Behavioral Effects of an Endogenous Morphine-Like Substance

The evidence for the presence of an endogenous ligand of the opiate receptor is now so convicing that it is important to compare known behaviorial properties of narcotics with the behaviorial activity of this substance.

The substance is called endorphin and it has been shown to block electrically evoked contractions of the mouse vas deferens and the guinea pig ileum and inhibit the stereo-specific receptor binding of the opiate antagonist (naloxone) in brain homogenates (Hughes et al. 1975). However, only very recently have any overt behaviorial changes been shown by administration of endorphin to intact animals.

King and co-workers (1976) showed that a partially puri-
fied enkephalin given to mice intracerebroventricularly caused
straub tail and piloerection. Morphine, 15 ug, given in the
same manner caused a similar response. The effects of the
opiate-like substance were immediately abolished by naloxone
given intravenously. Also Belluzzi and co-workers (1976)
showed that giving enkephalin in the same manner as King
caused analgesia which could be reversed by giving naloxone
subcutaneously and which could not be established again by
another injection of enkephalin. Only after naloxone wore
off could enkephalin again produce analgesia. Thus direct
evidence does exist for behavorial properties of this morphine-
like substance.

EXPERIMENTAL

Animals

Male hooded rats of the Long-Evans strain, random-bred,
weighing 250-300 grams at the beginning of the experiments,
were obtained from Charles River Breeding Farms (Canadian
Breeding Farm and Laboratories, Inc.), Wilmington, Massachu-
setts. 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 physio-
logical measurements.

Materials

1. Chemicals and Drugs

Analytical reagent grade chemicals or equivalent were
used throughout this study. The co-factors, adenosine tri-
phosphate (ATP), nicotinamide adenine dinucleotide phosphate
(NADP), hexokinase (yeast), and glucose-6-phosphate dehydro-
genase (G-6-PD) were obtained from Sigma Chemical, St. Louis,
Missouri.

N-butryl-acetate was obtained from Aldrich Chemical
Co., St. Louis, Missouri,

Morphine sulfate was obtained from Merck & Co., West
Point, Pa. Naloxone hydrochloride was from Endo Laboratories,
Inc., Garden City, New York. Apomorphine hydrochloride was
obtained from Mallinckrodt Chemical Works, St. Louis, Mo.
Pentobarbital sodium was obtained from Abbott Laboratories,

Chicago, Ill. Ethanol (95%) was obtained from U.S. Distillers. Saccharin was obtained from McKesson, Bridgeport, Conn. Oil of anise was obtained from Merck, New Jersey.

All drugs were dissolved in distilled water. Doses are presented in terms of salts. The volume of each injection never exceeded 0.7 cc, and all saline injections were equal in volume to their corresponding drug treatment injections.

2. Reagents

    a. Perchloric acid (0.4N): 34.4ml of concentrated perchloric acid (70%, Mallinckrodt Chemical Works) was diluted to one liter.

    b. Tris buffer (.05M): 4 gm of tris-(hydroxymethyl)- aminomethane (Calbiochem) was dissolved in 500 ml of deionized glass distilled water. The pH of tris solution was then adjusted to 8.5 by adding 0.5M HCl (J. T. Baker). The final volume was brought to 1 liter by deionized glass distilled water.

    c. L-cysteine (0.1%):100 mg of L-cysteine (Aldrich Chemical Co.) was dissolved in 100 ml of deionized glass distilled water.

    d. Potassium ferricyanide (0.02%): 20 mg of potassium (Aldrich Chemical Co.) was dissolved in 100 ml of deionized glass distilled water.

Conditioning Procedures

    a. Auditory: Conditioning consisted of giving an injection of morphine sulfate paired with a bell sound (78 db

20 KHZ SPL (sound pressure level) measured one meter from the
bell) twice daily at 0830 and 2030 according to the four dif-
ferent schedules appearing in Table 1. The injections of
morphine were spaced 12 hours apart beginning with 10 mg/kg/
injection, and were increased by 10 mg/kg every third injec-
tion until 100 mg/kg/injection (200 mg/kg/injection) or
200 mg/kg/day (400 mg/kg/day) was reached (Table 1). The
rats were maintained at this dose for 2-15 days and then with-
drawn.

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±2) 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 injec-
tion was given. Then the animal was again returned to the
plastic container, and after a total of 60 seconds had elpased,
the bell was turned off. The rat was then immediately re-
turned to his individual cage. Each day the order of animals
going through this procedure was changed.

The identical procedure was followed during the even-
ing session with the exception that the animal's weight was
not taken at that time.

b. Taste: Conditioning consisted of giving an injection of morphine sulfate paired with saccharin (0.1%) twice daily at 0830 and 2030 (Table 2). The injections of morphine were spaced 12 hours apart beginning with 10 mg/kg and were increased by 10 mg/kg every third injection until 100 mg/kg/injection or 200 mg/kg/day was reached. The rats were maintained at this dose for 15 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 single-pan balance to be weighed and then returned to the plastic container. The animal was then removed from the container, an intragastric tube (attached to a one ml syringe filled with 0.1% saccharin) was inserted into its mouth and 0.5 ml was slowly infused for 30 seconds. At this time the morphine injection was given by the same method discussed above. The animal was then placed back in its home 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 animal's weight was not taken at that time.

c. Intereoceptive (drug): Conditioning consisted of giving an injection of morphine sulfate paired with either pentobarbital (10 mg/kg/i.p.), apomorphine (1.25 mg/kg/i.p.) or alcohol (1 gm/kg/I.G.) twice daily at 0830 and 2030. The injections of morphine were given as described in Table 1.

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 single-pan balance to be weighed and then re-
turned to the plastic container. The animal was again removed
from the container and injected with one of the three above-
mentioned drugs. The animal was then placed back in its home
cage for a specific period of time (pentobarbital and apo-
morphine 10 min, alcohol 5 min). After waiting a designated
period of time the animal was taken from its home cage and
given an injection of morphine by the same method discussed
previously. The rat was placed back in its home cage immedi-
ately after being injected.

The identical procedure was followed during the even-
ing session with the exception that the animal's weight was
not taken at that time.

d. Olfactory (oil of anise): Conditioning consisted
of giving an injection of morphine sulfate paired with oil of
anise smell twice daily at 0830 and 2030. The injections of
morphine were given as described previously (Table 2).

The procedure for injection during the morning session
was as follows: each animal was taken out of its cage, placed
in a plastic container (5/container) and taken to a single-pan
balance to be weighed (each animal separately) and then re-
turned to the plastic container. The container was then taken
to a room, maintained at a constant temperature (21+C 1), where

they were placed (5/jar) into a plexiglass jar (30.5 cm x 30.5 cm).
The jar contained a 10 cm plexiglass cube filled with gauze
which had 12 drops of anise oil. After the animals were
placed in the jar a perforated stainless steel cover was
placed over the jar to prevent the animals from escaping.
Five minutes after the animals were placed in the jar each
was removed and injected with an appropriate dose of mor-
phine. The animals were returned to the jar after the in-
jections for the duration of the 30 min pairing session. At
30 min the animals were placed back into the plastic con-
tainer and returned to their home cages.

The identical procedure was followed during the even-
ing session with the exception that the animal's weight was
not taken at that time.

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 5 cm (Myers, 1971) into the rectum
for one min (Lomax, 1970). Each animal had his temperature
taken immediately before and 30 min after the appropriate
conditioning paradigm at 0830 on two successive days preced-
ing withdrawal. These four insertions of the probe allowed
the animals to adjust to the procedure. Also, the animals were
handled very carefully 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 index 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)
5 cm and held it in place for one min.

Measurement of Withdrawal Symptoms

Rats dependent on morphine were removed from their home
cages and placed in a novel environment (Wikler and Pescor,
1967) to observe their behavior. The animals were observed
for 30 min prior to test treatment and at 30 min following
treatment. The following bits of behavior (withdrawal symptoms)
were observed:

a. Shakes - These were 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 min session.

b. Ptosis - This condition was present when the animal's
eyelids were drooping but not closed tightly and he was cap-
able of movement. The animal moves periodically, and this
state is distinguishable from sleep. The amount of time spent
in this state was measured by elapsed timers and cumulated
over the 30 min observation period.

c. Writhing - This consisted of dragging the abdomen
on the floor of the observation cage or arching of the back,
neither of which was accompanied by yawning. Total number of
writhings were measured and cumulated during the 30 min ob-
servation period.

d. <u>Piloerection</u> - This symptom was scored when the rat's fur stood out from the body. If no piloerection was observed a score of 0 was given. Questionable piloerection was given a score of 1. If the hair stood out at about 45° then a score of 2 was given. The score of 3 was given if the animal's hair was perpendicular to his body. This measurement was made after the animal had time to groom following placement into the observation chamber. This was done so as not to report ruffed fur that might have resulted from handling.

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

All of the measurements were made at 0, 24 and 48 hours following the last morphine injection. These observations were always made between 0800 and 1100 hours.

e. <u>Aggression</u> - This was measured only in the oil of anise conditioned animals and the control groups for that experiment. This was done by observing (while the animals were in the plexiglass jars for 30 min) for vocalizations, rearing and attacking. Since no sophisticated equipment was used, aggression was considered present if vocalizations were heard and at least 20 attacks were seen accompanied by rearing behavior during the first 15 min of the test period. Thus aggression was either present or absent for each group tested.

<u>Serum Glucose</u>

The glucose level in the blood was evaluated by measuring the conversion of glucose to glucose-6-phosphate by

adenosine triphosphate in the presence of hexokinase, coupled
with the subsequent reduction of NADP to NADPH.

$$\text{Glucose + ATP} \xrightarrow{\text{hexokinase}} \text{G-6-P + ADP}$$

$$\text{G-6-P + NADP} \xrightarrow{\text{G-6-PD}} \text{6-PG + NADPH}$$
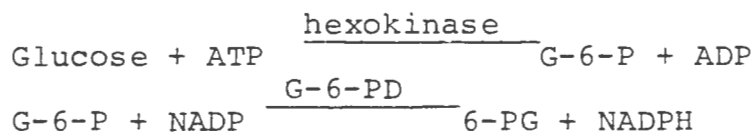
Abbrevations:

ADP - adenosine diphosphate

ATP - adenosine triphosphate

G-6-P - glucose-6-phosphate

G-6-PD - glucose-6-phosphate dehydrogenase

NADP - nicotinamide adenine dinucleotide phosphate

NADPH - nicotinamide adenine dinucleotide phosphate,
        reduced form.

Each animal was individually placed in a plexiglass
restraint with only its tail outside of the restraint. The
tail was submerged into a water bath (55 C) for 45 seconds
to dilate the tail vein. Upon removal from the bath the
tail was wiped dry and 2 mm was cut, using surgical scissors.
Blood was collected in a plastic centrifuge tube which con-
tained .1 ml sodium fluoroacetate (anticoagulant). After
blood was obtained from the animals it was taken to a clini-
cal centrifuge and spun at 4000 rpm for 10 min. Following
centrifugation serum was pipetted from each sample and placed
into small (5 ml) serum vials.

The serum was added (0.02 ml) to a cuvet which con-
tained 3.0 ml of mix (ATP .005 M, NADP .0002 M, Mg   .005M,

hexokinase 0.8 units/ml, G-6-PD 0.4 units/ml) and assayed spectrophotometrically (absorbance at 340 nm) (Barthelmai and Czok, 1962). The initial absorbance was taken prior to addition of the sample (mix alone) and the final absorbance was measured 5 min after the sample was added to the cuvet (mix and serum). Serum blank was prepared by adding .02 ml serum to 3 ml/0.85% NaCl in a cuvet and its absorbance read at 340 nm in a Gilford model no. 2400 spectrophotometer. The concentration of glucose in the experimental samples was determined by the following:

Absorbance=Final Absorbance-Initial Absorbance-Serum blank absorbance. Serum glucose (mg/100 ml)=Absorbance X 440.

The 440 is a factor derived by taking into account the volume of liquid in cuvet, weight of 1 micromole of glucose, absorbance at 340 nm of a solution containing 1 micromole of NADPH/ml and volume of specimen. The calculated concentration was expressed as mg % of glucose.

Homovanillic Acid

Homovanillic acid (HVA) was evaluated by measuring the levels in corpus striata spectrofluorometrically. The animals were allowed food and water ad libitum prior to being sacrificed by decapitation. The brains were rapidly removed from the cranium and placed in ice. The lateral ventricles were opened to expose the corpus striatum which was removed from each side and pooled. All of the above procedure was carried

out on ice using ice-chilled surgical apparatus. The striata were weighed and pooled with striata of another animal and homogenized in 6 ml of 0.4 N perchloric acid. The homogenate was transferred to a 15 ml plastic centrifuge tube and centrifuged at 10,000 g at 0°C for 15 min in a Servall RC 2B refrigerated centrifuge.

The supernatant from each centrifuge tube was transferred into a large 50 ml capable tube. The supernatant was then saturated with NaCl (2.5 g/sample). N-butylacetate (10 ml) was added to the samples and the mixture shaken for 30 min. Following the 30 min of shaking each sample was centriguged in a clinical centrifuge at 2000 g for 5 min. Then 9 ml of the butylacetate layer was transferred to another 30 ml capable tube containing 3 ml of 0.05 M tris buffer, shaken for 20 min and again centrifuged for 5 min in a clinical centrifuge. A 1.5 ml tris buffer extract was transferred to a test tube and assayed spectrophotometrically (Anden et al., 1962). To the test tube was added 0.5 ml of glass distilled water, 1.5 ml of 5 N ammonium hydroxide and 0.2 ml of 0.02% potassium ferricyanide. Exactly 4 min later was added 0.2 ml of 0.1% L-cysteine solution, the concentration of HVA was estimated by reading the samples at 425 nm while activating at 320 nm in an Aminco-Bowman Spectrofluorometer. The concentration of HVA in the experimental samples was determined by comparing the fluorescence with the known concentration of a standard HVA solution. The calculated concentration was expressed as nanograms of HVA per gram of tissue.

Statistics

The following statistical procedures were used in this study when the significance of a result was not clear. Student's "t" tests were used for determining the significance of a difference between two correlated means (i.e., pre-treatment and post-treatment temperatures). Both independent and dependent "t" tests were used as described in Goldstein (1964).

Where data required a comparison of an experimental group with a number of control groups, chi-square test was used according to Goldstein (1964).

RESULTS

The results of this investigation are divided into four
sections: effects of auditory stimulus on withdrawal signs
using different schedules of addiction; alteration of with-
drawal signs by gustatory and intereoceptive stimuli; effects
of olfactory stimulus on withdrawal signs; and reversal of
the effects of olfactory CS by a narcotic antagonist.

A. Auditory Stimulus Effects on Selected Withdrawal Signs
    Under Different Addiction Schedules

Previous work by this investigator established a bell-
sound as an auditory conditional stimulus (CS) capable of re-
versing morphine withdrawal hypothermia (Drawbaugh and Lal;
1974, 1976). It was then decided to determine if other with-
drawal signs could be altered by pairing the bell sound with
morphine administration. Changes in temperature were measured
along with five additional withdrawal signs: weight, shakes,
ptosis, piloerection, and writhing. The rats were addicted
by injecting morphine paired with bell as previously described.
Following addiction they were withdrawn and exposed to either
the bell sound or nothing every 24 hr and their withdrawal
signs were measured at 24 and 48 hr after their last morphine-
bell pairing. Other animals who received morphine alone during
addiction received either the bell or nothing following the
same procedure mentioned above.

Table 1

Schedules of Chronic Morphine Administration for Conditional Stimulus Experiment

DAY	DOSAGE OF Mg/Kg/INJECTION (per Day)			
	low level–long term	low level–short term	high level–long term	high level–short term
1	10 (20)	10 (20)	10 (20)	10 (20)
2	20 (40)	20 (40)	30 (60)	30 (60)
3	30 (60)	30 (60)	50 (100)	50 (100)
4	40 (80)	40 (80)	70 (140)	70 (140)
5	50 (100)	50 (100)	90 (180)	90 (180)
6	60 (120)	60 (120)	110 (220)	110 (220)
7	70 (140)	70 (140)	130 (260)	130 (260)
8	80 (160)	80 (160)	150 (300)	150 (300)
9	90 (180)	90 (180)	170 (340)	170 (340)
10	100 (200)	100 (200)	200 (400)	200 (400)
11	100 (200)	100 (200)	200 (400)	200 (400)
12	100 (200)	100 (200)	200 (400)	200 (400)
13	100 (200)	100 (200)	200 (400)	200 (400)
14	100 (200)	100 (200)	200 (400)	200 (400)
15	100 (200)	100 (100)[1]	200 (400)	200 (200)[1]
16	100 (200)		200 (400)	
17	100 (200)		200 (400)	
18	100 (200)		200 (400)	
19	100 (200)		200 (400)	
20	100 (200)		200 (400)	
21–25	100 (100)[1]		200 (200)[1]	

[1]Last injection of sequence (0830).

Table 2

Effect of the Auditory Stimulus on Withdrawal Signs in Rats, Short term (15 days) –
Low level (200 mg/kg/day) of addiction

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	Withdrawal Signs[4] (mean $\pm$ s.e.)					
			Weight[5]	Temperature[6]	Shakes	Ptosis[7]	Piloerection[8]	Writhing
			24 HOURS OF WITHDRAWAL					
Morphine	none	28	301.4 + 4.2 296.7 + 5.0 - 4.7 + 1.3	37.25 + .03 37.15 + .05 -0.10 + .06	2.71 + 0.43	37 + 18	25 / 28	0.68 + 0.26
Morphine	bell	10	302.2 + 6.8 297.2 + 8.1 - 4.9 + 2.0	37.28 + .05 37.27 + .04 -0.01 + .04	4.40 + 1.89	0	10 / 10	0.40 + 0.16
Morphine + Bell	none	9	301.0 + 6.3 298.9 + 6.6 - 2.1 + 1.0	37.26 + .07 37.31 + .06 +0.05 + .07	2.67 + 0.41	0	9 / 9	0
Morphine + Bell	bell	10	308.5 + 6.4 303.7 + 6.9 - 4.8 + 0.9	37.23 + .04 38.06 + .09 +0.82 + .09 [9]	3.30 + 0.78	0	10 / 10	0.40 + 0.16
			48 HOURS OF WITHDRAWAL					
Morphine	none	28	 276.4 + 4.3 -25.1 + 1.9	37.24 + .04 37.16 + .05 -0.08 + .05	5.50 + 0.60	5 + 5	28 /28	0.86 + 0.24
Morphine	bell	10	 275.4 + 6.5 -26.8 + 2.4	37.24 + .06 37.25 + .05 +0.02 + .04	4.60 + 1.58	0	10 /10	1.10 + 0.43
Morphine + Bell	none	9	 275.4 + 6.6 -25.6 + 1.8	37.18 + .07 37.23 + .05 +0.05 + .05	5.56 + 1.16	18 + 14	9 / 9	0.56 + 0.44

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Table 2 (continued)

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	Withdrawal Signs[4] (mean $\pm$ s.e.)					
			Weight[5]	Temperature[6]	Shakes	Ptosis[7]	Piloerection[8]	Writhing
Morphine + Bell	bell	10	280.8 $\pm$ 6.3 -27.7 $\pm$ 2.2	37.29 $\pm$ .05 37.85 $\pm$ .06 +0.56 $\pm$ .05	3.90 $\pm$ .050	4 $\pm$ 4	10 / 10	0.90 $\pm$ 0.73

[1] Terminal morphine dose, 200 mg/Kg/day

[2] Presented 30 min prior to withdrawal measurements.

[3] Number of animals in each group.

[4] Signs measured for 30 min., except for weight and temperature.

[5] Change is comparing zero time with 24 and 48 hr withdrawal measure.

[6] Temperature taken prior to and 30 min after treatment during withdrawal.

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

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

[9] P value .01 using Student's t-test (each animal used as own control)

The data presented in Table 2 demonstrate that the bell sound paired with morphine injections during addiction did not reduce any of the withdrawal signs other than changes in body temperature at either the 24 or 48 hr observation period ($p < .01$). Animals not receiving bell during addiction and receiving bell during withdrawal showed no effect on any withdrawal sign measured.

In order to see if number of pairings would play a role in increasing stimulus control of withdrawal signs the number of days morphine administered was extended from 15 to 25 days (long-term - low level, Table 3). This increase in number of days permitted 20 additional pairings of the bell with morphine. As can be seen in Table 3, 50 pairings of the bell and morphine did not reverse any of the withdrawal signs other than temperature ($p < .01$). However, the number of shakes were significantly increased and the variability of shakes between animals was reduced.

Since the increase in the number of pairings was ineffective in increasing the bell's effectiveness, the terminal dose of morphine was increased to 400 mg/Kg/day for 15 days (Table 4). Doubling of the terminal dose was also ineffective in altering the bell's ability to effect withdrawal signs other than temperature ($p < .01$). This schedule produced fewer shakes than the 200 mg/Kg/day for a 25-day schedule.

Therefore, an attempt was made to alter withdrawal signs by using the high terminal dose (400 mg/Kg/day) and increasing the number of days to 25 as was previously tried at

Table 3

Effect of the Auditory Stimuls on Withdrawal Signs in Rats Made Morphine Dependent
in 25 Days to 200 mg/Kg/day (long term – low level)

Treatment during Addiction [1]	Treatment during Withdrawal [2]	N [3]	Withdrawal Signs [4] (mean $\pm$ s.e.)					
			Weight [5]	Temperature [6]	Shakes	Ptosis [7]	Piloerection [8]	Writhing
			24 HOURS OF WITHDRAWAL					
Morphine	Nothing	13	349.2 + 6.0	37.05 + .04				
			338.7 + 6.0	36.98 + .06	6.23 + 1.14	75 + 34	13 / 13	2.46 + 1.02
			10.57 + 1.41	−0.07 + .08				
Morphine + Bell	Nothing	14	330 + 4.9	37.04 + .06				
			322.6 + 4.5	37.01 + .07	7.57 + 1.7	9 + 9	14 / 14	2.86 + .85
			6;43 + 1.78	−0.03 + .09				
Morphine + Bell	Bell	18	331.5 + 6.0	37.09 + .05				
			320.6 + 6.3	37.67 + .06	6.39 + .8	136 + 65	18 / 18	0.89 + .35 [10]
			10.58 + 1.65	−0.59 + .06 [9]				
			48 HOURS OF WITHDRAWAL					
Morphine	Nothing	13		37.16 + .08				
			324.5 + 7.2	37.17 + .06	6.31 + .83	88 + 50	13 / 13	2.15 + .6
			28.48 + 2.41	+0.01 + .02				
Morphine + Bell	Nothing	14		36.88 + .12				
			302 + 5.5	36.79 + .10	6.57 + .78	37 + 16	14 / 14	1.79 + .42
			27.86 + 1.86	−0.09 + .07				
Morphine + Bell	Bell	18		36.91 + .07				
			306.5 + 5.3	37.29 + .08	3.94 + .84 [10]	27 + 18	18 / 18	2.78 + .89
			24.28 + 1.63	+0.38 + .06 [9]				

1. Refer to No. 1 of Table 2.
2. Refer to No. 1 of Table 2.
3. Refer to No. 3 of Table 2.
4. Refer to No. 4 of Table 2
5. Refer to No. 5 of Table 2

6. Refer to No. 6 of Table 2.
7. Refer to No. 7 of Table 2.
8. Refer to No. 9 of Table 2
9. Refer to No. 9 of Table 2 ($p < .01$)
10. P value $< .05$ using Student's t-test
(each animal used as own control)

Table 4

Effect of the Auditory Stimulus on Withdrawal Signs in Rats Made Morphine Dependent in 15 Days to 400 mg/Kg/day (short term-high level)

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	Withdrawal Signs[4] (mean ± s.e.)					
			Weight[5]	Temperature[6]	Shakes	Ptosis[7]	Piloerection[8]	Writhing
			24 HOURS OF WITHDRAWAL					
Morphine	Nothing	10	305.3 ± 6.1 / 298.7 ± 5.8 / -6.6 ± 1.67	37.10 ± .07 / 37.17 ± .07 / +0.07 ± .06	4.4 ± .84	70 ± 43	10 / 10	0.5 ± .31
Morphine + Bell	Nothing	5	294.2 ± 13.1 / 288.2 ± 13.7 / -6.0 ± 0.71	37.27 ± .06 / 37.26 ± .05 / -0.01 ± .03	1.0 ± .71	0	5 / 5	0.5 ± .50
Morphine + Bell	Bell	6	314.3 ± 7.9 / 304.5 ± 6.7 / -9.83 ± 1.62	37.11 ± .08 / 37.87 ± .08 / +0.75 ± .08	1.67 ± .61	212 ± 96	6 / 6	0.17 ± .17
			48 HOURS OF WITHDRAWAL					
Morphine	Nothing	10	279.7± 6.6 / 25.6± 3.86	36.98 ± .08 / 36.95 ± .07 / -0.03 ± .09	3 ± .52	6 ± 6	10 / 10	2.7 ± .56
Morphine + Bell	Nothing	5	265 ± 15.1 / -28.5± 1.26	36.90 ± .15 / 36.94 ± .11 / +0.04 ± .08	4.5 ± 1.44	4 ± 4	5 / 5	2.25 ± 1.03
Morphine + Bell	Bell	6	282.5± 5.9 / -31.83±3.01	36.91 ± .07 / 38.00 ± .06 / +1.09 ± .06[9]	3.83 ± 1.58	0	6 / 6	1.67 ± .92

1. Refer to No. 1 of Table 2
2. Refer to No. 2 of Table 2.
3. Refer to No. 3 of Table 2.
4. Refer to No. 4 of Table 2.
5. Refer to No. 5 of Table 2
6. Refer to No. 6 of Table 2.
7. Refer to No. 7 of Table 2.
8. Refer to No. 8 of Table 2.
9. Refer to No. 9 of Table 2 (p < .01)
10. P value .05 using Student's t-test (each animal used as own control - Morphine nothing vs. Morphine + Bell

the lower terminal dose (200 mg/Kg/day). Table 5 shows that
again no signs were conditionable except for body temperature
(p < .01). These results show that the bell was ineffective
in altering withdrawal signs other than temperature even af-
ter altering the terminal dose and number of stimulus-drug
pairings.

B. Alteration of Withdrawal Signs by Gustatory and Intero-
   ceptive Stimuli

In order to determine the effects of a gustatory stim-
ulus paired with morphine on withdrawal signs, a saccharin
solution was infused into the rats' mouths prior to every
morphine injection and then the saccharin presented to the
animals at 24 and 48 hrs after the last pairing.

In the presence of saccharin, conditioned-withdrawn
rats did not show an alteration in withdrawal signs measured
except for temperature at 48 hr (Table 6). A slight, but
non-significant reduction of wet shakes occurred at 48 hr of
withdrawal after saccharin administration.

Since auditory and gustatory stimuli were unable to
significantly alter any of the withdrawal signs other than
temperature it was decided to use an interoceptive stimulus
(i.e., drug). In order to determine if an interoceptive
stimulus is effective, alcohol was selected to be paired with
each morphine injection. Control animals were given water
intragastrically with each morphine injection. During with-
drawal at 24 and 48 hrs all groups had their body weight,

Table 5

Effect of the Auditory Stimulus on Withdrawal Signs in Rats, Long term (25 days) –
High Level of Addiction (400 mg/Kg/day)

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	Withdrawal Signs[4] (mean $\pm$ s.e.)					
			Weight[5]	Temperature[6]	Shakes	Ptosis[7]	Piloerection[8]	Writhing
Morphine	None	5	288.8 $\pm$ 4.9	37.26 $\pm$ .12	24 HOURS OF WITHDRAWAL			
			279.6 $\pm$ 5.2	37.28 $\pm$ .05	5.00 $\pm$ 1.41	52 $\pm$ 33	5 / 5	1.60 $\pm$ 0.93
			−9.20 $\pm$ 2.27	+0.02 $\pm$ .05				
Morphine + Bell	None	10	299.1 $\pm$ 8.85	37.13 $\pm$ .05				
			289.8 $\pm$ 8.6	37.12 $\pm$ .06	5.60 $\pm$ 0.96	75 $\pm$ 41	10 / 10	2.50 $\pm$ 1.30
			−9.30 $\pm$ 1.70	0.00 $\pm$ .05				
Morphine + Bell	Bell	10	293.5 $\pm$ 7.1	37.13 $\pm$ .06				
			283.4 $\pm$ 6.5	38.02 $\pm$ .08[9]	5.20 $\pm$ 0.84	82 $\pm$ 35	10 / 10	1.70 $\pm$ 0.75
			−10.00 $\pm$ 1.56	−0.89 $\pm$ .06				
					48 HOURS OF WITHDRAWAL			
Morphine	None	5		37.15 $\pm$ .15				
			261.4 $\pm$ 6.5	37.16 $\pm$ .08	6.20 $\pm$ 1.07	56 $\pm$ 35	5 / 5	2.80 $\pm$ 1.24
			−27.40 $\pm$ 5.07	+0.01 $\pm$ .07				
Morphine + Bell	None	10		37.02 $\pm$ .08				
			273.3 $\pm$ 6.9	37.03 $\pm$ .06	6.20 $\pm$ 0.61	65 $\pm$ 30	10 / 10	3.30 $\pm$ 0.76
			−25.80 $\pm$ 4.02	+0.01 $\pm$ .05				
Morphine + Bell	Bell	10		36.95 $\pm$ .09				
			268.1 $\pm$ 7.0	37.85 $\pm$ .08	5.3 $\pm$ 0.79	72 $\pm$ 44	10 / 10	1.50 $\pm$ 0.50
			−25.40 $\pm$ 4.28	−0.90 $\pm$ .07				

1. Refer to No. 1 of Table 2
2. Refer to No. 2 of Table 2
3. Refer to No. 3 of Table 2
4. Refer to No. 4 of Table 2
5. Refer to No. 5 of Table 2

5. Refer to No. 5 of Table 2.
6. Refer to No. 6 of Table 2.
7. Refer to No. 7 of Table 2.
8. Refer to No. 8 of Table 2.
9. Refer to No. 9 of Table 2 (p .01)

Table 6

Effect of Gustatory Stimulus (Saccharine) on Withdrawal Signs in Rats During
the Primary Abstinence Period

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	Post Withdrawal Sign[4] (mean ± s.e.)					
			Weight[5]	Temperature[6]	Shakes	Ptosis[7]	Piloerection[8]	Writhing
			24 HOURS OF WITHDRAWAL					
Morphine + H_2O	H_2O	18	340.9 ± 5.7 334.1 ± 5.8 -6.78 ± 1.38	36.93 ± .08 36.87 ± .08 -0.07 ± .05	7.44 ± 1.86	46 ± 23	2.1 ± .15	1.61 ± .42
Morphine + SAC	SAC	18	353.7 ± 6.0 345.8 ± 6.6 -7.89 ± 1.83	36.99 ± .07 37.19 ± .09 +0.20 ± .05	6.89 ± 1.55	41 ± 21	1.9 ± .20	1.78 ± .46
			48 HOURS OF WITHDRAWAL					
Morphine + H_2O	H_2O	18	310.6 ± 6.2 30.44 ± 2.54	36.72 ± .08 36.61 ± .05 -0.11 ± .05	8.11 ± 1.67	38 ± 23	2.3 ± .10	2.5 ± .72
Morphine + SAC	SAC	18	326.0 ± 5.6 -28.50 ± 2.27	36.72 ± .06 37.00 ± .05[8] +0.29 ± .06[8]	5.11 ± .91	156 ± 40[9]	2.2 ± .15	3.93 ± .79

1. Refer to No. 1 of Table 2.
2. Refer to No. 2 of Table 2.
3. Refer to No. 3 of Table 2.
4. Refer to No. 4 of Table 2.
5. Refer to No. 5 of Table 2.
6. Refer to No. 6 of Table 2.
7. Refer to No. 7 of Table 2.

8. P value < .05 using student's t-test (each animal used as own control).

9. P value < .05 using student's t-test (comparing morphine + sac-sac vs. Morphine + H_2O-H_2O).

temperature, shakes, ptosis, piloerection and writhing mea-
sured.

In the presence of alcohol at 24 hrs withdrawal, con-
ditioned rats exhibited an increase in temperature and a
non-significant reduction in shakes (Table 7) compared to the
control groups. However, presentation of alcohol to the ex-
perimental group at 48 hrs of withdrawal had no effect on
temperature or shakes in addition to no effect on any of the
other measured symptoms.

Since the use of alcohol as an intereoceptive stimulus
was unable to reliably reduce signs other than temperature,
it was necessary to try another drug stimulus to check if an
intereoceptive stimulus could become a conditional stimulus.
Data presented in Table 8 show that the second drug chosen,
apomorphine (1.25 mg/Kg), was also ineffective in significant-
ly altering any of the withdrawal signs at either 24 or 48
hrs after the last apomorphine-morphine pairing.

One additional drug, pentobarbital, was used as an
intereoceptive stimulus. As the case with the other drugs,
pentobarbital was ineffective in altering any withdrawal
sign significantly except temperature at either 24 or 48 hrs
of withdrawal (Table 9). Pentobarbital affected the temper-
ature of both paired and unpaired animals; however, instead
of increasing temperature as the auditory and gustatory
stimuli did, pentobarbital caused a significant decrease in
body temperature. This drop in temperature of drug alone

Table 7

Effect of Alcohol (Intereoceptive) Stimulus on Withdrawal Sign in Rats During the
Primary Abstinence Period

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	Change in Withdrawal Signs[4] (mean ± s.e.)					
			Weight[5]	Temperature[6]	Shakes	Ptosis[7]	Piloerection[8]	Writhing
			24 HOURS OF WITHDRAWAL					
Morphine + H_2O	None	5	283.6 ± 10.3 272.2 ± 9.2 −11.4 ± 2.96	36.80 ± .15 36.74 ± .14 −0.05 ± .01	4.2 ± 1.28 4.2 ± 0.8 0 ± .89	0 194 ± 149 +194 ± 149[10]	1.8 ± .2 1.8 ± .2 0	0.4 ± .24 0 0.4 ± .24
Morphine + H_2O	H_2O	5	275.8 ± 7.5 271.2 ± 7.2 − 4.6 ± .60	36.84 ± .14 36.71 ± .13 −0.12 ± .05	6.0 ± 2.85 5.2 ± 1.62 −0.8 ± 2.40	12 ± 12 68 ± 13 +56 ± 19	2 2 0	0.4 ± .4 0.4 ± .4 +0.2 ± .2
Morphine + Alcohol	None	10	288.5 ± 9.6 278.5 ± 10.1 −10.0 ± 1.85	36.73 ± .06 36.63 ± .07 −0.10 ± .04	10.5 ± 3.13 12.9 ± 3.59 +2.4 ± 3.02	6 ± 6 221 ± 79 +215 ± 78[10]	1.9 ± .10 2 ± .15 +0.1 ± .1	0.4 ± .22 0.4 ± .31 0 ± .18
Morphine + Alcohol	Alcohol	10	276.9 ± 8.5 266.5 ± 7.7 −10.4 ± 2.15	36.82 ± .08 37.43 ± .14 +0.71 ± .11[9]	14 ± 2.67 7.9 ± 2.17 −6.1 ± 1.36	0 178 ± 54 +178 ± 54[10]	1.6 ± .16 1.6 ± .16 0	1.3 ± .45 1.4 ± .85 +0.1 ± .59
			48 HOURS OF WITHDRAWAL					
Morphine + H_2O	None	5	258.2 ± 10.1 −25.4 ± 4.06	36.98 ± .10 36.96 ± .04 −0.02 ± .07	4.8 ± 1.46 4.8 ± .97 0 ± 1.52	0 486 ± 167 +486 ± 167[10]	2.4 ± .4 2.2 ± .2 −0.2 ± .2	1.6 ± .75 2.2 ± .86 +0.6 ± .51

Table 7 (continued)

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	Change in Withdrawal Signs[4] (mean ± s.e.)					
			Weight[5]	Temperature[6]	Shakes	Ptosis[7]	Piloerection[8]	Writhing
Morphine + H_2O	H_2O	5	245.6 ± 3.8 -30.2 ± 5.43	36.89 ± .07 36.88 ± .10 -0.01 ± .09	7.2 ± 2.8 6.4 ± 1.03 -0.8± 1.98	0 232 ± 102 +232 ± 102	2.2 ± .2 2.2 ± .2 0	1.8 ± 1.11 2.0 ± .95 +0.2 ± 1.07
Morphine + Alcohol	Alcohol	10	246.8± 7.24 29.7±1.56	36.64 ± .23 36.75 ± .25 + 0.11 ± .09	11.6 ± 2.48 9.0 ± 1.83 -2.6 ± 1.33	0 298 ± 62 +298 ± 62[10]	2.4 ± .16 2.5 ± .17 +0.1 ± .18	3.2 ± .88 2.9 ± .25 -0.3 ± .91

1. Refer to No. 1 of Table 2.
2. Refer to No. 2 of Table 2.
3. Refer to No. 3 of Table 2.
4. Signs measured prior to treatment during withdrawal and 30 min following treatment. Difference was determined by using each animal as own control.
5. Refer to No. 5 of Table 2.
6. Refer to No. 6 of Table 2.
7. Measured in seconds (duration) prior to and 30 min. following treatment during withdrawal.
8. All animals showed piloerection, these numbers represent a rating score prior to and 30 min following treatment during withdrawal.
9. Refer to No. 9 of Table 2 (p <.05)
10. Refer to No. 10 of Table 3 (p <.05)

Table 8

Effect of Apomorphine (Intereoceptive) Stimulus on Withdrawal Signs in Rats, During the Primary Abstinence Period

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	Change in Withdrawal Signs[4] (mean ± s.e.)					
			Weight[5]	Temperature[6]	Shakes	Ptosis[7]	Piloerection[8]	Writhing
			24 HOURS OF WITHDRAWAL					
Apomorphine + Morphine	Apomorphine	10	275.5 ± 7.6	36.85 ± .09	4.1 ± .92	0	0	1.6 ± .87
			266.7 ± 8.5	36.92 ± .10	2.2 ± .49	126 ± 89	0	0.9 ± .35
			-8.8 ± 2.3	+0.07 ± .04	-1.9 ± .78	+126 ± 89[10]	0	-0.7 ± .84
Morphine	Apomorphine	10	282.5 ± 5.8	36.47 ± .07	9.5 ± 1.98	0	2	0.8 ± .43
			273.5 ± 6.3	37.01 ± .07	5.9 ± 1.15	110 ± 73[10]	23 ± .15	2.5 ± .50
			-9.0 ± 1.93	+0.54 ± .04	-3.6 ± 1.03	+110 ± 73	+0.3 ± .15	+1.6 ± .64
			48 HOURS OF WITHDRAWAL					
Apomorphine + Morphine	Apomorphine	10		35.04 ± .30	6.1 ± 1.82	296 ± 155	2.4 ± .16	1.2 ± .47
			246.2 ± 5.8	34.98 ± .93	4.2 ± 1.78	300 ± 113	2.2 ± .13	2.1 ± .75
			-28.5 ± 3.64	-0.06 ± .04	-1.9 ± .95	+4 ± 86	-0.2 ± .13	+0.9 ± .55
Morphine	Apomorphine	10		36.14 ± .38	8.1 ± 1.79	0	2.4 ± .16	2.5 ± .95
			255.9 ± 5.8	36.19 ± .34	7.4 ± 1.2	111 ± 54[10]	2.6 ± .16	4.4 ± .93
			-26.7 ± 2.97	+0.05 ± .05	-0.7 ± 1.27	+111 ± 54	+0.2 ± .13	+1.9 ± .99

1. Refer to No. 1 of Table 2.
2. Refer to No. 2 of Table 2.
3. Refer to No. 3 of Table 2.
4. Refer to No. 4 of Table 7.
5. Refer to No. 5 of Table 2.
6. Refer to No. 6 of Table 2.
7. Refer to No. 7 of Table 7.
8. Refer to No. 8 of Table 7.
9. Refer to No. 9 of Table 2 ($p < .01$).
10. Refer to No. 10 of Table 3 ($p < .05$)

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TABLE 9

Effect of Pentobarbital (Intereoceptive) Stimulus on Withdrawal Signs in Rats, During the
Primary Abstinence Period

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	Change in Withdrawal Signs[4] (mean $\pm$ s.e.)					
			Weight[5]	Temperature[6]	Shakes	Ptosis[7]	Piloerection[8]	Writhing
						24 HOURS OF WITHDRAWAL		
Morphine	None	10	290.6 + 6.1	36.87 + .07	7.0 + 1.36	0	1.7 + .15	1.5 + .50
			278.2 + 5.6	36.90 + .08	7.1 + 1.57	118 + 49	1.4 + .15	1.8 + .74
			−12.4 + 1.88	+0.03 + .03	+0.1 + 1.04	+118 + 49 [10]	−0.3 + .15	+0.3 + .79
Morphine	Pentobarb	10	284.1 + 8.2	36.90+ .06	7.2 + 2.08	6 + 6	1.7 + .15	1.3 + .62
			271.4 + 8.5	36.48+ .08	6.8 + 1.54	408 + 113	1.7 + .15	0.5 + .50
			−12.7 + 2.79	−0.43+ .08 [9]	−0.4 + 1.02	+402 + 112 [10]	0	−0.8 + .74
Pentobarb + Morphine	Pentobarb	10	314.2 + 6.1	37.00+ .06	3.9 + .85	0	1.4 + .16	0
			303.9 + 5.0	36.61+ .06	3.8 + .73	33 + 27	1.9 + .10	1.7 + 1.17
			−10.2 + 1.67	−0.39+ .05 [9]	−0.1 + .74	+33 + 27	+0.5 + .17	+1.7 + 1.17
						48 HOURS OF WITHDRAWAL		
Morphine	None	10		36.90 + .09	7.2 + 2.04	7 + 7	2	1.8 + .55
			254.9 + 8.14	36.92 + .1	7.3 + 1.84	132 + 60	1.7 + .15	2.1 + .55
			−29.2 + 2.64	+0.03 + .03	+0.1 + 1.04	+125 + 52 [10]	+0.3 + .15	+0.3 + .79
Pentobarb + Morphine	Pentobarb	10	288.8 + 5.9	37.04 + .04	4.2 + .89	1.5 + 1.5	2	1.3 + .50
			−29.2 + 2.64	36.66 + .06 [9]	5.5 + .90	40 + 28	1.9 + .18	2.3 + .99
				−0.38 + .04	+0.3 + 1.00	+39 + 20	+0.2 + .18	+1.0 + .90

1. Refer to No. 1 of Table 2.
2. Refer to No. 2 of Table 2.
3. Refer to No. 3 of Table 2.
4. Refer to No. 4 of Table 7.
5. Refer to No. 5 of Table 2.

6. Refer to No. 6 of Table 2.
7. Refer to No. 7 of Table 7.
8. Refer to No. 8 of Table 7.
9. Refer to No. 9 of Table 2 (p < .01).
10. Refer to No. 10 of Table 3 (p < .05).

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Table 10

Effect of the Conditional Stimulus (Oil of Anise) Duration on Withdrawal Sign During the
Primary Abstinence Period

Treatment during Addiction	Treatment During Withdrawal[1] (minutes)	N [2]	Change in Withdrawal Signs[3] (mean + s.e.)				
			Temperature[4]	Shakes	Ptosis [5]	Piloerection[6]	Writhing
			24 HOURS OF WITHDRAWAL				
CS-morphine	NO (CS)	10	$37.16 \pm .07$	11.7 ± 2.7	54 ± 36	$1.7 \pm .15$	$1.4 \pm .81$
			$37.70 \pm .06$	14.2 ± 3.22	264 ± 82	$1.7 \pm .15$	2.1 ± 1.04
			$-0.06 \pm .03$	$+2.5 \pm 1.32$	$+210 \pm 61$	$0 \pm .15$	$+0.7 \pm .52$
CS-morphine	CS (2)	10	$36.98 \pm .07$	8.2 ± 2.78	76 ± 59	$1.7 \pm .15$	$.9 \pm .6$
			$37.16 \pm .08$	7.4 ± 2.96	316 ± 116	$1.7 \pm .15$	$.6 \pm .22$
			$+0.18 \pm .08$	$-0.8 \pm .93$	$+240 \pm 87$	$0 \pm .21$	$-0.3 \pm .56$
CS-morphine	CS (30)	10	$37.17 \pm .08$	12.9 ± 3.73	21 ± 21	$1.5 \pm .17$	$1.4 \pm .54$
			$37.61 \pm .13$	7.8 ± 1.87	668 ± 119	$1.3 \pm .15$	$.5 \pm .31$
			$+0.44 \pm .14[7]$	-5.1 ± 3.03	$+647 \pm 114[7]$	$-0.2 \pm .13$	$-0.9 \pm .60$
CS-morphine	CS (60)	10	$36.98 \pm .07$	10.5 ± 2.01	0	$1.4 \pm .16$	$.5 \pm .22$
			$37.48 \pm .09$	$7.1 \pm .86$	556 ± 113	$1.4 \pm .16$	$.6 \pm .34$
			$+0.49 \pm .11[7]$	-3.4 ± 2.01	$+556 \pm 113[7]$	$0 \pm .15$	$+0.1 \pm .43$
			48 HOURS OF WITHDRAWAL				
CS-morphine	NO (CS)	10	$36.93 \pm .07$	9.3 ± 1.58	77 ± 38	$2.1 \pm .18$	$1.7 \pm .6$
			$36.97 \pm .05$	10.3 ± 2.23	164 ± 48	$2.2 \pm .2$	$2.3 \pm .62$
			$+0.04 \pm .04$	$+1.0 \pm 1.03$	$+87 \pm 59$	$+0.1 \pm .1$	$+0.6 \pm .97$
CS-morphine	CS (30)	10		11.5 ± 1.10	0	$1.95 \pm .05$	$1.6 \pm .46$
			$37.12 \pm .06$	$6.3 \pm .67[8]$	397 ± 76 [7]	$1.85 \pm .08$	$.8 \pm .29$
			$+0.37 \pm .05[7]$	-4.75 ± 1.04	$+397 \pm 76$	$-0.1 \pm .07$	$-0.8 \pm .51$

1. Refer to No. 2 of Table 2.
2. Refer to No. 3 of Table 2.
3. Refer to No. 4 of Table 2.
4. Refer to No. 6 of Table 2.
5. Refer to No. 7 of Table 7.

6. Refer to No. 8 of Table 7.
7. Refer to No. 9 of Table 2 ($p < .01$).
8. Refer to No. 10 of Table 3 ($p < .05$).

may mask the pentobarb's conditional effect. However, since
a control group which received pentobarb during withdrawal
also showed a drop in temperature of the same amount, this
idea seems unlikely (Table 9).

C. Olfactory Stimulus Effect on Withdrawal Signs: Physical,
    Behavioral and Biochemical

The effect of an olfactory stimulus odor is shown in
Table 10 as a more effective stimulus in being able to alter
not only temperature ($p < 0.01$) as the bell and saccharin
did, but also shakes ($p < 0.05$), and ptosis ($p < 0.01$). As can
be seen in Table 10, it was necessary to present the condi-
tional stimulus (CS) for a period of time exceeding 2 min,
but not more than 30 min. Also, the effectiveness of anise
oil as a CS was evident at both the 24 and 48 hr withdrawal
measurements.

In order to substantiate the effectiveness of anise
odor as a CS, a number of control groups were done (Table 11).
As shown in Table 11 the control groups included: 1) CS
presented to naive animals - CS presented during withdrawal;
2) CS-morphine pairing during addiction - no treatment during
withdrawal; 3) morphine alone during addiction - CS presented
during withdrawal; 4) random CS + morphine during addiction -
CS presented during withdrawal and 5) morphine alone during
addiction - no treatment during withdrawal. The anise odor
did not affect any withdrawal signs of the control groups
thus supporting the fact that the CS must be presented during
withdrawal to CS-morphine paired animals, if it is to be

Table 11

Effect of Conditional STimulus (Oil of Anise) on Rats Made Morphine-Dependent for 25 Days
to 200 mg/Kg/day

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	Change in Withdrawal Signs[4] (mean + s.e.)					
			Weight[5]	Temperature[6]	Shakes	Ptosis[7]	Piloerections[8]	Writhing
			24 HOURS OF WITHDRAWAL					
None	None		264.1 ± 5.3	37.81 ± .07	0.25 ± .12	0	0	0
			265.9 ± 6.1	37.70 ± .08	0.25 ± .25	0	0	0
			+ 1.8 ± 2.3	-.11 ± .07	0	0	0	0
CS	CS	20	280.0 ± 6.7	37.80 ± .07	0	0	0	0
			281.2 ± 8.0	37.76 ± .09	0.2 ± .1	0	0	0
			+ 1.2 ± 1.5	-0.04 ± .06	+0.2 ± .1	0	0	0
Morphine	N	10	290.6 ± 6.1	36.87 ± .07	7.0 ± 1.36	0	1.7 ± .15	1.5 ± .5
			278.2 ± 5.16	36.90 ± .08	7.1 ± 1.57	118 ± 49	1.4 ± .15	1.8 ± .74
			-12.4 ± 1.9	+0.03 ± .03	0.10± 1.04	+118 ± 49[10]	-0.30± .15	+0.30± .79
CS-Morphine	N	43	278.3 ± 4.9	37.31 ± .04	10.09± 1.14	36 ± 12	1.30± .07	1.33 ± .35
			265.0 ± 5.1	37.28 ± .05	11.12± 1.32	228 ± 48	1.44± .08	2.40 ± .51
			-13.2 ± 1.1	-0.03 ± .05	+1.03 ± 1.31	+192 ± 48	+0.14 ± .08	+1.07 ± .60
Morphine	CS	10	281.7 ± 6.3	37.07 ± .07	8.1 ± 2.39	0	1.4 ± .16	0.4 ± .16
			271.3 ± 4.9	37.11 ± .10	9.9 ± 1.95	98 ± 42	1.6 ± .16	1.5 ± .40
			- 10.4 ± 2.8	+0.04 ± .04	+1.80± .74	+98 ± 42[10]	+0.20± .20	+1.10 ± .35
Random CS Morphine	CS	10	285.2 ± 7.4	36.98 ± .07	8.2 ± 3.16	0	1.2 ± .13	0.4 ± .22
			276.3 ± 8.1	36.93 ± .09	9.1 ± 2.81	164 ± 83	1.5 ± .17	1.8 ± .76
			-8.9 ± .06	-0.05 ± .06	+0.90± 2.02	+164 ± 83[10]	+0.30 ± .15	+1.40± .61
CS-Morphine	CS	35	284.6 ± 5.1	37.24 ± .06	10.09± 1.45	34 ± 17	1.46± .08	1.11 ± .25
			271.8 ± 5.4	37.73 ± .08	6.71± 0.96	623 ± 68	1.51± .08	0.69 ± .17
			-12.8 ± 1.1	+0.49 ± .07[9]	-3.38± .61	+589 ± 65[10]	+0.05± .07	-0.42 ± .27

Table 11 - (Continued)

Effect of Conditional Stimulus (Oil of Anise) on Rats Made Morphine Dependent for 25 days to 200 mg/Kg/day

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	Change in Withdrawal Signs[4] (mean + s.e.					
			Weight[5]	Temperature[6]	Shakes	Ptosis[7]	Piloerection[8]	Writhing
			48 HOURS OF WITHDRAWAL					
CS	CS	20	280 + 6.7	37.75 + .08	0	0	0	0
			279 + 5.3	37.76 + .08	0	0	0	0
			-0.8 + 1.2	+ 0.01 + .05	0	0	0	0
Morphine	N	10		36.90 + .09	7.2 + 2.04	7 + 7	2	1.8 + .55
			254.9 + 8.4	36.92 + .10	7.3 + 1.84	132 + 60	1.7 + .15	2.1 + .55
			-29.2 + 2.64	+ 0.03 + .03	+0.10+ 1.04	+125 + 52[10]	+0.30 + .15	+0.03 + .75
CS-Morphine	N	19		36.94 + .09	7.74+ .99	103 + 64	2.42 + .14	3.26 + .95
			248.6 + 7.0	36.92 + .10	8.84 + 1.30	149 + 41	2.32 + .13	3.16 + .68
			-29.68 + 1.78	- 0.02 + .04	+1.10 + 0.62	+46 + 63	-0.10+ .13	-0.10 + .65
Morphine	CS	10		36.82 + .12	8 + 1.56	0	2.1 + .1	0.5 + .22
			252.4 + 6.6	36.88 + .14	11.8 + 1.89	188 + 55	2.1 + .1	2.1 + .57
			-29.30 + 0.86	+ 0.06 + .05	+3.8 + 1.22	+188 + 55[10]	0	+1.60 + .54
Random CS		10		36.90 + .10	9.7 + 2.26	12 + 12	1.7 + .15	1.3 + .47
Morphine	CS		260.7 + 7.3	36.95 + .14	11.6 + 3.07	162 + 62	1.8 + .13	1.8 + .33
			-24.50 + 2.02	+0.05 + .07	+1.9 + 1.52	+150 + 63	+0.10 + .18	+0.5 + .52
CS Morphine	CS	40		37.11 + .08	8.41 + .83	31 + 12	2 + .03	1.92 + .43
			258.2 + 5.7	37.58 + .06	5.50 + .57	551 + 65	1.85 + .09	1.18 + .33
			-26.38 + 1.27	+ 0.47 + .05[9]	-2.91 + 0.76	+520 + 61[10]	-0.15 + .08	-0.75 + .32

1. Refer to No. 1 of Table 2.
2. Refer to No. 2 of Table 2.
3. Refer to No. 3 of Table 2.
4. Refer to No. 4 of Table 7.
5. Refer to No. 5 of Table 2.
6. Refer to No. 6 of Table 2.
7. Refer to No. 7 of Table 7.
8. Refer to No. 8 of Table 7.
9. Refer to No. 9 of Table 2 ($p < .01$)
10. Refer to No. 10 of Table 3 ($p < .05$)

effective in reducing specific withdrawal signs. The effect on ptosis by the CS showed an increase rather than a decrease upon observation; this effect was significant ($p < .05$) (Table 11).

As can be seen in Table 12 the CS was able to significantly reduce spontaneous aggression in animals which had a history of CS-morphine pairing (Chi-square analysis, $p < .05$).

Anise odor, thus far, has been effective in altering physical (temperature) and behavioral withdrawal signs (aggression, ptosis, shakes and writhing). However, if anise oil were able to alter biochemical parameters of withdrawal, this would give conclusive support of its true effectiveness as a conditional stimulus.

Blood glucose and homovanillic acid measures were chosen as the biochemical parameter to be monitored in the conditioned and unconditioned animals. Data presented in Table 13 show the effect of the CS on blood glucose levels of conditioned and unconditioned rats. The CS was able to increase the glucose level in conditioned animals at 24 ($p < .01$) and 48 hr ($p < .05$) of withdrawal. But as can be seen in Table 13, the CS groups showed no significant increase in blood glucose when compared to saline-treated animals.

Data summarized in Table 14 shows that HVA was also increased following anise oil presentation to anise oil-morphine animals during withdrawal at 24 and 72 hr ($p < .05$). The increase in HVA was similar to that seen following morphine

Table 12

Effect of Conditional Stimulus (Oil of Anise) on Withdrawal
Induced Aggression

Treatment during Addiction[1]	Treatment during Withdrawal[2]	N[3]	% Groups Fighting
CS	CS	4	0
Morphine	None	5	80
CS-Morphine	None	8	88
Morphine	CS	5	100
Random CS-Morphine	CS	5	80
CS-Morphine	CS	12	42[4]

[1]Refer to No. 1 of Table 2.

[2]Refer to No. 2 of Table 2.

[3]Number of groups (5 animals/group)

[4]Chi square analysis χ^2 4.78; p < .05.

Table 13

Effect of the Conditional Stimulus (Oil of Anise) on Blood
Glucose Levels in Naive and Morphine Withdrawn Rats

Group [1]	Treatment[2]	N[3]	Blood glucose levels [4] (mg % $\pm$ s.e.)
Naive Rats	Saline	14	99 $\pm$ 3.8
	Morphine 20 mg/Kg	7	140.2 $\pm$ 4.9[5]
24 Hr Withdrawal	Saline	5	96 $\pm$ 4.2
	No CS	20	101.4 $\pm$ 3.0
	CS	10	118.4 $\pm$ 3.1[5]
48 Hr Withdrawal	Saline	5	96.1 $\pm$ 2.5
	Random CS + CS	5	99.4 $\pm$ 4.4
	No CS + CS	5	98.6 $\pm$ 4.9
	No CS	5	104.3 $\pm$ 3.0
	CS	5	115.7 $\pm$ 7.3[6]

[1] Terminal dose of morphine for withdrawn animals was 200 mg/Kg/day.
[2] Refer to No. 2 of Table 2.
[3] Refer to No. 3 of Table 3.
[4] Blood drawn 30 min following treatment.
[5] Refer to No. 9 of Table 2 (p $<$ .01).
[6] Refer to No. 10 of Table 3 (p $<$ .05)

Table 14

Effect of the Conditional Stimulus (Oil of Anise) on Striatal
Homovanillic Acid Levels in Naive and Morphine Withdrawn Rats

Group[1]	Treatment[2]	N[3]	HVA LEVELS [4] (ug HVA/g Striatum $\pm$ s.e.)
Naive Rats	Saline	5	0.446 $\pm$ .032
	Morphine 10 mg/Kg	5	0.728 $\pm$ .049 [5]
	Morphine 100 mg/Kg	5	0.855 $\pm$ .082 [5]
24 Hr Withdrawal	No CS	5	0.450 $\pm$ .014
	CS	5	0.591 $\pm$ .026 [6]
72 Hr Withdrawal	Morphine 10 mg/Kg	5	0.507 $\pm$ .017
	Morphine 100 mg/Kg	5	0.838 $\pm$ .084 [5]
	No CS	5	0.480 $\pm$ .042
	CS	5	0.613 $\pm$ .051 [6]

[1]Refer to No. 1 of Table 13.
[2]Refer to No. 2 of Table 2.
[3]Pairs of animals.
[4]Animals were sacrificed 30 min following treatment.
[5]Refer to No. 9 of Table 2 ($p < .01$).
[6]Refer to No. 10 of Table 3 ($p < .05$).

(10 mg/Kg) given to 72 hr withdrawn animals but not as great
as a 10-fold high morphine (100 mg/Kg) dose given at 72 hrs
(Table 14).

D. Reversal of the Action of Olfactory Stimulus as a CS by
    Naloxone

Data presented in Table 15 show the effects of four dif-
ferent doses of naloxone given to conditioned animals pre-
sented the CS during withdrawal. As can be seen, different
doses of naloxone were found to reverse the CS's effect on
specific withdrawal signs. Naloxone given at a dose of
0.04 mg/Kg was ineffective in blocking any of the signs mea-
sured. However, 0.16 mg/Kg of naloxone was able to block the
CS reduction of writhing. At 0.64 mg/Kg of naloxone the tem-
perature increase seen following the CS was not only blocked,
but a further reduction due to the narcotic antagonist was
observed. Also, the effect on shake behavior was completely
blocked at a dose of 0.64 mg/Kg (naloxone). Ptosis was in-
creased which exceeded CS's effect, but naloxone by itself
in morphine dependent animals causes an increase in ptosis
by itself, thus making it difficult to obtain any meaning
with relation to the CS at either 0.64 or 2.5 mg/Kg. These
data along with data previously reported by this lab
(Drawbaugh & Lal, 1974) using a bell sound (CS) suggest the
hypothesis that the CS and morphine may indeed work on the
same receptors. Present evidence that an endogenous morphine-
like substance exists may be the means of the CS effect
(this will be discussed in detail later).

Table 15

Effect of Naloxone Pretreatment on Conditioned Animals Presented the Conditional
Stimulus During Withdrawal

Dose of Naloxone[1]	N[2]	Change in Withdrawal Signs[3] (mean + s.e.)				
		Temperature[4]	Shakes	Ptosis[5]	Piloerection[6]	Writhing
None	43	$37.24 \pm .06$	10.09 ± 1.45	34 ± 17	$1.46 \pm .08$	$1.11 \pm .25$
		$37.73 \pm .08$	$6.71 \pm .76$	623 ± 68	$1.51 \pm .08$	$.69 \pm .17$
		$+0.49 \pm .07$[7]	$-3.38 \pm .61$	$+589 \pm 65$[7]	$+0.05 \pm .07$	$+0.42 \pm .27$
0.04	10	$36.95 \pm .04$	6.4 ± 1.3	31 ± 31	$2.1 \pm .1$	$1.3 \pm .6$
		$37.63 \pm .05$	$3.5 \pm .76$	283 ± 95	$2.3 \pm .16$	$.6 \pm .34$
		$+0.68 \pm .05$[7]	-2.90 ± 1.11	$+252 \pm 78$[7]	$+0.2 \pm .13$	$-0.70 \pm .68$
0.16	10	$36.97 \pm .06$	$8.5 \pm .95$	0	$2.1 \pm .1$	$0.8 \pm .39$
		$37.52 \pm .09$[7]	$5 \pm .7$	167 ± 54[7]	$2.2 \pm .13$	$1 \pm .3$
		$+0.55 \pm .09$	$-3.50 \pm .70$	$+167 \pm 54$	$+0.1 \pm .1$	$+0.20 \pm .42$
0.64	10	$36.88 \pm .1$	$8.1 \pm .78$	0	$1.8 \pm .13$	$.7 \pm .42$
		$36.45 \pm .14$[7]	7.8 ± 1.18	754 ± 63[7]	$2.1 \pm .15$	$.9 \pm .59$
		$-0.44 \pm .12$	-0.30 ± 1.50	$+754 \pm 63$	$+0.3 \pm .16$	$+0.20 \pm .63$
2.50	10	$36.94 \pm .04$	8.5 ± 2.17	0	$1.4 \pm .16$	1.8 ± 1.27
		$35.69 \pm .24$	8.2 ± 1.35	1166 ± 127	$2.4 \pm .16$	$1.1 \pm .60$
		$-1.25 \pm .26$[7]	-0.30 ± 1.49	$+1166 \pm 127$[7]	$+1.2 \pm .13$	$-0.70 \pm .76$

[1] mg/Kg given i.p.
[2] Refer to No. 3 of Table 2.
[3] Refer to No. 4 of Table 7.
[4] Refer to No. 6 of Table 2.
[5] Refer to No. 7 of Table 7.

[6] Refer to No. 8 of Table 7.
[7] Refer to No. 9 of Table 2 ($p < .01$)
[8] Refer to No. 10 of Table 3 ($p < .05$)

Data presented in Table 16 show that Naloxone's (2.5 mg/Kg) effect on blood glucose levels was very unpredictable. A slight, insignificant, decrease in blood glucose levels was observed at 24 hr and an increase at 48 hr. Thus, even though it seemed to block the CS effect as shown in this table it is very difficult to interpret because of naloxone's effects in animals not treated with the CS during withdrawal.

Table 16

Effect of Naloxone Pretreatment on Blood Glucose Levels in Conditioned Animals Presented the Conditional Stimulus During Withdrawal

Group[1]	Treatment[2]	N[3]	Blood glucose levels (mg % $\pm$ S.E.)[4]
Naive rats	Saline	14	99 $\pm$ 3.8
	Morphine[5]	7	140.2 $\pm$ 4.9
	Naloxone[6]	10	90.3 $\pm$ 4.1
24 Hr Withdrawal	Saline	5	96 $\pm$ 4.2
	CS	10	118.4 $\pm$ 3.1
	Naloxone[6]	10	89 $\pm$ 6.3
	CS + Naloxone[6]	10	93[4]$\pm$ 3.7
48 Hr Withdrawal	Saline	5	96.1 $\pm$ 2.5
	CS	5	115.7 $\pm$ 7.3
	Naloxone[6]	10	107.3 $\pm$ 4.8
	CS + Naloxone[6]	10	95.1 $\pm$ 8.4

[1]Refer to No. 1 of Table 13.-

[2]Refer to No. 2 of Table 2.

[3]Refer to No. 3 of Table 3.

[4]Refer to No. 4 of Table 13.

[5]20 mg/Kg, given intraperitoneally.

[6]2.5 mg/Kg, given intraperitoneally.

DISCUSSION

The major significance of this study is the demonstration that several morphine withdrawal signs can be reduced by environmental stimuli that have been repetitively paired with morphine administration. In this study, rats given an auditory (bell), gustatory (saccharin) or olfactory (anise oil) stimulus paired with morphine during addiction, exhibited an increase in temperature analogous to the effect of morphine when given the respective stimulus alone during withdrawal.

In addition to temperature, behavioral signs such as shakes, ptosis, writhing and aggression were altered by the olfactory stimulus during withdrawal. The auditory, gustatory, and interoceptive stimuli were found not to significantly alter any of the above-mentioned behavioral signs. Also, two biochemical measures were altered by presentation of anise oil during withdrawal: 1) blood glucose, and 2) homovanillic acid.

The following discussion will be divided into four parts. The first part will deal with the auditory, gustatory and interoceptive stimuli. The second part will include evidence establishing the ability of the olfactory stimulus to alter all three types of withdrawal measurements; 1) physical, 2) behavioral and 3) biochemical changes that normally occur following a morphine injection. The third part will deal with the ability of naloxone to reverse the anise

oil's conditional effects. The last part will deal with the significance of these findings.

Auditory, Gustatory and Interoceptive Stimuli

The addiction schedule used in conditioning morphine's effects to establish the bell as a conditional stimulus, with respect to hypothermia was used unsuccessfully in an attempt to alter other withdrawal signs. It was therefore decided to increase the number of CS-UCS pairings, as other investigators had done, notably Wikler & Pescor (1967) who paired the CS with morphine for nine weeks. The increased number of pairings, from 30 to 50 were still inadequate since no additional signs were controlled by the bell stimulus.

Since increasing CS-UCS pairings at the dose of 200 mg/Kg/day did not enhance CS effectiveness with regard to withdrawal signs other than hyperthermia, it was decided to try increasing UCS amplitude. Thus the dose was doubled to 400 mg/Kg/day, a dose used in this lab for other types of experiments (Puri & Lal, 1973; Gianutsos et al., 1974). Again no additional signs were altered at either 30 or 50 pairings. It is important, however, to recognize that in using the four different schedules, the temperature effect was always highly significant following such presentation during withdrawal.

The ineffectiveness of the bell to alter other signs beside temperature may have been due to a number of factors which were not tested or tested but not exhaustively analyzed. One possibility may be that even more than 50 pairings may

be required to be able to bring other withdrawal signs under stimulus control. Goldberg (1971) and Lynch et al. (1973) have shown that stimulus control is indeed dependent upon the number of CS-UCS pairings. Another possible parameter which may have contributed is the length of bell presentation for each trial. This looks especially important when one considers CS strength and CS-UCS optimal interval. In addition, the fact that latter experiments using an olfactory stimulus required the CS to be presented for a time period greater than two minutes lends support to this explanation. Along with the above-mentioned classical conditioning variables is motivation. Once considered only a parameter in instrumental conditioning, it is now known to play a major role in classical conditioning (Beecroft, 1966). Wikler & Pescor (1967) conditioned their animals by presenting the morphine only once daily, thus assuring that their rats were well into withdrawal.

Another area, separate from addiction procedures, which may have limited conditionability consists of the signs that were monitored. This problem of measuring withdrawal signs has caused a number of labs to measure only one sign (body weight (Kumar, 1972), or to rate a combination of selected signs following antagonist treatment (Wei, 1973)). The concern of this investigation was not to become too restrictive in the signs measured since it was felt that the more signs that could be shown to change following stimulus presentation the better the opportunity would be to show quantiative differences between different types of stimuli.

The behavioral and physical signs selected were easily measurable; however, many other signs exist for rats going through morphine withdrawal: salivation, rhinorrhea, restlessness, altered food and water consumption (Watanabe, 1971; Martin et al., 1963_. Since it would not be practical to monitor all the different signs, those that could be measured with ease and reliability were chosen. All of these variables must be considered in evaluating not only the auditory stimulus, but the other stimuli to be mentioned.

The use of a gustatory stimulus in morphine conditioning experiments has been used by others (Wikler & Pescor, 1967); Kumar & Stolerman, 1972); but, not in the same manner it was used in this study.

Saccharine was chosen as a gustatory stimulus because rats have been shown to drink very large amounts when given the opportunity and show preference for it over plain water. The results, however, did not bear out saccharine as a good choice, or that gustatory stimuli in general can serve as CS's. Again, the only sign alterable was temperature and the effect was only about 25% that of the auditory stimulus, possibly suggesting it was even weaker than the bell. However, many factors may have been involved in the ineffectiveness of saccharine's ability to become a strong conditional stimulus. One reason briefly, alluded to above was the selection of an appropriate gustatory stimulus. A bitter substance may have been a better choice. Or possibly a

different concentration of saccharine may have increased its effectiveness, since it has been shown that there is a relationship between concentration and the rat's desire for the substance. Also, as was mentioned for the auditory stimulus, the schedule of addiction and the number of pairings may not have been enough to allow for saccharine to control additional morphine-like effects when presented during withdrawal.

In addition to schedule of addiction, the CS-UCS interval was very important in this experiment and may indeed have been the biggest reason for saccharine failure to elicit morphine-like effects. The saccharine was infused for only 45 seconds because the withdrawn animal became very restless; since the only restraint was the experimenter's hand, he became difficult to manage. Thus the stimulus was very short and time of UCS onset was longer than might be desired. Also, the problems of motivation and signs monitored apply to the gustatory experiment.

The third type of stimulus, interoceptive, was shown to be even less effective than either auditory or gustatory stimuli. Not even temperature was increased by any of the drugs (apomorphine, alcohol or pentobarbital) paired with morphine, when later given by themselves during withdrawal. The use of a drug as a CS coupled with a UCS (drug) has not been shown to be effective (Lynch, et al., 1973) but few experimenters have tried such a combination.

The selection of apomorphine, alcohol and pentobarbital was based on their distinctive ability to be discriminated by the rat as being different from the UCS and thus a foundation for a CS-UCS pairing (Overton, 1972). However, combining drug effects can cause a number of problems, especially with the drugs selected. Apomorphine, it is thought, works opposite to morphine in the dopaminergic system (Kaariainen and Ahtee, 1976) and even at the dose given may cause an undesirable interaction apart from the gastrointestinal disturbances. This, unfortunately, was the case. Withdrawal signs were fewer in number without presentation of the CS and apomorphine affected temperature by itself in withdrawn animals, when it was not paired during addiction, thus confounding any temperature data that may have been obtained. Since the experiment was completed it has been shown that very small doses (0.16 mg/Kg) of apomorphine show major endocrine effects in withdrawn rats (Lal et al., 1976) thus suggesting that reducing the dose would be imperative so as not to confound the withdrawal signs by effects of apomorphine itself.

Alcohol and pentobarbital at the doses used also had effects of their own which confounded the withdrawal signs monitored. Unfortunately, no data existed prior to these attempts at observing the interaction of acute or chronic morphine with the drugs selected and what may happen during withdrawal when only pentobarbital or alcohol is administered alone. One problem that occurred with the other

stimuli which was circumvented by the drug stimuli was dura-
tion of CS and CS-UCS interval. The drugs were given so as
at least a 15-min overlap between CS-UCS existed, so as to
insure that the stimulus was present when morphine began to
have an effect.

From the data where an interoceptive stimulus was
paired with morphine it appears that this type of conditiion-
ing because of drug variables is probably the most difficult
(dose duration of action, interaction). This does not mean
that such an attempt should not be considered, but a number
of preliminary experiments should be done prior to selection
of a substance to be used (i.e., effect of substance on non-
paired morphine-withdrawn animals.

Use of an Olfactory Stimulus to Alter Morphine Withdrawal Signs in Addicted Rats

The following section contains evidence that a condition-
al stimulus, anise odor, is able to elicit effects during with-
drawal which normally are produced only by morphine.

In animals addicted to anise-oil-morphine:

1. To be effective as a CS, oil of anise must be pre-
sented for more than 2 min but not to exceed 30 min to CS-
morphine-paired animals during withdrawal.

2. In the presence of the CS, 24 and 48 hours after the
last CS-morphine pairing, the rats showed respectively a 30%
and 35% reduction in wet shakes. If no CS was presented the

conditioned animals exhibited a 10% increase in shakes at 24 and 48 hrs of withdrawal. If the CS was randomly presented to the rats during addiction and then presented anise oil during withdrawal, this group showed a 10% increase in shakes at 24 hr and 20% increase at 48 hr of withdrawal.

3. In the presence of the CS, 24 and 48 hr after the last CS-morphine pairing, the rats showed a significant increase in rectal temperature. If no CS was presented the conditioned animals showed no change in temperature comparing the temperature before and after observation. The group of random oil of anise-morphine when given the CS also showed no change in rectal temperature.

4. In the presence of the CS, 24 and 48 hours after the last CS-morphine pairing, the rats showed a significant reduction in writhing and an increase in ptosis time at 24 and 48 hr of withdrawal. Conditioned rats not receiving the CS showed an increase in writing at 24 hours and no change at 48 hours. Ptosis time increased for the conditioned animals not getting the CS but not nearly to the extent of the conditioned animals. The random CS-morphine group when given the CS during withdrawal exhibited increased writhing at 24 and 48 hours and their ptosis time increased but again not nearly as much as the conditioned animals following CS treatment.

5. In the presence of the CS, 48 hr after the last CS-morphine pairing, the rats exhibited a significant reduction in aggressive behavior. If no CS was presented, grouped

conditioned animals fought vigorously. Addicted animals
not given the CS during addiction and given the CS during
withdrawal, fought when grouped during withdrawal as did
the random CS-morphine group.

These data suggest the following:

1. The anise oil, when paired with morphine during
    addiction, was able to effectively alter a num-
    ber of withdrawal signs. Thus the anise oil
    had acquired conditional properties.

2. The conditional effect could be seen for at least
    48 hr after the last CS-UCS pairing.

3. Anise oil when not systematically paired with
    morphine had no significant effect when pre-
    sented during withdrawal on any signs monitored.

4. Anise oil presentation was adequate to reduce
    aggressive behavior of conditioned rats, thus
    giving strong support for an overall reduction
    of unstability due to narcotic withdrawal.

Behavorially the work using oil of anise (olfaction)
has a number of very important implications. The first
being, that smell seems to play a more important role in
conditioning of morphine's effects. Even though the bell was
somewhat effective in mimicking an action of morphine, only
olfaction was able to alter temperature, shakes, writhing,
and aggression. This becomes even more important as the
first systematic attempt at human conditioning [O'Brien, 1976)

used an uaidoty tone and olfaction (oil of peppermint) as a
compound CS, thus using the best two senses as found in this
study.

The O'Brien study was successful in conditioning with-
drawal signs by pairing the compound CS with naloxone and
after a number of days giving the CS alone, thus inducing
withdrawal without the antagonist. Five of the eight subjects
in the study showed that the auditory and olfactory stimuli
had acquired conditional properties.

In the present study it is difficult to assess other
factors which may cause the procedure used for conditioning
to involve a compound conditional stimulus. However, the
strongest factor in the chain of events must be the anise oil
as only the experimental group showed conditioning of mor-
phine's effects.

In addition to the physical and psychological changes
controlled by anise oil, HVA was observed to be brought under
stimulus control. Only one other lab has reported such a
find (Perez-Cruet; 1976) and he brought dopamine (DA) metabo-
lism under stimulus control using a buzzer as the CS and
methadone as a UCS. The present study shows the CS to increase
DA metabolism, thus increasing HVA formation at 24 and 72 hr
following the last CS-morphine pairing.

The increase in HVA levels following CS presentation
would not seem to be due to chance since conditioned animals
not receiving the CS showed no changes in DA metabolism. It
is also as unlikely that the changes observed are due to

stress as all controls were put through the same procedure as the experimental group except they did not get the CS. Since the classical conditioning paradigm utilized in this study has been used to establish drug conditioning with fairly good success, it is more likely to assume that the changes in HVA levels associated with the CS (olfactory-oil of anise) are the result of drug conditioning and this change can be considered as a conditional reflex of DA metabolism.

The ability to alter a transmitter of the brain should open new thinking into the complicated function of neurotransmitters in the brain as the activities of these transmitters are not only affected by direct stimuli, but also by conditioned experiences to which the organism has learned a reflex response. The fact that a transmitter can be brought under stimulus control may alter the thinking of individuals in the learning field who generally assumed that a neurotransmitter's function would be very difficult to alter by a psychological stimulus (Perez-Cruet, 1976). There are a number of good reasons to support the above statement: 1) neurotransmitter functions depend on enzymes which must travel from the cell body down the neuron, the process takes approximately 2.5 days; and 2) unless neurotransmitter functions are measured within specific neuronal pathways it will be very hard to determine what kind of impact the stimulus may have on neurotransmitter function. Also, it must be remembered that only one metabolite in one area was measured. Other

transmitters such as, acetylcholine, serotonin or norepine-phrine may be altered as well as other areas may be involved such as hypothalamus (endocrine and temperature) or amygdola (aggression). Many more studies will be required to eluci-date the activity of DA as being the only transmitter which can be conditioned or that others may be brought under stimulus control. It is most likely that a number of transmitters are involved in the conditioned response and not just DA.

Even though the changes in HVA levels has been defined as a conditional phenomenon, in terms of neurotransmitter function, this change may represent a much more complex activity because the stimulus-reflex association most likely occurs cortically, as well as striatally. Also, the drug may act at a receptor whereas the stimulus may cause something to be released which will then affect the receptor.

Considering all of the many possibilities discussed above in explaining how the conditional stimulus affects neurotransmitter function the fact remains that the process does occur with pairing an auditory stimulus with methadone (Pérez-Cruet, 1976) and pairing an olfactory stimulus with morphine. Thus it seems that the brain functions can be conditioned like those of other visceral organs.

A second biochemical change was brought under stimulus control, blood glucose. The changes due to CS presentation could be seen at 24 and 48 hr withdrawal in animals which had 50 pairings of CS-morphine. This conditional increase is

difficult to explain as it has been shown by Sable-Amplis (1972) that tolerance develops to the increase in blood glucose levels following morphine administration. Thus after only 3 or 4 injections the injection of morphine and CS are no longer paired with the biochemical change. Thus one explanation for this increase in blood glucose level during withdrawal following CS presentation may be that another system was conditioned and the resultant effect was expressed as increased blood glucose. There is no doubt that the glucose level increased, just that the system or systems causing this change remain to be elucidated.

Use of Naloxone to Reverse the Effects of a CS

The ability of naloxone to block the CS effects on temperature and shakes in conditioned animals as it does in morphine addicted animals given morphine has some very important implications. Since an opiate receptor was found by Pert and Snyder (1973) and a number of substances isolated which may affect this receptor (one endorphin associated with a reduction in pain) these bits of information may indeed be valuable in explaining how naloxone blocks a conditional stimulus from mimicking morphine's effects. Given that an endorphin exists (Goldstein, 1976; Hughes et al., 1975; Terenius and Wahlstrom, 1975; Simantov and Snyder, 1976), then the CS may be activating this substance which in turn acts on the opiate receptor and the resultant behaviorial

changes which were previously attributed to morphine, now occur following CS presentation.

To explain in detail the last statement it is important to understand how endorphin may be working. Normally, enkephalin may be assumed to control certain inhibitory mechanisms determining the level of neurotransmitter release. If an opiate is administered to increase this level of inhibition, to cause analgesia, then the control will be under the exogenous narcotic and not the endogenous enkephalin. A negative feedback may slow down or even stop the synthesis of the endogenous substance now that an exogenous opiate has taken control (Kosterlitz and Hughes, 1975). Now the central nervous system will be completely dependent on the injected narcotic to maintain the inhibitory mechanism.

When the narcotic is withdrawn suddenly, those inhibitory mechanisms become inactive, because what endorphin exists will not be able to stimulate the opiate receptor until the receptors regain their sensitivity or until enough enkephalin can be synthesized or both. Thus the withdrawal syndrome develops which may be attributed to the lack of control of the inhibitory system. Now if the CS were presented to conditioned animals it may cause any one or more of the following:

1) A transient alteration in receptor sensitivity; 2) release the peptide substance (enkephalin) in large enough quantities to partially alter a number of withdrawal signs, or 3) mobilize morphine which still can be found in tissue to affect opiate receptors.

The above mentioned phenomenon of the CS altering opiate receptors or releasing enkephalin can be borne out by the fact that naloxone was able to reverse the CS effect as it reverses morphin effects; thus, providing strong indirect evidence for a mechanism of how the CS works. Unfortunately, the attempt to block the CS's effect on blood glucose levels was very confusing, and little can be said. It looked as though naloxone was affecting baseline levels of blood glucose thus making it difficult to interpret the results, But, it must be remembered that the effect seen may not be a direct effect of the CS as hepatic glycogenolysis which occurs following morphine initially disappears after 3-4 injections. Also, glucose metabolism remains difficult to assess even after a month of withdrawal (Sable-Amplis, 1972). Thus the system itself is rather complex and its function not fully understood following narcotic administration.

It does look from Table 16 that the conditioned changes in blood glucose levels were blocked, but further work must be done before such a conclusion can be drawn. A better understanding of glucose metabolism following narcotics is necessary and naloxone's effects on glucose levels of both addicted and naive rats should be studied.

Significance of the Findings

Many researchers are approaching conditional aspects of narcotic addiction from the standpoint that human addicts take heroin in order to relieve withdrawal. However, this

study suggests that neutral stimuli which are paired frequently enough with morphine injections will come to elicit the same or similar physiological reactions as the drugs themselves. For example, when the addict sees a syringe and any other environmental factors associated with "shooting-up," it will begin to elicit physiological reactions to morphine before it is ingested. This can be seen more vividly by "needle freaks" who will stick themselves and shoot anything in order to postpone the onset of withdrawal.

What may be happening in the above situation of the "needle freak" is that he is able to release the morphine-like substance and relieve (temporarily) some withdrawal signs by sticking himself. The release of enkephalin may be exactly what the olfactory CS is releasing, thus in part an animal analog of activation has been creation. Thus if an animal analog of narcotic addiction has been developed the next and probably most important phenomenon can be observed - extinction. If the effects of a narcotic are really conditioned, then the resistance to extinction should be very high. Also, this opens the possibility of injecting enkephalin into the conditioned animals and see if enkephalin without the CS can reduce parts of the withdrawal syndrome similar to the CS. Also, since the areas which contain opiate receptors have been localized, naloxone could be injected into different areas to see which one(s) must be blocked in order to prevent the CS from working.

This study has provided additional groundwork for future work by: 1) neurochemists to determine if the condtioning of other neurotransmitter systems will be useful in establishing whether there is a general pattern of conditioned neurotransmitter functions in brain or specific conditioning of monoaminergic functions; 2) physiological psychologists to determine which area of the brain has the major responsibility for narcotic conditioning behavior; 3) psychiatrist to develop experiments utilizing human subjects to see if the major concern of the addict is to prevent withdrawal or the conditioning is of the drug effects as eluted to above; and 4) sociologist to better characterize the environmental cues that may be best associated with narcotic administration given that a better understanding of the conditional phenomenon is forthcoming.

This study also supports the use of narcotic antagonists in treatment of narcotic addicts. The problem that arises now is what part of drug taking behavior can be classified as operant and what part as classical conditioning. Thus all that really can be said from this study is that a narcotic antagonist would be useful in treatment of human addicts, as seen from the animal analog, but the extent of its usefulness may be just of limited value.

Many more systematic studies must be done to evaluate the components of classical conditioning such as CS-UCS interval, motivation, acquisition and extinction in drug conditioning experiments.

CONCLUSIONS

1) The conditional stimulus found best in development of an animal analog of narcotic addiction was oil of anise (olfactory) (see Table 17).

2) The olfactory conditional stinulus was able to alter both physical and psychological temperature withdrawal signs which include: temperature (similar to an auditory stimulus (bell)), shakes, writhing and aggression.

3) The olfactory conditional stimulus was able to alter two biochemical systems: 1) dopamine metabolism (HVA levels) and 2) blood glucose levels similar to the action of morphine.

4) Naloxone was able to block both physical and behavorial properties of the CS supporting the possibility that the CS may work by releasing an endogenous morphine-like substance.

Table 17

Summary of Conditioning Experiments

Stimulus	Signs Measured							
	Temperature	Shakes	Ptosis	Piloerection	Writhing	Aggression	BG[2]	HVA [3]
Morphine	++++	++++	++++	++++	++++	++++	+++	++++
Oil Anise	++	++	0	+	++	++	++	++
Bell	++	0	+	0	0	−	−	−
Saccharin	+	0	0	0	0	−	−	−
Pentobarbital	0	0	0	0	0	−	−	−
Apomorphine	0	0	0	0	0	−	−	−
Alcohol	0	0	0	0	0	−	−	−

[1]Withdrawal signs measured at 24, 48, 72 hr withdrawal

[2]Blood glucose level

[3]Homovanillic acid level

(Note: + = small effect, ++ = medium effect, +++ = large effect, ++++ = very potent effect, 0 = no effect, − = not measured.)

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