CONDITIONING OF DRUG INDUCED TEMPERATURE CHANGES

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ABSTRACT

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The nature and direction of conditioned drug effects were analyzed in terms of homeostatic, or regulatory, feedback systems. It was argued that where in such a system a drug acted would allow one to predict both the nature and direction of the conditioned response. A drug that acted prior to the "integrator" would result in a conditioned response similar to the observed drug effect, while a drug that acted after the integrator would result in a conditioned response that would correct for (or oppose) the observed drug effect.

This analysis of conditioned drug effects was then applied to drugs affecting the thermoregulatory system in the rat. Previous work had determined there were two conditioned temperature responses to morphine, a conditioned hypothermia that occurred in a pre-injection environment 23 hours after the daily morphine injection, and a conditioned hyperthermia that occurred in a distinctive drug-injection environment.

Experiments 1 and 2 attempted to establish the importance of temporal versus environmental cues as conditioning stimuli. The relevance of temporal stimuli was minimized in Experiment 1 by administering morphine at irregular times on alternate days. For one group (COND) morphine injections were preceded and followed by periods in distinctive environments. Group PSEUDO animals, though exposed to the environments, received morphine on the intervening days in the home cage; group SALINE received only saline. Tests for conditioning were carried out both during drug conditioning and after a drug-free period. All animals receiving morphine showed a nonspecific hypothermia when not under the direct influence of morphine. A conditioned hyperthermia was evident in group COND animals in the distinctive environments. In Experiment 2, in which animals remained in their home cages at all times, the relevance of temporal cues was emphasized by administering morphine at exactly 24-hour intervals. These animals became hypothermic only around the time of the expected injection. Animals in another group that received morphine at irregular times showed the nonspecific hypothermia seen previously. There was no evidence for a conditioned hyperthermia in the second experiment.

In Experiment 3, the conditioned effects produced by 20 mg/kg morphine sulfate, 5 mg/kg d-amphetamine sulfate (both of which produced hyperthermia) and by 20 mg/kg naloxone hydrochloride (which produced hypothermia) were compared. Four groups of rats received IP injections of either one of these drugs or of saline at exactly 24-hour intervals. Each injection was preceded and followed by a period in one of the two distinctive environments. Tests were made to determine the relative effectiveness of temporal and environmental stimuli as conditioned stimuli for the conditioned effects observed.

The conditioned responses to morphine and naloxone mirrored each other. The stimuli of the distinctive injection environment elicited conditioned hyperthermia in morphine-group animals and conditioned hypothermia in naloxone-group animals; temporal cues elicited conditioned hypothermia in morphine-group animals and conditioned hyperthermia in naloxone-group animals. The conditioned response to amphetamine was hyperthermia; a response that mimicked the unconditioned response and that was elicited by the environmental stimuli of the injection environment.

Experiment 4, similar in design to Experiment 3, studied the conditioned response to several doses of

d-amphetamine sulfate; 0, 1, 2 and 5 mg/kg. Unlike Experiment 3, animals were pre-exposed to the experimental procedures. Under these conditions two conditioned responses to amphetamine emerged; prior to the drug-free period temporal cues elicited conditioned hypothermia, while environmental stimuli elicited conditioned hyperthermia. Thus amphetamine and morphine resulted in similar conditioned responses. The result of these experiments were discussed in terms of the analysis of conditioned drug effects developed in the introduction.

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It has been stated that the doctoral thesis represents the culmination of the candidate's research program. Any person who has reached this stage in his or her career knows that in actual fact the doctoral thesis represents the work of many individuals. It is this group of people that I would especially like to thank.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

Physiological responses may become conditioned if a drug is repeatedly administered. These conditioned responses can be distinguished from other direct and indirect drug effects by the fact that under appropriate circumstances they can be elicited without administering the drug. Conditioned responses have been postulated to play a role in drug tolerance and sensitization (Siegel, 1975, 1977b), in drug abuse (Lynch, Stein & Fertziger, 1976; Wikler, 1948, 1973a; Grabowski & O'Brien, Note 1), and in behavioral medicine (Woods & Kulkosky, 1976). The importance of this form of classical conditioning can be underscored when one considers that it can occur whenever drugs are chronically administered. In these circumstances the administration ritual can become a conditioned stimulus and elicit a conditioned response. However even a cursory review of literature concerned with conditioned drug effects reveals that considerable confusion exists in the interpretation of these studies. Part of the problem may well be the complexity of the drug effects and the physiological systems being manipulated, but misunderstandings about classical conditioning also abound. An analysis of the conditioning of drug effects will therefore be presented in an attempt to permit a re-evaluation of research in the area, and to provide a framework for the experiments of the present thesis. Parts of this

analysis are based on suggestions made previously by Obal (1966) and Wikler (1973b).

Classical conditioning is among the simplest procedures studied in psychology. An animal is repeatedly presented with two successive events or stimuli normally in close temporal continuity. After repeated presentations to the animal of the two stimuli the occurrence of the first stimulus can be shown to result in an "expectancy" for the second stimulus. In most conditioning studies the second stimulus is an unconditioned stimulus, meaning that it reliably elicits a response, called the unconditioned response. When an unconditioned stimulus is used the first stimulus comes, after repeated pairing, to elicit a response similar to the unconditioned response. Under these circumstances the first stimulus is called the conditioned stimulus and the response it elicits the conditioned response. The prototypical experiment was done by Pavlov (1927) who presented first a bell and then food to a hungry dog. The food reliably elicited the response of salivation and after several pairings the bell also came to elicit salivation prior to, or in the absence of, presentation of the food.

Despite the simplicity of the conditioning procedure

there appears to be considerable confusion about the meaning of the terms unconditioned stimulus and unconditioned response. The confusion is particularly evident when drugs or hormones are used to induce physiological changes. This may be due partially to the casual use of terms, but it appears to reflect real misunderstandings about conditioning. For example, the administration of the drug is often referred to as the unconditioned stimulus. This has led to claims of backward conditioning in conditioned taste aversion (Boland, 1973; Domjan & Gregg, 1977). The backward conditioning procedure is to present the unconditioned stimulus followed by the conditioning stimulus and is in general considered to be an ineffective procedure for conditioning (Mackintosh, 1974). In the conditioned taste aversion studies, however, although the drug administration preceded the taste stimulus, the illness induced by the drug occurred after the conditioning, or taste, stimulus, making the relation between the events one that is normally found to be effective. The point is that the illness produced by the drug should be considered the unconditioned stimulus.

Very often in conditioning studies using drugs every physiological change produced by the drug has been considered to be the unconditioned response. In a review of

conditioning of changes in blood glucose levels Woods and Kulkosky (1976) refer to glucose administration as the unconditioned stimulus and the subsequent rise in blood glucose as the unconditioned response. They are, in effect, using these two terms to refer to the same phenomenon. Siegel (1975, 1977b) has suggested that in some cases, the response that becomes conditioned is one that acts to oppose the unconditioned response. He calls these compensatory conditioned responses. These responses would prove difficult to reconcile with a conditioning model that predicts that the conditioned response would be similar to the unconditioned response. It is apparent that the terms used in conditioning must be more precisely defined if contradictions and problems of this type are to be avoided.

In the study of conditioning and learning a stimulus has traditionally been defined as some physical event that results in neuronal consequences. For environmental or external stimuli this implies the existence of some type of receptor that registers the physical change and then transmits this information to the nervous system. A change in the environment, no matter how drastic, for which the animal has no such receptor cannot serve as a stimulus. In a similar manner changes in the internal

environment can be called stimuli if they can be shown to produce changes in the nervous system. Responses have, when considering conditioning and learning, been viewed as being some neurally induced change in non-neural tissue. For example, skeletal responses result from neurally induced muscle contractions. Clearly what is crucial in this definition is that the response be the outcome of neural activity. The emphasis on the neural component in these definitions is due to the fact that learning and conditioning are activities of the central nervous system. Stimuli can be viewed as inputs to, and responses as outputs from, the central nervous system.

The definition of a stimulus and a response presented here is more restrictive than that found in biology as a whole, and it becomes important, therefore, to note which general uses of these terms are excluded by these definitions. First, all internal events and changes that do not directly involve the nervous system are excluded from these definitions. For example, a rise in blood glucose level has a direct action on the pancreas causing the secretion of insulin (Gerich, Charles & Grodsky, 1976). This insulin in turn directly causes body cells to increase their uptake of glucose and results in a fall in glucose levels. In terms of conditioning processes none

of these steps entails either stimuli or responses as the nervous system is not involved. Secondly, events that occur completely within the central nervous system cannot be stimuli or responses according to these definitions. Stimuli are neural reactions to non-neural events and responses are non-neuronal reactions instigated by the nervous system. This is not to suggest that stimuli or responses do not activate many neurons in the central nervous system, but merely reflects the fact that their origin or termination must be outside the nervous system. Clearly central nervous system changes are the basis for conditioning and other forms of learning. It is, however, the relationship between stimuli that is learned, and learning is normally demonstrated through changes in responding.

There remains one class of events that has not been explicitly included in or excluded from these two definitions; experimenter-induced manipulations that act at the level of the central nervous system, such as administration of centrally acting drugs. Many of these drugs act by mimicking or interfering with the actions of neurotransmitters at particular synapses. Technically these manipulations can be viewed as inducing a neural reaction by non-neural means, and as such can be considered to be

stimuli. This may, however, be an oversimplification; for example, little would be gained by viewing the manipulation of the final set of neurons in the central nervous system before a non-neural effector organ as a stimulus or an input to the central nervous system. Resolution of this issue requires other concepts and will be returned to later.

Pavlov (1927) defined an unconditioned stimulus as one that reliably elicits a response. When studying the internal milieu it is necessary to consider the nature of the responses elicited by internal stimuli. This requires consideration of the concept of homeostasis (Cannon, 1932). It is clear that many things that physiologists measure (blood glucose levels, temperature, heart rate, to name a few) are maintained within definite limits. For example, despite exposure to a wide variety of environmental temperatures, animals maintain a stable internal temperature. This regulation implies some form of active control to maintain the internal equilibrium. Such systems are known as negative feedback systems, inasmuch as responses act to oppose the disturbance and to correct any imbalance. If, for example, the body temperature becomes elevated, the response of the negative feedback system regulating temperature acts to cool the body and to return

the temperature to normal. Obviously not all types of regulation involve the nervous system; the reaction by the pancreas to increased blood glucose mentioned above is such a regulatory mechanism acting to control glucose levels. While these non-neural regulatory mechanisms may be important they are not usually directly involved in conditioning and will not be considered further in this discussion.

A feedback system in its simplest form is shown in Figure 1 and consists of three elements: a sensor, an integrator, and an effector. Figure 2 shows an example of a system that would maintain blood glucose above a minimum level; the one shown in Figure 3 would regulate body temperature. Note that these diagrams of feedback systems make no suggestion as to how they are actually implemented in the nervous system. Further, in actual fact all feedback systems are more complex than the diagrams suggest. In many feedback systems there are several different types of sensors, several levels of integrators and multiple classes of effectors; the various systems are interrelated and any given effector may be activated by more than one system. For example, peripheral vascular tone is important both in control of blood pressure and in the regulation of body temperature. Indeed one of the major

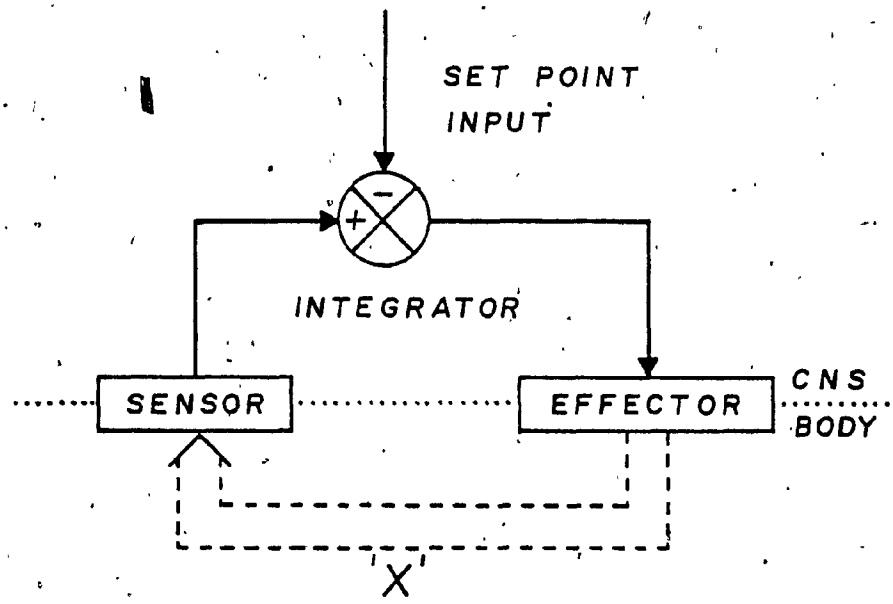

Figure 1. A schematic diagram of the elements of the feedback system that regulates 'x'. The sensor measures the level of 'x' and produces a stimulus, which goes to the integrator. The integrator compares this stimulus to a second signal, called the set point, to determine if 'x' is at the appropriate level. When 'x' is at the appropriate level the two signals are equal and the integrator does not produce any output signal. If 'x' is not at the appropriate level the integrator produces an error signal which activates the effectors. The effectors act in a manner to return 'x' to the appropriate level and thus reduce the imbalance at the integrator. Many of these feedback systems are found in the central nervous system (CNS).

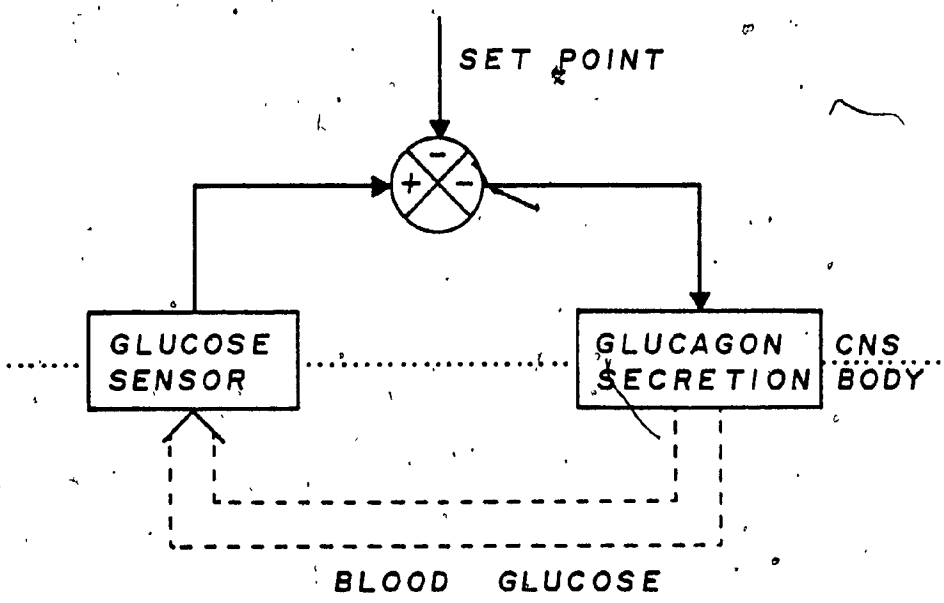

Figure 2. A feedback system that would maintain the amount of glucose in the blood above a particular level. As the glucose level falls the signal from the sensor decreases till it becomes smaller than the set point signal. The output of the integrator then becomes 'negative' and this activates the secretion of glucagon by the pancreas. Glucagon causes blood glucose levels to increase, which in turn increases the signal from the sensor. This changes the output of the integrator so it becomes zero or positive and stops the glucagon secretion. Note that this system will not correct the levels of blood glucose when they rise above the normal level.

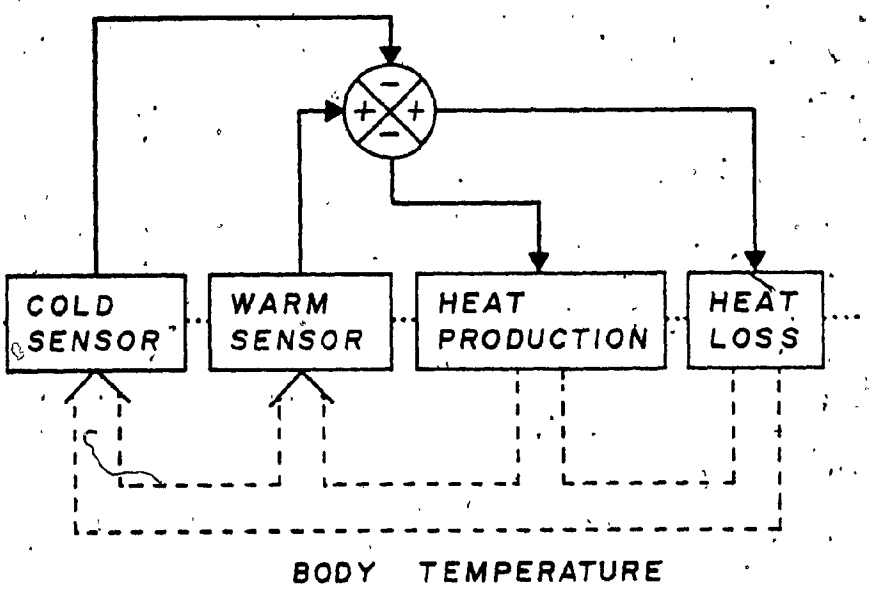

Figure 3. A feedback system that would regulate the body's temperature. If the body is at the normal temperature the inputs of the cold and warm sensors to the integrator will balance and no effectors will be activated. If the body temperature decreases the cold input will increase, and the warm input will decrease. This results in a net 'negative' output of the integrator and activates the heat production mechanisms which causes the body temperature to increase. Similarly, a hyperthermia will activate heat loss mechanisms, and return the body temperature to normal. Thus deviations of body temperature in either direction are corrected by this feedback system. Note that no 'set point' is required in the system.

problems in physiology is to determine the exact nature and interrelationship of these feedback systems. Satinoff (1978) has shown that despite extensive study of the thermoregulatory system the basic control mechanisms involved have still not been determined.

Traditionally feedback systems have been used in the study of regulatory mechanisms involved in such physiological systems as temperature control and nutrient and fluid balance. There is, however, no reason to limit this concept to physiological regulatory systems. Any system that involves feedback requires the three elements shown in Figure 1, whether it be to control muscular contractions or glandular secretions. Further, even systems that do not involve feedback may involve some neural integrator, if the essential feature of an integrator is that it compares or summates multiple inputs in to produce a single output. For purposes of this discussion, however, the simple model shown in Figure 1 will usually suffice to illustrate the link between the unconditioned stimulus and the unconditioned response.

As mentioned earlier, in conditioning studies using drugs, the unconditioned stimulus is usually considered to be the drug administration, while the unconditioned

response is viewed as being the observed drug effect. Drugs may, however, have effects that do not directly activate the nervous system and these, according to the present definitions, would not be considered as involving either stimuli or responses. Clearly these non-neuronal consequences of a drug should be differentiated from neurally mediated responses to the drug. Drugs can act in many different ways to produce their effects and it becomes necessary, therefore, to determine for each case the exact nature of the unconditioned stimulus and unconditioned response. Many drugs have multiple effects, each produced by a different mechanism, which must be individually analyzed. For example, morphine causes many different physiological effects, some by acting directly on peripheral organs, others through activation of central neural mechanisms. To complicate the situation further, morphine seems to have multiple central actions on some systems, such as thermoregulation, that vary with dose. Low doses of morphine act at the anterior hypothalamus changing some input to the thermoregulatory integrator causing an increase in body temperature (Clark, 1979). Large doses of morphine depress all thermoregulatory responses and consequently the body temperature becomes a function of the environmental temperature (Clark, 1979). Fortunately it appears possible to divide most drug

actions into two classes on the basis of whether their site of action is prior to or after the integrator.

Any feedback system can be viewed as a loop with the regulated measure providing both a convenient beginning and end to the loop. The sensor is thus prior to or "upstream" from the integrator, and the effectors after or "downstream" from the integrator. All drug actions on any particular system involving feedback can thus be classified on the basis of whether they act prior to or after the integrator. This scheme assumes that no drug acts directly on the integrator, an unproven assumption, but one that appears to cause no serious problem.

A drug action prior to the integrator will change one or more of the inputs to the integrator. This will have an effect at the integrator similar to that produced by an actual change in the regulated measure. Drug actions prior to the integrator will effectively, at the level of the integrator, be the same as a stimulus change. Previously a stimulus has been defined as the neural consequence of some non-neuronal change. This definition can now be seen to include all drug actions prior to the integrator. Thus even drugs that act within the nervous system can still be viewed as stimuli if they act prior to the integrator.

A change in the input to the integrator will result in an error signal that will activate an effector. A drug with a stimulus action, that is, a drug that acts prior to the integrator, will therefore result in the activation of an effector. This activation of the effector will produce the observed physiological effect ascribed to the drug. Thus, in the case of drugs that act prior to the integrator, the drug can be said to be the unconditioned stimulus and the observed drug effect to be the unconditioned response. Note that this is exactly the view that most investigators have taken, but here limited to a specific class of drug actions; those acting prior to the integrator.

The other class of drug actions to be considered is that of drugs that act after the integrator. Drug actions after the integrator include actions occurring directly on effector organs themselves, and actions within the central nervous system at points after the integrator. In neither case is the observed drug effect due to a change in the input to the integrator. Because a stimulus has been defined as a change in the input to the integrator, drugs that act after the integrator cannot be viewed as stimuli.

Such drug effects result in changes in the value of the regulated measure and represent deviations from

normal. These deviations, because of the feedback loop, will in turn act as stimuli at the sensor changing the input to the integrator. This imbalance at the integrator will result in the activation of effectors to restore the equilibrium. For example, a drug that acts to directly decrease metabolism will result in a non-neurally produced decrease in body temperature. By definition, this drug effect on temperature represents a drug action after the thermoregulatory integrator. The decrease in body temperature will, however, act as a stimulus for the thermoregulatory integrator. This stimulus will result in the activation of effectors that increase heat production and act to increase the body temperature, returning it to normal. Generally if the drug acts after the integrator, the observed drug effect will be a deviation from equilibrium that the feedback system will try to counteract by activating effectors to oppose the observed drug effect.

In this situation where the observed drug effect acts as the stimulus to the integrator, the observed effect of the drug changes the input to the integrator and can thus be called the unconditioned stimulus. The effectors activated by the integrator will be the unconditioned response. Note that in this case the unconditioned response acts to oppose the observed drug effect, a

consequence of the negative nature of the feedback. In summary then, when drug effects are viewed this way, that is in relation to an integrator, any observed effect can be either the unconditioned response or the unconditioned stimulus depending on whether the drug acts prior to or after the integrator, respectively.

As the specification of the unconditioned stimulus and unconditioned response depends on where the drug acts with respect to the integrator, it becomes necessary to be able to determine this by some independent means. Fortunately there are several techniques that can be used for this purpose. For example, if the drug can be shown to be having its effect without involving the nervous system, it can be assumed to be acting directly on an effector and after the integrator. One other fairly general method often used in the study of thermoregulation is to determine whether all or most of the effectors act together to induce the observed drug action. If all the effectors work together to induce the change, it can be concluded that the drug acts prior to the integrator because only the integrator would be able to activate all the effectors in concert. In contrast, if some of the effectors act to produce the effect and others to oppose the effect, then it can be assumed that the drug is acting

after the integrator. If the drug acts after the integrator it would act on only a few effectors and as the observed drug effect becomes a stimulus for the integrator this would result in responses by the other effectors that oppose the drug action. Thus it is possible to determine where, relative to the integrator, the drug acts by simultaneously looking at the action of several individual effectors involved in the feedback loop.

The reason that this method of looking at the action of several effector systems is often used to study drugs that affect thermoregulation, is that animals use behavioral as well as physiological means to regulate body temperature. Thus the behavior of the animals, under the appropriate circumstances, will reflect an output of the thermoregulatory integrator. The actual body temperature of the animal, in a thermoneutral environment, will reflect the activity of the physiological effectors. Thus here are two classes of temperature effectors that can be compared in order to determine where in relation to the integrator any given drug is acting. If the activity of the physiological effectors and the behavioral responses promote the same goal, then the drug is assumed to be acting prior to the integrator. If, however, the behavior of the animal acts to counteract the observed temperature

change, then the drug is assumed to be acting after the integrator. For example, morphine, in small doses, causes an increase in body temperature suggesting that physiological heat production effectors are being activated. The behavior of these animals also indicates that heat seeking mechanisms are being activated; animals receiving morphine stay under a heat lamp longer than those receiving saline (Cox, Ary, Chesarek & Lomax, 1976). Thus with morphine the physiological and behavioral mechanisms reinforce each other suggesting that this drug acts prior to the integrator. On the other hand, animals injected with N-methyldiphenhydramine exhibit a fall in body temperature, but stay under a heat lamp longer than saline injected animals (Cox, Green & Lomax, 1975). Thus the effect of the drug on body temperature and on thermoregulatory behavior oppose each other, suggesting that this drug acts after the integrator. This conclusion is consistent with the known peripheral site of action of N-methyldiphenhydramine.

Given that there exists a method of determining whether drugs act prior to or after the integrator, it then becomes possible to determine whether the drug itself or its observed effect is the unconditioned stimulus. Because the nature of the unconditioned stimulus and unconditioned response can be determined for any drug

action, it becomes possible to predict the form of the conditioned response. For drugs that act prior to the integrator the observed drug effect is the unconditioned response and it is predicted, therefore, that the conditioned response would be similar to this observed drug effect. In the case of drugs that act after the integrator, where the unconditioned response opposed the direct drug effect, the conditioned response would also be expected to oppose the observed drug effect. It has been noted that there are situations in which the conditioned response does oppose the observed drug effect. This has been labeled paradoxical conditioning (Finch, 1938a; Korol, 1973; Lang, Brown, Gershon & Korol, 1966). Clearly this "paradox' is due to the incorrect application of the term 'unconditioned response' and not to the existence of a different type of conditioning.

Siegel (1975, 1976, 1977b) in a series of conditioning studies using drugs has suggested that at least some cases of drug tolerance may be due to classical conditioning. Siegel starts with the finding that the conditioned response often opposes the observed drug effect. He suggests that if both the conditioned response and drug action occur together the net result will be a greatly reduced drug effect. As a conditioned response

develops gradually over trials, the observed result will be a gradually decreasing drug effect, which is by definition tolerance. In pharmacological studies of the repeated injection of drugs, the injection ritual reliably predicts the occurrence of drug, and as such acts as a conditioning stimulus. Thus conditioning can contribute to tolerance development even in experiments not explicitly studying conditioning.

In many ways Siegel's theory of drug tolerance is consistent with the present analysis, and indeed can be considered complementary to it. Siegel provides no explanation as to why some conditioned responses are similar to while others are different from the observed drug effect. The distinction made in the present analysis between drugs that act prior to and those that act after the integrator provides an explanation for this difference. Note that Siegel labels all drug induced changes as unconditioned responses to the drug and thus for him the conditioned response is sometimes similar to and sometimes different from the unconditioned response. According to the present analysis, if care is taken to determine the real unconditioned stimulus produced when a drug is administered, the conditioned response should be similar to the unconditioned response. Siegel's

explanation of drug tolerance should apply therefore only to drugs that act after the integrator, because only in these cases do the conditioned responses oppose the observed drug effects. A drug that acts prior to the integrator produces a conditioned response that mimics the observed effect of the drug and therefore should show no tolerance due to conditioning. If tolerance occurs with a drug that acts prior to the integrator it must, according to the present analysis, be of nonconditioning origin.

Siegel (1977a) also suggests that drug sensitization, that is increased responsiveness to a drug over repeated injections, may be due to the influence of conditioned responses. He suggests that if the conditioned response mimics the unconditioned response these two responses would summate to produce a greater observed effect. According to the present analysis this would happen only if the drug acts prior to the integrator. The conditioned response may therefore result in an apparent sensitization or tolerance depending on whether the drug acts prior to or after the integrator to induce its effect.

There are also many cases in which tolerance or sensitization can occur for reasons other than conditioning (Kalant, LeBlanc & Gibbins, 1971). It is therefore

necessary to consider the implications for a conditioning study of a drug effect that shows tolerance due to other, nonconditioning, mechanisms. If tolerance occurs extremely rapidly it is doubtful that conditioning would occur at all, because the unconditioned stimulus and response would soon become negligible. If, however, tolerance occurred slowly or were incomplete, it might still be possible to obtain conditioning. If the drug acts prior to the integrator and the observed drug effect is also the unconditioned response, the conditioned response will add to the magnitude of the observed effect. If, however, tolerance is developing over trials, while at the same time the conditioned response is getting stronger, the apparent result will be to slow down the rate of or to prevent the occurrence of tolerance to the drug. In contrast, if the conditioned response opposes the drug effect, that is if the drug acts after the integrator, it will increase the apparent development of tolerance to the drug. With drug sensitization the effects will be reversed; actions of drugs prior to the integrator will show increased amounts of sensitization because of conditioning, while for drugs that act after the integrator sensitization will be reduced by conditioning. Thus conditioned effects will either increase or decrease the development of tolerance or sensitization depending on

where, relative to the integrator, the drug has its effect.

Experimental evidence

Keeping this analysis of conditioning drug effects in mind, let us now examine and evaluate the experimental evidence. Conditioning studies using drugs to induce the unconditioned stimulus started, just as did the more traditional conditioning studies, by using the production of salivation as the unconditioned response. Pavlov (1927) was one of the first to report, in an experiment done by Krylov, one of his associates, the use of morphine as an unconditioned stimulus to elicit salivation in dogs. Krylov found that after five or six daily injections of morphine the events leading up to the injection were able to elicit the responses produced by morphine. Pavlov reported that the dog would salivate profusely and vomit prior to, or in the absence of, morphine administration. This method of eliciting conditioned salivation was soon studied by many other researchers (Collins & Tatum, 1925; Crisler, 1928, 1930; Gulliksen, 1931; Kleitman, 1929; Kleitman & Crisler, 1927). Their studies showed that conditioned salivation was readily obtained using morphine, and was similar to the more usual forms of conditioning using food or dilute acid in terms of acquisition and extinction.

Two other drugs were used in this period to study salivary conditioning, pilocarpine and atropine. Both drugs have direct effects on the salivary glands; pilocarpine elicits salivation, atropine blocks it. In contrast to morphine which induces salivation by a central stimulus action, these two drugs have a direct effect on the salivary gland and can be said to act after the integrator that controls salivation. Pilocarpine has been shown in three studies not to support conditioning (Crisler, 1930; Kleitman, 1927; Mulinos & Lieb, 1929). In contrast Finch (1938b) reported some minor success in eliciting conditioned salivation using pilocarpine. The small magnitude of his conditioned response (0.2 ml) and the fact that, as in most of these early studies, there were no controls for sensitization or pseudoconditioning, suggests that his results must be viewed with caution. Therefore, despite the ability of pilocarpine to produce large amounts of salivation, it appears to be difficult to obtain conditioned salivation to a stimulus previously paired with pilocarpine. In contrast atropine, which blocks salivation, appears able to support conditioning. A conditioned stimulus presented prior to atropine produces increasing amounts of salivation with repeated pairings (Finch, 1938a; Korol, 1973; Korol, Sletten & Brown, 1966; Lang, Brown, Gershon & Korol, 1966; Lang, Rush & Pearson, 1969;

Mulinos & Lieb, 1929; Wikler, 1948). The only reported failure to obtain conditioned salivation using atropine was by Crisler (1930), but the dose of atropine he employed may have been too small to support conditioning. Thus we have here an example of two drugs, both of which have direct effects on the salivary glands, but only one of which supports conditioning. Pilocarpine which induces salivation does not, whereas atropine which blocks it results in a conditioned response but one that is opposite to the observed drug effect.

Salivation suffers from one drawback as a model for this analysis of conditioning prescribed here in that it is largely a unidirectional response. While it is easy to get an increase in salivation above the baseline, this baseline appears to be so low that it would be hard to observe a decrease in salivation. In six dogs studied by Finch (1938a, 1938b), using equipment accurate to .01 ml saliva, the baseline salivation never exceeded .1 ml over 15 minutes and was usually below .05 ml, making it difficult to observe any decreases from the baseline. This finding suggests that the reason pilocarpine does not appear to support conditioning is that the conditioned response, which according to the present analysis should be a decrease in salivation, would be difficult to observe.

A test for conditioned inhibition would resolve this issue but has not been done. Because the effect of atropine is to block salivation, the conditioned response would be, as observed, salivation. Thus the difference between atropine and pilocarpine may not be in their ability to support conditioning, but rather in the observability of the conditioned response. A similar phenomenon may explain the negative results seen by Katzenelbogen, Loucks and Gantt (1939) who attempted to condition gastric secretions using histamine. Because histamine acts directly on the stomach lining to cause gastric secretions, the expected conditioned response, according to the present analysis, would be a suppression of gastric secretion. In the experiment, however, there were no measurable secretions from the Pavlovian pouch during the fifteen minute pre-experiment baseline. Thus it is not surprising that there was no evidence for conditioning in this study.

Various drugs that affect the cardiovascular system have been used in attempts to condition heart rate changes. Bykov (1957), reporting on a series of studies carried out in his laboratory, stated that morphine, nitroglycerin, strophanthin, epinephrine, and acetylcholine all resulted in conditioned responses similar to the drug

effect. The fact that some of these drugs act prior to and others after the integrator controlling heart rate appears to have made no difference in the direction of the conditioned response; in both cases the conditioned response was similar to the unconditioned response. While at first glance these findings appear to create problems for the present analysis, they have not been replicated by other investigations (see below); in fact Gantt (1972) has suggested that as Bykov's finding consisted of selected protocols they should be viewed with caution.

Two conditioning studies have been reported using epinephrine, a drug that directly increases the heart rate by acting on heart muscle (Russek & Pina, 1962; Subkov & Zilov, 1937). Both studies reported a conditioned decrease in heart rate, a result consistent with the present analysis, but contradictory to Bykov's (1957) findings. On the other hand, Mackenzie and Gantt (1950) and Rush, Pearson and Lang (1970) report that atropine, which also increases the heart rate directly, does not support conditioning. Examination of their results, however, suggests, as predicted by the present analysis, that in both cases there is a small decrease in heart rate in response to the conditioned stimulus. A similar failure to condition heart rate changes using acetylcholine, which has the direct effect of increasing the heart

rate, is reported by Teitelbaum, Gantt and Stone (1956). Again there are suggestions of a conditioned response which opposed the observed drug effect as the authors report "the heart rate during the CS was slower than or equal to that of the control rate during the 10 seconds preceding the CS" (p. 486). It appears, therefore, that these studies involving drugs with direct effects on the heart, contradict Bykov's (1957) findings and either support, or, at least, do not contradict, the present analysis.

When one examines the conditioned heart rate studies using drugs that act prior to the integrator it appears that their results are consistent with the present analysis; in these cases the conditioned responses are similar to the unconditioned responses elicited by the drugs. Perez-Cruet and Gantt (1964) report that bulbocarpine, a drug that acts centrally to increase the heart rate, results in conditioned increases in heart rate. Small doses of morphine act centrally to increase heart rate and again the conditioned response elicited is an increased heart rate (Rush et al, 1970).

When larger doses of morphine are used for conditioning the conditioned response is still an increase in heart

rate, despite the fact that a large dose of morphine results in a decreased heart rate (Rush et al, 1970). Large doses of morphine, however, are reported to have a non-specific depressant effect on the animal (Clark, 1979; Domino, Vasko & Wilson, 1976; Seevers & Deneau, 1963). Rush et al (1970) report that at the larger of two doses of morphine, dogs became sedated, drowsy, and hung limply in their supporting straps. This suggests that large doses or morphine may act to depress effector mechanisms, an action that would occur after the integrator. Thus, with large doses or morphine the expected conditioned response would be opposite to the observed drug effect as was reported by Rush et al (1970).

When large doses of morphine are administered a second process may also be active in producing the observed conditioned increase in heart rate. There have been several studies suggesting that the occurrence of the unconditioned response is not necessary for conditioning (Crisler, 1930; Finch, 1938a; Light & Gantt, 1936). Thus the stimulus action of morphine on heart rate may still be able to support conditioning despite the blockage of response production by the depressant effects of large doses of morphine. The recent discovery that morphine acts on several different opiate receptors, each of which,

produces different effects (Gilbert & Martin, 1976; Lord, Waterfield, Hughes & Kosterlitz, 1977; Martin, Eades, Thompson, Huppler & Gilbert, 1976) lends support to this possibility. As small doses of morphine have a stimulus action producing an increase in heart rate the conditioned response expected would be an increase in heart rate. Either of these mechanisms could explain why in the Rush et al (1970) study the conditioned response was an increase in heart rate, even though in large doses morphine produced a decreased heart rate.

Finally there is one study of conditioning of the cardiovascular system in which glyceryl trinitrate and phentolamine, both of which act directly on the vascular system to decrease the blood pressure, were used (Lang, Ross & Grover, 1967). This decrease in blood pressure acts as an unconditioned stimulus causing an increase in heart rate. After repeated pairings the conditioned stimulus, as expected, came to elicit an increase in heart rate. In general it appears that in studies in which attempts were made to condition the cardiovascular system, the results support the view presented here that the nature of the conditioned response is determined by whether the drug acts prior to or after the integrator.

Woods and Kulkosky (1976) have presented an extensive review of experiments in which classically conditioned changes in blood glucose levels were studied. While it is the opinion of the present author that they too make the same terminological confusions that others have made, their summary of the literature fits well into the present analysis. Drugs that act non-neuronally to directly effect blood glucose levels, that is drugs that act after the integrator, result in conditioned responses that oppose the observed drug effect. Manipulations that result in elevated blood glucose, such as glucose, epinephrine, or glucagon injections, result in a conditioned fall in blood glucose levels; in contrast, manipulations that decrease blood glucose levels, for example physiological doses of insulin which acts directly on cellular uptake mechanisms, result in a conditioned increase in blood glucose levels.

Woods and Kulkosky (1976) report one exception to this analysis of their results, but also propose a mechanism to explain this anomaly that is consistent with the present analysis. They report that there are a large number of studies showing that if high, coma-inducing, doses of insulin are used in conditioning, the conditioned response observed is a decrease in blood glucose (see

Woods & Kulkosky, 1976). It would appear, therefore, that in this case the conditioned response to a drug that acts after the integrator is similar to its observed effect. This would of course be counter productive for the animal, in that it would potentiate an already dangerous situation. Woods and Kulkosky suggested in 1976, however, that when these high, unphysiological doses of insulin were injected some of the insulin might have leaked into the cerebrospinal fluid. They postulated, and it has since been confirmed (Havrankova, Roth & Brownstein, 1978), that there were insulin sensitive neural cells near the ventricles. These cells act as sensors that provide stimuli within the central nervous system to regulate glucose levels. Insulin leaking into the cerebrospinal fluid might increase the uptake of glucose by these cells and would thereby create a false stimulus indicative of elevated glucose levels, whereas in reality body glucose levels were decreasing. The unconditioned response to this apparent elevation of blood glucose levels would be to release insulin, and consequently the conditioned response would also result in decreased blood glucose. This suggested account of these apparently contradictory findings is in accord with the present analysis.

Siegel (1975, 1976, 1977b) has suggested that conditioning factors may account for the tolerance to the

analgesic effect of morphine. He suggests that a compensatory hyperalgesic response is conditioned to the environment in which morphine is administered which counteracts the analgesic action of the morphine injection. Siegel (1975, 1976) showed that the analgesic action of morphine could be re-established if the morphine was presented in a novel place. He reasoned that, in the new environment, the conditioned response would not occur and therefore would not counteract the analgesic effect of morphine to provide evidence for tolerance. Furthermore, he reported that saline injections, administered instead of the expected morphine, resulted in a hyperalgesic response (Siegel, 1975). Manipulations that produce changes in conditioning, i.e. extinction, pre-exposure to the conditioning stimulus, partial reinforcement, all had the expected effects on tolerance development (Siegel, 1975, 1977b). Finally, Siegel, Hinson and Krank (1978) showed that an arbitrary stimulus, a noise light complex, repeatedly paired with a morphine injection could elicit analgesic tolerance even when animals were tested for analgesia at the end of the experiment. Despite the alternative accounts and the evidence to the contrary offered by Bardo and Hughes (1979) and Sherman (1979), Siegel has presented considerable evidence that a conditioned hyperalgesic response contributes to the tolerance of the analgesic effect of morphine.

Because, however, morphine analgesia represents by definition at least, a decrease in pain sensitivity it would appear to be an effect of morphine that occurs prior to the integrator of a pain regulatory system. Siegel's findings of a compensatory conditioned hyperalgesic would appear, therefore, to be inconsistent with the present analysis. A drug that acts prior to the integrator should produce a conditioned response that is similar to the observed drug effect. The pain regulatory system is a complex one and appears to involve an endogenous pain suppression mechanism that is brought into action as a response to a painful event (Basbaum & Fields, 1978). Morphine injected into the body could act at the same receptor sites as the endogenous opioid released in response to pain, that is, after the integrator. If at least part of morphine's analgesic action mimicks a post-integrator response a conditioned hyperalgesia in response to morphine would be predicted by the present analysis. Another possible explanation of Siegel's results may lie in the fact that under certain circumstances morphine has been shown to cause hyperalgesia (Jacquet & Lajtha, 1973; Kayan, Woods & Mitchell, 1971). In view of this finding it is interesting to note that in one experiment by Siegel (1976) animals treated with morphine had shorter latencies on the hot plate on the last six out of eight trials, than animals receiving saline and tested on the hot plate.

That is, even under the influence of morphine they appeared to be hyperalgesic. If such hyperalgesia represents a direct stimulus action of morphine then the expected conditioned response would also be hyperalgesia. Either or both of these suggested mechanisms would predict, in a manner consistent with the present analysis, conditioned hyperalgesia.

Several studies have been carried out in an attempt to condition the withdrawal symptoms of morphine that result when the drug is abruptly withdrawn following chronic administration in dependent animals. Because the constellation of physiological symptoms known as the withdrawal syndrome has been thought to play an important role in the maintenance of drug self-administration, a number of workers have been concerned with the conditionability of the syndrome (Lynch et al, 1976; Grabowski & O'Brien, Note 1). If the termination of morphine administration results in withdrawal, the animal is said to have been dependent on morphine. Withdrawal can be elicited by terminating morphine administration; under these conditions it is said to begin within 8 to 16 hours of the last injection and to last several days (Martin, Wikler, Eades & Pescor, 1963). Withdrawal symptoms can also be precipitated by administration of an opiate antagonist such as naloxone or

nalorphine; under these conditions the onset occurs within minutes (Blasig, Herz, Reinhold & Zieglgansberger, 1973; Martin, 1967).

The first experiment showing that withdrawal symptoms could be conditioned was done by Irwin and Seevers (1956). Rhesus monkeys dependent on various opioids received repeated presentation of a conditioned stimulus paired with a nalorphine injection that induced withdrawal symptoms. Long after morphine termination, presentation of the conditioned stimulus alone was able to elicit withdrawal-like symptoms. Similar conditioned withdrawal-like effects were reported by Goldberg and Schuster (1970) in post-abstinent monkeys. These conditioned responses included suppression of bar pressing for food, vomiting, and salivation, and could be elicited for several months post-abstinence until extinction finally occurred. Wikler and Pescor (1967, 1970) using 24-hour withdrawal showed that "wet-dog shakes", a withdrawal behavior in rats, could be elicited by the conditioned environmental stimuli two months after morphine termination. Recent work using naloxone to induce withdrawal symptoms in humans undergoing a methadone maintenance program shows that similar conditioned withdrawal changes can be elicited in humans (O'Brien, 1976; O'Brien, Testa, O'Brien, Brady & Wells,

1977). It thus appears that stimuli repeatedly paired with the occurrence of withdrawal are able to elicit withdrawal-like symptoms long after the termination of morphine administration. This suggests that withdrawal symptoms are due to an action that occurs prior to the integrator for these symptoms and is consistent with the view that withdrawal is due to an action at the opiate receptor prior to the integrator.

Drugs that affect the thermoregulatory system have been used in a number of conditioning studies. For several reasons the thermoregulatory system is a good one in which to study the conditioned effects of drugs; body temperature is easily measured, it can be obtained repeatedly without any surgical intervention, it shows both increases and decreases in response to drug action, and it is possible using techniques discussed earlier to determine if a drug acts prior to or after the integrator in the thermoregulatory system. While there have been a few studies using other drugs most studies have used morphine and it therefore seems appropriate to determine first what is known about morphine effects on body temperature. It will then be possible to evaluate in some detail the studies on conditioned temperature effects of morphine, and later to apply the present analysis of conditioning to the experiments of this thesis.

Morphine and body temperature

The effects of morphine on body temperature are complex and depend on such variables as dose administered, species studied, route of administration, degree of restraint, drug history, and environment conditions (Clark, 1979). The effects of morphine and naloxone on body temperature suggest a role for the endogenous opioid peptides in thermoregulation (Stewart & Eikelboom, 1979). The effects of morphine on the thermoregulatory system can best be described under four headings; the initial or acute effects, the chronic effects, the effects of terminating morphine administration, and the role of endogenous opioid peptides in thermoregulation. Because the effects of morphine may vary somewhat from species to species the present discussion will be limited for the most part to studies using rats.

The acute effects of morphine on body temperature are dose related; low doses produce hyperthermia while higher doses result in a biphasic response, hypothermia followed by hyperthermia (Gunne, 1960; Herrmann, 1942, Lotti, 1973; Oka, Nozaki & Hosoya, 1972). Microinjections of morphine directly into brain tissue cause essentially the same dose related changes in body temperature as do systemic injections, suggesting that the temperature effects of morphine

are brought about by the direct action of morphine on the central nervous system. Lotti, Lomax and George (1965a, b) showed that administration of 50 ug of morphine sulfate into the anterior hypothalamus resulted in a marked hypothermia. A smaller dose, 4 ug, of morphine injected into the same site induced a rise in body temperature (Cox, Ary, Chesarek & Lomax, 1976). Systemic administration of N-methyl-morphine, a drug that does not cross the blood brain barrier, has no effect on body temperature; but its intrahypothalamic administration results in hypothermia (Foster, Jenden & Lomax, 1967). Similarly, central administration of the opiate antagonist nalorphine is able to to block the hypothermic effect of an intravenous injection of morphine.

In order to determine the eventual conditioned temperature response elicited by morphine, it is necessary to determine whether it produces its effect by acting prior to or after the integrator. If the drug depresses the effectors of the thermoregulatory system, the animal will no longer be able to regulate its temperature. Environmental conditions will then influence body temperature; hypothermia will result in a cold environment and hyperthermia in a warm environment. This is what happens following administration of large doses of morphine in

mice and rats (Cochin, Rosow & Miller, 1978; Herrmann, 1941; Oka, 1977; Paolino & Bernard, 1968) suggesting that large doses of morphine depress or incapacitate the thermoregulatory system (Clark, 1979). This is therefore an extreme case of a drug action after the integrator acting on all effectors.

The hyperthermic effect of small doses of morphine, in contrast, appears to be independent of environmental temperature (Rudy & Yaksh, 1977). As discussed earlier, thermoregulatory behavior studies suggest that morphine in small doses acts prior to the integrator (Cox, Ary, Chesarek & Lomax, 1976). In the past it has been suggested that morphine hyperthermia may be a function of its activity increasing effects (Lotti, 1973), but the behavioral studies make this unlikely (Cox, Ary, Chesarek & Lomax, 1976). Also the hyperthermia can occur without an increase in activity (Rudy & Yaksh, 1977). Thus the hyperthermia appears to be due to a direct temperature effect of morphine due to an action prior to the thermoregulatory integrator.

With chronic administration the initial effects of morphine change. Gunne (1960) reported that animals rapidly become tolerant to the hypothermia produced by

large doses of morphine; after two or three injections morphine results in a marked hyperthermia. This finding has since been replicated many times using both a peripheral route of administration (Fernandes, Kluwe & Coper, 1977; Goldberg, 1972; Martin, Pryzbylik & Spector, 1977; Martin et al, 1963; Mucha, Kalant & Linseman, 1979; Rosenfeld & Burks, 1977), and with administration of morphine directly into the anterior hypothalamus (Lotti et al, 1965b). In contrast, tolerance does not appear to develop to the hyperthermic effects produced by morphine; the peak of the hyperthermic response does, however, occur earlier in time after the injection when animals are injected repeatedly (Chodera, 1966; Goldberg, 1972; Gunne, 1960; Lal, Miksic & Drawbaugh, 1978, Martin et al, 1977; Martin et al, 1963; Maynert & Klingman, 1962, Mucha et al, 1979; Oka et al, 1972; Rosenfeld & Burks, 1977; Sherman, 1979; Thornhill, Hirst & Gowdey, 1978). Thus it appears that irrespective of the initial or acute effects of a given dose of morphine, after chronic administration, the reponse is hyperthermia. Fernandes et al (1977) report a slight tolerance to the hyperthermic effect but this may also reflect the changing time course of morphine effects mentioned above. In contrast to these studies reporting little or no tolerance, Siegel (1978) has reported complete tolerance of the hyperthermic response to a 5 mg/kg

subcutaneous injection of morphine over a series of ten injections. In only one other study is a brief statement made to the effect that complete tolerance occurred to the hyperthermia effect of morphine (Rudy & Yaksh, 1977). At present there is no explanation for those discrepant findings.

When morphine is administered chronically by means of an implanted pellet that maintains high blood levels of morphine for at least 100 hours (Berkowitz, Cerreta & Spector, 1974), it has been noted that after 50 hours animals are no longer hyperthermic. This finding led Ary and Lomax (1979) to conclude that in this preparation tolerance of morphine hyperthermia does occur. These same workers, however, observed that seventy-two hours after pellet implantation the animals remained under a heat lamp twice as long as untreated animals causing their body temperature to rise far above normal (Ary & Lomax, 1979). This latter finding suggests that the disappearance of the hyperthermia is due to a failure of the mechanisms required to maintain the elevated temperature while at the same time morphine continues to be effective prior to the integrator.

When the chronic administration of large doses of morphine to rats is terminated, hypothermia is evident

for several days (Martin et al, 1963; Roffman, Reddy & Lal, 1973). Similarly, withdrawal hypothermia can be elicited by administration of naloxone to animals implanted with morphine pellets (Ary & Lomax, 1979). The degree of hypothermia appears to be a function both of the dose of morphine chronically administered (Mucha et al, 1978) and the dose of naloxone used to induced withdrawal (Ary & Lomax, 1979). Martin et al (1963) reported that the hypothermia seen initially after morphine termination was followed by a second phase, a slight hyperthermia that lasted several months.

Work with the endogenous opioid peptides suggests that their initial or acute effects on body temperature are similar to the acute effects of morphine; low doses result in a hyperthermia and larger doses produce a hypothermia (Blasig, Bauerle & Herz, 1979; Ferri, Arrigo, Reina, Santagostino, Scoto & Spadaro, 1978; Huidobro-Toro & Way, 1979). This dose-related pattern has been reported using intraventricular administration of beta-endorphin in mice (Huidobro-Toro & Way, 1979), and with rats (Blasig et al, 1979; Ferri et al, 1978). Systemic administration of FK 33-824, a synthetic opioid peptide that is not readily degraded, also results in these dose-related temperature changes (Blasig et al, 1979). As does morphine, the

spinal administration of beta-endorphin results in hyperthermia (Martin & Bacino, 1979). Both the hypothermia and the hyperthermia produced by beta-endorphin are naloxone reversible, but larger doses of naloxone are necessary to block the hyperthermia (Blasig et al, 1979; Ferri et al, 1978; Huidobro-Toro & Way, 1979; Martin & Bacino, 1979). The hyperthermic action of beta-endorphin, like morphine, is independent of environmental temperature (Huidobro-Toro & Way, 1979) suggesting it reflects an action prior to the thermoregulatory integrator. Finally, cross tolerance between temperature effects of morphine and the opioid peptides has been found (Blasig et al, 1979; Ferri et al, 1978; Huidobro-Toro & Way, 1979). It appears that the actions of morphine on body temperature can be mimicked by the opioid peptides, suggesting that they act through common mechanisms.

The effects of morphine and the opioid peptides on body temperature has led to the speculation that endogenous opioid peptides may play a role in thermoregulation. If the opioid peptides are involved in tonic thermoregulation it would be expected that blockade of the opiate receptor by naloxone would result in some temperature change in opiate-naive animals. Early reports, however, indicated that administration of the specific opiate antagonists naloxone or naltrexone resulted in little or no

change in body temperature (Ferri et al, 1978; Goldstein & Lowery, 1975; Lal, Miksic & Smith, 1976; Rudy & Yaksh, 1977; but see Cowan & MacFarlane, 1976). This led many to conclude that opioid peptides did not play an important role in tonic thermoregulation. In contrast Stewart & Eikelboom (1979) reported that in unstressed animals, naloxone induced a dose-related hypothermia. In animals that had been stressed, either by handling or by a noise stressor, naloxone had little effect on body temperature. They suggested that previous researchers, because they were doing acute pharmacological studies, were using animals that were stressed by the introduction of experimental procedures, and that the action of the naloxone was masked by the stress induced release of endogenous opioids. Both Blasig, Hollt, Bauerle and Herz (1978) and Stewart and Eikelboom (1979) found that if naloxone was administered prior to stressing the animal it could prevent or attenuate the stress induced rise in temperature, leading to the speculation that the stress induced hyperthermia was mediated by an opiate mechanism. Additional evidence was provided by Blasig et al (1978) who found that intraventricular administration of only the active enantiomer (-) naloxone and not the inactive (+) naloxone caused hypothermia providing evidence that naloxone acts by competitive blockade at a central opiate receptor. It

therefore appears that the opioid peptides may play a role both in tonic thermoregulation and in stress induced temperature changes.

Conditioned temperature changes

Before reviewing the studies on the conditioning of temperature changes induced by morphine it seems appropriate to make a prediction, on the basis of the present analysis, about the nature of the conditioned temperature response that should be expected in these studies. Morphine after chronic administration produces a hyperthermic response due to its action prior to the thermoregulatory integrator. According to the present analysis morphine thus acts as an unconditioned stimulus producing a hyperthermia as the unconditioned response. Therefore the predicted conditioned response would be a conditioned hyperthermia similar to the observed effect of morphine. The hyperthermia seen initially after large doses of morphine shows tolerance and should not, therefore, be expected to result in conditioning. Because, however, the hypothermia appears to be due to a depressive effect of morphine on the thermoregulatory effectors any conditioning that did occur should also result in a conditioned hyperthermia. Note in addition that the predicted conditioned hyperthermia might act both to increase the rate of

tolerance of any morphine-induced hypothermia and to delay any tolerance of the morphine-induced hyperthermia.

In the first conditioning study of the temperature effect of morphine large dependence inducing doses of morphine were used as the unconditioned stimulus (Roffman et al, 1973). A well-lit injection environment and a one minute bell were paired with each of four daily intraperitoneal injections of morphine for all animals. The morphine dose was increased until the animals were receiving 200 mg/kg/day, a dose that was maintained for three days before the morphine injections were terminated. When the morphine injections were terminated animals not exposed to the conditioned stimuli exhibited a withdrawal hypothermia lasting approximately three days. Animals presented with the conditioned stimuli (the environment, bell and saline injection) maintained a normal temperature. The authors suggested that the conditioned stimuli were able to prevent the occurrence of the withdrawal hypothermia. It was not clear from their study, however, whether the conditioned response was the prevention of the withdrawal related hypothermia or was a conditioned hyperthermia that summated with the withdrawal hypothermia, resulting in approximately normal temperature. If the withdrawal-induced hypothermia was being prevented by

presenting the conditioned stimuli this conditioned response would only be evident for a short period after morphine termination. In contrast, a conditioned hyperthermic response should be elicitable long after morphine termination. Obviously the way to differentiate between these two hypotheses would be to test the animals after the primary withdrawal symptoms had disappeared but this was not done.

Subsequently Drawbaugh and Lal (1974) studied the interaction between the opiate antagonist naloxone and the conditioned response preventing the withdrawal hypothermia. They found that in animals made dependent in the manner described in the previous study and then withdrawn from morphine 24 hours previously, naloxone induced only a slight additional drop in temperature, relative to similarly dependent animals injected with saline. While the presentation of the conditioned stimuli resulted in a return to normal of the body temperature of animals injected with saline, the animals injected with naloxone remained hypothermic. This is similar to the finding of Tye and Iversen (1975) who reported that conditioned stimuli, previously paired with morphine injections in dependent animals, would prevent the withdrawal-induced depression of operant responding but were ineffective if

withdrawal was induced by a naloxone injection rather than by terminating morphine administration. Because naloxone blocked the conditioned response Drawbaugh and Lal (1974) concluded that the conditioned stimuli caused an increase in the body temperature of rats undergoing withdrawal by acting at the same site as morphine.

In an attempt to determine the neural substrate of this form of conditioning Drawbaugh and Lal (1976) tested a series of neurotransmitter blockers in animals exhibiting the conditioned response. They compared the effects of various blockers in animals that were undergoing withdrawal hypothermia and that were either injected with morphine or presented with the conditioned stimuli. While most blockers had the same effect on both groups of animals some blockers had different effects in animals injected with morphine than in animals presented with the conditioned stimuli. Haloperidol blocked the temperature rise induced by presentation of the conditioned stimuli to animals undergoing withdrawal but not the temperature rise induced by the unconditioned stimulus; the morphine injection. This suggested that dopamine neurons were involved only in the conditioned response. By contrast cyproheptidine, an anti-serotonergic agent, did the reverse, blocking only the unconditioned temperature response to morphine

but not the conditioned temperature response. These results suggest that there are differences in the neural mechanisms involved in the conditioned and unconditioned response.

Miksic, Smith, Numan and Lal (1975) extended these results using smaller, 20 mg/kg (route of administration unspecified), doses of morphine. They found that following conditioning the conditioned stimulus alone, a tone presented in a distinctive environment, would elicit hyperthermia, mimicking the morphine effect. In their study each animal served as its own control and the measure of hyperthermia was the change in temperature produced by presentation of the conditioned stimulus. Using this design it is not possible to determine whether the experimental group animals were hypothermic before or hyperthermic after presentation of the conditioned stimulus compared to animals in some appropriate control group. It is possible that the injections of morphine could have produced a degree of dependence in these animals and consequently resulted in a withdrawal hypothermia during tests for conditioning. Thus again in this study it is not clear if this conditioned response represents a conditioned hyperthermic response or a conditioned prevention of a withdrawal hypothermia.

Lal et al (1976) showed that an even smaller dose of morphine, 10 mg/kg, could support conditioning and produce a conditioned hyperthermia. While in this case they present both the pre-treatment and post-treatment temperature data, their description of the control group treatment is so vague that it is impossible to determine whether animals in the experimental groups were hypothermic before or hyperthermic after presentation of the conditoned stimulus. It is interesting, however, that the pre-treatment temperatures of the experimental group animals were similar to those seen in animals undergoing withdrawal hypothermia in their previous studies (Drawbaugh & Lal, 1974, 1976; Roffman et al, 1973). Just as in earlier studies using larger doses of morphine (Drawbaugh & Lal, 1974) Lal et al (1976) reported that naloxone could block the conditioned hyperthermia leading them to suggest that the conditioned response was elicited by endogenous opiate-like substances. There are two arguments that can be made against this suggestion. Because both morphine and the opioid peptides act at the same opiate receptor it might be expected that neural manipulations using various blockers should effect the conditioned and unconditioned response equally, which is not the case (see above, Drawbaugh & Lal, 1976). Secondly, their argument that the conditioned hyperthermia involves an endogenous opioid rests on the fact that while

naloxone blocked the conditioned response it had no unconditioned effect on the temperature of naive animals. Subsequent work has suggested that while naloxone has no effect on body temperature in naive animals it causes a dose-related hypothermia in well habituated animals (Stewart & Eikelboom, 1979). Thus the lack of conditioned hyperthermia, when animals are pre-treated with naloxone, may be due to the summation of the naloxone action and the conditioned response elicited through an entirely different mechanism.

In contrast to the studies of Lal and his associates who report a conditioned hyperthermia to stimuli paired with either large or small amounts of morphine, Siegel (1978) found a conditioned hypothermia to conditioned stimuli paired with small doses of morphine. Siegel compared the temperature readings of three groups of animals all of which were injected daily and placed in a distinctive environment every second day. One group of animals received ten subcutaneous injections of 5.0 mg/kg morphine always in the distinctive environment, while a second group of animals received equivalent morphine injections in the home cage. The third group of animals received saline on all occasions. Temperature measurements taken every ten minutes in the distinctive environment indicated

that tolerance to the hyperthermic effects of morphine developed in animals receiving morphine in that room. Animals from both morphine groups were given an eleventh morphine injection in the distinctive environment. While both groups had equivalent pharmacological history only the group that had been receiving morphine in the distinctive environment showed tolerance to the hyperthermic effects of morphine. When all animals were injected with saline in the distinctive environment, the animals that had been receiving morphine in the distinctive environment became hypothermic relative to the two other groups. Siegel argues that tolerance of the hyperthermic effect of morphine is due to the development of a conditioned hypothermia elicited by the distinctive environment. Additional evidence for this conditioning explanation comes from his report that extinction trials reversed the hyperthermic tolerance and that partial reinforcement delayed the acquisition of tolerance. However, as mentioned earlier, in contrast to Siegel (1978) most studies find that morphine's hyperthermic effects show little or no tolerance.

Recently Sherman (1979) attempted to replicate Siegel's (1978) findings and failed. In contrast to Siegel and in agreement with the other reports mentioned

earlier he found that the morphine-induced hyperthermia did not tolerate, but rather that there was an enhancement of the hyperthermia over several injections, probably due to the shift in the time course of this effect. Sherman (1979) also reported a conditioned hyperthermia, similar to that found by Lal and associates, that showed extinction over nonreinforced trials. At present there is no good explanation for Siegel's (1978) findings but Sherman suggests that the stress induced by Siegel's repeated temperature measurements may have interacted with the unconditioned temperature effects of morphine and resulted in a conditioned hypothermia rather than the more usual conditioned hyperthermia.

THE ORIGINAL EXPERIMENT

In 1977 in an attempt to evaluate the basis of some of these divergent results, the present author carried out an experiment designed to investigate several aspects of the conditioned temperature response to morphine under a single set of experimental conditions. Because previous studies had used only single doses of morphine, it was decided to use a wide range of doses, 5, 25 and 200 mg/kg of morphine within one experiment. In addition, in order to differentiate between any anticipatory conditioned responses and conditioned responses that might occur after the injection of morphine, distinctive environmental stimuli were reliably associated with the period prior to the injection and other environmental stimuli were associated with the period following the injection. Secondly, it seemed important, because withdrawal can result in temperature changes, to make tests for conditioning both before and after a drug-free period. This original experiment was the basis of the present author's masters thesis and has been published in full (Eikelboom & Stewart, 1979). Because subsequent work to be reported in this thesis grew out of the finding of this experiment the method and results will be presented in some detail.

Figure 4 presents a schematic summary of the design used both in the original experiment and in all others reported in this thesis. In all experiments only one drug injection, or trial, was given each day. Although the experiments lasted different numbers of days, each was divided into three distinct treatment phases. First, there was a short period of habituation to the procedures, lasting 3 to 6 days, when the conditioning stimuli were presented but when all animals were injected with saline. This was followed by a conditioning phase during which the conditioned stimuli were paired with the unconditioned stimulus induced by the drug injection. During the latter part of the conditioning phase various tests were interspaced between the conditioning trials. The nature of these tests varied with the experiment, but usually they consisted of a home-cage day when the animals were not presented with the conditioned stimuli, but remained in their home cages, and a conditioning test day when the conditioned stimuli were presented and all the animals were injected with saline. The conditioning phase of the experiment was followed by a drug-free period during which the animals remained in the home cage and the conditioned stimuli were not presented. After the drug-free period further tests for conditioning were made. Thus there were tests for conditioning both during 24 h withdrawal and after a period of abstinence.

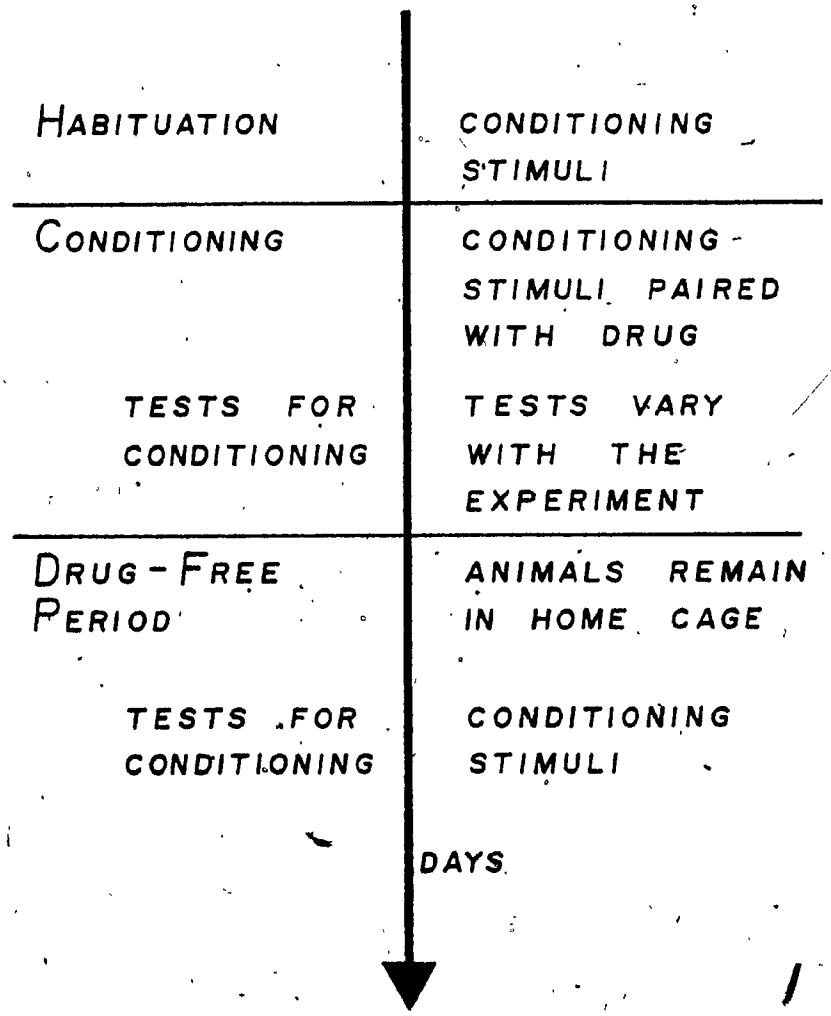

Figure 4. An overview of the design of the experiments presented in this thesis.

Figure 5 shows the daily routine for the original experiment. Although the precise daily routines for the later experiments were different, they always included temperature measurements taken both before and after the daily drug injection. In the original experiment each daily injection occurred at 12:00 h and was preceded by two hours in a distinctive, dark, quiet, pre-injection environment and was followed by three hours in a distinctive, bright, noisy, injection environment.

Two different conditioned temperature changes were observed in this study. One, a conditioned hypothermia was elicited in the pre-injection environment prior to the daily morphine injection during the conditioning period, but no longer occurred when tests were made after the drug-free period. The other, a conditioned hyperthermia that mimicked the unconditioned hyperthermia, was evident in the injection environment and manifested itself most clearly in tests made after the drug-free period.

Figure 6 shows the mean body temperature of animals at 11:00 h in either the pre-injection environment or the home cage. It was clear that animals in the morphine groups showed a dose-related hypothermia relative to animals in the saline group, and further that this effect was

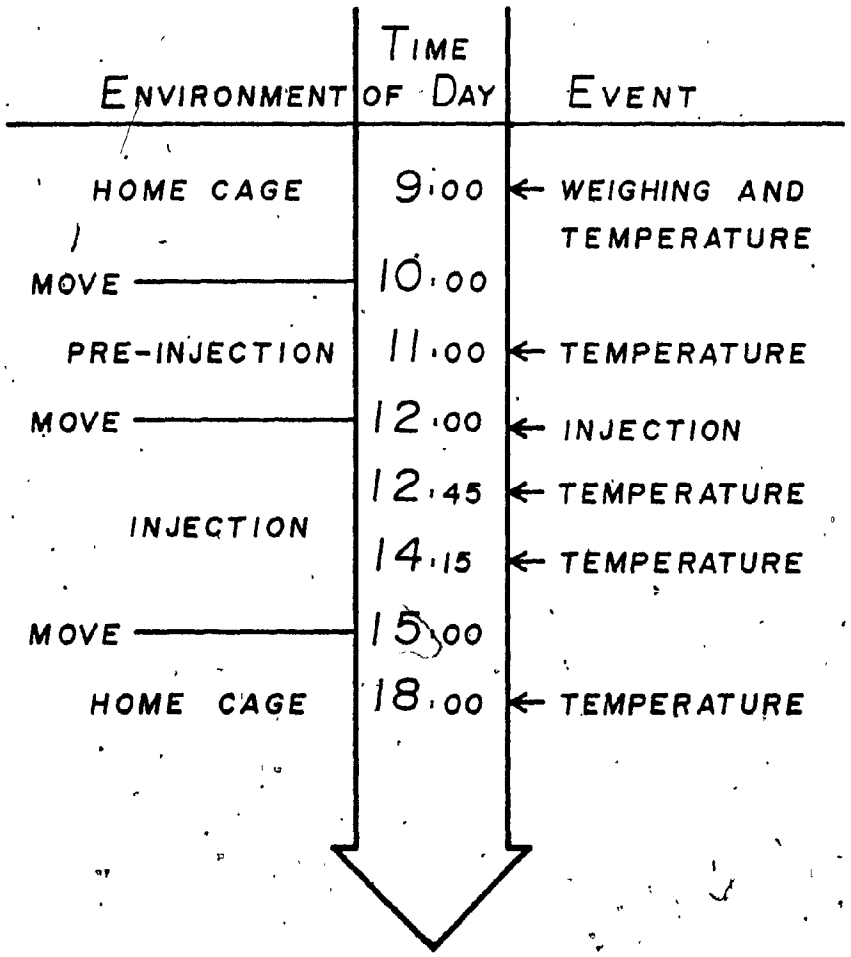

Figure 5. Daily routine followed on conditioning days in the original experiment.

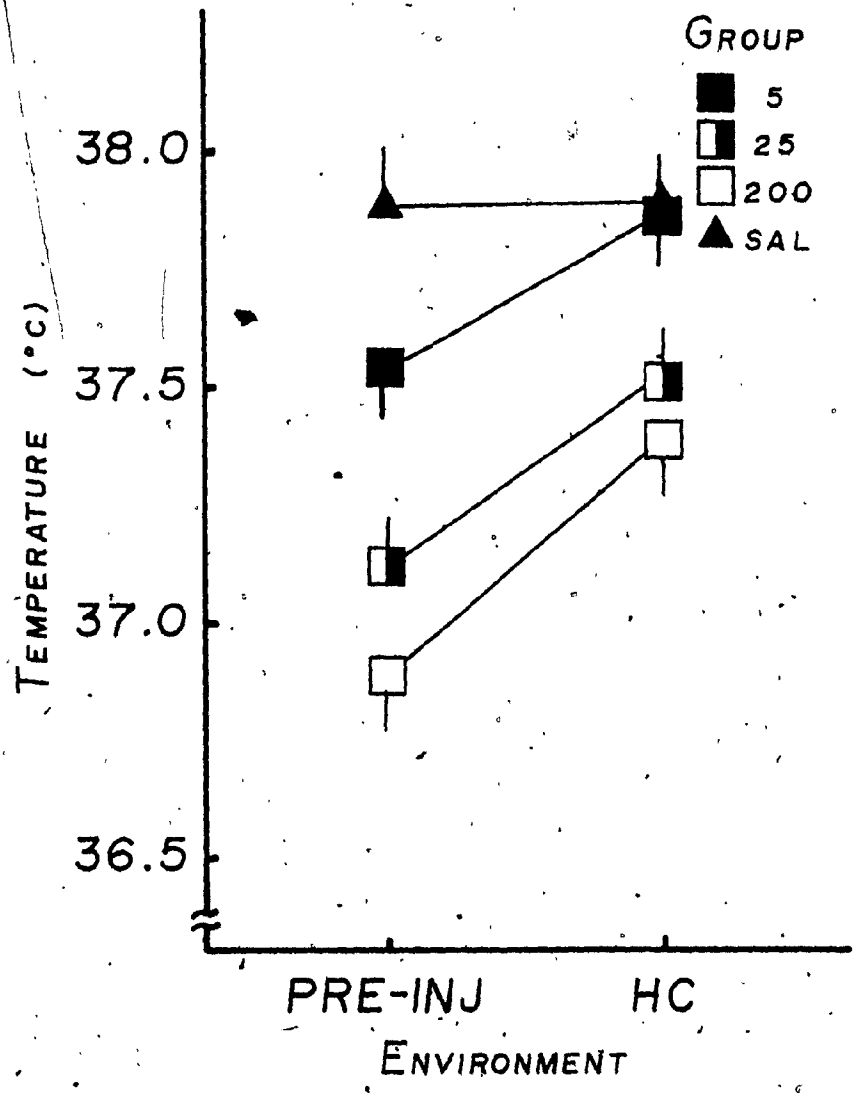

Figure 6. Average mean body temperature of animals in each of the four groups taken at 11:00 h either in the pre-injection environment (PRE-INJ) or in the home cage (HC). Each point represents an average of four test days. Vertical bars equal one SEM.

stronger in the pre-injection environment than in the home cage. Because the hypothermia was stronger in the pre-injection environment it was concluded that this hypothermia of animals in the morphine groups relative to animals in the saline group was a conditioned response that occurred in anticipation of the morphine injection.

One finding that poses some difficulty for the conditioning explanation of the pre-injection hypothermic effect is that after the drug-free period when animals were again placed in the pre-injection environment, no group differences were found. Thus it seemed that the conditioned effect had disappeared without explicit extinction trials, which would be an unusual finding. A careful evaluation of the procedure suggested another explanation. Because the morphine injections always occurred at 12:00 h, it was possible for temporal cues to act as conditioned stimuli. Throughout the whole experiment there were never any group differences at 9:00 h but at 11:00 h, even in the home cage, there was a statistically significant group effect during the period of morphine administration (Figure 6). The animals receiving morphine were hypothermic relative to those receiving saline even on the home-cage days. More importantly, although there continued to be no group differences at

9:00 h, during the drug-free period animals in the morphine groups showed a hypothermia relative to saline group animals at 11:00 h that gradually disappeared over days in a dose-related fashion (see Figure 7). This suggests that temporal cues may have been acting as conditioned stimuli and that extinction of the response to these cues could have occurred during the drug-free period.

In the injection environment after the drug-free period, animals in the morphine groups were hyperthermic relative both to animals in the saline group and to their own temperatures taken in the home cage (see Figure 8). Direct testing for this effect during the period of morphine administration was confounded by the group differences at 11:00 h (see Figure 6). This conditioned hyperthermia is similar to that found by Lal and his associates and by Sherman (1979) as discussed earlier.

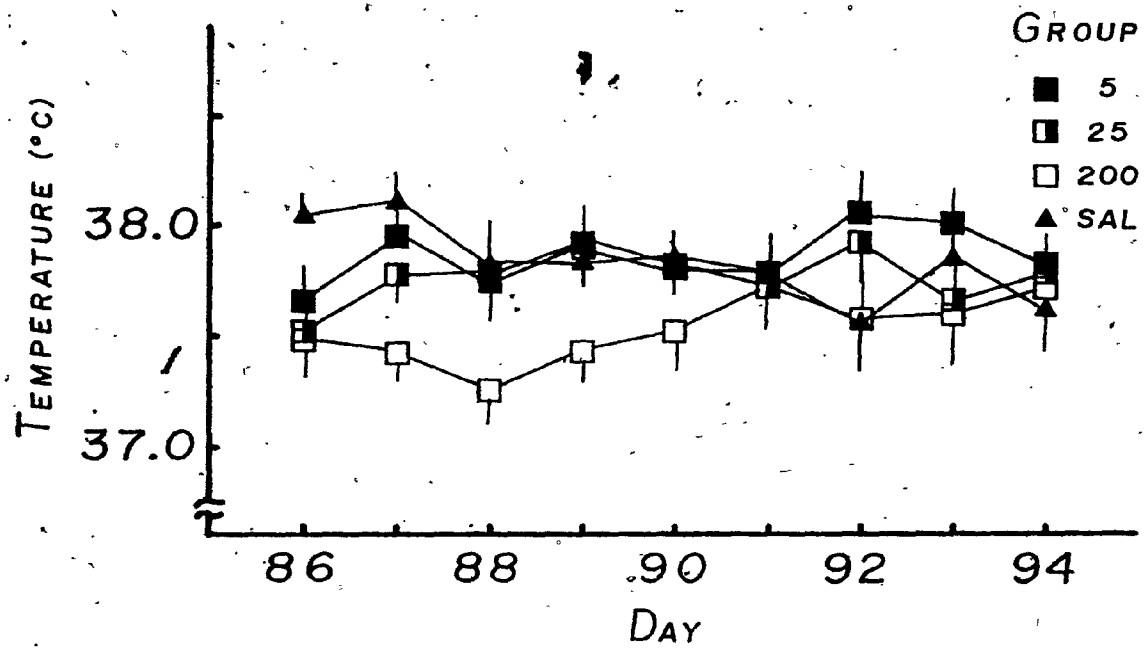

Figure 7. Mean body temperature of animals in each of the four groups taken at 11:00 h in the home cage during the drug-free period, Days 86 to 94.

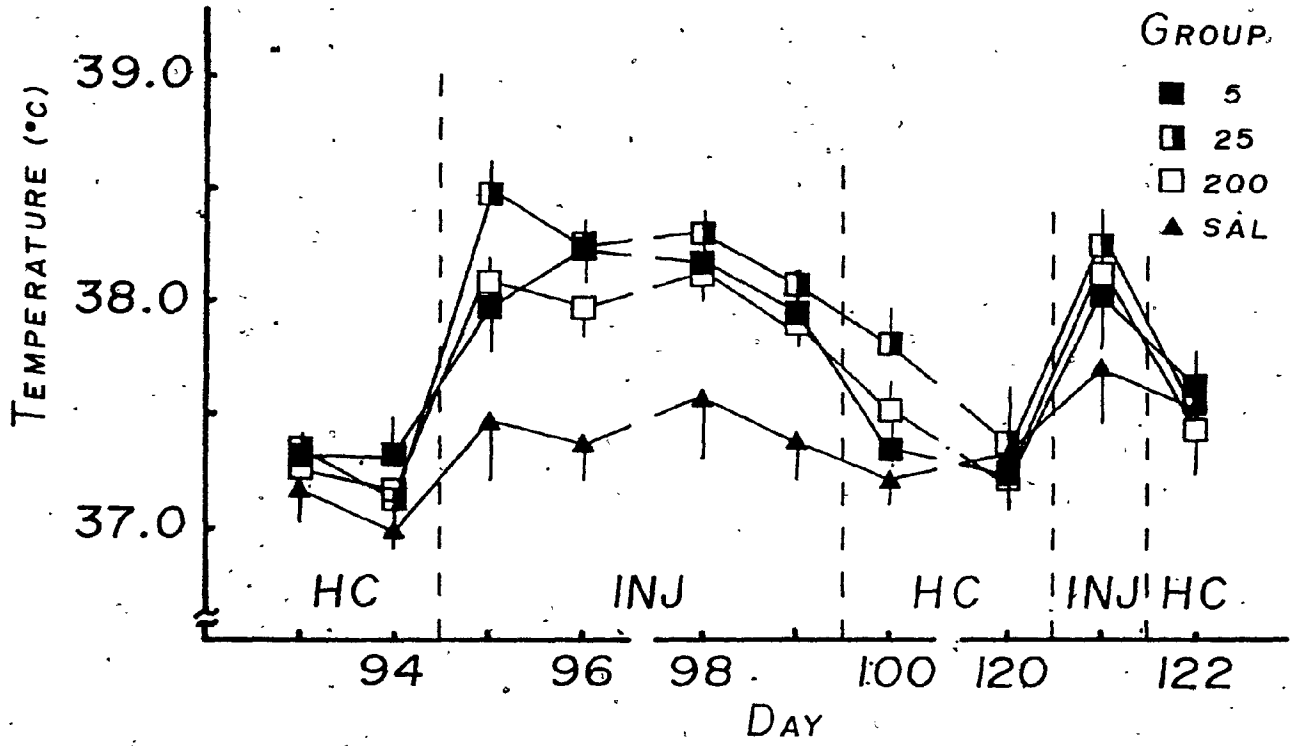

Figure 8. Mean body temperature of animals in each of the four groups taken at 14:15 h on Days 93 to 122 in either the home cage (HC) or the injection environment (INJ).

EXPERIMENT I

One unexplained finding from the original study was that the pre-injection conditioned hypothermia disappeared over the period of abstinence when no explicit extinction trials were given; during this time animals were left in their home cages. Inspection of the data suggested a testable explanation; it appeared that during the drug-free period the conditioned hypothermic response might have been elicited even in the home cage by circadian or temporal stimuli, and thus, animals were in fact experiencing extinction trials. As injections of morphine always occurred 24 h apart, at noon, daily temporal or circadian stimuli were part of the conditioned stimulus complex. It seemed possible that the two different conditioned responses, hypothermia in the pre-injection period and hyperthermia in the post-injection period, were being elicited by different classes of stimuli. However, in the original experiment both temporal and situational stimuli were confounded; both were explicitly paired with the morphine injection on every trial.

In order to differentiate between the effectiveness of these two classes of stimuli, two experiments were designed attempting to make one set of stimuli irrelevant

while explicitly pairing the other with the injections. In the first experiment the relevance of circadian or temporal stimuli was minimized while distinctive environmental stimuli were paired with the injection of morphine. This was accomplished by administering morphine at irregular times on alternate days, while regularly placing the animal in distinctive pre-injection and injection environments whenever the drug was given. In the second experiment the converse was attempted by administering morphine at a fixed time each day in the home cage where animals remained throughout the day.

Method

Subjects

Thirty-one male Wistar rats, weighing 175-200 g on arrival, obtained from Canadian Breeding Farms and Laboratories, St. Constant, Quebec, were housed individually in stainless steel cages (18 cm x 25 cm x 18 cm) for the duration of the study. Purina Lab Chow and water were available to the animals at all times in the home cage. The animal room was lit from 7:00 h to 21:00 h and was maintained at a constant temperature of 22±1°C. Animals were randomly assigned to one of three groups, a conditioning group (COND), n = 11, a pseudo-conditioning group (PSEUDO), n = 10, and a saline group (SALINE), n = 10.

Design

In order to minimize the relevance of temporal cues as signals for morphine, all animals were given fluid injections once daily at random times between 11:00 h and 18:00 h six days a week. On the first, third and fifth days of the week injections were given in the injection environment; the injections were preceded by a two-hour stay in a pre-injection environment, a dark, quiet room, and were followed by a three-hour stay in the injection environment, a bright, noisy room. On the second, fourth and sixth days injections were given to animals in the home cage. Animals in Group COND received morphine injections on the days the animals were in the distinctive environments and saline injections on the home-cage days. Group PSEUDO animals received morphine injections on the home-cage days and saline injections on days spent in the distinctive environments. Animals in Group SALINE received saline injections throughout. Thus animals in Group COND and Group PSEUDO received equal numbers of morphine injections but morphine injections were paired with distinctive environmental stimuli only for animals in Group COND. Temperature measurements were taken one hour before, and both one and two hours after every injection.

Prior to the beginning of the conditioning period, all animals experienced one week of habituation to the experimental procedures during which they received only saline injections. Conditioning lasted twelve weeks and involved a total of 32 morphine injections for animals in Groups COND and PSEUDO. After six weeks of conditioning, tests for conditioned effects were made by replacing morphine with saline once a week. Saline substitutions were made for animals in Group PSEUDO on home-cage days, and for animals in Group COND on days when animals were moved to the distinctive environments. Each group had saline substituted for morphine on three occasions. A ten-day drug-free period followed, during which animals remained in their home cage. Though the animals were not injected during this period their temperatures were measured three times a day. This was followed by a further two and a half weeks, 16 days, when the conditioning procedures were reinstated with the exception that no morphine was administered. Every second day during this post-drug period the animals were reintroduced to the conditioning environments, had their temperatures measured, but were injected with saline. On the intervening days the animals remained in their home cages, were injected with saline and had their temperatures measured.

Procedure

Temperature Measurement. Rectal temperature was measured by means of a small animal probe (Yellow Springs model 402) and a Yellow Springs Tele-Thermometer model 46 TUC (accuracy = ±.15°C). The rats were placed in a small rectangular trough closed at one end (7 cm x 22 cm x 8 cm), and were held down with one hand while the probe was inserted a minimum of 6 cm as recommended by Lomax (1966) for approximately 30 s until the temperature reading stabilized. After two or three measurements the rats accepted the procedure with little objection (no biting, squealing or kicking and only minimal struggle against the momentary restraint).

Drugs and Injection Procedure. Throughout the study all drugs were injected IP in a volume of 1 ml/kg. Solutions were made using the physiological saline which was also used for saline injections. Morphine sulfate was administered at a dose of 20 mg/kg.

Pre-injection Room. Animals were transported from the home-cage room in groups of three or four to the pre-injection room where they were individually housed in wooden boxes (17 cm x 28 cm x 13 cm) with wire tops and

wood shavings on the floor. The pre-injection room was dimly lit, quiet, and maintained at a temperature of 22 ±1°C throughout the experiment.

Injection Room. In contrast, the injection room was brightly lit, had a continuous 75 db white noise background, and was maintained at a temperature of 23±1°C. The animals remained in their boxes in both environments, were moved in them, and were only taken out for the temperature measurement and injection. Food and water were not available in either the pre-injection room or the injection room. The animals were always handled and treated in the same order, keeping the time between events the same for all animals.

Results

Each day the first body temperature reading was taken one hour prior to the daily injection. These temperatures were analyzed to determine whether they varied as a function either of group treatment or of where they were taken, that is, in the home cage or in the pre-injection environment. Figure 9 shows that the animals in Groups COND and PSEUDO were both hypothermic relative to animals in Group SALINE during the pre-injection period. The hypothermia

was present during tests when animals remained in their home cages and during tests when animals were taken to the distinctive pre-injection environment. A Group x Environment analysis of variance was carried out using individual mean scores for each set of three test days; both the main effects were significant (see Appendix, Table 1). From Figure 9 it is evident that animals in Group PSEUDO and Group COND did not differ in temperature and that the Group effect reflects the fact that animals in both these groups were hypothermic relative to animals in Group SALINE. Though the Group x Environment interaction was not significant, it did approach significance indicating a trend towards a greater hypothermia in all morphine-group animals relative to animals in Group SALINE in the pre-injection environment. The hypothermia appeared to be a nonspecific unconditioned effect common to animals receiving morphine. The significant Environment effect was due to the fact that, in general, all three groups of animals had somewhat higher body temperatures in the pre-injection environment than in the home cage.

Animals in the two morphine groups did not, however, have similar temperatures in the injection environment on the three test days when all animals received saline. An

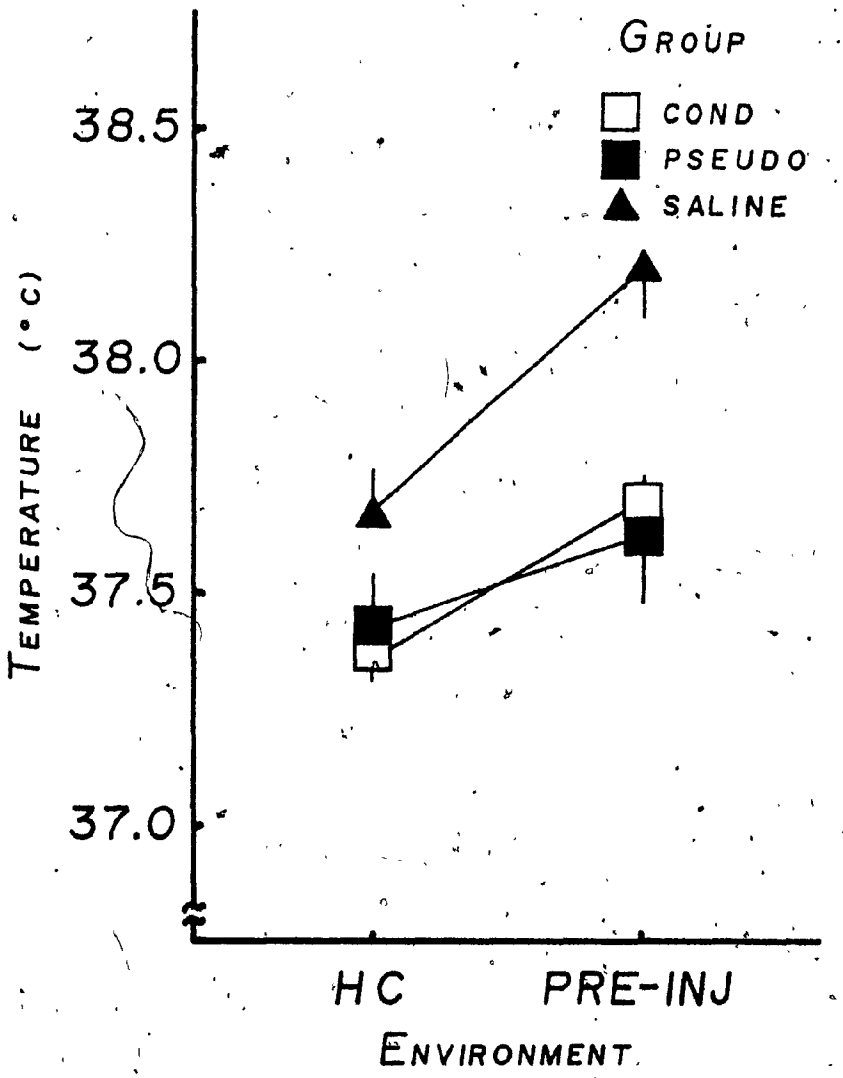

Figure 9. Average mean body temperature of animals in each of the three groups of Experiment 1 taken one hour before the daily injection in either the home cage (HC) or in the pre-injection environment (PRE-INJ). Each point represents the average of three test days.

analysis of variance of body temperature, measured one hour after the saline injection, using individual animal means for the three days, revealed significant group differences (see Appendix, Table 2). In the injection environment, animals in Group PSEUDO (37.0°C) were hypothermic relative to animals in Group SALINE (37.6°C) and Group COND (37.5°C), but animals in Group COND were no longer hypothermic relative to Group SALINE animals.

After the drug-free period, prior to the daily injection, animals in the two morphine groups were no longer hypothermic relative to animals in Group SALINE. There were two types of test days after the drug-free period: days the animals remained in their home cage, and days they were placed in the distinctive environment. On days the animals remained in their home cage, their temperatures were measured three times, once before and twice after the saline injection. These temperatures, averaged over the eight home-cage days, are shown in Figure 10A. Three Group x Day anaylses of variance, one for each of the temperature measurements, revealed that at no time after abstinence did the groups differ in the home cage (see Appendix, Tables 3-5).

Figure 10B, however, shows that group differences were evident after abstinence, when animals were returned

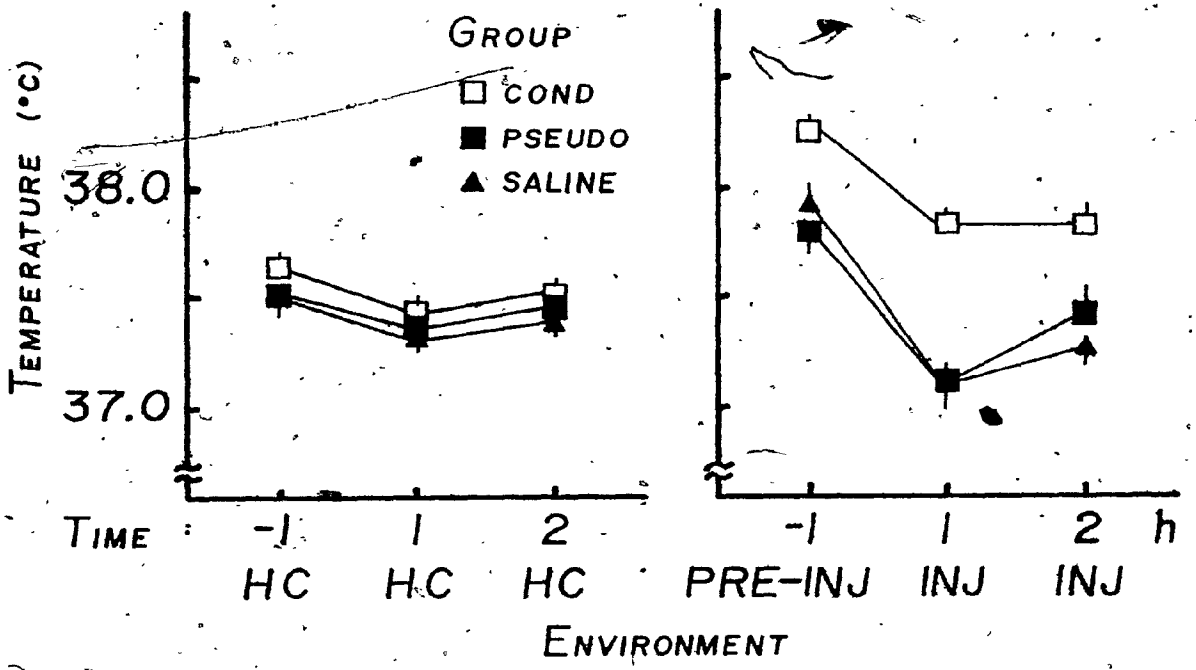

Figure 10. Average mean body temperature of animals in each of the three groups of Experiment 1 taken on test days after abstinence. A Days when the animals remained in their home cage (HC) and had their temperatures measured before and after the saline injection. B Days when the animals were placed in the pre-injection environment (PRE-INJ) before and in the injection environment (INJ) after the saline injection. Each point represents the average of eight test days.

to the distinctive environments. For each of the three daily temperature measurements averaged over the eight conditioning test days, animals in Group COND were hyperthermic relative to animals in either Group PSEUDO or Group SALINE. Because there were absolute temperature differences for all animals in the two environments, Group x Day analyses of variance were done separately for each of the three daily temperature measurements. As expected, in each analysis the Group effect was significant, but in no case was the Group x Day interaction significant (see Appendix, Tables 6-8). The lack of interactions suggests that the group differences were consistent over the tests after abstinence and that this difference resists extinction. Thus only the animals that had had morphine administration paired with environmental stimuli, Group COND animals, showed a conditioned hyperthermia after abstinence, both in the pre-injection and injection environment.

The data obtained from this experiment also bear on the question of whether tolerance develops to the unconditioned hyperthermic effects of morphine. In animals in Group COND, morphine induced a hyperthermia of about 2.5°C both one and two hours after administration. To test for tolerance, the hyperthermia induced by the first two

injections of morphine was compared to that induced by the last two morphine injections, numbers 31 and 32. At one hour after morphine administration there was no significant difference between the hyperthermia induced by the first two and the last two injections; 39.9°C and 39.7°C ($t(10) = 1.50$, $p > .15$). At two hours after morphine administration there was a significant difference between the hyperthermia induced by the first two and the last two injections, 40.1°C and 39.7°C ($t(10) = 3.90$, $p < .05$). Note, however, that even two hours after the last two morphine administrations animals in Group COND were clearly hyperthermic compared to animals receiving saline on those days both in Group SALINE (37.5°C) and in Group PSEUDO (37.0°C) (see Appendix, Table 9). Thus, it appears that after repeated administration morphine induces a hyperthermia comparable to that which it induces on the first few occasions; the only change may be that the hyperthermia does not last as long on later occasions.

Discussion

In this experiment morphine injections were administered at irregular intervals, but were preceded and followed by two-hour and three-hour stays in distinctive pre-injection and injection environments for animals in

Group COND. Animals in Group PSEUDO received equivalent amounts of morphine under similar temporal schedules but unpaired with the distinctive environmental stimuli. No evidence was obtained for a conditioned pre-injection hypothermia like that seen in the original experiment when animals were always injected at the same time of day. Figure 9 shows that while animals in the conditioning group (COND) appear hypothermic relative to animals in Group SALINE both in the home cage and in the pre-injection environment, in both environments the hypothermia was equivalent to that seen in animals in the pseudo-conditioning group (PSEUDO). Thus the hypothermia seen in this experiment appears to have been an effect of the repeated morphine administrations rather than a conditioned response. Note that in the present experiment animals in Group COND were placed in a distinctive environment for two hours preceding the morphine injection, yet no conditioned hypothermia occurred. Thus, when the predictive value of the daily temporal cues paired with the morphine injection were reduced, distinctive environmental stimuli were unable to act as conditioned stimuli and to elicit a conditioned hypothermia.

After abstinence, when the animals were returned to the distinctive environments, a conditioned hyperthermia

was evident, similar to that seen in the original experiment. This hyperthermia can be called conditioned in that it was specific to animals in Group COND; animals in Group PSEUDO that had received an equivalent amount of morphine in the home-cage environment were never hyperthermic after abstinence. One finding of interest was that the conditioned hyperthermia was evident both in the pre-injection and in the injection environment. In the original experiment the hyperthermia was not evident in the pre-injection environment after abstinence. This difference may be due to the absence of a conditioned hypothermia in the present study.

There are two clear findings from the present experiment. First, in the absence of daily temporal cues that accurately predict the morphine injection, a hypothermia did develop, but it was not elicitable by pre-injection environmental stimuli. Second, conditioned hyperthermia was seen after abstinence and was elicited by the environmental stimuli associated with morphine administration.

EXPERIMENT 2

In this experiment, in contrast to the first, an attempt was made to maximize the predictive value of daily temporal cues and to reduce the effectiveness of environmental stimuli as cues for morphine. This was done by injecting animals with morphine at the same time each day and by keeping the animals in their home cages throughout the experiment.

Method

Subjects

Twenty-five male Wistar rats, weighing 175-200 g on arrival, obtained from the same supplier, were housed and maintained under the same conditions as animals in Experiment 1. Animals were randomly assigned to one of three groups, a conditioning group (COND), n = 9, a pseudo-conditioning group (PSEUDO), n = 8, and a saline group (SALINE), n = 8.

Design

As this experiment was designed to study the effectiveness of temporal cues as conditioned stimuli and to minimize the predictive value of environmental cues, the

animals remained in their home cages throughout. Each day all animals received two injections, one always administered at 10:30 h while the second was administered at random, on the half hour, between 8:30 h and 20:30 h. Animals in Group COND received morphine every day at 10:30 h and saline at the random time injection. Animals in Group PSEUDO received saline at 10:30 h and morphine at the random time. Group SALINE animals received saline on both occasions. For animals in Group COND only the temporal, or circadian, cues were predictive of the morphine injection. Animals in Group PSEUDO while receiving an equal number of morphine injections as animals in Group COND, had no explicit stimuli predictive of the morphine injection. Rectal temperatures were measured at 9:30 h, 11:30 h, and a random time, on the half hour, between 8:30 h and 20:30 h.

Days 1 to 4 of the experiment served as a habituation phase, during which all animals received saline injections. From Day 5 to Day 40 all animals received the appropriate injections, except for the three test days, Days 28, 34 and 40, when all animals received saline injections. This was followed by a further eight test days, a drug-free period, when temperatures were measured at the appropriate times but when all animals received saline. Drug dose,

injection procedure, and temperature measurement were the same as in Experiment 1.

Results

There were three days, Days 18, 26 and 33 when the random time temperature measurements were taken at 10:30 h, just prior to the time of the injection when animals in Group COND received their daily morphine. Animals in Group COND and Group PSEUDO did not differ in temperature (37.9°C to 37.7°C) at this time, but both were hypothermic relative to animals in Group SALINE (38.3°C). A one-way analysis of variance, using individual animal means for the three days, revealed that there were significant group differences (see Appendix, Table 10). Scheffé tests revealed that, as in the first experiment, animals receiving morphine were hypothermic relative to animals in Group SALINE ($p < .05$). At 10:30 h, however, there was no difference between animals in Group COND and Group PSEUDO.

On three days, Days 22, 27 and 31, the animals' temperature was measured both at 8:30 h and at 9:30 h, one and two hours before the 10:30 h injection time. The 8:30 h and 9:30 h group mean temperatures are shown in Figure 11.

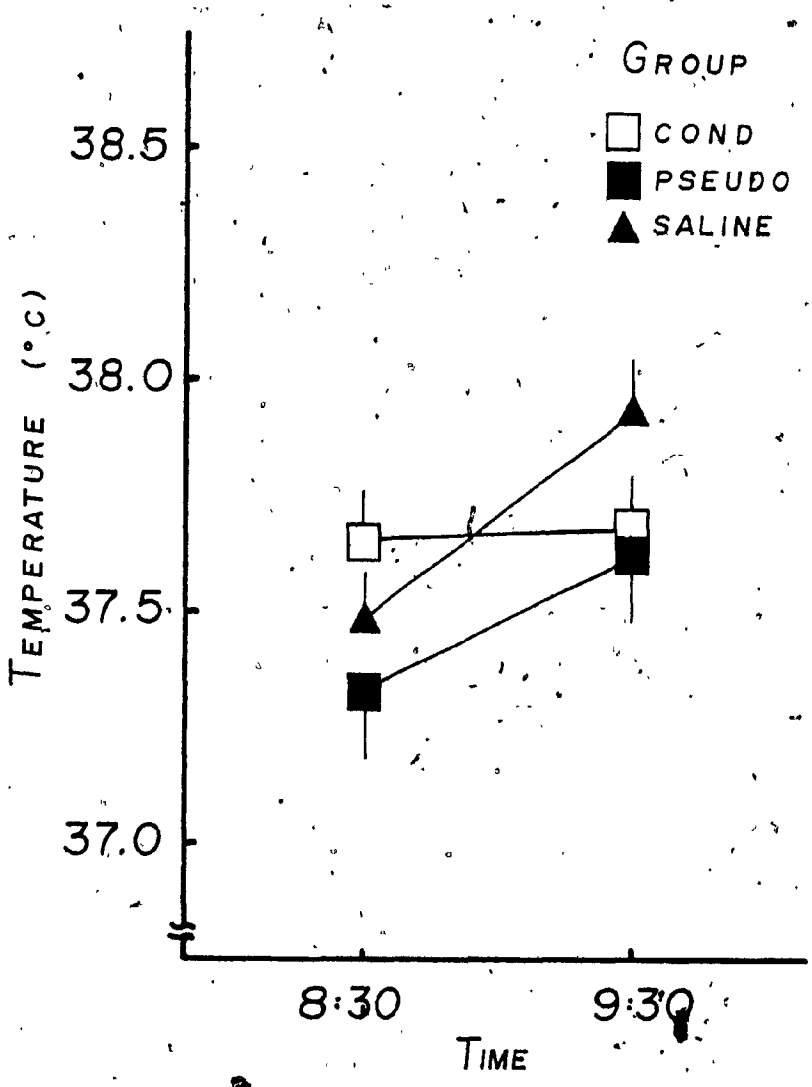

Figure 11. Average mean body temperature of animals in each of the three groups of Experiment 2 taken in the home cage two hours, 8:30 h, or one hour, 9:30 h, before the daily injection. Each point represents the average of three test days.

It can be seen that while animals in Group PSEUDO are hypothermic relative to animals in Group SALINE at both 8:30 h and 9:30 h, animals in Group COND are hypothermic, relative to Group SALINE animals only at 9:30 h. A Group x Time analysis of variance, using individual animal means for the three days, revealed that only the Time effect and the Group x Time interaction were significant (see Appendix, Table 11). While the Time effect reflects a general rise in temperature seen from 8:30 h to 9:30 h, the interaction reflects the change in body temperature of animals in Group COND relative to the other two groups. Thus, for animals of Group COND that were receiving morphine at the fixed time each day, 10:30 h, the hypothermia was evident only around the time of the injection, while for animals that were receiving morphine at random times, Group PSEUDO, the hypothermia was evident at all times tested.

A comparison was made of the 9:30 h and 11:30 h temperature measurements for the three saline tests done during the morphine conditioning period, Days 28, 34 and 40 and is shown in Figure 12. Both one hour before and one hour after the 10:30 h injection animals in the two groups receiving morphine, Group PSEUDO and Group COND, were hypothermic relative to animals in Group SALINE. Individual animal measurements were averaged across the

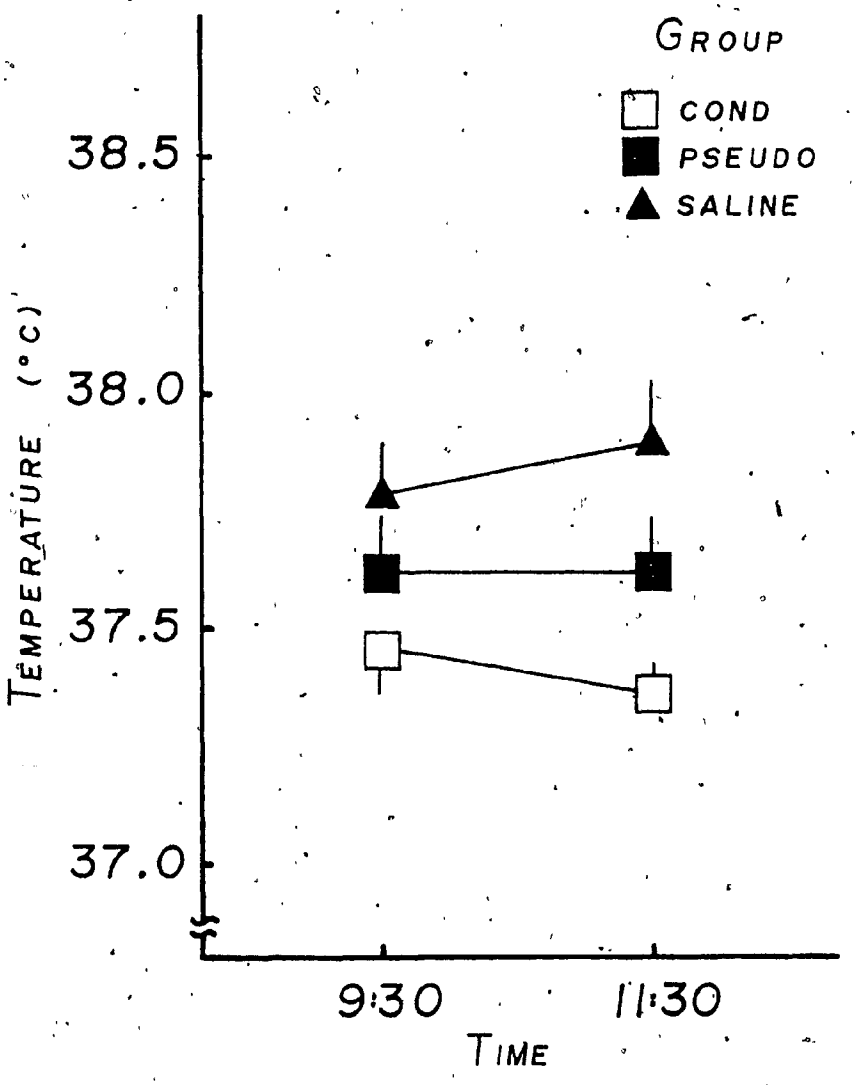

Figure 12. Average mean body temperature of animals in each of the three groups of Experiment 2 taken in the home cage one hour before, 9:30 h, and one hour after, 11:30 h, the saline injection. Each point represents the average of three test days.

three days for each time and a Group x Time analysis of variance was carried out. Only the Group effect was significant (see Appendix, Table 12). The lack of a significant interaction suggests that the hypothermia was just as strong one hour before and one hour after the 10:30 h injection and provides no evidence for a conditioned hyperthermia.

To test for conditioned hyperthermia after a six day drug-free period the 9:30 h and 11:30 h temperatures were analyzed for the last three days of the experiment, Days 46, 47 and 48. Two one-way analyses of variance using individual mean temperatures for the three days, showed that the groups did not differ at either 9:30 h or at 11:30 h (see Appendix, Tables 13 and 14). Thus, there was no evidence of hypothermia after the drug-free period.

As in the first experiment there was no evidence for tolerance of the unconditioned hyperthermic effect of morphine. Indeed, the temperature measurement taken one hour after injection produced a greater hyperthermia after the 32nd and 33rd injections of morphine, 39.4°C, than after the first and second injections, 38.3°C ($t(8) = 4.61$, $p < .001$), probably reflecting a shift forward in the time of the peak effect.

Discussion

As in the first experiment animals receiving daily morphine injections were hypothermic relative to Group SALINE animals. Unlike the first experiment, however, there was a difference between animals in Group COND and in Group PSEUDO. Animals in Group PSEUDO, as did morphine-group animals in the first experiment, received morphine at irregular times of the day and thus had no temporal cue predictive of the morphine injection. The hypothermia seen in these animals, as in the first experiment, was nonspecific in that it was evident at all times during the morning. In contrast, animals in Group COND that received morphine at 10:30 h every day were hypothermic only around the time of the injection. At 8:30 h there was no evidence of hypothermia in these animals. This finding is similar to that of the original experiment in which animals receiving morphine at a fixed time of day were hypothermic one hour before, but not three hours before, the daily injection. Because in the present experiment only the daily temporal cues were predictive of the morphine injection the hypothermia seen in animals in Group COND around the time of the injection can be viewed as a temporally conditioned response. Furthermore, in the present experiment, in which only daily temporal cues

were predictive of the morphine injection and in which the importance of environmental cues was minimized, there was no evidence for conditioned hyperthermia in any of the tests.

EXPERIMENT 3

In Experiments 1 and 2 environmental stimuli and temporal cues were separately paired with the morphine injection. Each of these conditioned stimuli was able to elicit one of the two conditioned responses observed in the original experiment. A conditioned hyperthermia was observed when conditioning involved the use of environmental stimuli as conditioning stimuli, while the use of temporal cues resulted in a conditioned hypothermia. Rather than testing the relevance of these two types of stimuli in separate experiments, it should be possible to pair both with morphine administration and then test them separately. For example, the effect of environmental stimuli could be isolated from that of temporal cues by testing the animals in the conditioning environment at the "wrong" time of day. The role of temporal cues could be evaluated by testing the animals in their home cage at the time they would usually be in the conditioning environment. This should make it possible to observe both conditioned responses, in the same animal, before the start of the drug-free period.

In the original experiment and in Experiments 1 and 2 morphine was used as the unconditioned stimulus. In the present experiment two additional drugs were investigated;

naloxone amd amphetamine. Naloxone, a pure opiate antagonist, causes a decrease in body temperature (Blasig et al, 1978; Stewart & Eikelboom, 1979). Amphetamine, like morphine, causes a hyperthermia but, unlike morphine, tolerance occurs to the hyperthermic effect of amphetamine (Harrison, Ambrus & Ambrus, 1952).

The use of amphetamine or morphine, drugs which have excitatory effects on behavior, as unconditioned stimuli has been shown to result in conditioned increases in activity (Kamat, Dutta & Pradham, 1974; Pickens & Dougherty, 1971; Tilson & Rech, 1973; Trost, 1973). Increases in the motor activity of animals, whether conditioned or unconditioned, could result in increased body temperature. This raises the possibility that the conditioned hyperthermia observed in the previous experiments may have been due to conditioned increases in activity and may not directly involve the thermoregulatory system. Note that while a drug may have a direct effect on body temperature it is possible that the conditioned temperature changes may be due to conditioned activity changes. Alternatively the conditioned temperature and activity changes may be two completely separate conditioned responses. An attempt was made to address this issue by investigating the conditioning of both temperature and activity changes in the same animal.

Method

Subjects

Thirty-two male Wistar rats, weighing 175-200 g on arrival, were obtained from the same supplier, and housed under the same conditions as animals in previous studies. Animals were randomly assigned to one of four groups of eight, differing only in the drug administered during conditioning, a morphine group (MOR), a naloxone group (NAL), an amphetamine group (AMP), and a saline control group (SAL).

Design

This experiment was similar to the original experiment in that during conditioning both environmental stimuli and temporal cues were paired with each drug injection. On conditioning days all animals were placed in the distinctive pre-injection environment for 90 minutes, from 10:15 h to 11:45 h, then moved to, and injected in, the injection environment where they remained a further 90 minutes, from 11:45 h to 13:15 h. Body temperatures were measured daily at 9:30 h in the home cage, at 11:00 h in the pre-injection environment, and at 12:30 h in the injection environment. On several days during conditioning, and on all test days, the motility of all animals was

measured. This was done by using time-sampling techniques and observing the animals from 11:30 h to 11:40 h in the pre-injection environment and from 12:15 h to 12:25 h in the injection environment.

Days 1 to 3 served as the habituation phase of the experiment; the conditioning procedures were followed, but animals in all groups were injected with saline. Conditioning trials, when all animals received the appropriate drug injections, took place daily from Day 4 to Day 32, with the exceptions noted below. In order to test for tolerance, each drug was administered once to animals in the saline group. Saline-group animals were injected with naloxone on Day 15, with amphetamine on Day 18, and with morphine on Day 21. Thus the temperature effect of the drugs could be compared, on the same day, in animals with and without previous drug experience. On Day 31, an otherwise normal conditioning day, the temperature of all animals was measured in the home cage at 15:30 h, 17:00 h and 18:30 h to determine the duration of the drug effect.

Day 26 was a conditioning test day; the usual conditioning procedures were followed, but all animals were injected with saline. On this day the temperature of all animals was also measured in the home cage at 15:00 h,

17:00 h and 18:30 h. On Day 33 the temporal cues and the environmental stimuli were presented separately. In the morning animals remained in their home cages, were not injected, and had their temperatures measured at the normal times: 9:30 h, 11:00 h and 12:30 h. Temperatures were measured again, in the home cages, at 15:00 h and 18:30 h. At 19:15 h the animals were moved to the pre-injection environment, nine hours later than normal. The animals were treated as if this was a normal conditioning trial, but when moved to the injection environment were all injected with saline. The temperatures were measured at 20:00 h in the pre-injection environment and at 21:30 h in the injection environment.

During the drug-free period, Days 34 to 48, animals remained in the home cages and were not injected. On the first eight, and last three, days of this period the temperatures of all animals were measured at the usual times. After the drug-free period further tests for conditioning occurred. Animals were placed in the conditioning environment, had their temperatures measured, and were injected with saline; all at the usual time. These tests occurred on Days 49, 50, 56, 61, 67, 68 and 69. On Days 55 and 60 animals had their temperatures measured in the home cage, while on the remaining days the animals stayed in their home cage and were not handled.

Procedure

The distinctive environments and the temperature measuring equipment were the same as used in previous experiments. As in previous experiments all drugs were injected intraperitoneally in a volume of 1 ml/kg. Solutions were made up using the physiological saline used for saline injections. The drug doses administered were as follows: morphine sulfate, 20 mg/kg; d-amphetamine sulfate, 5 mg/kg; and naloxone hydrocholoride, 20 mg/kg.

Activity measurement. The motility of each animal was scored, using a time sampling technique, in both the pre-injection and injection environment. Each day the motility of the animals was measured; the animals were observed five times in the pre-injection environment and six times in the injection environment. Each observation lasted three seconds and the animals' behavior, as one of six mutually exclusive categories, recorded. These behaviors were assigned a weight from 0 to 4 on the basis of the amount of muscular activity involved in each behavior. These behaviors and their weighting are listed in Table 1. From these behavioral records two motility scores, ranging from 0 to 10, were derived for each animal.

TABLE 1

The behaviors and weighting used to score the motility of animals.

Behavior	Weighting	Description
no activity	0	no visible movement; sleeping
sniffing	1	moving of vibrissa or flaring of nostrils
grooming	2	any form of grooming behavior
rearing	2	lifting body on hind legs and pressing head against the wire top of cage
bobbing	3	head bobbing vigorously and much sniffing; a stereotypical behavior usually seen in response to amphetamine
locomotion	3	moving whole body from one side of box to another

Results

The first three days of the experiment were habituation trials when all animals received saline injections. Three Group x Day analyses of variance, one for each of the three daily temperature measurements taken during habituation, were carried out. In no analysis did the Group effect or the Group x Day interaction approach significance (see Appendix, Tables 15-17). There was, however, always a significant Day effect that merely reflected the fact that temperature variations occurred from day to day. These fluctuations in day to day body temperature have been observed before, and have tended to be the same for all groups of animals.

Three Group x Day analyses of variance, one for each of the three daily temperature measurements, were carried out on the data from the first five conditioning days, Days 4 to 8 (see Appendix, Tables 18-20). In the home cage at 9:30 h and in the pre-injection environment at 11:00 h the Group effect and the Group x Day interaction were not significant. Figure 13 shows the temperatures of animals in the four groups at 12:30 h in the injection environment for these first five days of drug administration. A Group x Day analysis of variance revealed that while the

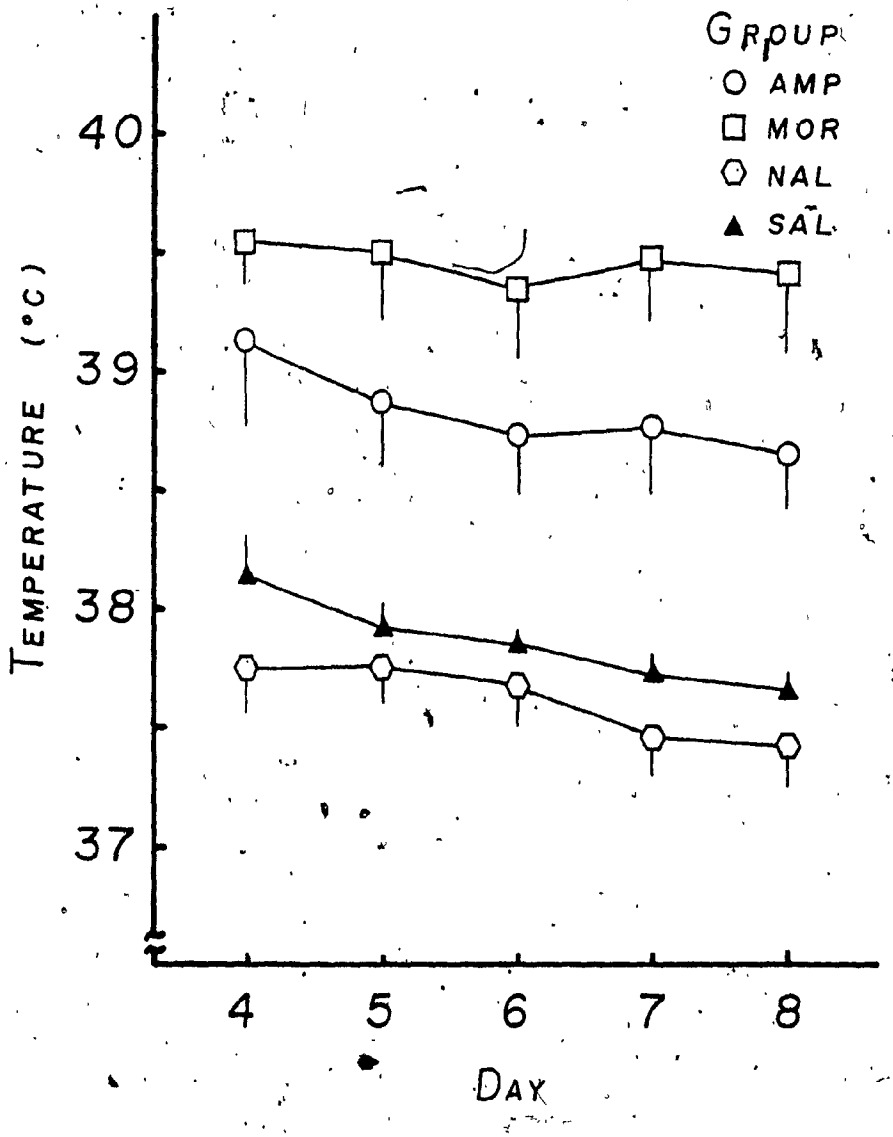

Figure 13. Mean body temperature of animals in each of the four groups taken at 12:30 h in the injection environment following drug administration. Drugs were administered for the first time on Day 4.

Group effect was significant the Group x Day interaction was not (see Appendix, Table 20). It is evident from Figure 13 that relative to animals in the saline group, morphine and amphetamine group animals were clearly hyperthermic. Animals in the naloxone group, though hypothermic relative to animals in the saline group on each day, were not significantly different from animals in the saline group (Scheffe, $p > .1$). The lack of a significant interaction suggests that the unconditioned drug effects did not change over the first few injections.

Animals in the saline group were administered each of the three drugs once during conditioning. A comparison could, therefore, be made between the temperature effects in animals with previous drug experience, animals in the different drug groups, and drug-naive animals, the saline-group animals. Student t-tests were carried out to compare the body temperatures of the two groups of animals. On Day 15 when saline-group animals were injected with naloxone their mean body temperature was not significantly different from that of naloxone-group animals ($t(14) = 1.90$, $p > .05$). There was also no significant difference between the hyperthermia in saline-group animals and in amphetamine-group animals when on Day 18 animals in both groups were injected with amphetamine ($t(14) = 1.43$,

$p > .15$). Finally, on Day 21 no difference was found between temperatures of animals in the morphine group and animals in the saline group receiving morphine for the first time ($t(14) = .76$, $p > .2$). In no case did the temperature effect of these drugs change significantly with repeated administration.

On Day 31 the body temperature of all animals was measured an extra three times, after the animals were returned to their home cage. Figure 14 shows the mean body temperature of animals in the four groups taken after the drug injection; first at 12:30 h in the injection environment, and later in the home cage at 15:30 h, 17:00 h and 18:30 h. Four analyses of variance, one for each temperature measurement, revealed that the groups differed significantly on the first three temperature measurements, but not on the last one taken at 18:30 h (see Appendix, Tables 21-24). It is evident from Figure 14 that while morphine and amphetamine both produced a hyperthermia, the amphetamine-induced hyperthermia was longer lasting. Naloxone, however, had some unexpected effects on body temperature. While at 12:30 h naloxone produced a slight hypothermia, later in the afternoon the naloxone-group animals were clearly hyperthermic relative to the saline-group animals.

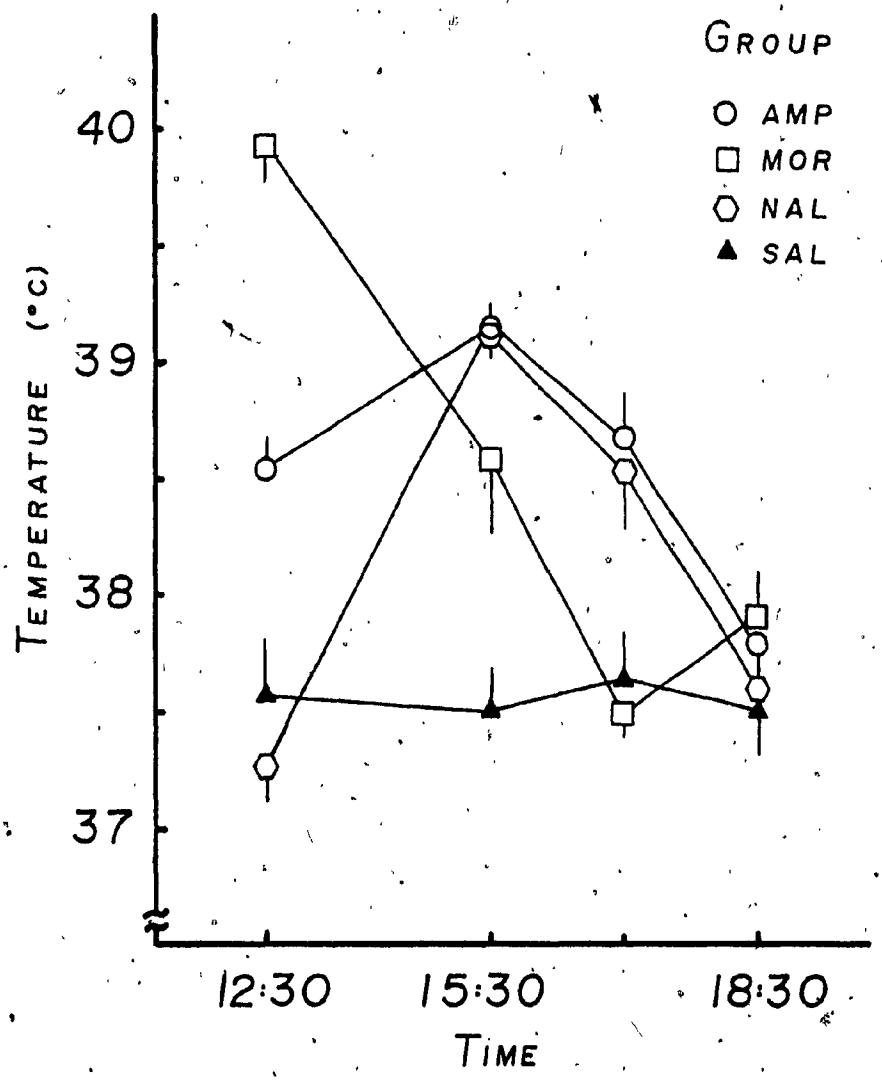

Figure 14. Mean body temperature of animals in each of the four groups on Day 31 taken at various times after the 11:45 h drug injection.

On Days 25, 27 and 32, otherwise normal conditioning days, animals had their motility measured both in the pre-injection environment and in the injection environment. Figure 15 shows the mean motility scores of animals in the four groups in each environment averaged over the three days. A Group x Day analysis of variance of the motility scores taken in the pre-injection environment revealed that the motility of animals in the four groups did not differ significantly (see Appendix, Table 25). A similar Group x Day analysis of variance of the motility scores taken in the injection environment revealed that the groups differed significantly (see Appendix, Table 26). In the injection environment, animals in the morphine and amphetamine groups were more active than animals in the naloxone and saline groups.

Prior to the drug-free period, there were three different conditioning tests. On Day 33 in the morning animals were left in the home cage and were not injected; this constituted a test for the effectiveness of temporal cues. On the evening of that day animals were placed in two conditioning environments as a test for the effectiveness of environmental stimuli in the absence of temporal cues. On Day 26 both types of conditioning stimuli were presented to animals together. This was done by

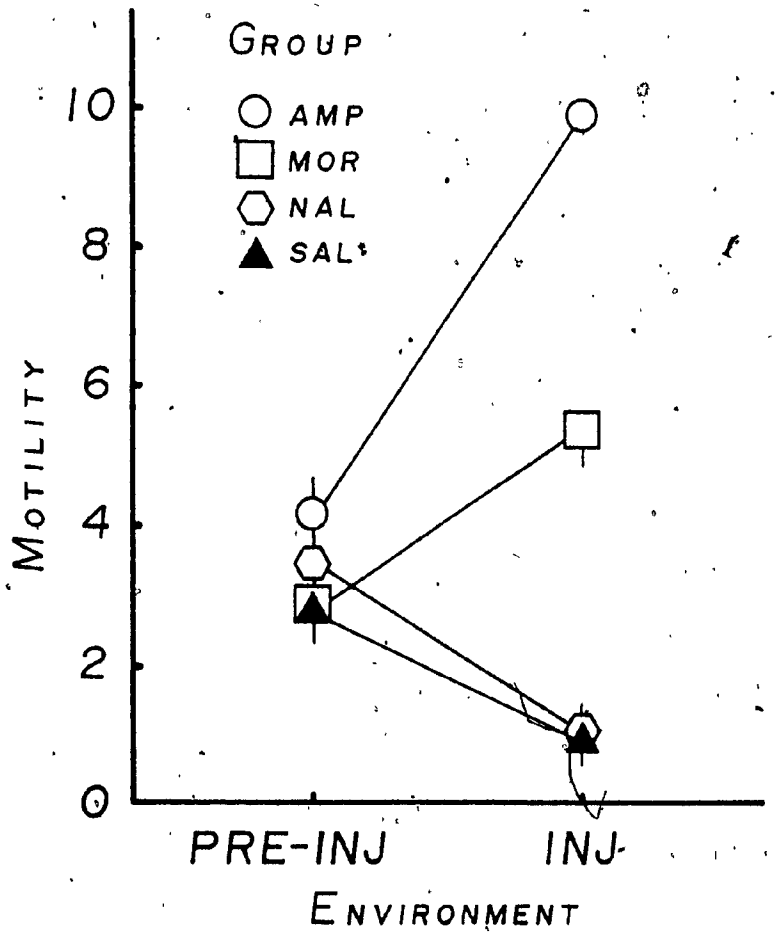

Figure 15. Mean motility score of animals in each of the four groups, averaged over Days 25, 27 and 32, three normal conditioning days, in the pre-injection environment (PRE-INJ) and in the injection environment, (INJ).

administering saline to the animals instead of the usual drugs on an otherwise normal conditioning day.

An anyalsis of variance revealed that on Day 33 at 9:30 h, in the home cage, the temperature of animals in the four groups did not differ (see Appendix, Table 27). Figure 16 shows the temperatures of animals in the four groups, in the home cage, at times they would normally have been in the conditioning environments. A Group x Time analysis of variance revealed that only the main effects were significant (see Appendix, Table 28). Relative to saline-group animals morphine group animals were hypothermic and naloxone group animals hyperthermic, at both times, in the home cage. The significant Time effect reflects the fact that animals were warmer at 11:00 h than at 12:30 h.

Figure 17 shows the temperatures of animals in the four groups when they were placed in the two conditioning environments on the evening of Day 33. A Group x Environment analysis of variance revealed only a significant Group effect (see Appendix, Table 29). Both the morphine- and amphetamine-group animals appeared to be hyperthermic relative to animals in the saline group. It is interesting that naloxone, the morphine antagonist, again appeared to

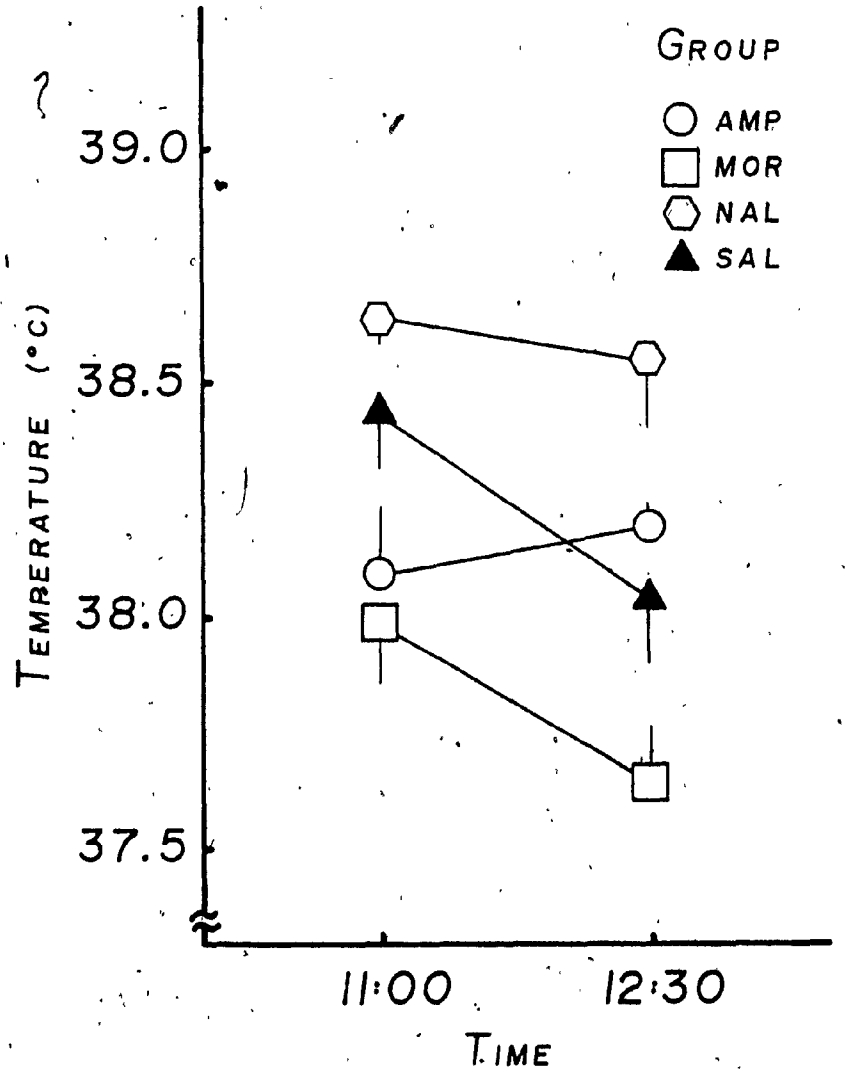

Figure 16. Mean body temperature of animals in each of the four groups taken in the home cage on Day 33, at times the animals would normally have been in the conditioning environment. Animals were not injected between these temperature measurements.

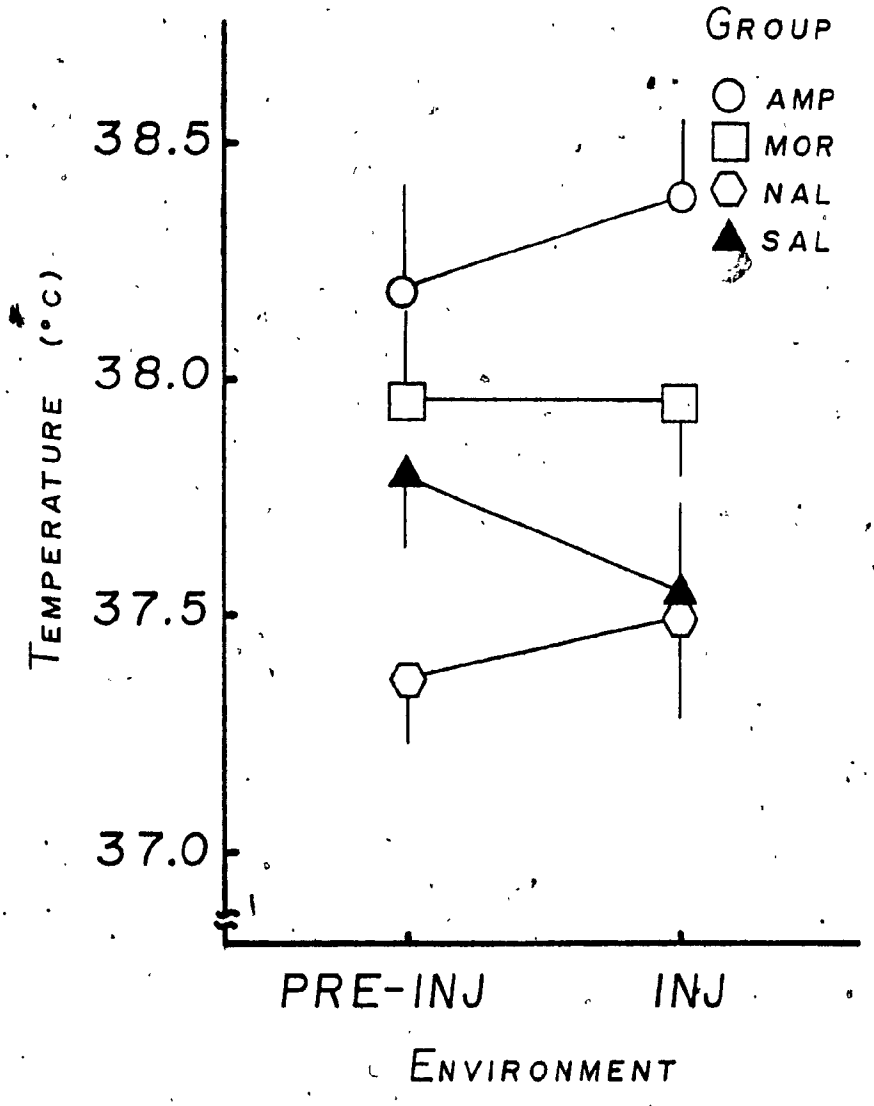

Figure 17. Mean body temperature of animals in each of the four groups taken in the pre-injection environment (PRE-INJ) and in the injection environment (INJ) on the evening of Day 33. All animals received saline injections.

result in a conditioned effect opposite in direction to morphine; naloxone-group animals were hypothermic relative to saline-group animals.

When both temporal cues and environmental stimuli were presented together it appeared that, just as in the original experiment, the conditioned effects summate and only a net effect was evident. Figure 18 shows the Day 26 temperatures of animals in the four groups, in the two conditioning environments, at the normal time in the morning. A Group x Environment analysis of variance revealed that both the group effect and the interaction were significant (see Appendix, Table 30). Simple main effects for a repeated measure design (Winer, 1971) revealed that, while in the pre-injection environment the group differences were not significant ($F < 1$), in the injection environment there were significant differences between the groups ($F(3, 44) = 10.50$, $p < .01$). It is evident from Figure 18 that these effects are due to the marked hyperthermia of amphetamine-group animals, relative to animals in the other groups, in the injection environment. Since both morphine- and naloxone-group animals environmental stimuli induced a conditioned temperature response in the opposite direction from the conditioned response induced by temporal cues, it is not

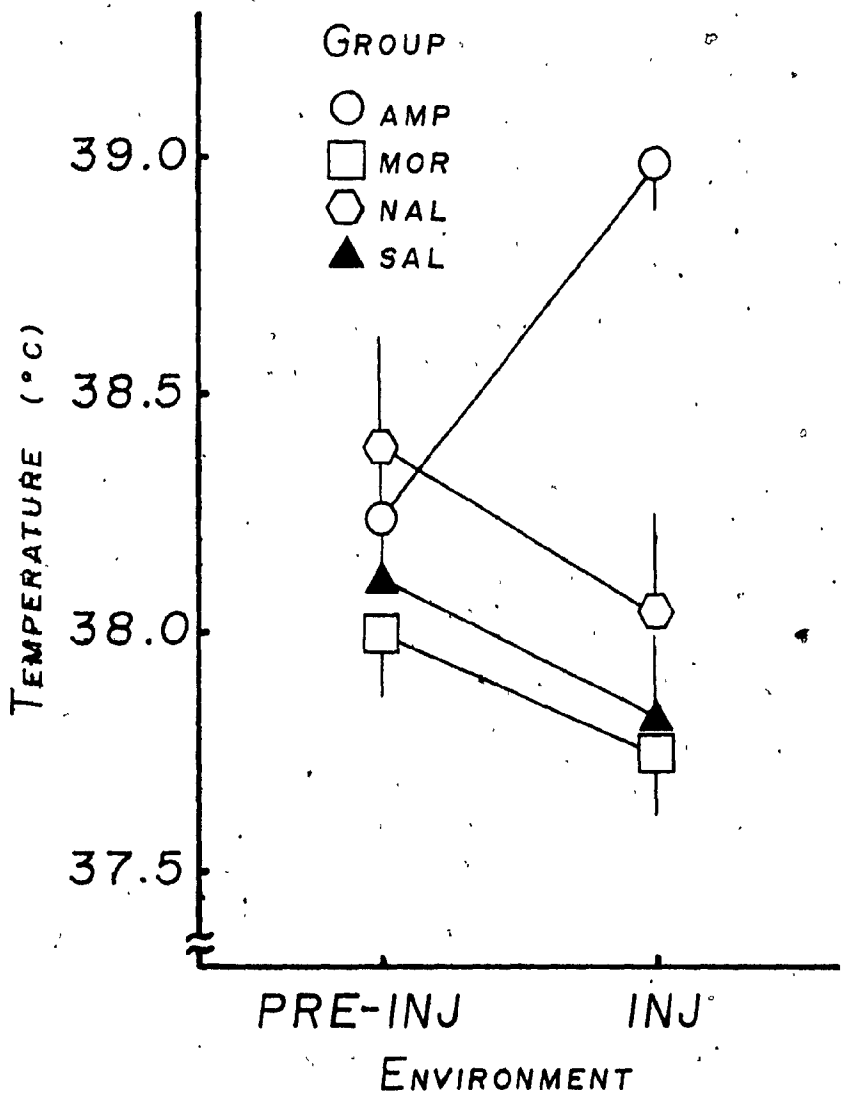

Figure 18. Mean body temperature of animals in each of the four groups taken in the pre-injection environment (PRE-INJ) and in the injection environment (INJ) at the usual times on Day 26. All animals received saline injections.

surprising that when presented together these stimuli appear to have so little effect on temperature in these animals.

On both the conditioning test days, Days 26 and 33, the animals' temperature was measured in the home cage at 15:30 h and 18:30 h. Figure 19 shows the temperatures of animals in the four groups at these times, averaged over the two test days. Two Group x Day analyses of variance revealed that only at 15:30 h did the temperatures of animals in the groups differ significantly (see Appendix, Tables 31 and 32). A comparison between Figure 14, showing the unconditioned drug effect, and Figure 19 revealed that at 15:30 h the temperatures of animals in the four groups after saline injections were similar to, but not as pronounced as, the unconditioned drug effect.

To test whether the activity-increasing effects of these drugs are conditionable the motility of all animals was determined on Day 33 when the animals received saline in the conditioning environment at the wrong time of day. Figure 20 shows the mean motility of animals in the four groups in both the pre-injection and injection environments. A Group x Environment analysis of variance revealed that both the Group effect and the Group x Environment interaction were significant (see Appendix, Table 33).

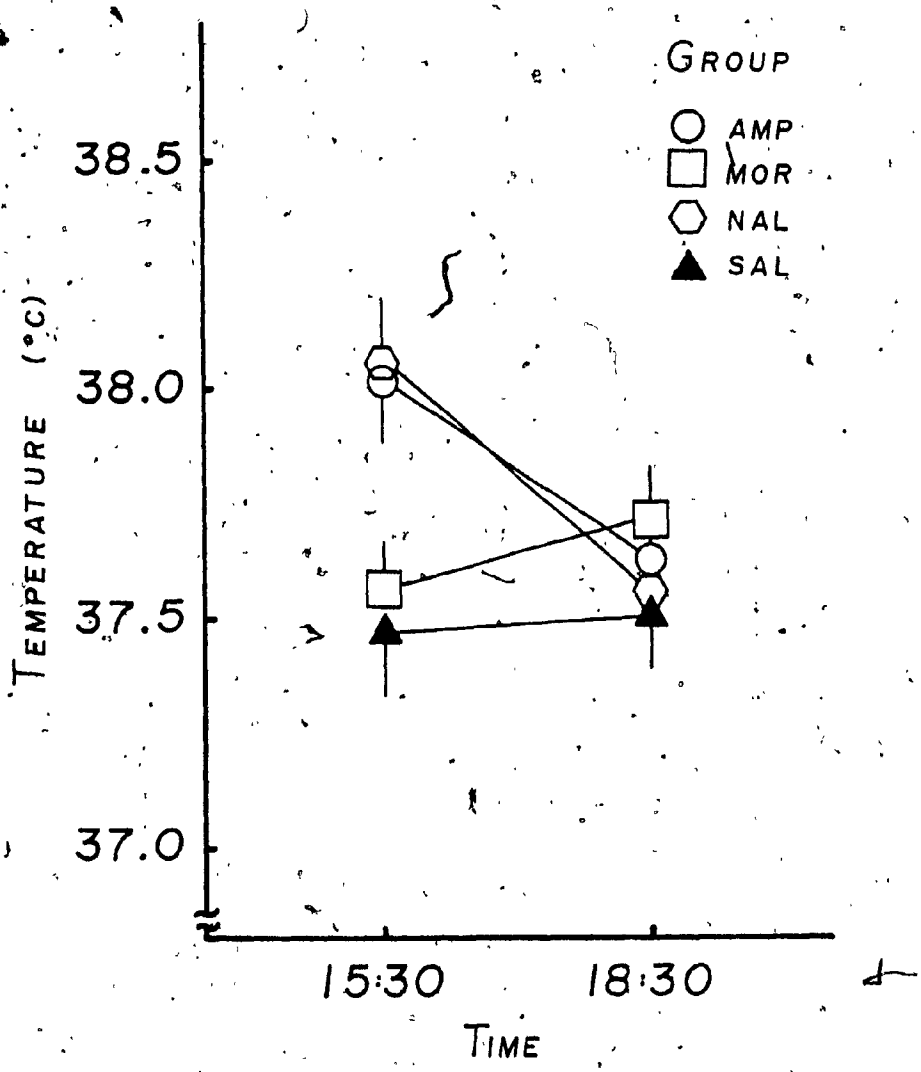

Figure 19. Mean body temperature of animals in each of the four groups averaged over the two conditioning test days, Days 26 and 33, when no drugs were administered, taken at 15:30 h and 18:30 h in the home cage.

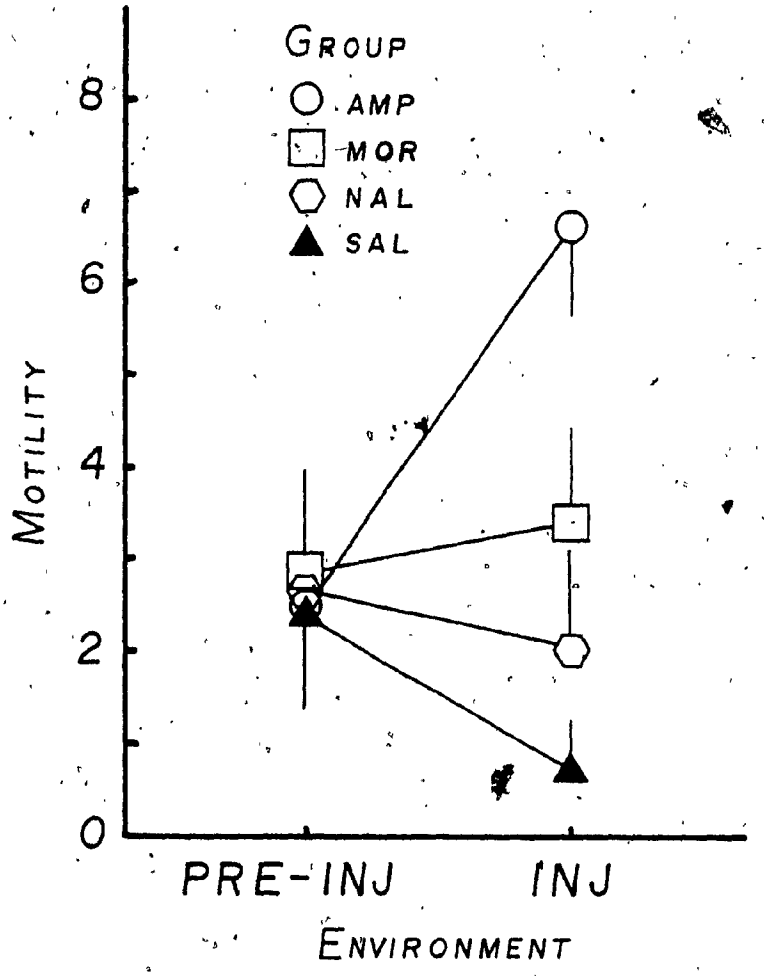

Figure 20. Mean motility score of animals in each of the four groups in the pre-injection environment (PRE-INJ) and in the injection environment (INJ) on the evening of Day 33. All animals received saline injections.

Simple main effects for a repeated measure design (Winer, 1971) revealed that in the pre-injection environment the group differences were not significant ($F < 1$), while in the injection environment there were significant group differences ($F(3, 31) = 6.68$, $p < .01$). It is clear from Figure 20 that this reflects the fact that only in the injection environment were the amphetamine- and morphine-group animals more active than animals in the saline group. In contrast, temperature measurements taken at the same time revealed that the groups differed in both the pre-injection and injection environment (see Appendix, Table 29 and Figure 17). Thus in the pre-injection environment, while morphine- and amphetamine-group animals were hyperthermic relative to the saline group animals, they were not more active than the saline-group animals.

At the end of the drug-free period animals in all four groups had similar body temperatures in the home cage. Three Group x Day analyses of variance, one for each of the three daily temperature measurements, were carried out for the last two days of the drug-free period, Days 47 and 48. At no time was the Group effect or the Group x Day interaction significant (see Appendix, Tables 34-36).

On Day 49 animals were reintroduced to the two conditioning environments. They were treated as on a normal

conditioning day, but received saline injections. Figure 21 shows the temperatures and the motility scores of animals in the four groups in both the pre-injection and injection environments for this day. Analyses of variance revealed that in both environments the body temperatures of the four groups differed significantly (see Appendix, Tables 37 and 38). Animals in the amphetamine and morphine groups were hyperthermic relative to animals in the saline group in both environments. In contrast, analyses of variance of the motility scores of the animals revealed that the groups only differed significantly in the injection environment (see Appendix, Tables 39 and 40). Animals in the morphine and amphetamine groups were more active than saline-group animals in the injection environment. Note that, again in the pre-injection environment, while there were significant temperature differences between animals in the four groups, there were no significant differences in the activity of the animals.

On the second test day after the drug-free period, Day 50, the temperatures of animals in the four groups no longer differed in the pre-injection environment (see Appendix, Table 41). On this day, as on the previous day, the motility scores of the animals in the four groups did not differ significantly in the pre-injection environment

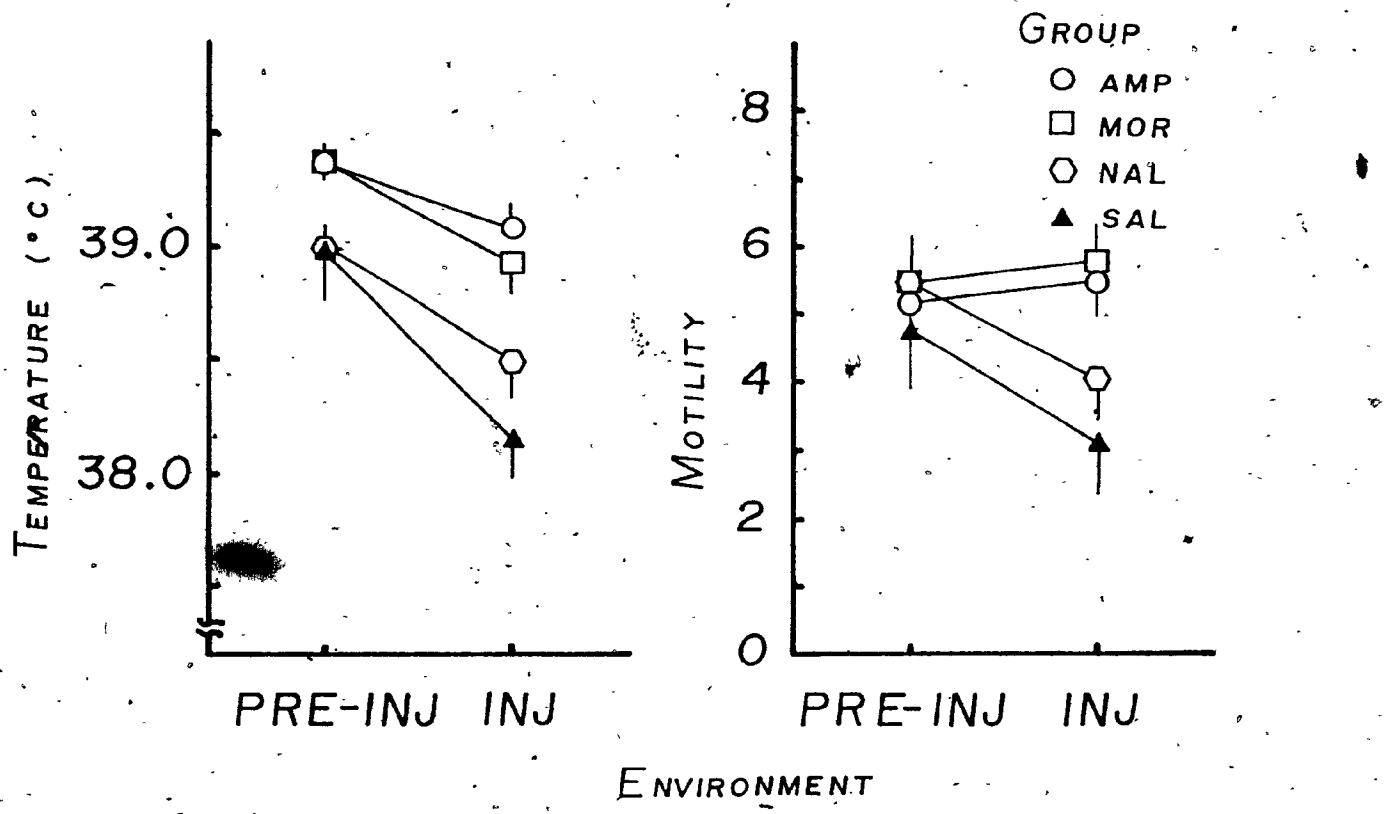

Figure 21. Mean body temperature and motility score of animals in each of the four groups taken in the pre-injection environment (PRE-INJ) and in the injection environment (INJ) on the first conditioning test day after the drug-free period, Day 49.

(see Appendix, Table 42). Thus the temperature of the animals in the four groups differed in the pre-injection environment only on the first test day after the drug-free period, but in this environment there were never any significant activity differences.

The group differences in the injection environment, both in temperature and activity, lasted several days before gradually disappearing. Figure 22 shows the temperature and motility scores of animals in the four groups over the seven extinction trials that took place after the drug-free period. Analyses of variance for each day revealed that there were significant group differences in temperature until Day 61 (see Appendix, Tables 43-48). The group differences in motility score were evident only until Day 56 (see Appendix, Tables 49-54). Thus the group differences in body temperature appeared to extinguish more slowly than the group differences in motility.

Discussion

The direction of the conditioned temperature responses observed in this experiment was a joint function of the drug used as the unconditioned stimulus and of the type of conditioned stimulus used to elicit the response.

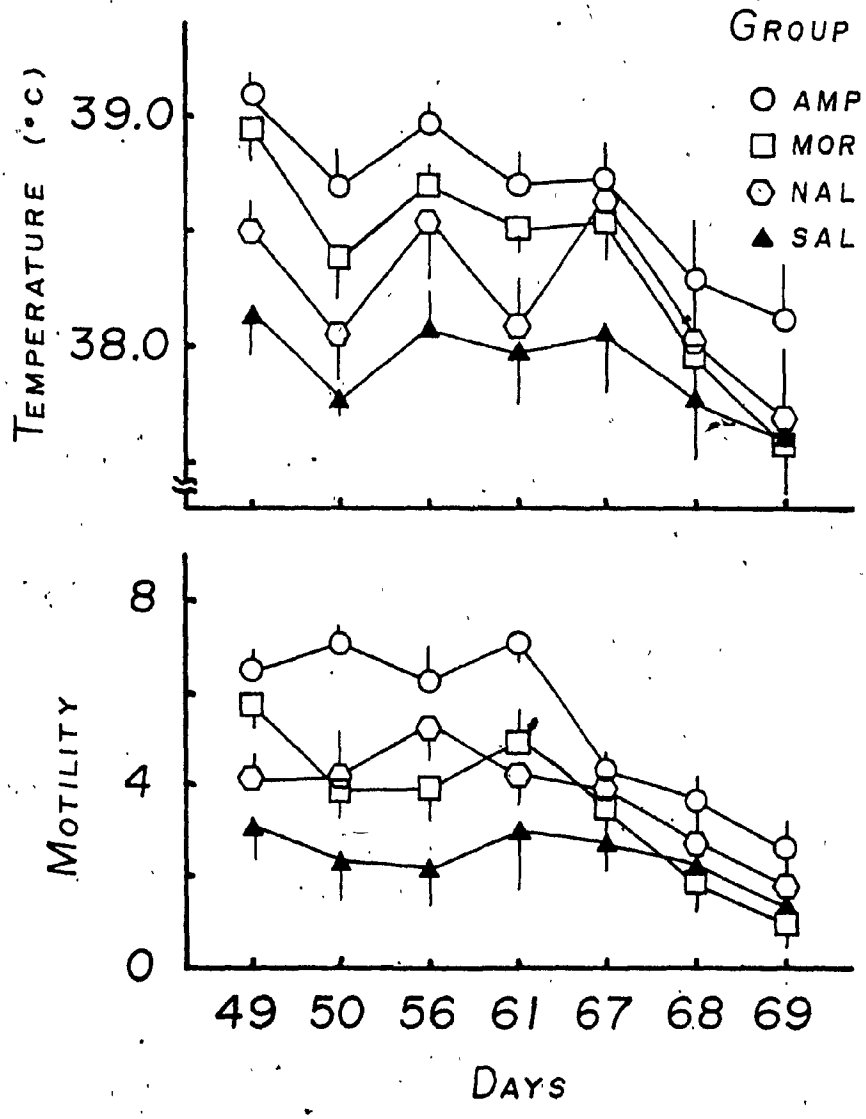

Figure 22. Mean body temperature and motility scores of animals in each of the four groups taken in the injection environment over the seven days that extinction trials were carried out.

Morphine and amphetamine both produced an unconditioned hyperthermia that lasted several hours. For morphine- and amphetamine-group animals a conditioned hyperthermia was evident in response to environmental stimuli, both prior to and after the drug-free period. As seen in previous experiments, morphine-group animals also become hypothermic in the presence of conditioned temporal cues. Naloxone administration resulted in a hypothermia that, while it was not very large, was evident after every injection. Later in the day, several hours after naloxone administration, animals in the naloxone group were hyperthermic relative to animals in the saline group. This finding has since been replicated (Eikelboom & Stewart, Note 2). The hyperthermia does not appear to be a direct effect of naloxone inasmuch as this drug has a plasma half life of only twenty minutes (Misra, Pontani, Vadlamini & Mule, 1976; Weinstein, Pfeffer & Schor, 1974). In naloxone-group animals, before the drug-free period, temporal cues elicited a conditioned hyperthermia while presentation of environmental cues resulted in a conditioned hyperthermia. After the drug-free period no conditioned responses were evident in naloxone-group animals.

Both morphine- and amphetamine-group animals were more active after their drug injections than animals

receiving saline. Conditioned increases in activity were observed in these two groups of animals but did not appear to be correlated with conditioned temperature changes. The conditioned hyperthermia could be elicited in morphine- and amphetamine-group animals in the pre-injection environment where there were no conditioned activity changes. During extinction the conditioned increases in activity disappeared before the conditioned hyperthermia. This suggests that conditioning of temperature changes occurs separately from conditioning of changes in activity.

EXPERIMENT 4

In Experiment 3 amphetamine administration resulted in a strong conditioned hyperthermic response. This conditioned response was elicited by environmental stimuli. In an attempt to study the conditioning of temperature responses further an experiment was done using several doses of amphetamine. The animals used had previously been in a naloxone dose-response study (Stewart & Eikelboom, 1979, Experiment 2).

Method

Subjects

Twenty-nine male Wistar rats, weighing 175 to 200 g at arrival, were obtained from the same supplier and housed under the same conditions as animals in the previous experiments. Animals were randomly assigned to one of four groups differing in the daily dose of d-amphetamine administered. Group SAL animals, n = 7, were injected with physiological saline; Group-1 animals, n = 7, were injected with 1 mg/kg of d-amphetamine; Group-2 animals, n = 7, were injected with 2 mg/kg of d-amphetamine; and Group-5 animals, n = 8, were injected with 5 mg/kg of d-amphetamine.

Two animals from Group-5 died after several days of amphetamine injections. All data collected from these animals were excluded from the various analyses. Thus Group-5 consisted of only 6 animals.

The naloxone experiment

Once every second day, for eight test days, animals were moved to a room where they were housed for three and a half hours in the boxes used in these conditioning studies, they were injected with saline most days and had their temperatures measured four times at 45 min. intervals. The test room was quiet and well lit. On the first, sixth and eighth day of this experiment animals were randomly assigned to groups receiving either 0, 1, 2.5, 10 or 25 mg/kg of naloxone. After this experiment finished animals remained in their home cage for ten days prior to starting the present experiment. Animals were then randomly assigned to the groups of the present study.

Design

Experiment 4 was similar to Experiment 3 in design and methodology. On conditioning days at 11:15 h, after having spent 90 min in the pre-injection environment, animals were moved to, and injected in, the injection environment where they remained for 90 min. Body temperature

was measured daily at 9:00 h in the home cage; at 10:30 h in the pre-injection environment, 45 min before the injection; and at 12:00 h in the injection environment, 45 min after the injection.

As in previous experiments there was a two day habituation phase when the animals underwent the conditioning procedures, but were injected with saline. With the exceptions noted below, Days 3 to 44 were conditioning days. On Days 7 and 8, after four conditioning days, animals were left unhandled in their home cages. Tests for conditioning, when all animals underwent the normal routine but were injected with saline, occurred on Days 25 and 31. On Days 19 and 45 animals remained in their home cages, were injected with saline, and had their temperatures measured at the usual times. Day 39 was a similar home-cage test day but included an additional test in the afternoon. Animals were placed in the conditioning environments, were injected with saline, and had their temperatures measured, all in the normal temporal sequence, but 6.5 hours later than usual.

On Days 45 to 49, the drug-free period, animals remained in their home cages, were not injected, but had their temperatures taken at the usual times. Two conditioning test days followed, Days 50 and 51, when the

normal conditioning routine was followed, but all animals were injected with saline.

Procedure

Throughout the study all drugs were injected intraperitoneally at a volume of 1 ml/kg. Solutions were made up in the physiological saline used for the saline injections. During conditioning, animals in Group SAL, Group-1, Group-2 and Group-5 received daily injections of 0, 1, 2 and 5 mg/kg d-amphetamine sulfate, respectively. Temperature measuring procedure and the conditioning environments were the same as those used in the earlier experiments.

Results

The habituation days' data for all animals were analyzed to see if groups differed because of their previous experience. Three Group x Day analyses of variance, one for each of three daily temperature measurements, were done using the two habituation days of the experiment (see Appendix, Tables 55-57). At no time was the Group effect or the Group x Day interaction significant. Thus it was concluded that the groups were equivalent at the start of this experiment.

Figure 23 shows the temperature of animals in all groups, forty-five minutes after the daily drug injection, for the first eight conditioning trials, Days 3 to 12. A Group x Day analysis of variance for the first four days, Days 3 to 6, revealed that both main effects and the Group x Day interaction were significant (see Appendix, Table 58). It is clear from Figure 23 that the interaction is due to the change, relative to animals in other groups, in Group-5 animals over the four days; from 1.5°C more hyperthermic on Day 3 to no different from other amphetamine groups on Day 6. The tolerance of the amphetamine-induced hyperthermia is most evident in Group-5 animals but can also be seen in Group-2 animals. Figure 23 shows that the two-day break resulted in a reinstatement of the amphetamine hyperthermia. A Group x Day analysis of variance of Days 9 to 12 revealed that both main effects and the Group x Day interaction were significant (see Appendix, Table 59). These effects again reflect the decrement over days of the amphetamine hyperthermia in Group-5 and Group-2 animals. Note, however, that in both periods tolerance was not complete.

Figure 24 shows the temperatures of animals in the four groups in the pre-injection environment forty-five minutes before the daily injection for the same eight

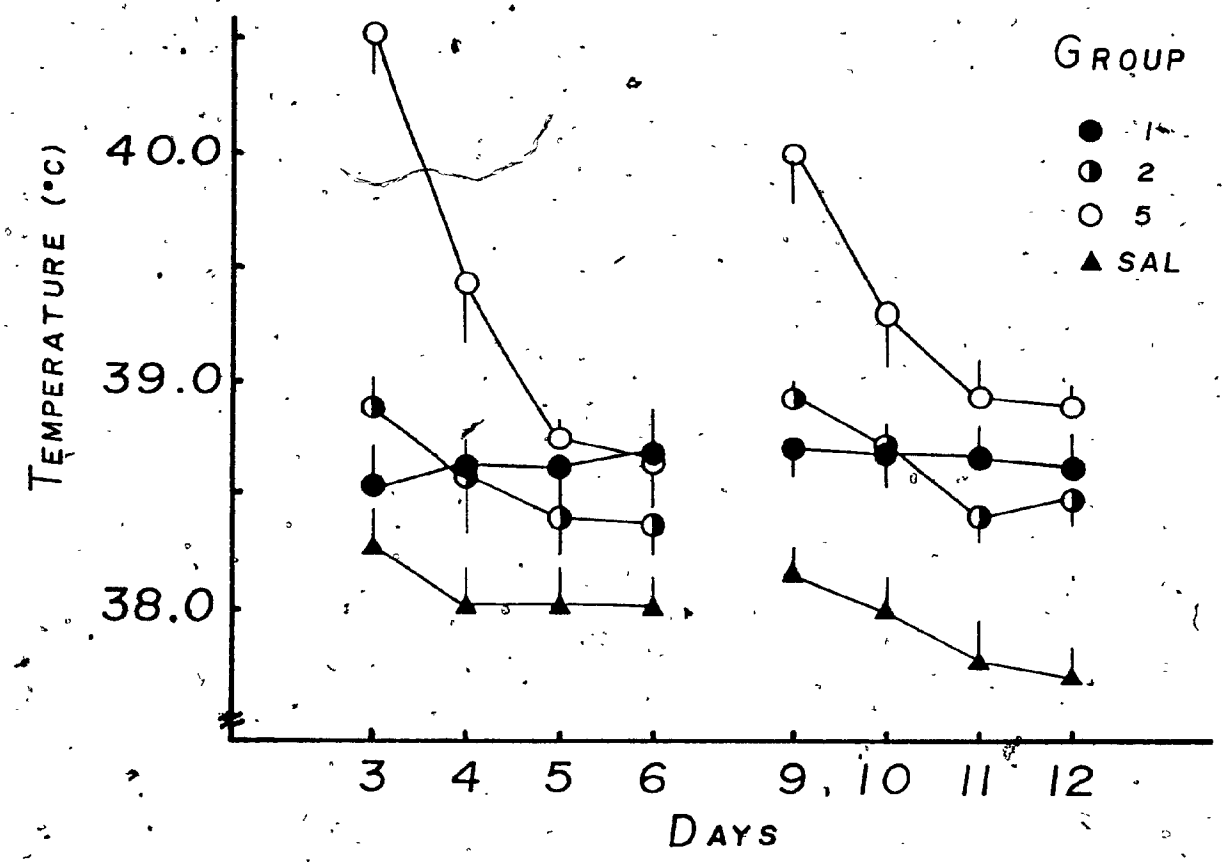

Figure 23. Mean body temperature of animals in each of the four groups taken at 12:00 h in the injection environment on the first eight days amphetamine was administered, Days 3 to 12.

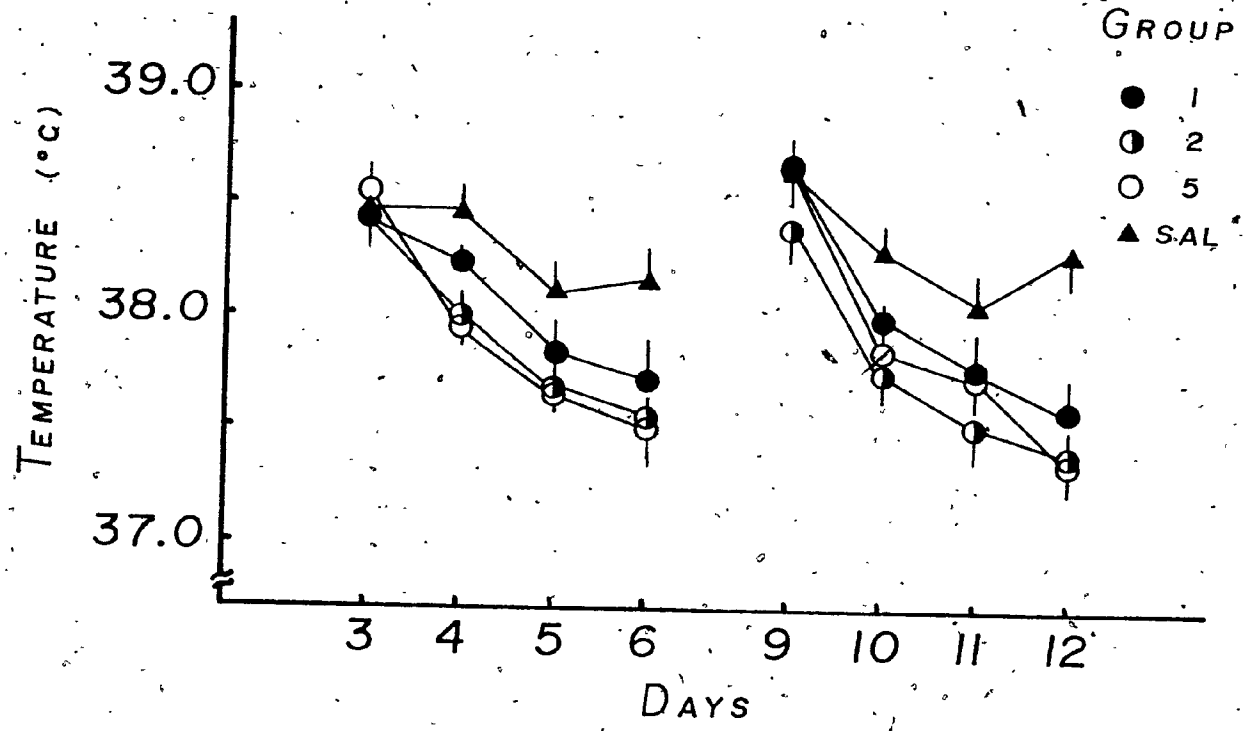

Figure 24: Mean body temperature of animals in each of the four groups taken at 10:30 h in the pre-injection environment on the first eight conditioning days of the experiment, Days 3 to 12.

days; Group x Day analyses of variance for Days 3 to 6 and Days 9 to 12 revealed that in both cases the main effects and interaction were significant (see Appendix, Tables 60 and 61). It is clear from Figure 24 that both interactions reflect the fact that over the two four-day periods animals in the amphetamine groups gradually became hypothermic relative to animals in the saline group. This hypothermia disappeared over the two-day home-cage period.

Figure 25 shows the 10:30 h temperature of animals in the four groups averaged over the two home-cage test days, Days 19 and 45, and over the two test days in the pre-injection environment, Days 25 and 31. A Group x Environment analysis of variance, using individual animal mean temperatures for each set of two test days, revealed that only the Group effect was significant (see Appendix, Table 62). The lack of a significant interaction means that the hypothermia seen in the amphetamine-group animals at 10:30 h was just as strong in the home cage as in the pre-injection environment. This suggests that environmental stimuli are not involved in eliciting the hypothermia in amphetamine-group animals.

The hypothermia in the amphetamine-group animals could be due to an indirect aftereffect of amphetamine

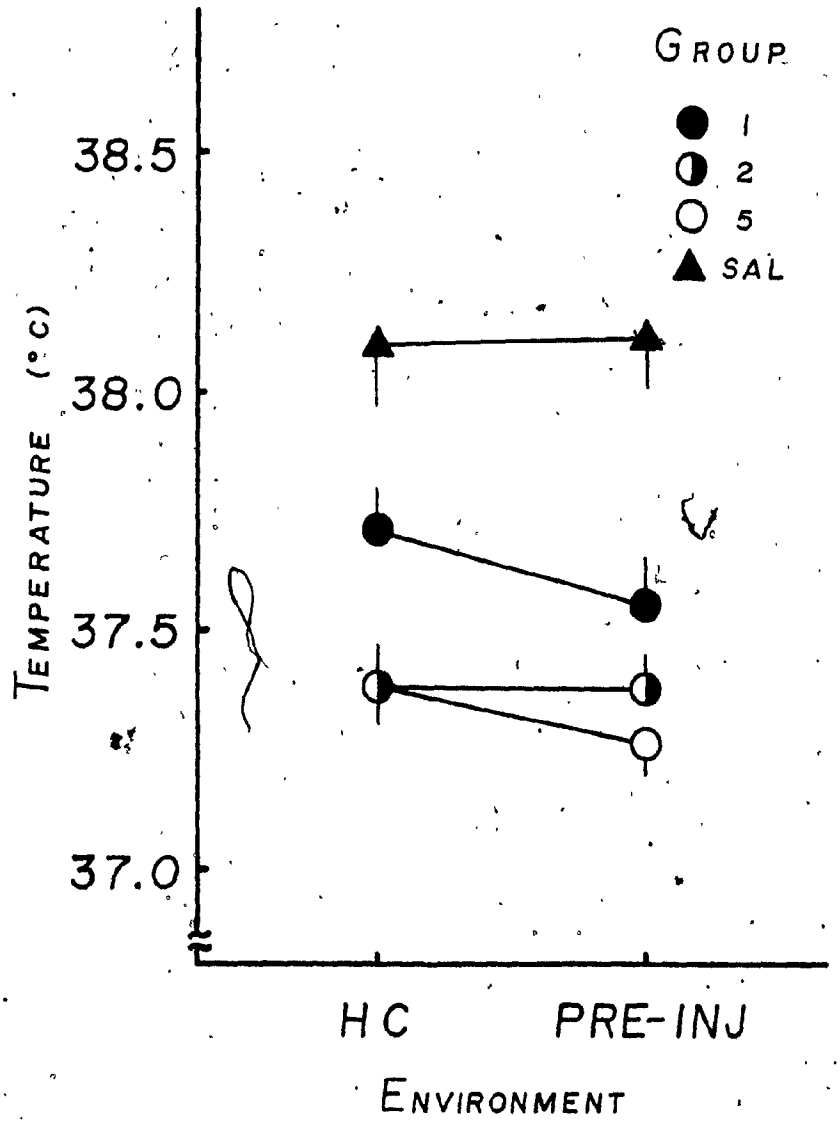

Figure 25. Average mean body temperature of animals in each of the four groups taken at 10:30 h in the home cage (HC) or in the pre-injection environment (PRE-INJ) on saline test days during the period of conditioning. Each point represents the average of two test days.

or it could reflect conditioning to temporal cues. If the hypothermia were due to an aftereffect of the amphetamine injection, then once gone it should not reoccur until after the next amphetamine injection. If, however, temporal cues are eliciting the hypothermia, it should reoccur daily around the time of the injection whether or not the animals received an injection 23 hours earlier. On Day 39, animals had their temperatures measured in the home cage at 10:30 h and in the pre-injection environment at 17:00 h; approximately 23 and 29 hours after the last drug injection. No injection of amphetamine was given on Day 39. On Day 40 at 10:30 h, approximately 47 hours after the last amphetamine injection, animals again had their temperatures measured in the pre-injection environment. Figure 26 shows the temperatures of animals in the four groups taken on these three occasions. A Group x Time analysis of variance revealed that both main effects and the interaction were significant (see Appendix, Table 63). Simple main effects for a repeated measure design (Winer, 1971) revealed that while at 17:00 h the groups did not differ ($F < 1$) at 10:30 h on both days there was a significant group difference ($F(3, 54) = 4.31$, $p < .01$; $F(3, 54) = 4.98$, $p < .01$). The hypothermia that was evident in amphetamine-group animals at 10:30 h disappeared by 17:00 h only to reappear the next day at 10:30 h.

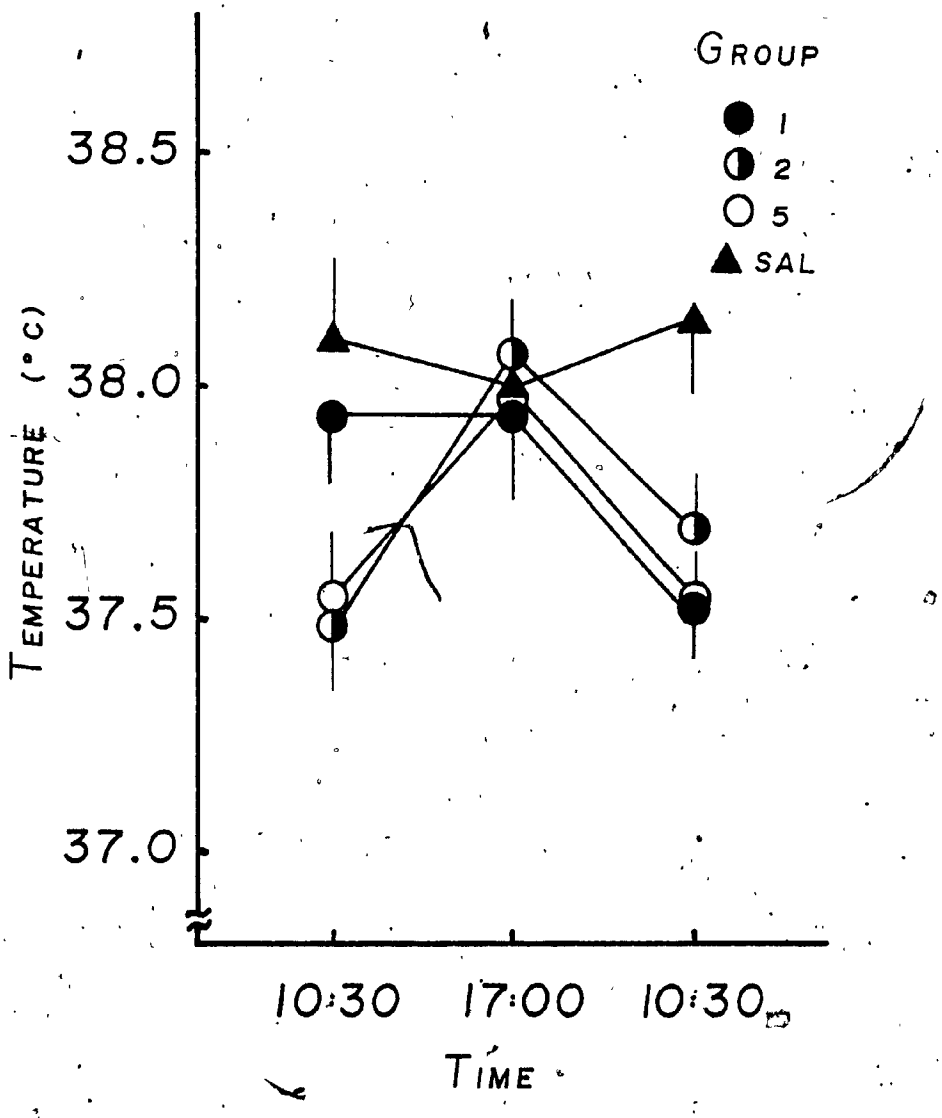

Figure 26. Mean body temperature of animals in each of the four groups taken at 10:30 h, 17:00 h and 10:30 h, 23, 30, and 47 hours respectively after the last amphetamine injections, Days 39 and 40 of the experiment.

This suggests that the hypothermia was a conditioned effect elicited by temporal cues.

The temperatures of animals taken at 10:30 h, 45 min before the saline injection, in the home cage at the end of the drug-free period and in the pre-injection environment after the drug-free period is shown in Figure 27. Each point represents the means for two test days, Days 48 and 49 in the home cage and Days 50 and 51 in the pre-injection environment. Two Group x Day analyses of variance revealed that in both the home cage and pre-injection environment only the Day effect was significant (see Appendix, Tables 64 and 65). It appears that after the drug-free period, as in previous experiments with morphine, the conditioned hypothermia in amphetamine-group animals is no longer evident either in the home cage or in the pre-injection environment.

As in Experiment 3, the stimuli of the injection environment elicited a conditioned hyperthermia in amphetamine-group animals. Figure 28 shows the 12:00 h body temperature of animals in all groups averaged over two test days in the home cage, Days 19 and 45, and over two test days in the injection environment, Days 25 and 31. Note that these tests occurred prior to the drug-free

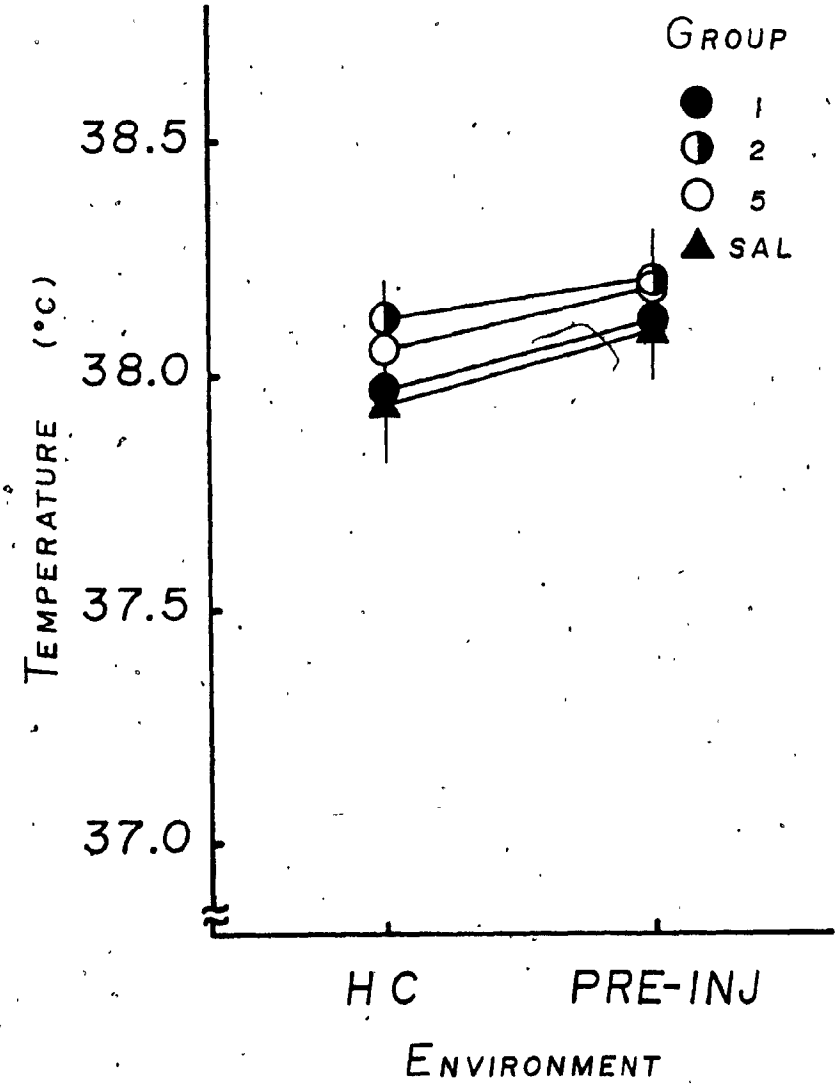

Figure 27. Average mean body temperature of animals in each of the four groups taken at 10:30 h in the home cage (HC) or in the pre-injection environment (PRE-INJ) on saline test days after the drug-free period. Each point represents the average of two test days.

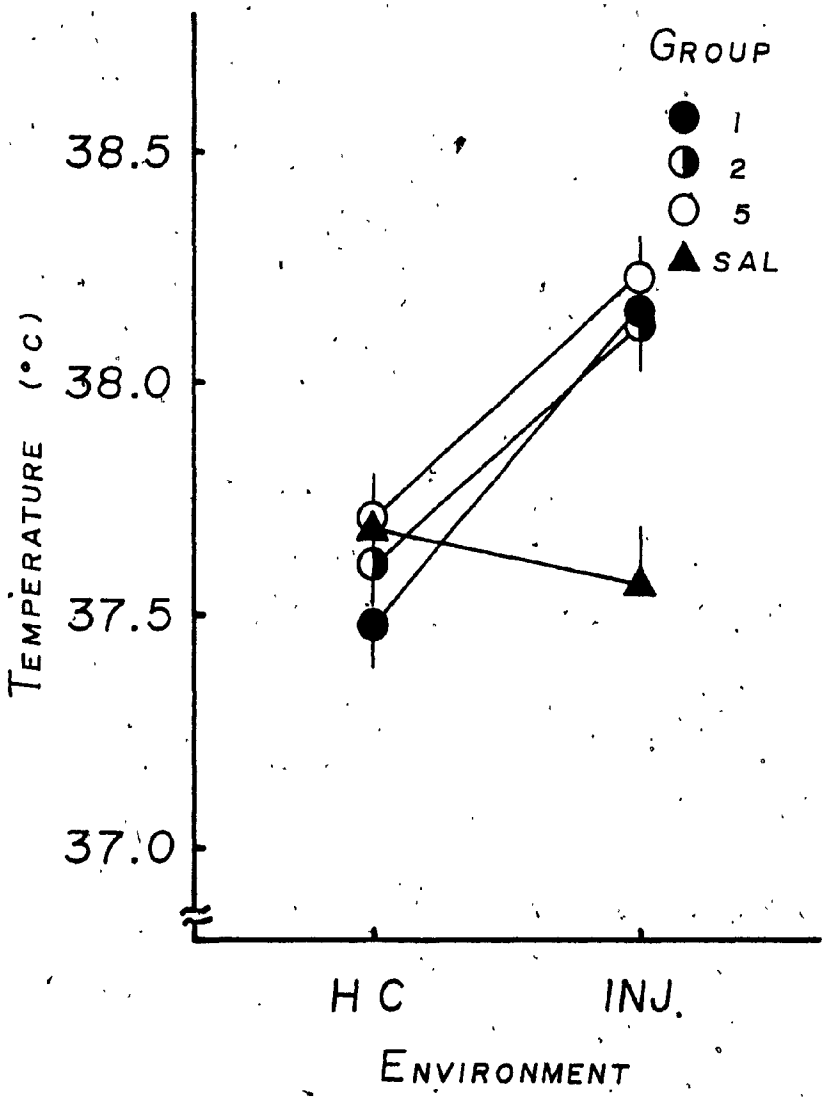

Figure 28. Average mean body temperature of animals in each of the four groups taken at 12:00 h in the home cage (HC) or in the injection environment (INJ) on saline test days during the period of conditioning. Each point represents the average of two test days.

period. A Group x Environment analysis of variance, using individual animal means for each set of two test days, revealed that the Environment effect and the Group x Environment interaction were significant (see Appendix, Table 66). This reflects the fact that at 12:00 h there were no differences between the groups in the home cage, whereas in the injection environment amphetamine-group animals were hyperthermic relative to saline-group animals.

On Day 39 animals had their temperatures measured in the injection environment at 18:30 h. An analysis of variance revealed that even at this time there were significant group differences (see Appendix, Table 67). Animals in the amphetamine groups were hyperthermic relative to animals in the saline group. Thus the injection environment was able to elicit a conditioned hyperthermia even in the absence of temporal cues.

After the drug-free period the conditioned hyperthermia could be elicited by placing the amphetamine group animals in the injection environment. Figure 29 shows the 12:00 h temperature of animals in the four groups averaged over the last two home-cage days of the drug-free period, Days 48 and 49, and over the two

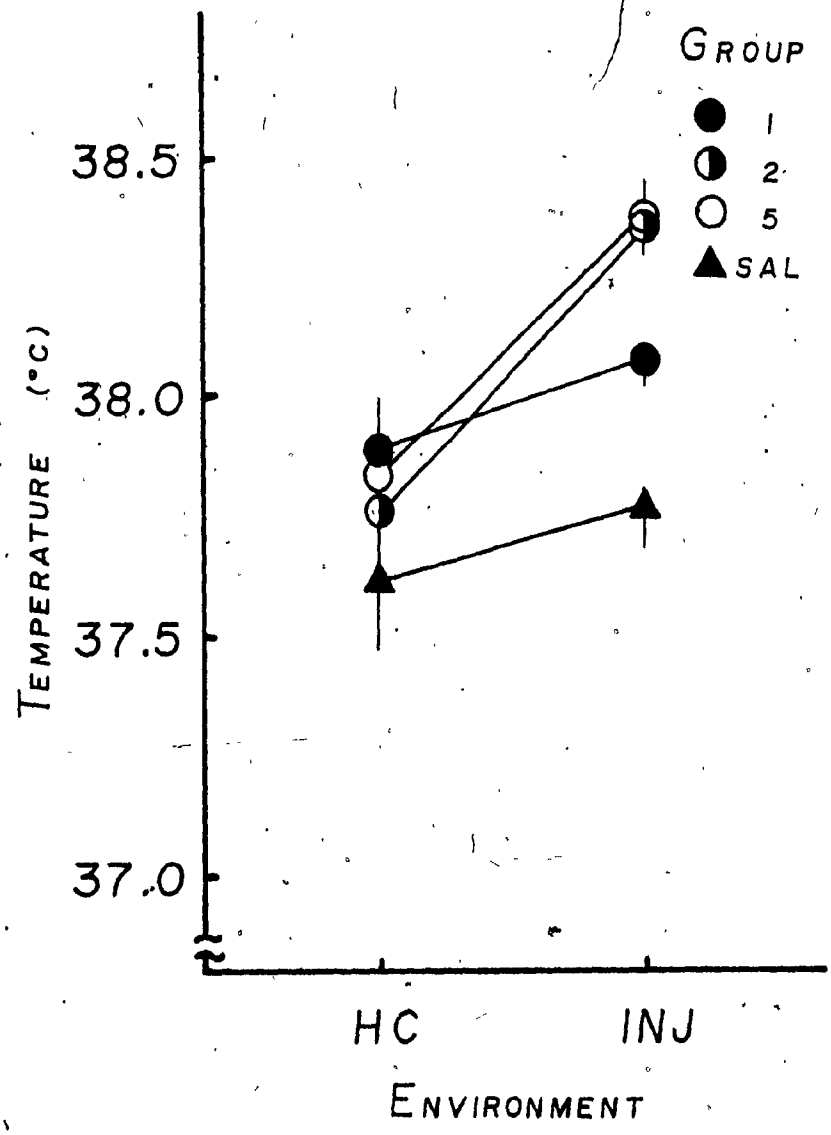

Figure 29. Average mean body temperature of animals in each of the four groups taken at 12:00 h in the home cage (HC) or in the injection environment (INJ) on saline test days after the drug-free period. Each point represents the average of two test days.

conditioning test days in the injection environment, Days 50 and 51. Group x Day analyses of variance revealed that while the groups of animals did not differ in the home cage, there were significant group temperature differences in the injection environment (see Appendix, Tables 68 and 69). After the drug-free period the amphetamine-group animals still became hyperthermic, relative to saline-group animals, when returned to the injection environment.

Discussion

The presentation of environmental stimuli associated with the injection of amphetamine resulted in the elicitation of a conditioned hyperthermic response. Amphetamine-group animals were hyperthermic relative to saline-group animals in the injection environment both before and after the drug-free period. This conditioned response mimicked the unconditioned hyperthermic effects of amphetamine. There was no evidence in this study that the conditioned hyperthermic response could be elicited in the pre-injection environment, either prior to or after the drug-free period. In Experiment 3 amphetamine-group animals showed a conditioned hyperthermia even in the pre-injection environment. This difference

may be due to the conditioned hypothermia, evident only in the present experiment, which, though under the control of temporal cues, normally occurred when the animals were in the pre-injection environment.

In this experiment amphetamine administration resulted in a second conditioned response, a conditioned hypothermia; under the control of temporal cues. As the hypothermia always occurred approximately 23 hours after the amphetamine injection it was necessary to demonstrate that this hypothermia was not due to a delayed amphetamine aftereffect. On days when amphetamine was not administered, the animals in the amphetamine groups were hypothermic around the time of the injection but not later in the day. The next day, however, around the time of the daily injection, approximately 47 hours after the amphetamine injection, the amphetamine-group animals were again hypothermic relative to saline-group animals. This suggests that the hypothermia is being elicited by temporal cues. Further, if this hypothermia was an amphetamine aftereffect, it should also have been evident in the third experiment in which a similar dose of amphetamine was administered to amphetamine-group animals. While there may be several reasons for the nonoccurrence of a conditioned response, a drug aftereffect being a

consequence of the drug itself should always occur. Thus it appears that the hypothermia seen in the amphetamine-group animals in the present experiment is a conditioned response under the control of temporal cues.

The magnitude of the hyperthermia caused by amphetamine administration was dose-related. However, unlike in Experiment 3, the magnitude of the hyperthermia showed some decrement over trials, at least at the higher doses. This tolerance of the amphetamine hyperthermia appeared to be due to the rapid development of the conditioned hypothermia. Over the first four days in the pre-injection environment, just prior to the injection, the amphetamine-group animals became hypothermic relative to saline-group animals. This conditioned hypothermia developed over the same period that the hyperthermic effect of amphetamine decreased. When the conditioned hypothermia was extinguished by not injecting the animals for two days, the magnitude of the amphetamine hyperthermia also returned to its initial level. In Experiment 3 where there was no evidence of the amphetamine hyperthermia decreasing over days there was also little evidence for a conditioned hypothermia. This strongly suggests that tolerance of the amphetamine hyperthermia is due to the development of a conditioned hypothermic response.

The major discrepancy between Experiment 3 and the present experiment was that the conditioned hypothermia was evident, in amphetamine-group animals, only in the present experiment. The major procedural difference in these two experiments was that in the present experiment animals were not naive at the start of the experiment. In fact the animals all had extensive pre-exposure to the procedures and environmental stimuli used in this experiment. It may be that this pre-exposure decreased the salience of the environmental stimuli, permitting conditioning to temporal cues. It is interesting that in Experiment 3 the conditioned hyperthermia observed in the amphetamine-group animals was stronger that that observed in the same dose group in the present experiment, both before and after the drug-free period. This suggests that when environmental stimuli are not degraded, they may result in a stronger conditoned response. These differences between Experiment 3 and Experiment 4 may also explain why, though in most studies tolerance occurs to amphetamine hyperthermia (Brodie, Cho, Stefano & Gessa, 1969; Gessa, Clay & Brodie, 1969; Harrison et al, 1952; Lewander, 1971; Lewander, Moliis & Brus, 1975), there are several reports that there is no tolerance evident to the hyperthermic effects of amphetamine (Obal, 1966; Thornhill, Hirst & Gowdey, 1977; Ulus & Kiran, 1975; Ziem, Coper, Broermann & Strauss, 1970).

GENERAL DISCUSSION

A consistent, but complex, picture emerges from these four experiments. Each of the drugs tested, morphine, naloxone, and amphetamine, supported two different and opposing conditioned responses. These two conditioned responses were elicited by different types of stimuli; one response was controlled by environmental stimuli, the second by temporal cues. The conditioned response elicited by the environmental stimuli had the same effect on body temperature as did the drug that initially elicited it. The conditioned response that occurred in the presence of the temporal cues had an effect on body temperature opposite to that directly produced by the drug administration. Conditioning experiments involving amphetamine and morphine, both of which had a hyperthermic effect on animals, resulted in a conditioned hyperthermia when animals were presented with the environmental stimuli, and in a conditioned hypothermia in the presence of temporal cues. Naloxone, which causes hypothermia, resulted in a conditioned hypothermia elicited by environmental stimuli and in a conditioned hyperthermia elicited by temporal cues.

The results of the experiments using morphine are consistent with most previous work in this area but

suggest the situation is not as simple as previous results would imply. As discussed earlier, most conditioning studies using morphine have reported a conditioned hyperthermic response. In all of these studies either environmental or other discrete external stimuli were used as conditioning stimuli. The results of these previous studies and of the present experiments are consistent with the finding that morphine acts prior to the thermoregulatory integrator to produce its hyperthermic effects; the conditioned response is similar to the observed drug effect as would be expected with a drug that acts prior to the integrator.

Only one other investigator has reported a conditioned hypothermia following repeated morphine administration (Siegel, 1978). Although at first sight the conditioned hypothermia seen in the present experiments appear similar to that reported by Siegel (1978), there are several reasons for suggesting that it may be different. In the present experiments the conditioned hypothermia seen in the morphine-group animals was not elicited by environmental stimuli but was under the control of daily temporal cues; Siegel (1978) was able to elicit a conditioned hypothermia using environmental stimuli and a 48-hour inter-injection interval. In the

present experiments the conditioned hypothermia was never evident after a drug-free period. Siegel (1978) found that unless explicit extinction trials were given, the conditioned response survived a drug-free rest period. More importantly, Siegel (1978) related the conditioned hypothermia observed in his experiments to tolerance of the hyperthermic effects of morphine. In the present experiments morphine continued to produce a marked hyperthermia even after repeated administrations. At present there is no good explanation for these differences.

The conditioned hypothermia observed in the morphine-group animals in the present experiments appears to be a conditioned withdrawal effect. There have been several studies showing that the physiological changes observed during withdrawal could be conditioned (see introduction). In rats hypothermia is one of the most reliable symptoms of opiate withdrawal (Ary & Lomax, 1979; Mucha et al, 1979). The interval between morphine injections in the present experiments was always twenty-four hours or more, an interval sufficient to produce the withdrawal symptoms (Martin et al, 1963). Indeed Wikler and Pescor (1967, 1970) used a 24-hour inter-injection interval to condition the "wet-dog shakes" that occur during morphine withdrawal. In the original study it was found that the

conditioned hypothermia was stronger in animals that were injected daily with 200 mg/kg of morphine, than in animals receiving smaller doses of morphine, suggesting the conditioned hypothermia was a function of the degree of dependence induced. It has recently been demonstrated, however, that withdrawal symptoms can be elicited without the administration of such large doses of morphine (Kosersky, Kowolenko & Howes, 1980). As in other conditioned withdrawal studies, the conditioned temperature responses in these experiments mimicked the withdrawal hypothermia. This suggests that withdrawal symptoms, like morphine effects, reflect changes occurring prior to the integrator.

Naloxone is a pure opiate antagonist and blocks the receptor to which morphine binds. With the discovery of the endogenous opioid peptides, a search for direct effects of naloxone itself was begun. Naloxone should block actions of these endogenous opioid peptides as well as actions of exogenous opiates. Simplistically it might therefore be expected that naloxone would result in changes opposite to those produced by morphine. While this is not always the case, it does seem to be true for thermoregulation; small doses of morphine produce a hyperthermia, and naloxone results in a hypothermia (Stewart &

Eikelboom, 1979). Chronic administration of naloxone or naltrexone (a longer lasting opiate antagonist) results in a supersensitivity of the opiate receptors (Amir & Amit, 1979; Lahti & Collins, 1978; Schulz, Wuster & Herz, 1979; Tang & Collins, 1978). This supersensitivity could result in rebound effects when the naloxone administration is terminated; a phenomenon that would be conceptually similar to, but probably opposite in direction to, the withdrawal effects seen on morphine termination.

Thus it may not be surprising that naloxone administration results in conditioned responses that are the mirror image of those obtained with morphine. In Experiment 3, the presentation to naloxone-group animals of the environmental stimuli used as the conditioning stimuli resulted in a conditioned hypothermia while in the same circumstances morphine-group animals showed a conditioned hyperthermia. In response to temporal cues naloxone-group animals became hyperthermic, and morphine-group animals became hypothermic. The fact that the conditioned responses observed in naloxone-group animals were weak would be expected on the basis that the unconditioned hypothermic effects of naloxone were not very pronounced.

Only one conditioning study has reported conditioned temperature changes using amphetamine as the unconditioned stimulus. Obal (1966) paired a discrete visual and auditory stimulus with hyperthermia-inducing injections of amphetamine. After several pairings, presentation of the conditioned stimulus alone resulted in a conditioned hyperthermic response. The amphetamine results of Experiment 3 and Experiment 4 are consistent with Obal's findings; the amphetamine-group animals showed a conditioned hyperthermia in the presence of conditioned environmental stimuli. These results imply that amphetamine produces its hyperthermic effect by an action prior to the thermoregulatory integrator.

While it might not be surprising that a hyperthermia-inducing drug such as amphetamine should have resulted in a conditioned hyperthermia, the development, in Experiment 4, of a conditioned hypothermia was unexpected. However, amphetamine, like morphine, appears to have multiple effects on body temperature. Some investigators report, as found here, that amphetamine administration results in hyperthermia (Brodie et al, 1969; Gessa et al, 1969; Harrison et al, 1952; Lewander, 1971; Lewander et al, 1975; Thornhill et al, 1977; Ulus & Kiran, 1975; Ziem et al, 1970), but others have reported an amphetamine-induced hypothermia (Cox & Lee, 1979; Jellinek, 1971; Kurk, 1972;

Kurk & Brittain, 1972; Yehuda & Frommer, 1978; Yehuda & Wurtman, 1972a, b, 1974). It appears that the dose of amphetamine administered is important in determining the direction of the response; low doses are more likely to result in a hypothermia, but other factors have also been implicated (Lewander, 1977; Yehuda & Frommer, 1978; Yehuda & Wurtman, 1972a). Although amphetamine affects several neurotransmitters, its hypothermic effects appear to be due to an action on the dopamine system. A major effect of amphetamine is to effectively increase the potency of the dopamine system (Lewander, 1977). In rats, dopamine has been implicated in heat loss mechanisms. Central administration of dopamine or administration of apomorphine, a dopamine receptor agonist, produces a hypothermia (Ary, Lomax & Cox, 1977a; Cox & Lee, 1977, 1979; Cox, Kerwin & Lee, 1978). Pimozide, a dopamine receptor antagonist, blocks these dopamine and apomorphine effects on temperature (Ary, Lomax & Cox, 1977a; Cox & Lee, 1977; Cox et al, 1978) and it also blocks amphetamine-induced hypothermia (Cox & Lee, 1977). This suggests that the amphetamine hypothermia is due to the activation of the dopamine system. It has been noted that the actual manifestation of the unconditioned response during conditioning is not necessary for development of the conditioned response (Crisler, 1930, Finch,

1938a; Light & Gantt, 1936). This suggests that though, in Experiment 4, only a hyperthermia was evident after the amphetamine injection, the conditioned hypothermia may have resulted from the effects of amphetamine on the dopamine neurons.

The finding that identical conditioned responses were observed to the two types of conditioned stimuli in amphetamine- and morphine-group animals suggests that both drugs may be acting through common mechanisms. It has become increasingly evident that the endogenous opioid peptide systems, the substrate on which morphine acts, interact at many levels with the catecholamine systems, the systems affected by amphetamine (Deyo, Swift & Miller, 1979; Moleman & Bruinvels, 1979; Pickel, Joh, Reis, Leeman & Miller, 1979; Schwartz, 1979). It has been suggested that chronic morphine administration results in a dopamine supersensitivity (Ary & Lomax, 1979; Schwartz, 1979; Smee & Overstreet, 1976). Specifically it appears that the hypothermia evident during morphine withdrawal involves a dopaminergic mechanism (Ary, Cox & Lomax, 1977). Pimozide, the specific dopamine antagonist, which itself has no direct effects on temperature, is able to block withdrawal hypothermia (Ary, Lomax & Cox, 1977b; Cox, Ary & Lomax, 1976). Pimozide also reverses the

effects, in morphine dependent animals, of naloxone on thermoregulatory behavior (Cox, Ary & Lomax, 1976) suggesting that this dopamine receptor involved in the withdrawal hypothermia occurs prior to the integrator. Apomorphine and dopamine greatly potentiate the hypothermia seen during withdrawal from morphine (Ary et al, 1977b). The reverse is also true; after chronic morphine administration the hypothermic effects of lateral ventricular injections of dopamine are potentiated (Ary & Lomax, 1979). Because the conditioned hypothermia seen in morphine group animals is assumed to be a conditioned withdrawal response, it most likely involves dopaminergic neurons. Similarly, as discussed earlier, the conditioned hypothermia, seen in amphetamine group animals, is also assumed to involve dopaminergic neurons. This suggests that both drugs may be having at least some of their effects on the same input pathway to a thermoregulatory integrator, and might be expected to result in similar conditioned responses.

There have also been studies demonstrating conditioned increases in dopamine turnover using drugs as unconditioned stimuli. Perez-Cruet (1976) reported that after repeated pairings of morphine or methadone with a conditioned stimulus, a 30 second buzzer, the presentation

of the conditioned stimulus alone resulted in increases in dopamine turnover. (Morphine and methadone administration produced unconditioned increases in dopamine turnover.) Subsequent work has shown that this conditioned increase in turnover was specific to dopamine; under similar circumstances there were no changes in serotonin turnover (Perez-Cruet, 1978). What makes these results particularly interesting is that conditioned increases in dopamine turnover have been observed in studies using amphetamine (Schiff, Bridger & Sharpless, 1978). It therefore appears that both amphetamine and morphine result in similar conditioned changes in dopamine turnover, and this may provide a common mechanism for the conditioned temperature responses seen in the present studies using these two drugs.

In light of the results of the present experiments the analysis of the conditioning of drug effects provided in the introduction seems oversimplistic. In the introduction the analysis revolved around a three-element feedback loop, consisting of sensor, integrator and effector. It was suggested that where in the system a drug had its effect, whether prior to or after the integrator, was critical in determining the nature of the unconditioned stimulus and unconditioned response. If the drug were to

act prior to the integrator, it would change an input to the integrator, and would be considered to be an unconditioned stimulus. The observed drug effect would result from the activation of an effector by the integrator, and would therefore be called the unconditioned response. Drugs that acted after the integrator would produce their observed effect without directly activating the integrator and would not, therefore, be labeled stimuli. In this case, however, because such a system involves feedback, the observed drug effect would act as an unconditioned stimulus and thus result in an unconditioned response, mediated via the integrator, that would oppose the observed drug effect. In either case, after conditioning, the conditioned response should be similar to the unconditioned response. Thus the conditioned response could be either similar to, or opposite to, the observed drug effect depending on whether the drug acted prior to or after the integrator. Note that for this analysis to have any predictive value, there must be some independent method for determining whether the drug acts prior to or after the integrator in any particular system. This analysis of the conditioning of drug effects implies that for any observed drug effect only a single conditioned response should be evident, a suggestion consistent with most previous work in this area. In the present experiments, drugs affecting body temperature resulted in two

different and opposing conditioned temperature responses. This suggests that a reappraisal of the present analysis of conditioned drug effects is necessary.

The analysis of the conditioning of drug effects revolved around three areas or concepts; the nature of the drug actions, the feedback systems involved, and the nature of conditioning. In the introduction the simplest model was analyzed; a drug with one effect, a three-element feedback system, and an idealized view of conditioning. It became apparent from the experiments, however that drugs can have multiple effects on temperature and that feedback systems can be more complex than the simple models presented in the introduction would suggest. In addition, the fact that the two responses conditioned in these experiments were elicited by different conditioned stimuli suggests that there can be selectivity of association between stimuli and responses.

Traditionally conditioning studies have measured only a single conditioned response, with the assumption that the choice of response is fairly arbitrary. It has long been known, however, that when using drugs that have multiple effects, conditioning and extinction of the various conditioned responses may occur at different rates, a

phenomenon Gantt (1953, 1966) labeled schizokinesis. One major point made by the present analysis is that the conditioning stimulus is paired with the unconditioned stimulus produced either directly or indirectly by the drug. Such a view makes it possible to handle the fact that a drug injection can result in several different unconditioned stimuli and unconditioned responses. Any drug action that changes an input to an integrator either directly, or indirectly through feedback, can be viewed as an unconditioned stimulus and can, therefore, support conditioning. If a drug changes the input to several integrators, it might result in several independent conditioned responses. Drugs that act prior to some integrators and after others would result in some conditioned responses that mimic and others that oppose the observed drug effect. Because these various conditioned responses are viewed as resulting from separate conditioning processes, there is no need to assume that the conditioning and the extinction of these various conditioned responses should occur at a uniform rate.

While most studies of the conditioning of drug effects have measured a single conditioned response, some have measured several different conditioned responses in the same animal. Morphine is a drug that has multiple

unconditioned effects. Rush et al (1970) showed that these morphine effects could be conditioned concurrently; conditioning with morphine as an unconditioned stimulus resulted in conditioned salivation, conditioned increases in gastric secretions. Korol et al (1966) studied the development of two conditioned responses using atropine, a drug that has the effect of blocking salivation and of causing a dilatation of the pupils. Over conditioning trials the animals developed both a conditioned salivation and a conditioned dilatation of the pupils. These two conditioned responses showed comparable onsets, peak effects, and extinction rates. Ditran, a drug similar to atropine, supported the same two conditioned responses (Lang et al, 1966). Thus atropine and ditran resulted in one conditioned response that mimicked and another that opposed the drug effects. Such results are consistent with the knowledge that these drugs act directly on the salivary gland (that is, after the integrator) to block salivation; they suggest, in addition, that the effect of these drugs on the pupil is due to an action prior to the integrator. Clearly both the present analysis and the results of such studies make it necessary to reject the view that a drug injection is a single unitary event or stimulus.

The feedback systems of the body are clearly more complex than the simple three-element system outlined in the introduction would suggest. Such systems often involve multiple feedback loops, each with several inputs and effectors, some of which can interact in unexpected ways. Satinoff (1978) has recently reviewed the evidence for thermoregulatory control and has contrasted several models of temperature regulation. It has long been known that there are multiple thermal sensors in the skin, spine and brain and many different effectors involved in the control of temperature. Until recently, however, it was assumed that all the sensors fed into a single complex thermal integrator which in turn activated the necessary effectors. Satinoff (1978) points out that this view is no longer consistent with the experimental evidence. She suggests that there are multiple integrators, each with a few inputs and outputs, organized in a hierarchical fashion.

A feedback system involving multiple integrators may have integrators that are linked either in series, where the output of one integrator acts as an input to a second, or in parallel, where the two integrators are independent of each other. In the latter case, however, both may be under the control of a higher level integrator. If

integrators were to be linked in series it is possible that a drug might act at a point in the system between two integrators, that is, after the first but prior to the second. If such a drug action resulted in a temperature change, to use thermoregulation as an example, the observed effect would be said to act as an unconditioned stimulus for the first integrator producing an unconditioned response opposing the observed drug effect. Because the drug acts prior to the second integrator, however, it could in theory also act as an unconditioned stimulus to produce the observed temperature change which would be by definition an unconditioned response. Thus in this case it might be said that there are two unconditioned stimuli, both the drug and its observed effect, as well as two unconditioned responses, the observed drug effect, and the activation of effectors to counteract the observed drug effect. Thus in this situation where there is a single drug action, conditioning might result in two different conditioned responses.

Two conditioned responses might also result if a drug were to act prior to an integrator in a system involving integrators acting in parallel. Because the drug acts prior to one integrator it serves as an unconditioned stimulus and the observed drug effect, produced by the

integrator, would be the unconditioned response. This observed drug effect might in turn act as a stimulus for the second integrator resulting in a second unconditioned response that opposed the observed drug effect. Because there would be two unconditioned stimuli and two unconditioned responses in these hypothetical situations conditioning could result in two conditioned responses. If such complex mechanisms do exist within the thermoregulatory system they could account for the findings of the present experiments in which two conditioned responses were observed.

The situation is further complicated, however, by the fact that morphine and amphetamine appear to have multiple effects on the thermoregulatory system. Just as a drug may have effects on more than one feedback system, a drug might have multiple effects on one complex feedback system. In this case a drug might result in several unconditioned stimuli and unconditioned responses within any one feedback system.

It should be noted, however, that the two conditioned responses observed in the present experiments would not normally have been detected in a standard conditioning paradigm. It became evident that different stimuli were

controlling the different responses only because body temperature was measured both prior to and after the daily drug injection and because tests for conditioning were carried out both prior to and after a drug-free period. Prior to this time conditioning studies with drugs had used a single conditioned stimulus, usually a discrete light or noise, with little concern about the nature of that stimulus. Recent experiments using taste stimuli and administering toxins, the conditioned taste aversion studies, suggested that not all stimuli were equivalent. In particular the bright-noisy water experiment of Garcia and Koelling (1966) showed that while rats could associate a discrete light-noise stimulus with shocks and taste cues with illness, these conditioned stimuli could not be reversed; animals did not learn to associate taste with shocks or a bright-noisy stimulus with illness. This has led to many subsequent experiments investigating the constraints that are involved in learning. It is now evident that not all stimuli are equally associable with any given unconditioned stimulus, but the nature of these constraints on learning is not yet clear (Seligman, 1970; Shettleworth, 1972).

In particular there have been studies showing that the different unconditioned effects of morphine, and also

of amphetamine, associate selectively with different types of stimuli. The pairing of taste cues with either morphine or amphetamine results in the development of a conditioned taste aversion suggesting that drugs are aversive (Cappell & LeBlanc, 1973; Cappell, LeBlanc & Endrenyi, 1973; Jacquet, 1973; LeBlanc & Cappell, 1974; Parker, Failor & Weidman, 1973). However, these drugs are also self-administered by the animal indicating they are positively reinforcing (Pickens & Harris, 1968; Schuster & Thompson, 1969; Weeks, 1962). In fact it has been demonstrated that rats will continue to self-administer the apomorphine that simultaneously results in a taste aversion (Wise, Yokel & DeWit, 1976). Similarly injections of morphine or amphetamine produce both a place preference and a taste aversion, when taste and place stimuli have been paired with the drug injection (Switzman, Amit, White & Fishman, 1978; White, Sklar & Amit, 1977; Sherman, Roskam & Holman, Note 3). It appears that approach and avoidance behavior are simultaneously associated with these two types of conditioned stimuli when drugs such as morphine or amphetamine are used to produce unconditioned stimuli.

In the present experiments environmental stimuli and temporal cues were able to elicit different conditioned

temperature responses. While environmental stimuli have been used in many different conditioning experiments, the use of temporal cues has not been as extensively analyzed. In the present studies drugs were injected at twenty-four hour intervals. While no explicit tests were done, it seemed reasonable to assume that it was the circadian interval of these injections which was important for conditioning. The conditioned responses elicited by temporal cues may have been under the control of some circadian rhythm. It is well known that body temperature fluctuations follow a circadian rhythm. Boulos, Rosenwasser and Terman (1980) reported that there appears to be a circadian system that could produce physiological changes in anticipation of events, such as feeding, occurring at twenty-four hour intervals. In particular, it has been noted that if animals were fed once every twenty-four hours, there were anticipatory changes in body temperature (Bolles & Duncan, 1969; Nelson, Scheving & Halberg, 1975). It may be that similar conditioned anticipatory changes in temperature are seen in the present experiments. It has recently been shown that the endogenous opioid peptide systems also show a circadian rhythm (Davis, Buchsbaum & Bunney, 1978; Frederickson, Burgis & Edwards, 1977). Thus it may not be surprising that some conditioned temperature responses to morphine are under the control of temporal or circadian cues.

While it may be premature, it is interesting to speculate about the functional significance of the conditioning processes evident in these experiments. It should be apparent that these processes do not exist only for the conditioning of drug effects. Rather these conditioning processes must have some survival value for the animal. Animals must learn about and respond to different contingencies in their environment, many of which include temperature information. To maintain a constant body temperature while the external temperature fluctuates animals must react to these fluctuations by changing the activity of their heat production or heat loss mechanisms. Two particularly useful correlations that exist in the environment are between place and temperature, some places are routinely warmer than others, and between time of day and temperature, nights are usually cooler than days. If the animal could learn about these environmental temperature regularities it would be in a better position to maintain a constant body temperature. Because time of day temperature contingencies are usually independent of environmental temperature relations it would be useful if these relationships could be learned separately. The findings of the present experiments imply that these two suggested learning mechanisms may involve links sensitive to morphine and amphetamine. It might, however, be

interesting to test these mechanisms more directly by using external heat loads as unconditioned stimuli.

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Appendix

Analyses of Variance

Tables

Tables 1 to 7 are from Experiment 1

Tables 10 to 14 are from Experiment 2

Tables 15 to 54 are from Experiment 3

Tables 55 to 69 are from Experiment 4

In all tables

* p .05

** p .01

*** p .001

TABLE 1

A Group x Environment analysis of variance of the temperatures of animals in all groups taken one hour before the daily injection averaged over the three test days in the pre-injection environment and over the three test days in the home cage.

Source	df	MS	F
Group (G)	2	3.11	8.67**
Subjects (S)	28	.36	
Environment (E)	1	6.07	43.40***
G x E	2	.44	3.17
S x E	28	.14	

TABLE 2

A one-way analysis of variance of the temperatures of animals in all groups taken one hour after the saline injection averaged over the three test days in the injection environment.

Source	df	MS	F
Group	2	1.06	10.35***
Subjects	28	.10	

TABLE 3

A Group x Day analysis of variance of the temperatures of animals in all groups taken one hour before the saline injection on the eight test days after the drug-free period when animals remained in their home cages.

Source	df	MS	F
Group (G)	2	.41	.69
Subjects (S)	28	.60	
Days (D)	7	1.30	7.74***
G x D	14	.18	1.09
S x D	196	.17	

TABLE 4

A Group x Day analysis of variance of the temperatures of animals in all groups taken one hour after the saline injection on the eight test days after the drug-free period when animals remained in their home cages.

Source	df	MS	F
Group (G)	2	.24	.54
Subjects (S)	28	.44	
Days (D)	7	.92	7.78***
G x D	14	.11	.93
S x D	196	.12	

TABLE 5

A Group x Day analysis of variance of the temperatures of animals in all groups taken two hours after the saline injection on the eight test days after the drug-free period when animals remained in their home cages.

Source	df	MS	F
Group (G)	2	.25	.58
Subjects (S)	28	.43	
Days (D)	7	1.05	6.33***
G x D	14	.08	.49
S x D	196	.17	

TABLE 6

A Group x Day analysis of variance of the temperatures of animals in all groups taken one hour before the saline injection for the eight test days after the drug-free period when animals were in the pre-injection environment.

Source	df	MS	F
Group (G)	2	4.32	6.99**
Subjects (S)	28	.62	
Days (D)	7	.67	4.51***
G x D	14	.06	.43
S x D	196	.15	

TABLE 7

A Group x Day analysis of variance of the temperatures of animals in all groups taken one hour after the saline injection for the eight test days after the drug-free period when animals were in the injection environment.

Source	df	MS	F
Group (G)	2	14.37	21.36***
Subjects (S)	28	.67	
Days (D)	7	1.88	13.47***
G x D	14	.16	1.17
S x D	196	.14	

TABLE 8

A Group x Day analysis of variance of the temperatures of animals in all groups taken two hours after the saline injection for the eight test days after the drug-free period when animals were in the injection environment.

Source	df	MS	F
Group (G)	2	7.13	7.53**
Subjects (S)	28	.95	
Days (D)	7	3.16	15.06***
G x D	14	.14	.64
S x D	196	.21	

TABLE 9

A one-way analysis of variance of the temperatures of animals in all groups taken two hours after the drug injection in the injection environment, averaged over the last two drug days.

Source	df	MS	F
Group	2	22.24	152.46***
Subjects	28	.15	

TABLE 10

A one-way analysis of variance of the temperatures of animals in all groups taken at 10:30 h, the time of the drug injection, in the home cage, averaged over Days 18, 26 and 33.

Source	df	MS	F
Group	2	2.02	10.47***
Subjects	22	.19	

TABLE 11

A Group x Time analysis of variance of the temperatures of animals in all groups taken at 8:30 h and at 9:30 h in the home cage averaged over Days 22, 27 and 31.

Source	df	MS	F
Group (G)	2	.71	1.00
Subjects (S)	22	.71	
Time (T)	1	2.26	14.53**
G x T	2	.58	3.73*
S x T	22	.16	

TABLE 12

A Group x Time analysis of variance of the temperatures of animals in all groups taken at 9:30 h and at 11:30 h in the home cage averaged over the three saline test days, Days 28, 34 and 40.

Source	dl	MS	F
Group (G)	2	2.55	5.21*
Subjects (S)	22	.49	
Time (T)	1	.00	.01
G x T	2	.18	1.50
S x T	22	.12	

TABLE 13

A one-way analysis of variance of the temperatures of animals in all groups taken at 9:30 h in the home cage, after the drug-free period, averaged over the last three days of the experiment, Days 46, 47 and 48.

Source	df	MS	F
Group	2	.07	.13
Subjects	22	.51	

TABLE 14

A one-way analysis of variance of the temperatures of animals in all groups taken at 11:30 h in the home cage, after the drug-free period, averaged over the last three days of the experiment, Days 46, 47 and 48.

Source	df	MS	F
Group	2	.30	1.16
Subjects	22	.26	

TABLE 15

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 9:30 h in the home cage for the three habituation days, Days 1 to 3.

Source	df	MS	F
Group (G)	3	.39	1.17
Subjects (S)	28	.33	
Days (D)	2	1.59	18.13***
G x D	6	.07	.81
S x D	56	.09	

TABLE 16

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 11:00 h in the pre-injection environment for the three habituation days, Days 1 to 3.

Source	df	MS	F
Group (G)	3	.60	1.92
Subjects (S)	28	.31	
Days (D)	2	.76	9.80***
G x D	6	.10	1.34
S x D	56	.08	

TABLE 17

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 12:30 h in the injection environment for the three habituation days, Days 1 to 3.

Source	df	MS	F
Group (G)	3	.43	1.46
Subjects (S)	28	.29	
Days (D)	2	.03	.56
G x D	6	.06	1.13
S x D	56	.05	

TABLE 18

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 9:30 h in the home cage for the first five conditioning days of the experiment, Days 4 to 8.

Source	df	MS	F
Group (G)	3	.50	.71
Subjects (S)	28	.70	
Days (D)	4	.04	.51
G x D	12	.12	1.40
S x D	112	.09	

TABLE 19

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 11:00 h in the pre-injection environment for the first five conditioning days of the experiment, Days 4 to 8.

Source	df	MS	F
Group (G)	3	.69	.76
Subjects (S)	28	.91	
Days (D)	4	.73	9.13***
G x D	12	.12	1.49
S x D	112	.08	

TABLE 20

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 12:30 h in the injection environment for the first five conditioning days of the experiment, Days 4 to 8.

Source	df	MS	F
Group (G)	3	29.27	27.42***
Subjects (S)	28	1.07	
Days (D)	4	.59	3.16*
G x D	12	.06	.30
S x D	112	.19	

TABLE 21

A one-way analysis of variance of the temperatures of animals in all groups taken at 12:30 h in the injection environment, after drug administration, on Day 31.

Source	df	MS	F
Group	3	11.40	56.25***
Subjects	28	.20	

TABLE 22

A one-way analysis of variance of the temperatures of animals in all groups taken at 15:30 h, in the home cage, after drug administration, on Day 31.

Source	df	MS	F
Group	3	4.66	16.67***
Subjects	28	.28	

TABLE 23

A one-way analysis of variance of the temperatures of animals in all groups taken at 17:00 h, in the home cage, after drug administration, on Day 31.

Source	df	MS	F
Group	3	2.99	10.64***
Subjects	28	.28	

TABLE 24

A one-way analysis of variance of the temperatures of animals in all groups taken at 18:30 h, in the home cage, after drug administration, on Day 31.

Source	df	MS	F
Group	3	.27	1.19
Subjects	28	.22	

TABLE 25

A Group x Day analysis of variance of the motility scores of animals in all groups taken in the pre-injection environment, before drug administration, on three conditioning days, Days 25, 27 and 32.

Source	df	MS	F
Group (G)	3	9.29	.80
Subjects (S)	28	11.62	
Days (D)	2	39.80	11.93***
G x D	6	4.49	1.35
S x D	56	3.34	

TABLE 26

A Group x Day analysis of variance of the motility scores of animals in all groups taken in the injection environment, after drug administration, on three conditioning days, Days 25, 27 and 32.

Source	df	MS	F
Group (G)	3	443.69	121.39***
Subjects (S)	28	3.66	
Days (D)	2	.54	.18
G x D	6	2.35	.77
S x D	56	3.04	

TABLE 27

A one-way analysis of variance of the temperatures of animals in all groups taken at 9:30 h in the home cage on Day 33, a conditioning test day.

Source	df	MS	F
Group	3	.36	2.23
Subjects	28	.16	

TABLE 28

A Group x Time analysis of variance of the temperatures of animals in all groups taken in the home cage, on a conditioning test day, at times animals would normally have been in the conditioning environments, 11:00 h and 12:30 h, on Day 33.

Source	df	MS	F
Group (G)	3	1.68	9.03***
Subjects (S)	28	.19	
Time (T)	1	.51	5.53*
G x T	3	.22	2.34
S x T	28	.09	

TABLE 29

A Group x Environment analysis of variance of the temperatures of animals in all groups taken in the pre-injection environment and in the injection environment on the evening of Day 33, a conditioning test day.

Source	df	MS	F
Group (G)	3	2.15	6.00**
Subjects (S)	28	.36	
Environment (E)	1	.01	.07
G x E	3	.16	.88
S x E	28	.18	

TABLE 30

A Group x Environment analysis of variance of the temperatures of animals in all groups taken in the pre-injection environment at 11:00 h and in the injection environment at 12:30 h on Day 26, a conditioning test day.

Source	df	MS	F
Group (G)	3	1.72	4.57**
Subjects (S)	28	.38	
Environment (E)	1	.03	.23
G x E	3	1.08	9.43***
S x E	28	.11	

TABLE 31

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 15:30 h, in the home cage, on conditioning test days, Days 26 and 33.

Source	df	MS	F
Group (G)	3	1.46	5.54**
Subjects (S)	28	.26	
Days (D)	1	.01	.02
G x D	3	.33	1.07
S x D	28	.31	

TABLE 32

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 18:30 h, in the home cage, on conditioning test days, Days 26 to 33.

Source	df	MS	F
Group (G)	3	.13	.55
Subjects (S)	28	.24	
Days (D)	1	.11	1.10
G x D	3	.20	1.94
S x D	28	.10	

TABLE 33

A Group x Environment analysis of variance of the motility scores of animals in all groups taken in the pre-injection environment and in the injection environment on the evening of Day 33, a conditioning test day.

Source	df	MS	F
Group (G)	3	36.49	3.54*
Subjects (S)	28	10.30	
Environment (E)	1	2.14	.41
G x E	3	17.11	3.28*
S x E	28	5.21	

TABLE 34

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 9:30 h, in the home cage, at the end of the drug-free period, Days 47 and 48.

Source	df	MS	F
Group (G)	3	.38	.77
Subjects (S)	28	.49	
Days (D)	1	.01	.03
G x D	3	.13	.67
S x D	28	.20	

TABLE 35

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 11:00 h, in the home cage, at the end of the drug-free period, Days 47 and 48.

Source	df	MS	F
Group (G)	3	.30	.55
Subjects (S)	28	.55	
Days (D)	1	.26	2.35
G x D	3	.08	.69
S x D	28	.11	

TABLE 36

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 12:30 h, in the home cage, at the end of the drug-free period, Days 47 and 48.

Source	df	MS	F
Group (G)	3	.37	.80
Subjects (S)	28	.46	
Days (D)	1	.03	.22
G x D	3	.28	2.26
S x D	28	.12	

TABLE 37

A one-way analysis of variance of the temperatures of animals in all groups taken in the pre-injection environment, at 11:00 h, on the first conditioning test day after the drug-free period, Day 49.

Source	df	MS	F
Group	3	.41	3.10*
Subjects	28	.13	

TABLE 38

A one-way analysis of variance of the temperatures of animals in all groups taken in the injection environment, at 12:30 h, on the first conditioning test day after the drug-free period, Day 49.

Source	df	MS	F
Group	3	1.49	8.86***
Subjects	28	.17	

TABLE 39

A one-way analysis of variance of the motility scores of animals in all groups taken in the pre-injection environment on the first conditioning test day after the drug-free period, Day 49.

Source	df	MS	F
Group	3	1.01	.26
Subjects	28	3.83	

TABLE 40

A one-way analysis of variance of the motility scores of animals in all groups taken in the injection environment on the first conditioning test day after the drug-free period, Day 49.

Source	df	MS	F
Group	3	12.94	4.62*
Subjects	28	2.80	

TABLE 41

A one-way analysis of variance of the temperatures of animals in all groups taken in the pre-injection environment, at 11:00 h, on the second conditioning test day after the drug-free period, Day 50.

Source	df	MS	F
Group	3	.29	1.39
Subjects	28	.21	

TABLE 42

A one-way analysis of variance of the motility scores of animals in all groups taken in the pre-injection environment on the second conditioning test day after the drug-free period, Day 50.

Source	df	MS	F
Group	3	6.09	.89
Subjects	28	6.84	

TABLE 43

A one-way analysis of variance of the temperatures of animals in all groups taken in the injection environment, at 12:30 h, on the second conditioning test day after the drug-free period, Day 50.

Source	df	MS	F
Group	3	1.29	5.04**
Subjects	28	.26	

TABLE 44

A one-way analysis of variance of the temperatures of animals in all groups taken in the injection environment, at 12:30 h, on the third conditioning test day after the drug-free period, Day 56.

Source	df	MS	F
Group	3	1.10	7.46***
Subjects	28	.15	

TABLE 45

A one-way analysis of variance of the temperatures of animals in all groups taken in the injection environment, at 12:30 h, on the fourth conditioning test day after the drug-free period, Day 61.

Source	df	MS	F
Group	3	.93	3.33*
Subjects	28	.28	

TABLE 46

A one-way analysis of variance of the temperatures of animals in all groups taken in the injection environment, at 12:30 h, on the fifth conditioning test day after the drug-free period, Day 67.

Source	df	MS	F
Group	3	.71	2.39
Subjects	28	.30	

TABLE 47

A one-way analysis of variance of the temperature of animals in all groups taken in the injection environment, at 12:30 h, on the sixth conditioning test day after the drug-free period, Day 68.

Source	df	MS	F
Group	3	.38	.75
Subjects	28	.50	

TABLE 48

A one-way analysis of variance of the temperatures of animals in all groups taken in the injection environment at 12:30 h, on the seventh conditioning test day after the drug-free period, Day 69.

Source	df	MS	F
Group	3	.49	1.24
Subjects	28	.39	

TABLE 49

A one-way analysis of variance of the motility scores of animals in all groups taken in the injection environment on the second conditioning test day after the drug-free period, Day 50.

Source	df	MS	F
Group	3	18.97	4.37*
Subjects	28	4.34	

TABLE 50

A one-way analysis of variance of the motility scores of animals in all groups taken in the injection environment on the third conditioning test day after the drug-free period, Day 56.

Source	df	MS	F
Group	3	16.47	3.82*
Subjects	28	4.32	

TABLE 51

A one-way analysis of variance of the motility scores of animals in all groups taken in the injection environment on the fourth conditioning test day after the drug-free period, Day 61.

Source	df	MS	F
Group	3	13.67	2.83
Subjects	28	4.83	

TABLE 52

A one-way analysis of variance of the motility scores of animals in all groups taken in the injection environment on the fifth conditioning test day after the drug-free period, Day 67.

Source	df	MS	F
Group	3	3.99	1.24
Subjects	28	3.21	

TABLE 53

A one-way analysis of variance of the motility scores of animals in all groups taken in the injection environment on the sixth conditioning test day after the drug-free period, Day 68.

Source	df	MS	F
Group	3	4.96	1.24
Subjects	28	4.01	

TABLE 54

A one-way analysis of variance of the motility scores of animals in all groups taken in the injection environment on the seventh conditioning test day after the drug-free period, Day 69.

Source	df	MS	F
Group	3	3.68	.89
Subjects	28	4.13	

TABLE 55

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 9:00 h in the home cage for the two habituation days, Days 1 and 2.

Source	df	MS	F
Group (G)	3	.13	.34
Subjects (S)	23	.39	
Days (D)	1	.06	.52
G x D	3	.07	.58
S x D	23	.13	

TABLE 56

A Group x Day analysis of variance of the temperatures of animals in all groups taken ~~at 10:30 h in the pre-~~ injection environment for the two habituation days, Days 1 and 2.

Source	df	MS	F
Group (G)	3	.10	.87
Subjects (S)	23	.11	
Days (D)	1	.07	1.08
G x D	3	.01	.09
S x D	23	.07	

TABLE 57

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 12:00 h in the injection environment for the two habituation days, Days 1 and 2.

Source	df	MS	F
Group (G)	3	.05	.23
Subjects (S)	23	.23	
Days (D)	1	.48	5.94*
G x D	3	.02	.20
S x D	23	.08	

TABLE 58

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 12:00 h in the injection environment for the first four days of drug administration, Days 3 to 6.

Source	df	MS	F
Group (G)	3	6.07	14.00***
Subjects (S)	22	.43	
Days (D)	3	1.61	18.99***
G x D	9	.89	10.55***
S x D	66	.08	

TABLE 59

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 12:00 h in the injection environment for the second four days of drug administration, Days 9 to 12.

Source	df	MS	F
Group (G)	3	8.01	23.86***
Subjects (S)	21	.34	
Days (D)	3	1.48	24.98***
G x D	9	.19	3.19**
S x D	63	.06	

TABLE 60

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 10:30 h in the pre-injection environment for the first four days of drug administration, Days 3 to 6.

Source	df	MS	F
Group (G)	3	.90	4.54*
Subjects (S)	22	.20	
Days (D)	3	2.85	57.27***
G x D	9	.13	2.59*
S x D	66	.05	

TABLE 61

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 10:30 h in the pre-injection environment for the second four days of drug administration, Days 9 to 12.

Source	df	MS	F
Group (G)	3	1.46	5.36**
Subjects (S)	21	.27	
Days (D)	3	3.79	72.80***
G x D	9	.21	4.09***
S x D	63	.05	

TABLE 62

A Group x Environment analysis of variance of the temperatures of animals in all groups taken at 10:30 h averaged over two test days in the home cage, Days 19 and 45, and over two test days in the pre-injection environment, Days 25 and 31.

Source	df	MS	F
Group (G)	3	3.53	15.06***
Subjects (S)	23	.23	
Environment (E)	1	.09	1.10
G x E	3	.05	.53
S x E	23	.09	

TABLE 63

A Group x Time analysis of variance of the temperatures of animals in all groups taken at 10:30 h in the home cage, at 17:00 h in the pre-injection environment, both on Day 39, a conditioning test day, and at 10:30 h in the pre-injection environment on Day 40.

Source	df	MS	F
Group (G)	3	.64	2.86
Subjects (S)	23	.22	
Time (T)	2	.52	6.68**
G x T	6	.28	3.58**
S x T	46	.08	

TABLE 64

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 10:30 h in the home cage at the end of the drug-free period, Days 48 and 49.

Source	df	MS	F
Group (G)	3	.11	.96
Subjects (S)	23	.11	
Days (D)	1	2.54	33.27***
G x D	3	.02	.21
S x D	23	.08	

TABLE 65

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 10:30 h in the pre-injection environment on conditioning test days after the drug-free period, Days 50 and 51.

Source	df	MS	F
Group (G)	3	.04	.43
Subjects (S)	23	.09	
Days (D)	1	3.03	65.31***
G x D	3	.02	.34
S x D	23	.05	

TABLE 66

A Group x Environment analysis of variance of the temperatures of animals in all groups taken at 12:00 h averaged over two test days in the home cage, Days 19 and 45, and over two test days in the injection environment, Days 25 and 31.

Source	df	MS	F
Group (G)	3	.58	2.81
Subjects (S)	23	.21	
Environment (E)	1	4.12	42.71***
G x E	3	.84	8.67***
S x E	23	.10	

TABLE 67

A one-way analysis of variance of the temperatures of animals in all groups taken at 18:30 h in the injection environment on Day 39, a conditioning test day.

Source	df	MS	F
Group	3	1.37	8.80***
Subjects	23	.16	

TABLE 68

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 12:00 h in the home cage at the end of the drug-free period, Days 48 and 49.

Source	df	MS	F
Group (G)	3	.19	.66
Subjects (S)	23	.29	
Days (D)	1	.71	10.15**
G x D	3	.15	2.21
S x D	23	.07	

TABLE 69

A Group x Day analysis of variance of the temperatures of animals in all groups taken at 12:00 in the injection environment on conditioning test days after the drug-free period, Days 50 and 51.

Source	df	MS	F
Group (G)	3	1.18	14.99***
Subjects (S)	23	.08	
Days (D)	1	.60	14.44***
G x D	3	.06	1.32
S x D	23	.04	