CHANGES IN LOCOMOTOR ACTIVITY AND BODY TEMPERATURE INDUCED BY MORPHINE IN THE VENTRAL TEGMENTAL AREA OF THE RAT BRAIN: CONDITIONED AND UNCONDITIONED EFFECTS

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ABSTRACT

Changes in Locomotor Activity and Body Temperature Induced by Morphine in the Ventral Tegmental Area of the Rat Brain: Conditioned and Unconditioned Effects

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The conditionability of changes in locomotor activity and body temperature induced by morphine administration into the ventral tegmental area was studied in rats. Morphine was found to induce both an increase in locomotor activity and hyperthermia. Both effects were reversed by a systemic injection of naloxone. Systemic injections of pimozide also blocked the morphine-induced increase in locomotor activity but did not affect the hyperthermia suggesting dopamine mediation of the former but not the latter. The increase in locomotor activity showed sensitization with repeated morphine administration and this sensitization was found to be specific to the environment in which morphine was administered. Conditioning tests also revealed that, in the absence of morphine, increased locomotor activity was elicited by the administration environment. Pimozide blocked the development of both conditioning and conditioned sensitization of the increased locomotor activity. It did not, however, completely block the expression of the already established conditioned sensitization. No evidence for conditioning of the morphine-induced hyperthermia was found in any of the conditioning tests conducted. The implications of these results for incentive-motivation theory, for relapse to drug-use, and for ideas about the biochemical substrates of learning are discussed.

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The study of the conditioning of morphine-induced responses is as old as the study of classical conditioning itself. Pavlov (1927/1960) himself first reported that the "symptoms" seen following an acute injection of morphine in the dog (nausea, salivation, vomiting, and then sleep) could come to be elicited, after repeated injections, by the preinjection procedures alone. Since this original observation, numerous investigators have demonstrated the classical conditioning of morphine-induced responses. The phenomenon is generally accepted as being robust, although not without methodological and conceptual problems (for reviews, see Lynch, Stein, and Fertziger, 1976; Wikler, 1973).

After an initial period in which investigators used morphine as one of many experimental tools to explore the newly described phenomenon of conditioning, the main concern of those studying morphine effects shifted, in the 1940's, to problems of morphine addiction and to questions about the role of conditioned withdrawal symptoms in relapse to drug use. Although one of the main factors behind this shift was surely the growing social concern with drug addiction, it would appear not to be a coincidence that interest in morphine withdrawal symptoms and their possible role in drug relapse developed as it did during the heyday of the drive reduction theory of reinforcement (i.e.: Hull, 1943). Indeed, the fundamental hypothesis developed in this period was that drug-seeking behavior was maintained in order to reduce or avoid the traumatic experience involved in withdrawal (Lindesmith, 1968). Classical conditioning was used to explain relapse to drug-taking after long-term abstinence. Conditioned withdrawal symptoms were thought to provide the acquired drive

necessary for the reinforcing effects of the drug injection (Wikler, 1948).

The situation today has changed little; need- or drive-reduction views continue to figure prominently in the thinking about drug-seeking behavior. For example, in a recent series of papers, Siegel (1975, 1977a, 1979) has concentrated on the observation that conditioned responses elicited by stimuli associated with drug injections are often opposite in direction to the direct effects of the drug. In the case of opiates, these conditioned opponent responses often resemble the physiological responses that are observed during drug withdrawal. Siegel(1975, 1977a, 1979) has suggested, therefore, that such conditioned opponent or compensatory responses may play an important role in both drug tolerance and dependence. According to this view, the conditioned opponent or compensatory responses act in a preparatory manner to reduce the effectiveness of the anticipated drug, that is, to produce tolerance. In the case when the anticipated drug is not administered, the conditioned opponent responses achieve full expression and the animal experiences withdrawal symptoms. The inference is that such conditioned withdrawal reactions initiate relapse to drug taking in the abstinent animal or individual.

Siegel has to date, however, made no direct test of the critical link between the conditioned opponent response and its ability to initiate "craving" and drug-seeking behavior. Furthermore, although several reports have been made of the successful classical conditioning of withdrawal reactions (Goldberg and Schuster, 1967; Irwin and Seevers, 1956; Trost,

1973; Wikler and Pescor, 1967), the ability of these conditioned responses to reinitiate drug-seeking behavior has not yet been demonstrated unequivocally (see Thompson and Oslund, 1965; Wikler and Pescor, 1967).

As previously mentioned, the classical conditioning of withdrawal reactions is thought to be the basis of conditioned drug-taking "drive." Hence, withdrawal reactions are assumed to be uncomfortable or aversive and the reduction of this discomfort is presumed to reinforce drug taking. The "drive-to-take-morphine" is inferred from the observable withdrawal reactions displayed by the organism. When withdrawal reactions become conditioned, therefore, the inference is that the "drive-to-take-morphine" has become conditioned. The ultimate test of this hypothesis is staightforward. If drive-reduction or the reduction of unconditioned withdrawal reactions is assumed to lead to drug-taking behavior then so too must the reduction of conditioned drive or conditioned withdrawal reactions lead to drug-taking behavior. This is not what has been found. Interestingly, attempts to condition other "drives" such as "hunger" or "thirst" have failed as well (for reviews, see Cravens and Renner, 1970; D'Amato, 1974; Mineka, 1975).

According to such a need view of drive, reduction of drive is the reinforcing event and the stimuli and responses that bring on reduced drive are strengthened in specific stimulus-response associations (i.e: Hull, 1943). Such a view is by no means without criticism and, indeed, objections have been raised on several theoretical and empirical grounds. Since there have already been more than adequate reviews of these objections

(Bindra, 1969, 1978, Note 1; Bolles, 1972), only a brief survey will be made here.

Two main issues have repeatedly plagued drive-reduction views of behavior. One is the inability of such views to adequately translate "drive" into behavior. The other concerns the concept of "drive" itself.

The idea that adaptive behavior results from the reinforcement by drive-reduction and ,therefore, the conditioning of rigid and specific stimulus-response associations has been criticized on several points. For example, there have been several demonstrations of conditioning without the reinforcement of any specific responses in latent learning and sensory preconditioning experiments (see Kimble, 1961, chapter 8). Other experiments have demonstrated that conditioning may occur without responding as when an animal is paralyzed (Solomon and Turner, 1962). Thus, neither the response nor the reinforcement of responses by drive-reduction seems to be necessary for conditioning to occur. Other behavioral objections have stemmed from the issue of "motor equivalence" (the substitutionality of movements involved in a response, a finding irreconcilable with the notion that drive-reduction reinforces specific stimulus-response associations) and from demonstrations that certain interim behaviors seem to be isolated from any reinforcement mechanism (i.e.: Staddon and Simmelhag, 1971). It is not clear, therefore, that the reinforcement and the subsequent conditioning of specific stimulus-response associations can account for many of the behaviors that animals exhibit including goal-directed and consummatory responses. Of course, without a functional response

mechanism, drive views are at a loss to explain adaptive behavior. The notion that the prevailing drive and the appropriate response "naturally" get matched is obviously insufficient (Bindra, Note 1).

In view of such criticism, it is not surprising that theorists also questioned the integrity of the notion that "drive" is solely a matter of internal "need" states. For example, a popular view has been that bodily depletion results in neural changes or signs of bodily need and that these neural changes comprise the drive that somehow instigates goal-directed actions. Such a view has been contraindicated by evidence that eating or drinking does not normally wait for the buildup of bodily needs for food and water and may occur in the absence of any marked sensory or chemical signs of depletion (Fitzsimmons, 1971; LeMagnen, 1971; Toates, 1981). These results pose the obvious problem of trying to explain consummatory behavior in the absence of "internal drive". Additional problematic data came, ironically, from demonstrations that electrical stimulation of different hypothalamic regions seemed to produce specific drive states. Soon various hypothalamic sites were identified as drive sites for eating, drinking, sexual activity and so on (Glickman and Schiff, 1967). In a series of experiments, however, Valenstein, Cox, and Kakolewski (1968) demonstrated that a rat stimulated at a site that would normally elicit eating (i.e.: excitation of the "hunger drive" site) would attend to other available stimuli (drink water, for example) if food was removed or not available in the experimental situation. In as much as it was generally agreed that the electrical stimulation of

different hypothalamic sites did in fact produce genuine "drives" under given experimental conditions, such results made it increasingly difficult to think of the instigation of specific goal-directed actions by a particular "drive." For example, why should an animal drink or engage in sexual activity when it presumably is hungry? Indeed, such results rendered the concept of "drive" as internal need state increasingly vague. Conversely, the suggestion that the generation of drives or, more appropriately, of motivational states is highly influenced by the nature of the incentive objects present in the environment has become increasingly prevalent.

The cumulative thrust of experiments such as those reviewed above has, in the past decade, resulted in the formulation and wider acceptance of an alternative view of motivation and learning. Such a view (Bindra, 1976, 1978) ascribes a primary role to incentive stimuli as the generators of motivational states and elicitors of actions. According to this view, internal bodily changes or signs of bodily need do not generate motivational states but rather "gate" the effectiveness of particular incentive stimuli to do so by modulating both the salience of these stimuli and therefore the quality and magnitude of the motivational states they generate. The generation of a motivational state, in turn, further enhances the salience and effectiveness of the incentive stimuli. One implication of this view is that the environmental (incentive) stimulus that generates a motivational state can also serve as the goal stimulus to which the organism's behavior is directed. Thus, an eating motivational state, for example, would direct an animal to

the very environmental stimulus that played a part in generating that state. This approach clearly circumvents many of the problems inherent in a drive-reduction explanation of goal-directed behavior.

Learning is viewed primarily in terms of the classical conditioning of stimulus-stimulus associations (see also Bolles, 1972). In the typical experimental setting, the stimuli of interest are usually the incentive or unconditioned stimulus (UCS) and the neutral or conditioned stimulus (CS). According to this theory, learning of a CS:UCS association is viewed as the acquisition by the central representation of the CS of the ability to excite the central representation of the UCS and thereby to generate a motivational state similar to that normally generated by the UCS. This motivational state in turn enhances the salience of the CS and the animal is likely to act in relation to it (as when the UCS is withheld in a test trial). This idea, that the CS acquires the ability to activate a motivational state similar to that activated by the UCS, is central to this incentive-motivational view of learning and motivation (Bindra, 1976, 1978).

It should be clear at this point that the role of classical conditioning in relapse to drug use is now open to two fundamentally different interpretations. On the one hand and as has been described earlier, the drive-reduction view argues that relapse to morphine use is due to the classical conditioning of the "drive-to-take-morphine." This conditioned drive is inferred from the conditioned withdrawal reactions displayed by the animal and must be accompanied by learned stimulus-response associations

that allow the animal to engage in the appropriate behaviors to reduce the drive. The incentive-motivational view, on the other hand, regards morphine as a rewarding incentive stimulus. Thus, relapse to morphine-use is viewed as being due to the classical conditioning of the appetitive motivational state generated by morphine. This motivational state in turn enhances the salience of the CS's and increases the likelihood that the animal will approach and interact with them (i.e.: the self-administation lever, the syringe, and so on). This view has been elaborated recently by Stewart, deWit, and Eikelboom (Note 2).

Thus, the disagreement between the two views centers on the particular association that must be learned. Simply stated, one view asserts that the CS must be paired with the absence of morphine or morphine-withdrawal (the "drive-to-take-morphine") while the other asserts that the CS must be paired with the presence of morphine (and, therefore, with the ensuing appetitive motivational state).

Clearly, the initial and crucial test for the incentive-motivational account of relapse to morphine-use is to determine whether the CS does in fact acquire the ability to generate an appetitive motivational state similar to that generated by morphine itself. Morphine is a drug with multiple unconditioned effects or responses. If these responses are presumed to underly or to be manifestations of the motivational state generated by morphine, then the extent to which a CS can come to elicit these responses reflects its ability to generate the same motivational state. This of course is classical conditioning: the CS is repeatedly paired with the UCS (morphine) and comes to elicit

conditioned responses that mimic the unconditioned responses elicited by the UCS.

Identification of the Unconditioned and Conditioned Responses to Morphine

As simple as the testing of the above prediction may appear, it is no small task. The situation is complicated somewhat by the fact that morphine has both depressant and excitatory actions on most of the behavioral and physiological responses studied to date and that these actions are both dose and species dependent. (Because of the species variable, the following discussion will be limited to those data obtained from the rat.) In general, small doses of morphine can be said to produce excitatory effects, while larger doses cause biphasic effects consisting first of a depressant phase and then of an excitatory phase (Domino, Vasko, and Wilson, 1976; Seevers and Deneau, 1963). The question then becomes which of these responses represents the unconditioned response (UCR) to morphine? Attempts to classify the UCR to morphine as either the excitatory or the depressant phase of the biphasic response do little to resolve the problem: a quick review of the literature will reveal that both have been used to specify different UCR's to morphine. For example, analgesia (Siegel, 1975) and hypoactivity (Mucha, Volkovskis, and Kalant, 1981), both of which represent depressant effects of morphine, are considered by some to be UCR's to morphine. On the other hand, hyperthermia, an excitatory effect of morphine, is also considered by many to be an UCR to morphine (i.e., Eikelboom and Stewart, 1979; Miksic, Smith, Numan, and Lal, 1975; Sherman,

1979; Siegel, 1978).

The situation is further complicated by the problem of the directionality of the conditioned response (CR). Classical conditioning, as originally conceptualized by Pavlov (1927/1960) and to a wide extent today (Mackintosh, 1974, chapter 3), is viewed as the acquisition by the CS of the ability to elicit CR's that mimic the UCR's normally elicited by the UCS. However, Siegel (1975), drawing largely from experiments on analgesia, has accumulated evidence to suggest that the CR to morphine is one that opposes the UCR (hence, the "conditioned compensatory response"). This finding at one time lead to claims of "paradoxical conditioning" and figures prominently in opponent-process theories of motivation (i.e., Solomon, 1977). Siegel (1975, 1976,1977b) has interpreted his results as providing an explanation for the finding that tolerance to morphine analgesia is specific to the environment in which morphine is administered. The suggestion is that the development of tolerance to the analgesic effect of morphine reflects the development of a conditioned compensatory hyperalgesic response which comes to be elicited by the administration environment (the CS). And indeed, Siegel (1975) has reported that tolerant rats, when tested with saline in the administration environment, show hyperalgesic responses.

To someone whose objective it is to demonstrate the elicitation by a CS of responses that mimic the UCR, the above findings are obviously problematic. There are several reasons, however, to reconsider their interpretation. In the above formulation, the UCS is viewed as morphine and the UCR as

analgesia. Eikelboom and Stewart (1982) make an elegant argument that this need not necessarily be the case (see also Kesner and Baker, 1981, p. 488). They suggest that if one views morphine as acting on the efferent arm of a regulatory feedback algesia system, the observed effect of morphine (analgesia) may be seen as constituting the UCS and a response to the UCS seen as the UCR (hyperalgesia). Conditioned hyperalgesia responses would therefore mimic the unconditioned hyperalgesia responses to analgesia (the UCS). There is, however, another possibility. Morphine has been reported to have, under certain conditions, unconditioned hyperalgesic effects (Jacquet and Lajtha, 1973; Kayan, Woods, and Mitchell, 1971). The possibility then arises that Siegel's results may reflect the conditioning of independently elicited hyperalgesic responses, that is, responses that are elicited by morphine action at opiate receptors that are independent of morphine's analgesic actions. In this case, morphine would be the UCS and the CR would mimic the UCR (hyperalgesia). These possibilities remain to be elucidated.

The generality of Siegel's (1975) position rests to a large extent on the tenet that tolerance develops to the effects of morphine. There is considerable evidence, however, that while the depressant effects of morphine show tolerance, the excitatory effects do not (Seevers and Deneau, 1963). Furthermore, not all CR's oppose the initially observed effects of morphine. (It is interesting to note that, by and large, reports of compensatory CR's have come from studies of depressant effects while reports of mimicking CR's have come from studies of the excitatory effects of morphine).

Most of the evidence for isodirectional CR's comes from studies of the thermic effects of morphine. In these studies, morphine is considered the UCS, hyperthermia the UCR, and the CR reported mimics the UCR (i.e., a conditioned hyperthermia response is obtained; Eikelboom and Stewart, 1979; Lal, Miksic, and Smith, 1976; Miksic et al., 1975; Sherman, 1979). In one study, Siegel (1978) reported a conditioned hypothermia, a CR which opposed the hyperthermia UCR. Furthermore, he reported that tolerance developed to the hyperthermia. Explicit attempts to replicate these results, however, have failed (i.e.: Sherman, 1979). Moreover, this finding of tolerance development to the hyperthermic effect of morphine is unique in the literature in this area (for a review see, Eikelboom and Stewart, 1982). There is at present no good explanation for Siegel's (1978) discrepant findings.

Because the effect of morphine on temperature is dose dependent (small doses produce a hyperthermic response whereas large doses produce a biphasic effect, first hypothermia followed by hyperthermia: Cox, Ary, Chesarek, and Lomax, 1976; Gunne, 1960), it is at first not clear why hyperthermia (and not hypothermia) is considered the UCR to morphine by so many. In their review, Eikelboom and Stewart (1982) suggest that independent lines of evidence point to a site of action of morphine which is on the afferent arm of the thermoregulatory system (see also Clark, 1979a). Evidence for this view comes from experiments which demonstrate that both behavioral and physiological responses to small doses of morphine will promote a rise in body temperature; not only will animals become

hyperthermic, but they will stay under a heat lamp longer than control animals (Cox et al., 1976). To the extent that behavioral and metabolic responses may represent two different classes of temperature effectors, the finding that both promote the same goal (hyperthermia) suggests that morphine is acting as an UCS on the afferent side of the thermoregulatory system.

The initial hypothermia obtained from high doses of morphine, on the other hand, is less well understood. It may be that high doses of morphine initially incapacitate the thermoregulatory system and render the animal unable to regulate its temperature; the animal becomes poikilothermic (Clark, 1979a). Because animals are usually tested in environments that are cooler than their core temperature, such a view would explain the initial hypothermia obtained from high doses of morphine. It is important to remember, however, that high doses of morphine also produce hyperthermia (the second phase of the biphasic response). Indeed, experiments in which animals have been administered morphine in a variety of ambient temperatures have demonstrated that although hypothermia is obtained in cool environments it is not obtained in a thermoneutral environment (Cochin, Rosow, and Miller, 1978; Paolino and Bernard, 1968). Hyperthermia, on the other hand, is obtained regardless of the ambient temperature (Rudy and Yaksh, 1977). Thus, whatever the mechanism of morphine-induced hypothermia is ultimately found to be, the above data strongly suggest that hyperthermia is an UCR to morphine. Experiments which have reported conditioned hyperthermia, therefore, demonstrate that a CS may come to elicit responses which mimic the UCR's to morphine.

The experiments in this thesis represent an attempt to extend these findings to locomotor activity. There are several reasons why this may be fruitful. First, the extension of findings from one measure to another is necessary if they are to have any generality. Locomotor activity is a simple behavior to record reliably in the rat and may provide such an extension. Second, it has been suggested that changes in locomotor activity levels may confound the concurrent measurement of other morphine effects such as analgesia (Mucha et al., 1981) and temperature (Lotti, Lomax, and George, 1965; Martin, Pryzbylik, and Spector, 1977). This possibility makes the study of morphine-induced changes in locomotor activity important in its own right. Finally, and perhaps most important, is the accumulation, in the past decade, of neurophysiological, neuroanatomical, and biochemical data which suggest a strong relationship between locomotor activity and the rewarding qualities of morphine. This possibility, of an intrinsic relationship between locomotor activity and reward, introduces obvious advantages to the use of locomotor activity as a response system with which to study the conditionability of the central motivational state induced by morphine. These ideas will become clearer in the following sections.

Morphine-Induced Changes in Locomotor Activity

Acute systemic injections of morphine have both depressant and excitatory actions on locomotor activity. Typically, medium to high doses result in an initial decrease in activity followed one to two hours later by an increase in activity (Babbini and Davis, 1972; Sloan, Brooks, Eisenman, and Martin, 1962; Vasko and

Domino, 1978). Low doses, on the other hand, result only in an increase in activity (Babbini and Davis, 1972; Fog, 1970; Vasko and Domino, 1978). With repeated injections of high doses, the decrease in activity shows development of tolerance and the increase in activity appears to become stronger and to occur earlier in time (Babbini and Davis, 1972; Martin, Wikler, Eades, and Pescor, 1963; Vasko and Domino, 1978).

The predominant explanation of these findings has been that morphine interacts with the mesolimbic dopamine system. Pollard and his colleagues (Pollard, Llorens, Bonnet, Costentin, and Schwartz, 1977) have argued that morphine acts initially to inhibit dopamine (DA) release by acting on opiate receptors at the terminals of mesolimbic DA neurons. In response to the inhibition, DA synthesis is increased. This increase in synthesis acts to overcome the inhibitory effect and ultimately acts to bring about the increase in locomotor activity. When morphine is administered intracranially to the nucleus accumbens (one of the terminal regions of mesolimbic DA neurons), the result is an initial period of decreased locomotor activity followed two to four hours later by a period of increased activity (Costall, Fortune, and Naylor, 1976, 1978; Dill and Costa, 1977; Pert and Sivit, 1977). When DA or DA agonists are administered directly to the nucleus accumbens, the result is an increase in locomotor activity which is blocked by DA receptor blocking agents (Costall and Naylor, 1976; Pijnenburg, Honig, Van Der Heyden, and Van Rossum, 1976; Wachtel, Ahlenius, and Anden, 1979). Costall, Naylor, Cannon, and Lee (1977) found, in addition, that DA injected into the nucleus accumbens resulted in locomotor

activity increases, but that similar injections into the caudate-putamen did not. Rather, these latter injections resulted in an increase in stereotyped behavior.

At first glance, this explanation of the effects of morphine on locomotor activity sits quite well with Siegel's (1975, 1977a, 1979) suggestions about the role of compensatory responses in drug tolerance. Here, morphine would be the UCS, the environment in which morphine is repeatedly administered the CS, and the initial decrease in locomotor activity obtained from high systemic doses the UCR. In this view, therefore, tolerance to the initial decrease in locomotor activity is seen as reflecting the development of a conditioned compensatory increase in locomotor activity which comes to be elicited by the CS.

These predictions were recently tested in a series of experiments by Mucha et al. (1981). They found that with high systemic doses of morphine, the unconditioned effect was an initial decrease in locomotor activity. Furthermore, when morphine was repeatedly administered in a distinctive environment (the CS), the resulting CR was an increase in locomotor activity. It was concluded, therefore, that tolerance to the initial activity decrease was probably due to the development of a conditioned compensatory increase in locomotor activity (the CR). The problem for this view is that Mucha et al. (1981) also found conditioned increases in locomotor activity when they used low doses of morphine as the UCS, even though these doses produced no unconditioned decreases in activity. A similar CR when low doses of morphine are used as the UCS was also reported earlier by Kamat, Dutta, and Pradhan (1974) and Perez-Cruet (1976).

Although it is generally agreed that the initial decrease in locomotor activity obtained from high systemic doses of morphine is due to an effect on opiate receptors at the terminals of mesolimbic DA neurons, the finding that small doses of morphine elicit only increases in locomotor activity suggests that morphine may have an independent excitatory effect on DA neurons. First, the opiate receptor antagonist, naloxone, blocks not only the depressant effect on locomotor activity of high systemic doses of morphine but also the excitatory effect of both low and high doses (Holtzman, 1976; Oka and Hosoya, 1976; Ostrowski, Hatfield, and Caggiula, 1982; Vasko and Domino, 1978). Thus, if the increases in locomotor activity were merely a "rebound" effect of the depressant action of high systemic doses of morphine, one would not expect them to be reversed by opiate receptor blockade. Second, Johnson, Sar, and Stumpf (1980) have demonstrated the presence of enkephalin terminals in close relation to DA mesolimbic cell bodies and opiate receptors have been located either on or proximal to these cell bodies (Elde, Hokfelt, Johannsson, Lungdahl, Nilsson, and Jeffcoate, 1978; Schwartz, 1979). These neurons project to the nucleus accumbens. Noting this, Joyce and Iversen (1979) injected morphine directly into the ventral tegmental area (VTA, the site of the cell bodies of the mesolimbic DA system). They found that these injections produced only an increase in locomotor activity and, furthermore, that this increase became enhanced with repeated injections. The increase in locomotor activity was reversed by naloxone and blocked by the DA receptor antagonist, haloperidol. Similar results have since been obtained from injections into the VTA of

enkephalin (Broekkamp, Phillips, and Cools, 1979) and beta-endorphin (Schwartz, Ksir, Koob, and Bloom, 1981). This excitatory effect on locomotor activity of opiate administration into the VTA seems to be due to an independent excitatory effect of opiates on mesolimbic DA neurons which results in enhanced release of DA in the region of the nucleus accumbens. Support for this view comes from demonstrations that morphine injected into the VTA causes an increase in the single-unit activity of mesolimbic DA cells (Gysling and Wang, 1982; Matthews and German, 1982). Furthermore, bilateral administration of haloperidol into the nucleus accumbens (Pijnenburg, Honig, and VanRossum, 1975) and selective 6-hydroxydopamine-induced lesions of the mesolimbic DA neuron terminals in the nucleus accumbens (Kelly and Iversen, 1976) both inhibit d-amphetamine-induced increases in locomotor activity, an effect thought to depend on DA action in the nucleus accumbens.

Thus, although the mechanism of morphine action proposed by Pollard et al. (1977) might appear to account for the effect of systemic injections of high doses of morphine, it cannot accomodate the increases in locomotor activity obtained with low systemic doses or from opiate administration directly into the VTA. Indeed, the Joyce and Iversen (1979) results suggest that another more direct action of morphine on the cell body region of mesolimbic DA neurons could be responsible both for the increases in locomotor activity obtained with low doses of morphine and the enhancement of these activity increases seen following repeated systemic injections of morphine. If one returns to the conditioning study of Mucha et al. (1981), it becomes clear that

an alternative explanation of their findings is possible. Because both low and high doses of morphine, when administered repeatedly in a distinctive environment, resulted in conditioned increases in locomotor activity, the possibility arises that what they reflect is the independent conditioning of the excitatory effects of morphine acting on opiate receptors in the VTA. In this case, the UCR to morphine would be the increases in locomotor activity and not the decreases in activity as suggested by Mucha et al. (1981) and required by Siegel (1975, 1977a, 1979). It cannot be denied, however, that morphine may also have unconditioned depressant effects on locomotor activity (as with high doses). Furthermore, it is not known whether the development of tolerance to these depressant effects is due to the direct activation of DA neurons by morphine action in the cell body region or to the negative feedback effect of morphine inhibition of DA release from terminals that initiates increased DA synthesis or both. (Another possibility, of course, may be decreasing affinity for morphine of opiate receptors at DA neuron terminals.) It is nonetheless clear that the excitatory effect of morphine on the cell body region of mesolimbic DA neurons is a real and independent effect and that it may provide the UCR for the conditioned increases in locomotor activity reported by Mucha et al. (1981).

One implication that arises from these findings is that the opiate receptors in the DA mesolimbic cell body region responsible for the low dose effects are more sensitive than those in the terminal regions that are activated by higher doses. It is well known that opiate receptors are not homogenous and may

differ in their sensitivities to morphine according to their location (Lord, Waterfield, Hughes, and Kosterlitz, 1977; Wood, 1982). The possibility then exists that the opiate receptors in the cell body and terminal regions of mesolimbic DA cells are of different types. This possibility has not been elucidated to date. It remains, however, that the net behavioral effect of systemic injections of morphine may reflect a difference in the properties of these two populations of receptors.

The VTA and Morphine Reward

There is now convincing evidence that the VTA not only mediates the excitatory effects of morphine on locomotor activity, but also mediates the rewarding effects of morphine (for a review, see Bozarth, 1983). Morphine injected into the VTA produces facilitation of brain stimulation reward (Broekkamp, Van den Boggard, Hiejnen, Rops, Cools, and Van Rossum, 1976). Rats will self-administer morphine into the VTA but not into other brain regions (Bozarth and Wise, 1980, 1981a). Self-administration is blocked by naloxone and does not seem to be due to behavioral arousal since animals in a yoked control group lever press significantly less often than experimental animals. Animals administered morphine into the VTA in a distinctive place in their environment will show a shift in preference for that place, that is, a conditioned place preference (Phillips and LePiane, 1980). That opiate action on opiate receptors in the VTA is not only sufficient but also necessary for reward was recently demonstrated by Britt and Wise (1983). They found that injection of an opiate receptor blocker into the VTA but not into

other brain regions attenuated heroin reward as evidenced by compensatory increases in intravenous heroin self-administration.

These data, therefore, when considered together with reports of opiate receptors in the VTA and the findings of Joyce and Iversen (1979) reviewed earlier, suggest that the rewarding effects of morphine may be mediated by mesolimbic DA neurons. Thus, DA receptor antagonists have been found to block not only the increases in locomotor activity induced by injections of morphine into the VTA but also to block morphine reward as evidenced by the lack of development of the conditioned place preference normally produced by systemic heroin (Bozarth and Wise, 1981b). It is worthwhile noting that since animals are tested for conditioned place preference in a drug-free state, it is unlikely that the proposed sedative effects of neuroleptics could account for the latter findings.

It is important also to note that the VTA is a site which does not seem to be associated with the elicitation of withdrawal reactions upon termination of morphine administration. This was demonstrated in an elegant experiment by Bozarth and Wise (1983). They found that the abrupt termination of morphine administration obtained by a naloxone injection precipitated withdrawal reactions in animals that had received morphine continually for three days into the periventricular gray region but not in animals that had been administered morphine into the VTA. It is quite apparant, therefore, that animals will self-administer morphine into the VTA because of the rewarding effects of the drug and not to relieve withdrawal discomfort as required by drive-reduction theory. Recent studies have also made it clear

that neither excessive drug experience nor physical dependence is necessary to obtain conditioned place preference. Indeed, significant place preference has been reported after one injection of morphine (Mucha, Van der Kooy, O'Shaughnessy, and Bucenieks, 1982) and heroin (Bozarth and Wise, 1983). Since these studies involved only one injection, there was no opportunity for the animals to learn about the effects of a drug injection on any possible withdrawal reaction.

The Present Experiments

The finding that both the locomotor activity and the rewarding effects of morphine appear to be mediated by the same substrate suggests a unique way to study the conditionability of the central motivational state induced by morphine. Iversen (1983), for example, has suggested that the increased locomotor activity induced by morphine in the VTA is indicative of a state of motivational arousal associated with activation of mesolimbic DA neurons. Conditioned increases in locomotor activity may therefore reflect the elicitation by conditioned cues of the same motivational state.

In the following experiments, therefore, the conditionability of the increases in locomotor activity induced by morphine administration into the VTA was studied. In all experiments, with the exception of Experiment 4, animals were bilaterally implanted with cannulae aimed at the VTA and their locomotor activity measured subsequent to morphine administration. It should be remembered that this site of administration permits the study of morphine induced increases in

locomotor activity unconfounded with either the decreases in locomotor activity or the withdrawal reaction liability seen to be possible with systemic administrations.

Body temperature was also measured and represents a more exploratory function of the experiments. First, it is easy to measure and did not interfere with the monitoring of locomotor activity. Second, little is known of the anatomical sites which mediate the temperature effects of morphine (see Clark, 1981). Several studies have demonstrated a dose-dependent effect following intracranial injections of morphine into the preoptic-anterior hypothalamus. Low doses of morphine (Cox et al., 1976; Teasdale, Bozarth, and Stewart, 1981) and beta-endorphin (Martin and Bacino, 1979) administered to this site result in hyperthermia whereas injections of high doses of morphine result in hypothermia (Lotti et al., 1965). Tseng, Wei, Loh, and Li (1980) also found that intermediate doses of beta-endorphin injected into this site induced a biphasic response: an initial hypothermia followed by hyperthermia. In this laboratory, pilot studies have indicated that a mild hyperthermia may be elicited from administration of morphine into the VTA and it was decided to study this effect more systematically. The following experiments, furthermore, provided the opportunity to study both the unconditioned effect and its potential for conditioning.

Experiment 1 was designed to examine whether the increases in locomotor activity induced by morphine administration could come to be elicited by the environment in which morphine was repeatedly administered (the CS).

In Experiment 2, the effects of the DA receptor antagonist,

pimozide, both on the unconditioned increases in locomotor activity and on the development of conditioning of these activity increases were examined.

Experiment 3 was designed to investigate the possibility that the sensitization of the activity increases reported by Joyce and Iversen (1979) could be a conditioned phenomenon specific to the injection environment. The effect of DA receptor blockade on the development and expression of this sensitization was also investigated.

In Experiment 4, the anatomical specificity of the locomotor activity and body temperature effects of morphine in the VTA were assessed by comparing the response levels of animals with cannulae placements dorso-lateral to the VTA to those of animals with cannulae placements in the VTA.

In Experiments 5 and 6, the pharmacological specificity of the morphine effect on locomotor activity and body temperature, respectively, was assessed with challenging injections of the opiate receptor blocker naloxone.

EXPERIMENT 1

This experiment was designed to determine whether the increases in locomotor activity induced by morphine administration into the VTA could come to be elicited by the environment in which morphine was repeatedly administered. Accordingly, animals were given a number of daily morphine administrations in a distinctive environment. On subsequent conditioning test days, animals were returned to the distinctive environment without morphine administration and their locomotor activity measured. If these animals showed higher levels of locomotor activity on conditioning test days than animals that had not received the explicit morphine-distinctive environment pairings, conditioning could be said to have occurred.

Body temperature was also measured subsequent to each morphine administration and on conditioning test days. Thus, both the unconditioned effect of morphine administration into the VTA and its potential for conditioning were investigated.

Methods

Subjects

Thirty-five male Wistar rats, obtained from Charles River Canada Inc. (St. Constant, Quebec) and weighing 275-300 g on arrival, were used. They were housed individually in stainless steel cages (18 cm x 24 cm x 18 cm) located in a reverse cycle room lit from 22:00 to 10:00 h and maintained at a constant

temperature of 21±1 degrees C. Purina Lab Chow and water were available to the animals ad libitum for the duration of the study.

Surgery

One to two weeks after arrival, animals were anaesthetized with sodium pentobarbital (.85 ml/kg Somnotol, M.T.C. Pharmaceuticals Ltd.) and stereotaxically implanted with chronic bilateral guide cannulae (22 guage, Plastic Products Company) aimed at the ventral tegmental area (VTA) and positioned 1 mm above the final injection site. The VTA coordinates were: A/P -3.8, L ± 0.6, and D/V -8.9 from skull (Pellegrino, Pellegrino, and Cushman, 1977). The guide cannulae were implanted at 16 degrees to the vertical. This permitted the use of the Plastic Products blocker and injector cannulae (both 28 guage) and furthermore steered the guide cannulae around the periventricular gray region (PVG) thus preventing damage to it and penetration of the cerebral ventrical. It is well known that one problem with intracranial drug administration is that the drug may diffuse up the cannula shaft (see Routtenberg, 1972). The angled implants used in this and the following experiments, therefore, helped circumvent the problem of drug reaching the PVG and the cerebral ventrical (merely 2 mm dorsal to the VTA) and helped ensure the neuroanatomical specificity of the drug effects under study. Cannulae placements were verified by the use of standard histological techniques.

Following surgery and for the remainder of the experiment, the animals' home cages were fitted with an aluminum plate floor covered with beta chip and wire screen covering the front of the

cage. Access to the food hopper remained unimpeded. These precautionary measures successfully prevented operated animals from pulling off their implants and contributed to distinguishing the home cage from the test environment.

Apparatus

A bank of 12 activity boxes was used to measure locomotor activity. Each box (20 cm x 41 cm x 25 cm) was constructed of white pressed wood (rear and two side walls), a wire screen ceiling, a Plexiglas front hinged door, and a floor consisting of 24 stainless steel rods. In addition, stainless steel plates covered the inside upper half of each side wall and the upper half region extending 11 cm from each end of the rear wall. The plates and the left, center, and right thirds of the floor each supported an interrupted current of 1.5 uA. One count was registered when an animal completed any break in the circuit. This permitted recording of both horizontal locomotion and rearing.

The activity boxes were kept in a room lit dimly with red light and maintained at a constant temperature of 21±1 degrees C. White noise (75 decibels) was continuously present to mask extraneous noise. The recording apparatus was situated in an adjacent room.

Rectal temperature was measured by means of a small probe (Yellow Springs model 402) and a Yellow Springs TeleThermometer model 46 TUC (accuracy = ± .15 degrees C). Animals were placed in a small rectangular trough closed at one end (9 cm x 27 cm x 10 cm) and were lightly restrained by the tail while the probe was

inserted a minimum of 6 cm as recommended by Lomax (1966) for approximately 30 s until the temperature reading stabilized.

Design

The experimental design involved giving daily morphine administrations to one group of animals in a distinctive environment, the activity box (Group Sham-HC/Morphine-AB), and to another group in the home cage (Group Sham-AB/Morphine-HC). Each group also received daily sham administrations in the other environment. Locomotor activity and temperature were recorded in the distinctive AB environment. Sham administrations were given first followed three hours later by morphine administrations. As a result, Group Sham-AB/Morphine-HC experienced the AB administration first followed by the HC administration while the reverse was true for Group Sham-HC/Morphine-AB. To control for possible order effects on the locomotor activity and temperature measures, two control groups were included. Each received daily sham administrations in both environments but in the opposite order (i.e.: Sham-AB/Sham-HC and Sham-HC/Sham-AB). Consequently, animals were randomly assigned to one of four groups: a conditioning group (Group Sham-HC/Morphine-AB), n=9; a conditioning control group (Group Sham-HC/Sham-AB), n=8; a pseudo-conditioning group (Group Sham-AB/Morphine-HC), n=10; and a pseudo-conditioning control group (Group Sham-AB/Sham-HC), n=8. Figure 1 summarizes the daily routine for all groups on conditioning days. Animals remained in their respective administration environments for 90 min. Following a three hour period in which all animals remained in their home cage, the procedure was reinstated for another 90 min period as per Figure

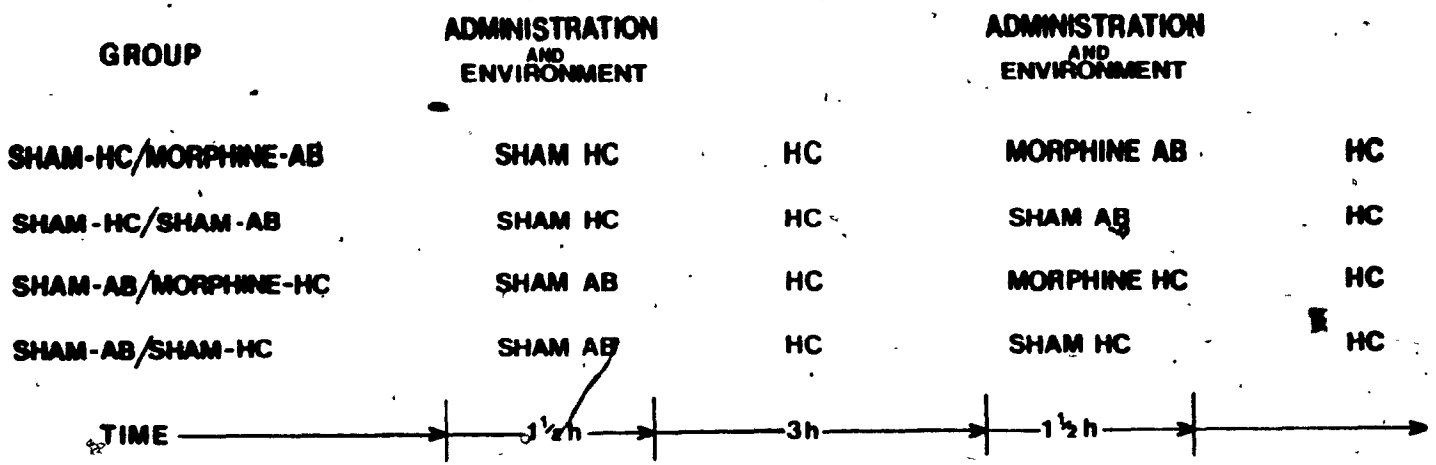

Figure 1. Summary of daily routine followed for all groups on conditioning days in Experiment 1. Locomotor activity and temperature were measured in the distinctive activity box environment. AB: activity box. HC: home cage.

1. Animals were run during their dark cycle (10:00-22:00).

This procedure was maintained five days a week for three weeks. Group Sham-HC/Morphine-AB and Group Sham-AB/Morphine-HC received daily morphine administrations four days a week. On day five of each week, all animals received sham administrations in both environments (the intermittent conditioning tests). Thus, animals in Group Sham-HC/Morphine-AB and Group Sham-AB/Morphine-HC received an equal number of morphine administrations (12) but these were paired with the distinctive environment (AB) only for animals in Group Sham-HC/Morphine-AB. Following a 10-day procedure- and drug-free period in which all animals remained in their home cage, the procedure was reinstated and all animals received sham administations in both environments (the final conditioning test).

Procedure

Animals were given two to three weeks to recover from the surgical trauma. During the last week of recovery, they were habituated to the temperature measurement procedure. After one or two measurements, animals accepted the procedure with little objection. This habituation was done in the animal room and represents the only training animals received prior to the start of the experiment.

Administration Procedure. Morphine was administered in the form of morphine sulphate crystal (B.D.H. Chemicals Canada Ltd.). The crystal was tapped into the tip of 28 gauge injector cannulae (10-15 taps) and these applied intracranially. As a result, only one dose (18±2 ug/injector cannula) was used for all animals. Both sham and morphine administration involved bilateral lowering

of 28 gauge cannulae 1 mm beyond the tip of the guide cannulae. Morphine loaded injector cannulae were used for morphine administration and long blocker cannulae were used for sham administration. The tips of the blocker cannulae normally in place were flush to the guide cannulae tips. Administration cannulae remained in place for 90 min.

Activity Box Procedure. Animals were transported from the animal room in groups of eleven and twelve. Once in the AB room, they were given their respective administrations and placed individually in the AB's for 90 min. During this time, the locomotor activity of each animal was recorded for nine sequential 10 minute periods. Animals were then taken out of the AB's and their body temperature measured. Administration cannulae were removed and the animals returned to their home cages. No food or water was available in the activity boxes.

Home Cage Procedure. This procedure was identical to the activity box procedure except that animals did not leave the animal room. Animals were transported in groups to a counter in the animal room. They were given their respective administrations and returned to their home cages. After 90 min, the animals were taken out of their home cages, rectally probed, their administration cannulae removed, and returned to their home cages.

Results

Locomotor Activity-Conditioning Days.

Figure 2 shows the group mean activity counts obtained on the

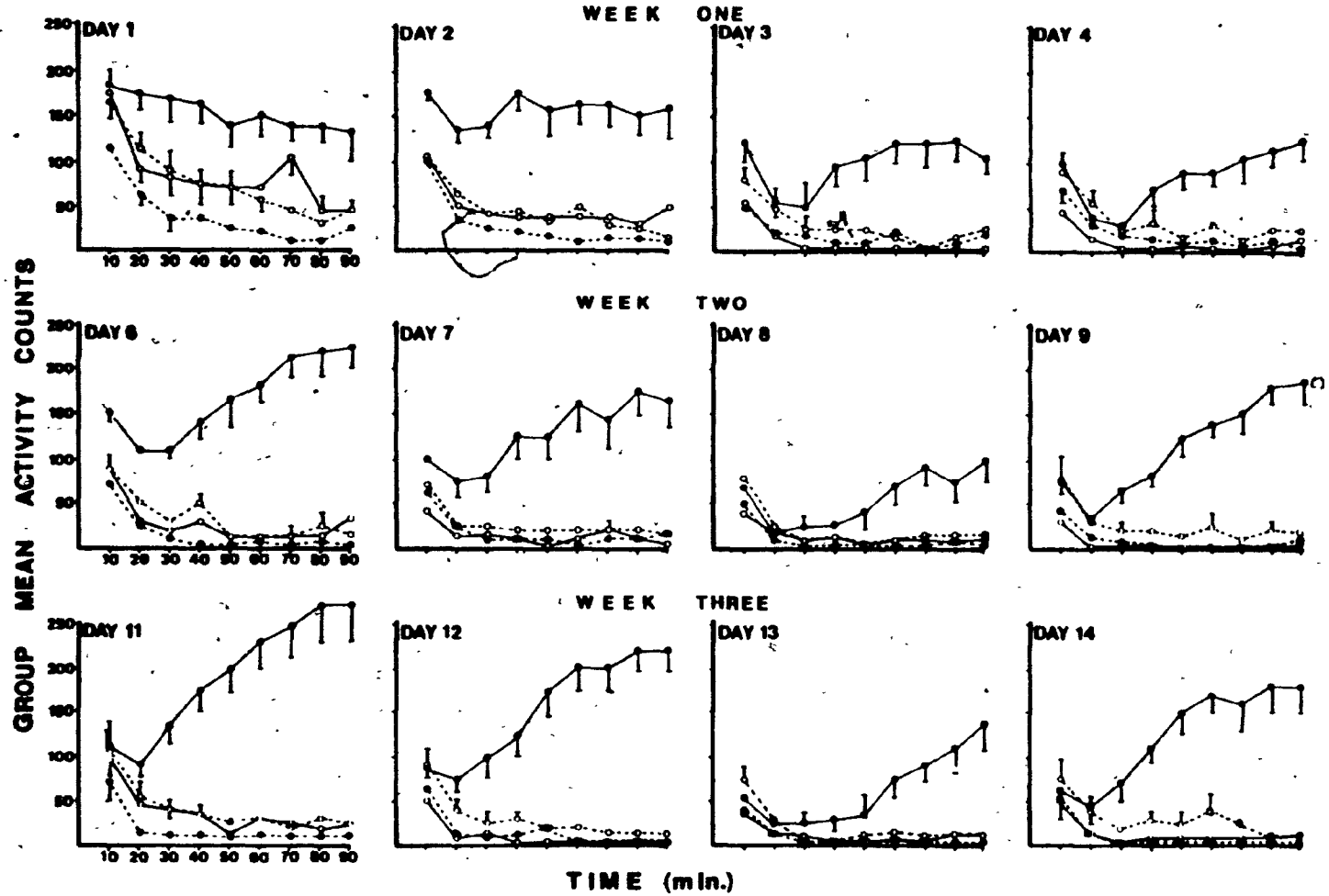

Figure 2. Group mean activity counts (± 1 S.E.M.) obtained on the 12 conditioning days for each of the four groups in Experiment 1. (●——●) Group Sham-HC/Morphine-AB, n=9, (●- - -●) Group Sham-HC/Sham-AB, n=8, (O——O) Group Sham-AB/Morphine-HC, n=10, (O- - -O) Group Sham-AB/Sham-HC, n=8. S.E.M.'s not shown in this and subsequent figures were too small to include.

12 conditioning days for each of the four groups. As can be seen, Group Sham-HC/Morphine-AB was consistently more active than all three other groups. On Day 1, this group showed a high level of activity which gradually decreased slightly. Over days, the increased locomotor activity of this group became characterized by lower initial values during the first 10 to 30 min after which activity increased progressively for the remainder of the session. All three other groups showed levels of activity which were higher at the beginning of each session and diminished with time.

Because of the extent of the data, differences between groups, days, and weeks were analyzed by using the day total activity counts obtained for each group. These are shown in Figure 3. The higher level of activity of Group Sham-HC/Morphine-AB relative to the three other groups is clear in this figure. Moreover, this group showed dramatic fluctuations in activity from one day to the next: first decreases from days one to three of each week followed by increases on the fourth day of weeks two and three. Activity levels for the three remaining groups were high on Day 1 and diminished to remain low on subsequent days. A repeated measures analysis of variance conducted on the day total activity counts revealed significant effects of groups, $[F(3,31)=101.08, p<.001]$, weeks $[F(2,62)=10.41, p<.001]$, days, $[F(3,93)=68.29, p<.001]$, and weeks x groups $[F(6,62)=2.31, p<.05]$, days x groups $[F(9,93)=14.53, p<.001]$, weeks x days $[F(6,186)=3.45, p<.005]$, and weeks x days x groups $[F(18,186)=3.38, p<.001]$ interaction. Multiple comparisons between means in this and the following experiments were

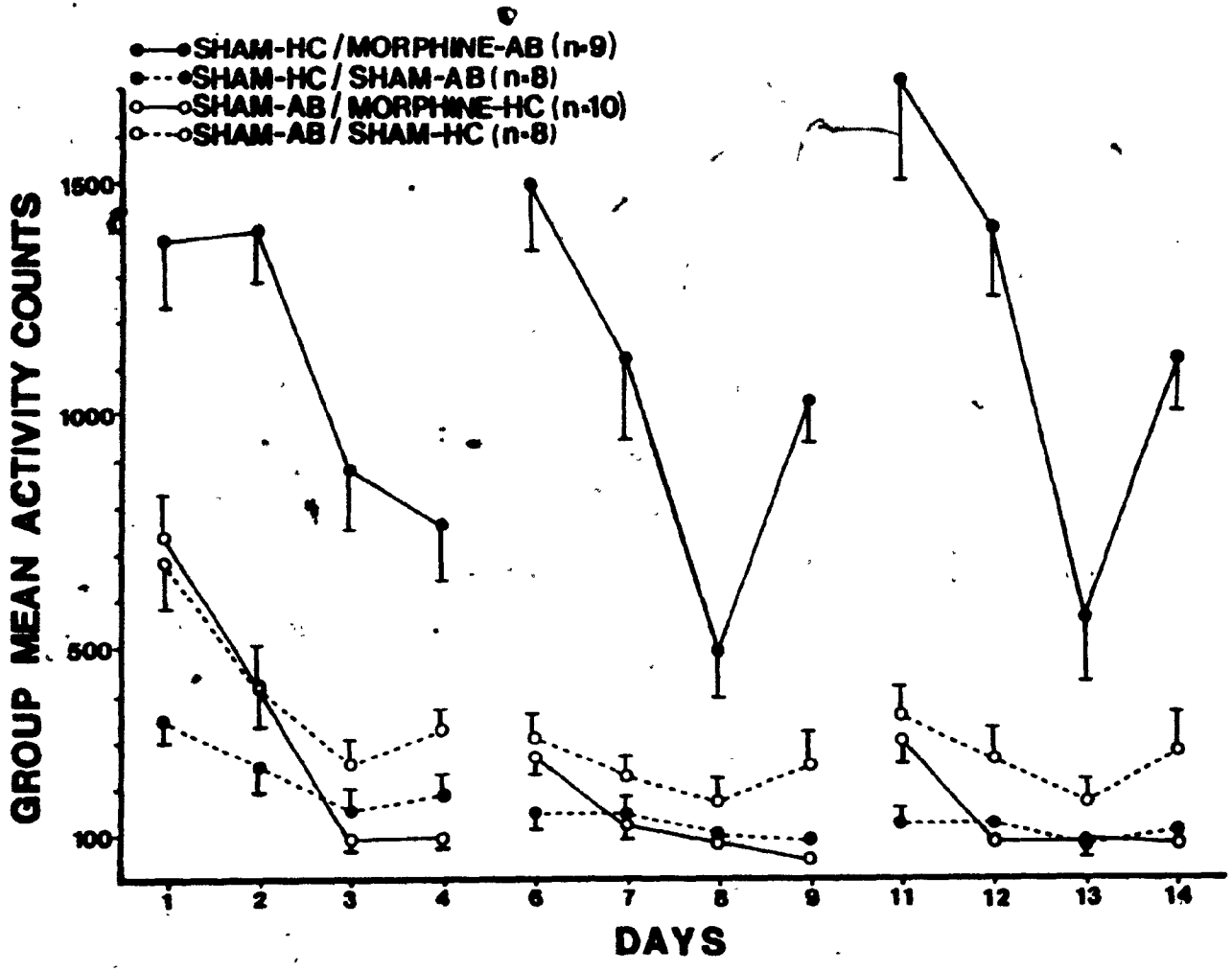

Figure 3. Group mean activity counts (± 1 S.E.M.) calculated from day totals obtained on the 12 conditioning days for each of the four groups in Experiment 1.

subsequently conducted with the Scheffe test. The appropriate error terms used were as specified by Kirk (1968).

These comparisons revealed that Group Sham-HC/Morphine-AB was significantly more active overall than all three other groups combined [$F(3,31)=99.13$, $p<.001$]. The latter three groups did not differ significantly from each other.

Group Sham-HC/Morphine-AB showed a slight increase in activity level from week one to week three although this was not significant [$F(2,62)=1.18$, $p>.05$]. The activity levels of the remaining three groups combined diminished significantly during this same period [$F(2,62)=9.19$, $p<.001$].

Although the significant groups x days interaction was clearly due to the much greater magnitude of fluctuations in Group Sham-HC/Morphine-AB relative to the other groups, a significant difference in activity level was also found between Group Sham-HC/Sham-AB and Group Sham-AB/Sham-HC on the combined first days of each week [$F(3,372)=2.79$, $p<.05$]. This difference was no longer present on the combined second days of each week [$F(3,372)=.76$, $p>.05$]. Thus, a significant order effect was manifest on the first day of the week and dissipated on subsequent days.

Locomotor Activity-Conditioning Tests.

Figure 4 shows the group mean activity counts obtained on the three intermittent conditioning test days (i.e., Days 5, 10, and 15) for each of the four groups. As can be seen, there is no evidence of conditioning. All groups had very low levels of activity. Repeated measures analyses of variance conducted on the data from each day revealed a significant effect of time [$F(8,248)=30.45$, $p<.01$] for Day 5, significant effects of groups

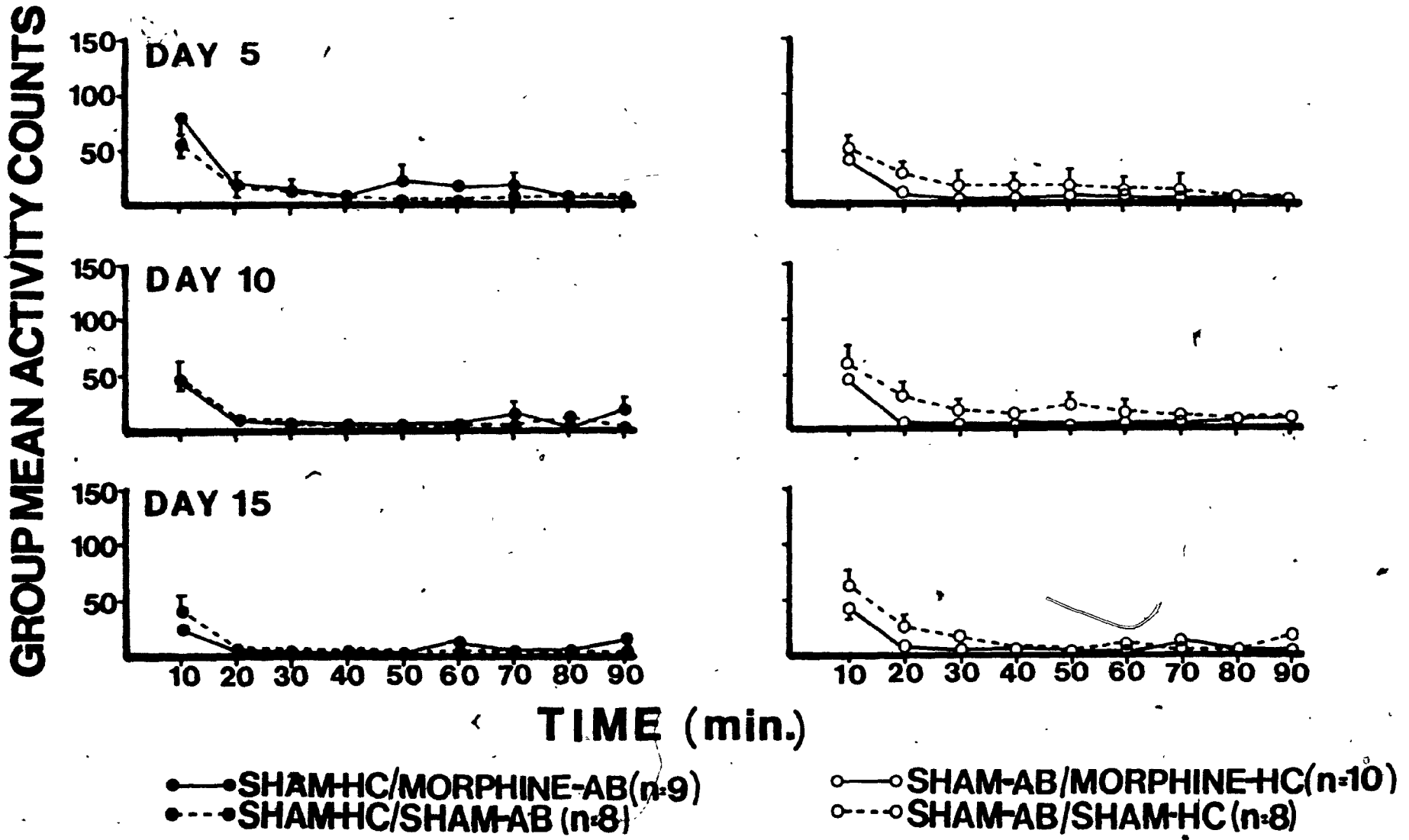

Figure 4. Group mean activity counts (± 1 S.E.M.) obtained on the three intermittent conditioning test days for each of the four groups in Experiment 1.

[F(3,31)=3.34, p<.05] and time [F(8,248)=27.71, p<.05] for Day 10, and significant effects of groups [F(3,31)=3.44, p<.05], time [F(8,248)=29.87, p<.05], and groups x time interaction [F(24,248)=1.85, p<.05] for Day 15. The significant effect of groups on Day 10 was due to Group Sham-AB/Morphine-HC having a slightly but significantly lower overall activity level than Group Sham-AB/Sham-HC [F(3,31)=2.91, p<.05]. No other group comparisons were significant on this day. On Day 15, Group Sham-AB/Sham-HC was slightly but significantly more active than the three remaining groups combined [F(3,31)=3.03, p<.05].

Figure 5 shows the group mean activity counts obtained on the final conditioning test day. It is clear that conditioned locomotor activity was obtained on this day. A repeated measures analysis of variance conducted on these data revealed significant effects of groups [F(3,31)=7.59, p<.001], time [F(8,248)=61.15, p<.001], and groups x time interaction [F(24,248)=1.75, p<.025]. Group Sham-HC/Morphine-AB was significantly more active overall than its control, Group Sham-HC/Sham-AB, [F(3,31)=4.71, p<.01] whereas Group Sham-AB/Morphine-HC did not differ significantly from its control, Group Sham-AB/Sham-HC, [F(3,31)=.7, p>.05]. The order effect found on the first day of each week during conditioning was also found on this day. Although Group Sham-HC/Morphine-AB did not differ significantly from Group Sham-AB/Morphine-HC and Group Sham-AB/Sham-HC combined [F(3,31)=.07, p>.05], these latter two groups combined were significantly more active overall than Group Sham-HC/Sham-AB [F(3,31)=17.81, p<.001].

Thus, Group Sham-HC/Morphine-AB, which had received morphine

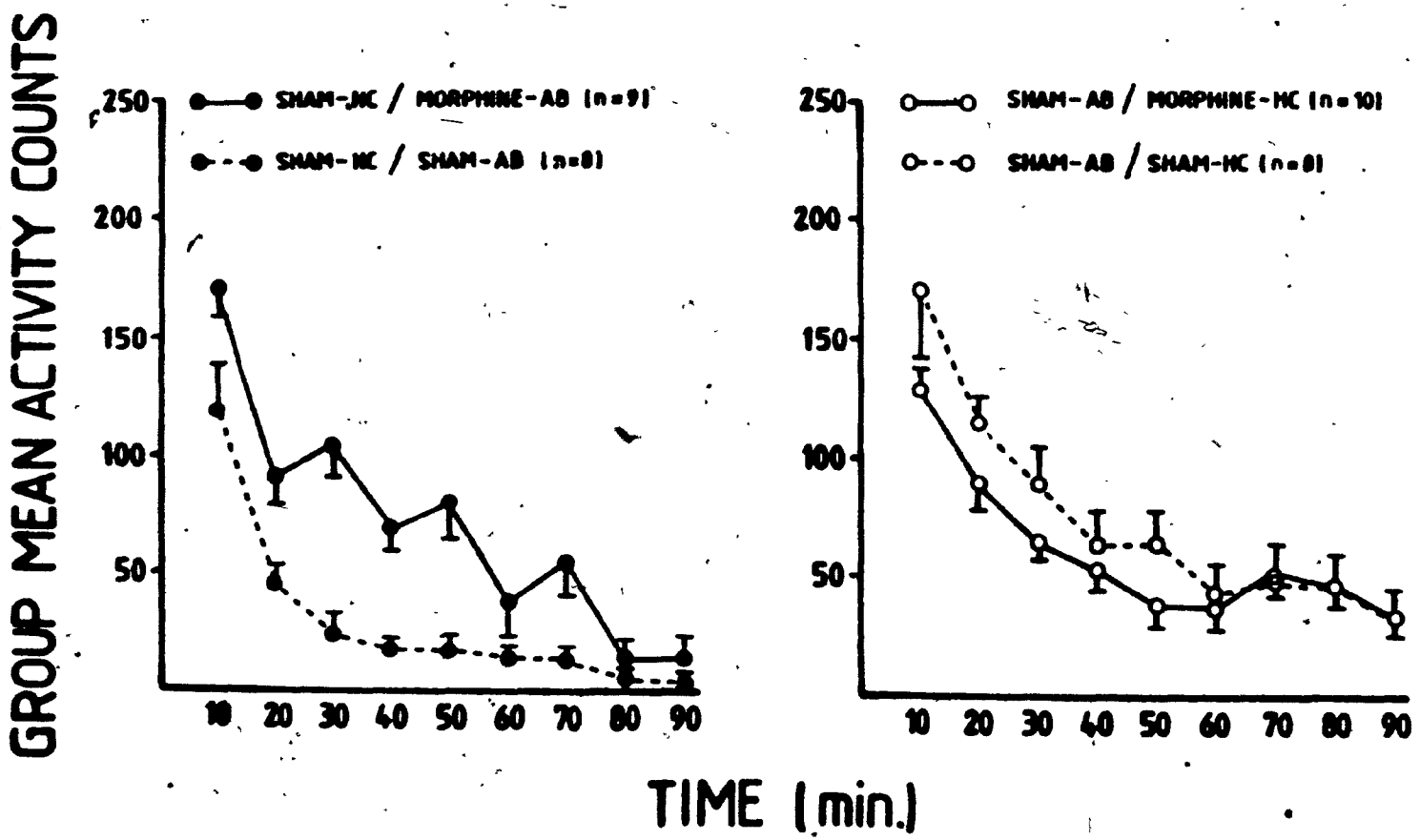

Figure 5. Group mean activity counts (± 1 S.E.M.) obtained on the final conditioning test day for each of the four groups in Experiment 1.

administrations repeatedly in the AB, was significantly more active than its sham control group whereas Group Sham-AB/Morphine-HC, which had received an equal number of morphine administrations but never paired with the AB, did not differ significantly from its sham control group. Furthermore, a strong order effect was evident in that Group Sham-HC/Sham-AB was significantly less active than Group Sham-AB/Morphine-HC and Group Sham-AB/Sham-HC combined.

Body Temperature-Conditioning Days.

Figure 6 shows the group mean rectal temperatures obtained on the 12 conditioning days for each of the four groups. As can be seen, Group Sham-HC/Morphine-AB was consistently hyperthermic relative to the other three groups. As with the locomotor activity measure, this group showed considerable fluctuation in body temperature from one day to the next. Body temperature in the remaining three groups was slightly elevated during week one and gradually diminished on weeks two and three.

A repeated measures analysis of variance conducted on these data revealed significant effects of groups [$F(3,31)=55.72$, $p<.001$], weeks [$F(2,62)=23.57$, $p<.001$], days [$F(3,39)=22.91$, $p<.001$], and weeks x groups [$F(6,62)=2.53$, $p<.01$] and days x groups [$F(9,93)=3.01$, $p<.001$] interaction. Multiple comparisons between means revealed that Group Sham-HC/Morphine-AB had significantly higher body temperatures overall than all three other groups combined [$F(3,31)=54.68$, $p<.001$]. The latter three groups did not differ from each other. Furthermore, while Group Sham-HC/Morphine-AB showed no change in body temperature from week one to week three, the body temperatures of the remaining

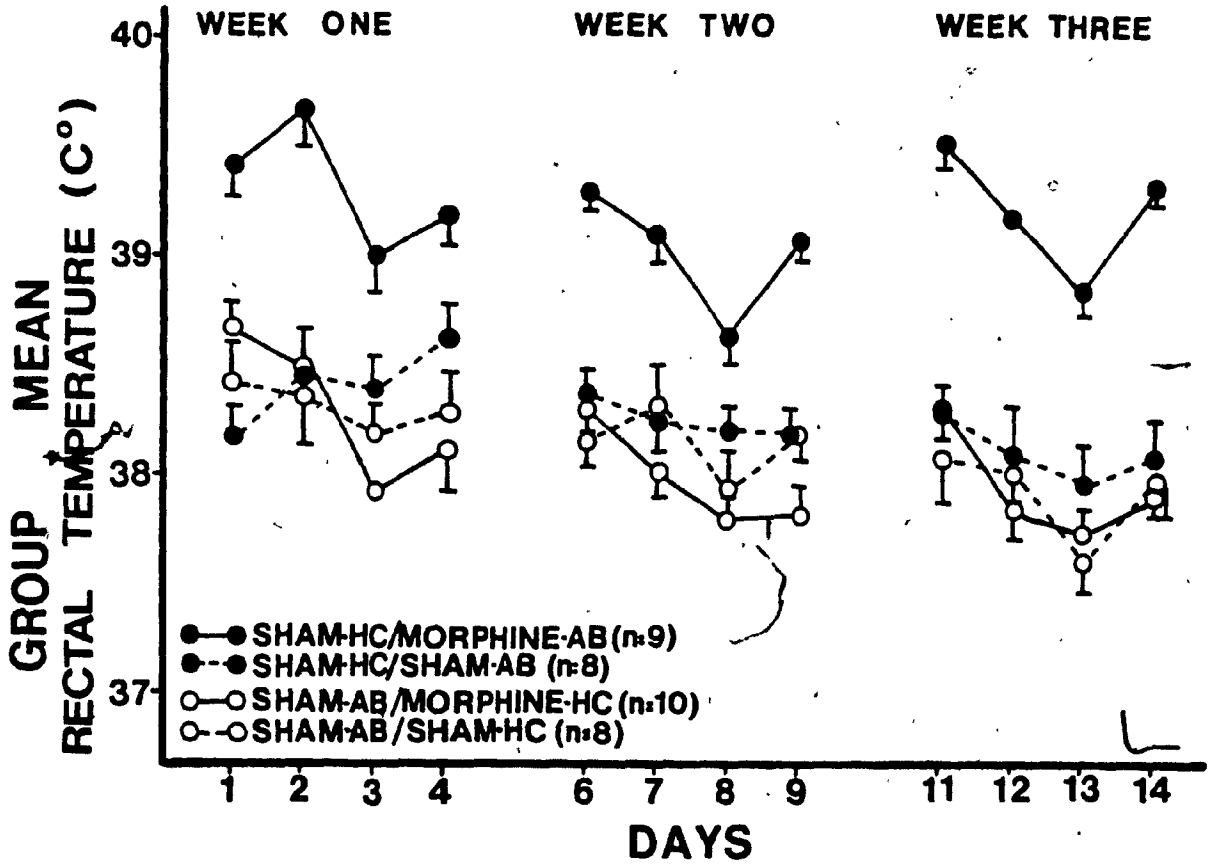

Figure 6. Group mean rectal temperatures (± 1 S.E.M.) obtained on the 12 conditioning days for each of the four groups in Experiment 1.

three groups combined diminished significantly in this period [$F(2,62)=24.75$, $p<.001$]. As can be seen from Group Sham-AB/Sham-HC and Group Sham-HC/Sham-AB in Figure 6, no order effect on the body temperature measure was apparent.

Body Temperature-Conditioning Tests.

The group mean rectal temperatures obtained on the three intermittent conditioning test days and on the final conditioning test day for each of the four groups are shown in Figure 7. As can be seen, there is no evidence for conditioning on any of the test days including the final conditioning test day. None of the one-way analyses of variance conducted on the data from each day even approximated a significant F ratio [$F(3,31)=.16$ for Day 5; .47 for Day 10; 1.23 for Day 15; and .58 for the final conditioning test day; $p>.05$].

Discussion

This experiment clearly demonstrated that morphine administration into the VTA results in both increases in locomotor activity and body temperature. Furthermore, the final conditioning test day revealed that the morphine induced increases in locomotor activity could come to be elicited by the environment in which morphine was repeatedly administered. This suggests, therefore, that a learned association developed between morphine (the UCS) and the distinctive AB environment (the CS). There was no evidence for conditioning of the morphine-induced hyperthermia.

It might be observed from Figure 5 that on the final conditioning test day Group Sham-HC/Morphine-AB was not

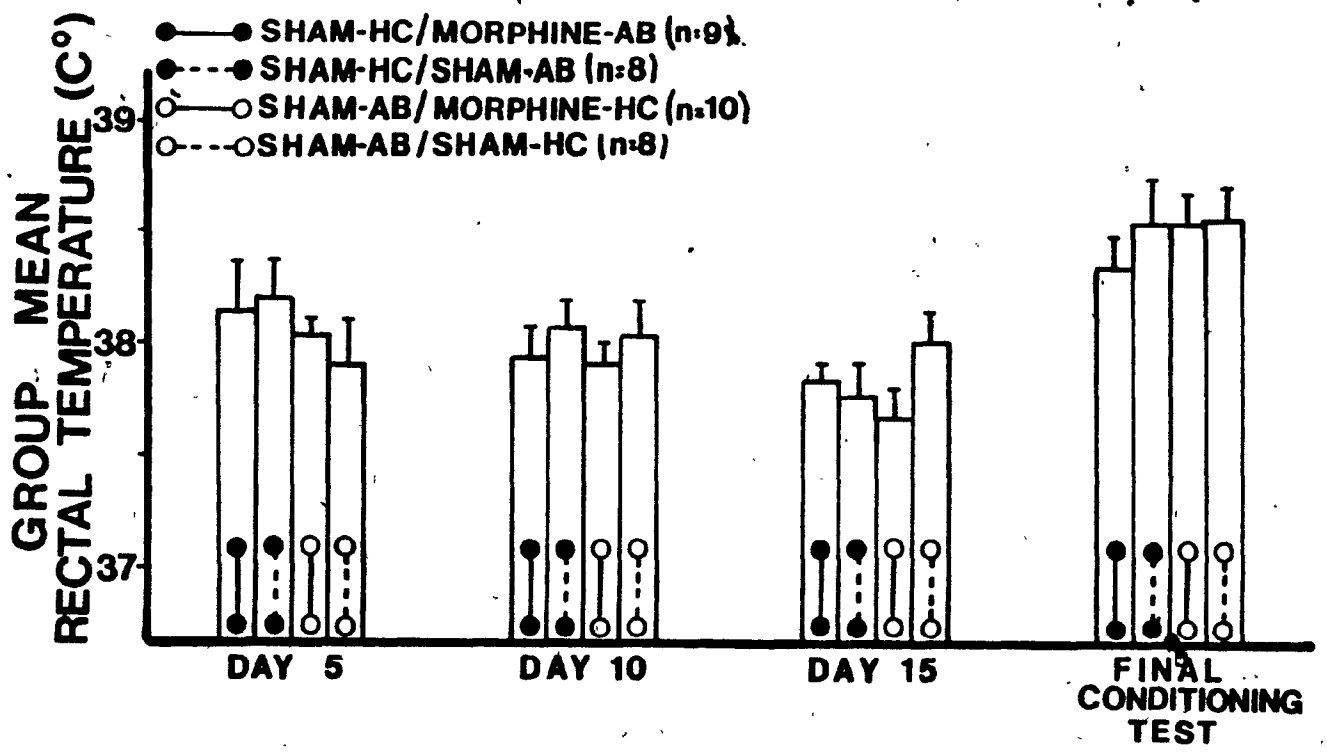

Figure 7. Group mean rectal temperatures (+ 1 S.E.M.) obtained on the three intermittent and on the final conditioning test day for each of the four groups in Experiment 1.

significantly more active than Group Sham-AB/Morphine-HC. It should also be noted that the important comparison to make is that between Group Sham-HC/Morphine-AB and its sham control group (Group Sham-HC/Sham-AB) and further that Group Sham-AB/Morphine-HC did not differ from its sham control group (Group Sham-AB/Sham-HC). A significant effect of the order in which animals were tested in the AB was found as demonstrated by the activity levels of the two sham control groups. Group Sham-AB/Sham-HC, which was given the AB treatment first and the HC treatment second, was significantly more active than Group Sham-HC/Sham-AB which was given the two treatments in the reverse order. This order effect was also manifest during conditioning on the first day of each week. Thus, it is clear that the increased level of locomotor activity shown by Group Sham-HC/Morphine-AB as compared to its sham control group on the final conditioning test day was due to classical conditioning, that is, to a learned association between morphine and the distinctive AB environment and not to pseudo-conditioning (see Mackintosh, 1974, chapter 2).

It is less clear, meanwhile, why no evidence of conditioning was found on the intermittent conditioning test days (Days 5, 10, and 15). It was expected that the development of the morphine-distinctive AB environment association would be manifested by progressively increasing levels of conditioned locomotor activity. Figure 4 shows that this was not found. One possible explanation for this finding is that the aftereffect of repeated morphine administrations masked the conditioned effect on test days that immediately followed daily morphine administrations. Similar results have come from experiments in which animals were

given repeated daily injections of morphine and subsequently challenged with DA receptor agonists. For example, while rats tested three or more days after the last morphine injection have been found to be supersensitive to apomorphine (Gianutsos, Hynes, Puri, Drawbaugh, and Lal, 1974), animals tested 24 hours after the last morphine injection have been found to be subsensitive to apomorphine (Christie and Overstreet, 1979). Also, Eikelboom and Stewart (1979) reported that the conditioned hyperthermia they obtained manifested itself most clearly after a drug-free period of several days. Such findings, however, have no well established biochemical explanation and there is evidence from other studies with morphine that conditioned effects sometimes are manifest on the day following the last morphine administration (Mucha et al., 1981; Stewart, 1981). It is possible, nonetheless, that such a phenomenon may have had an effect on the manifestation of conditioning on the intermittent test days in this experiment.

Finally, Group Sham-HC/Morphine-AB showed large fluctuations in locomotor activity levels during conditioning days (Figure 3). These may have been due in part to varying latencies to morphine action at opiate receptors on different days (Figure 2) which could have occurred because of damage to opiate receptors proximal to the injector cannulae tips. The fact that morphine was administered daily may not have provided enough time for regeneration of damaged receptors. For this reason, the regimen of daily morphine administrations was discontinued. In the remaining experiments, successive morphine administrations were separated by at least one drug-free day.

EXPERIMENT 2

The accumulation of demonstrations of classical conditioning of morphine responses has prompted attempts to elucidate the neural mechanisms underlying the conditioned response (e.g.: Drawbaugh and Lal, 1974; Lal et al., 1976). The approach to this issue taken in this experiment was to first attempt to identify the biochemical basis of the UCS for increased locomotor activity when morphine is administered into the VTA. It may appear at first sight that one should regard the action of morphine at opiate receptors in the VTA as the UCS. It is considered by most workers in the field that the increased levels of locomotor activity that result from morphine administration into the VTA are caused by increased DA release at mesolimbic DA neuron terminals (Experiment 1; Broekkamp et al., 1979; Joyce and Iversen, 1979; Schwartz et al., 1981). This increase in DA release might then be viewed as the UCR to morphine's UCS action at opiate receptors in the DA cell body region. An alternative possibility, however, is that the action of released DA at receptors postsynaptic to DA terminals may constitute the UCS.

The present experiment sought to distinguish between these two possibilities by investigating the effect of the DA receptor blocker, pimozide, on the development of conditioned locomotor activity induced by morphine administration into the VTA. Pimozide does not block the action of morphine at opiate receptors (Leysen, Tollenaere, Koch, and Laduron, 1977) or the ensuing release of DA (in fact, it is thought to

contribute to it, Seeman, 1980). If, therefore, these actions of morphine were in fact the UCS and UCR respectively, the conditioning of increased locomotor activity should proceed unimpeded under the influence of pimozide. In this case, pimozide would merely block the behavioral manifestation of the UCR and it is well established that the occurrence of the behavioral UCR is not necessary for conditioning to occur (Mackintosh, 1974, chapter 3; Solomon and Turner, 1962).

If, on the other hand, the UCS action of morphine necessary for the conditioning of locomotor activity were effects subsequent to DA release at receptors postsynaptic to mesolimbic DA cell terminals, then DA receptor blockade would be expected to interfere with the conditioning of locomotor activity. The action of pimozide in this case would be, in effect, to block the UCS.

This experiment also investigated the effect of pimozide on the hyperthermia induced by morphine administration into the VTA. Animals might become hyperthermic following morphine administration into the VTA because of their increased locomotor activity. If animals showed hyperthermia to morphine administration following pretreatment with pimozide, another mechanism for the hyperthermia would be suggested. Furthermore, if hyperthermia were elicited by morphine following pimozide pretreatment, it is unlikely that DA systems were involved.

Methods

Subjects

Eleven male Wistar rats, obtained from Charles River Canada Inc. (St. Constant, Quebec) and weighing 275-300 g on arrival, were used. One to two weeks after arrival, they were stereotaxically implanted with chronic bilateral guide cannulae aimed at the VTA. Surgery and housing details were as specified in Experiment 1.

Apparatus

The apparatus and methodology used to measure locomotor activity and body temperature and the sham and morphine administration procedures were all as specified in Experiment 1. The dose of pimozide used was .5 mg/kg and was injected intraperitoneally (i.p.) in a volume of 1 ml/kg. The pimozide solution was prepared by dissolving pimozide (Janssen Pharmaceuticals) in a 3% solution of heated tartaric acid (Fisher Scientific Company). This dose of pimozide has been found to have no effect on body temperature when animals are tested in the ambient temperatures used in these experiments (Clark, 1979b).

Design and Procedure

Animals were randomly assigned to one of two groups: Group Pimozide-Morphine (n=6) and Group Pimozide-Sham (n=5). All animals were first injected with pimozide in their home cages. Four hours later, animals were carried to the distinctive AB room where animals in Group Pimozide-Morphine were given a morphine administration and animals in Group Pimozide-Sham were given a sham administration. All animals were then placed individually in

the AB's for 90 min and their locomotor activity measured. Following this period of time, animals were taken out of the AB's and their body temperature measured. Administration cannulae were removed and the animals returned to their home cages.

This procedure was repeated seven times once every other day. Following an eight day procedure- and drug-free period in which all animals remained in their home cages, the procedure was reinstated and all animals received a sham administration in the AB (the conditioning test day). On this day, animals received a tartaric acid vehicle injection (i.p., 1 ml/kg) four hours prior to testing.

Results

Locomotor Activity-Conditioning Days.

Figure 8 shows the group mean activity counts obtained on the seven conditioning days for each of the two groups. As can be seen, both groups showed very low levels of activity throughout. These were slightly higher at the beginning of Day 1 but diminished to stay low on subsequent days. There was no significant difference between levels of activity in the two groups. A repeated measures analysis of variance conducted on the day total activity counts revealed only a significant effect of days [$F(6,54)=2.61$, $p<.05$]. Thus, pimozide completely blocked the increases in locomotor activity normally induced by morphine administrations into the VTA.

Locomotor Activity-Conditioning Test.

Figure 9 shows the group mean activity counts obtained on the

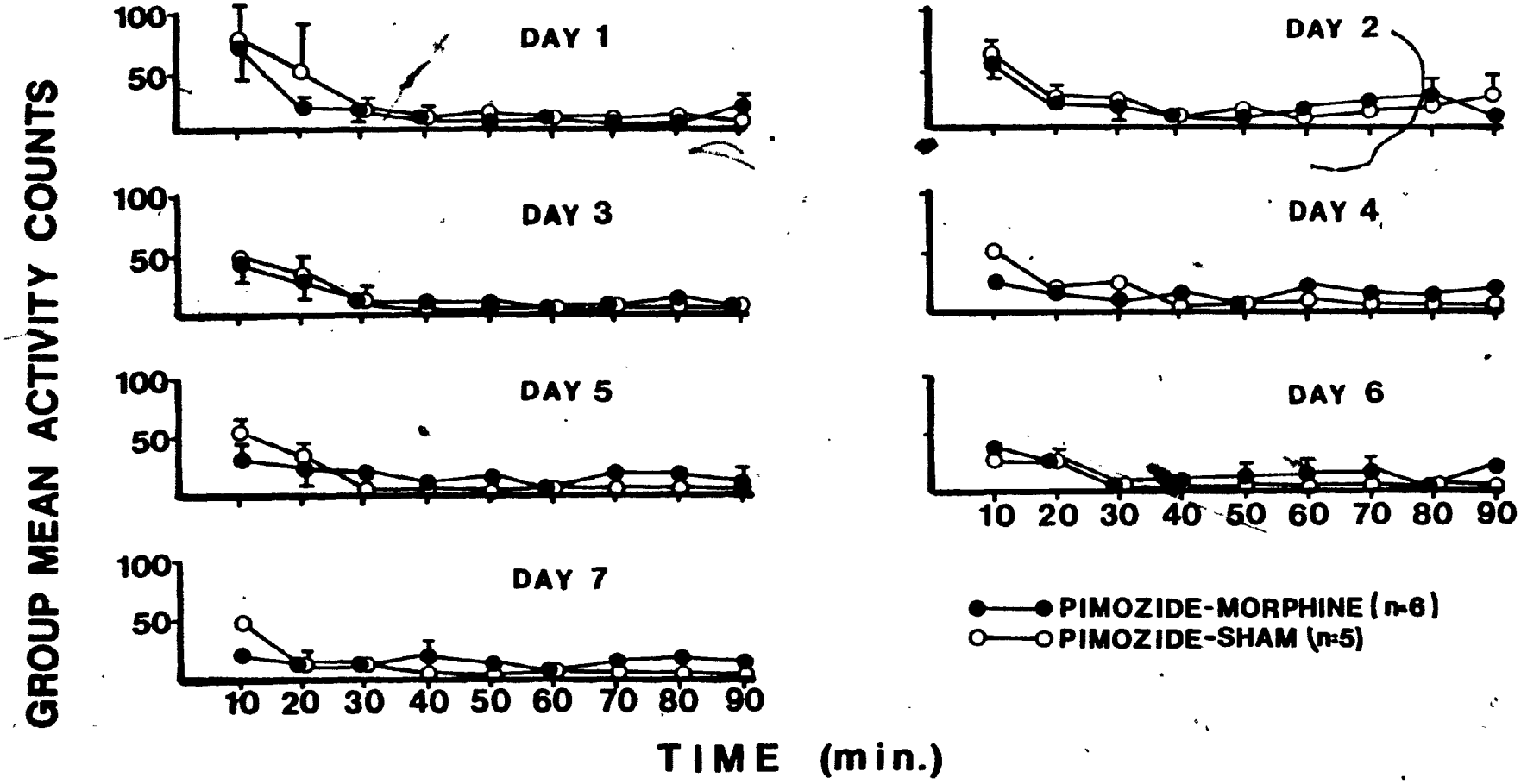

Figure 8. Group mean activity counts (± 1 S.E.M.) obtained on the seven conditioning days for each of the two groups in Experiment 2.

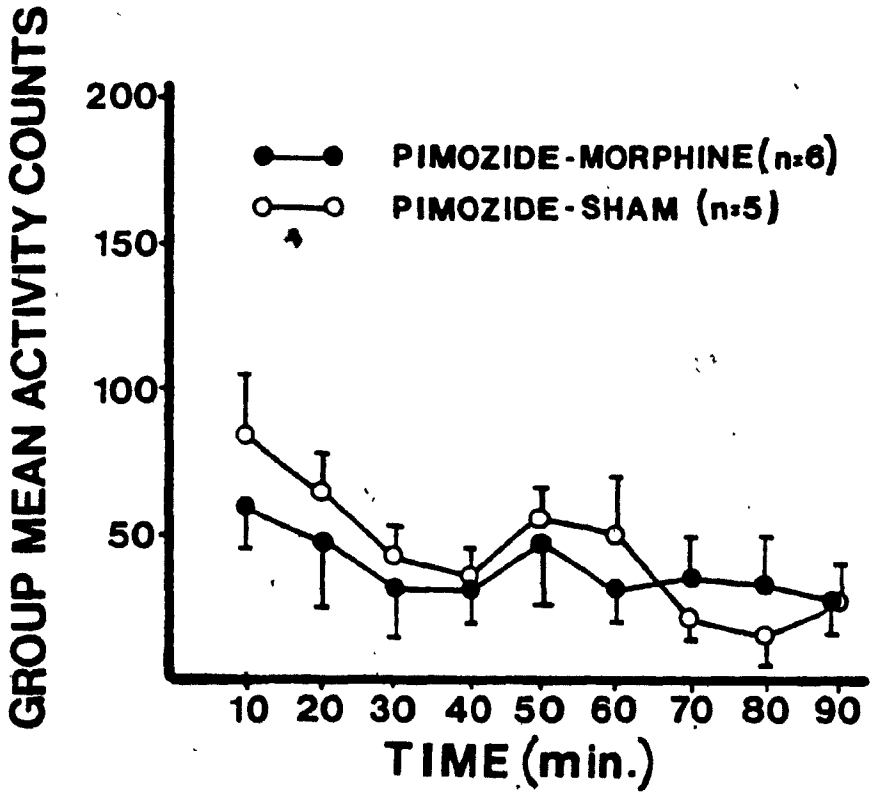

Figure 9. Group mean activity counts (± 1 S.E.M.) obtained on the conditioning test day for each of the two groups in Experiment 2.

conditioning test day for each of the two groups. Group Pimozide-Morphine was not significantly more active than Group Pimozide-Sham. A repeated measures analysis of variance conducted on these data revealed only a significant effect of time [$F(8,72)=4.01$, $p<.001$]. Activity levels for both groups were slightly higher at the beginning of the session and diminished gradually with time.

Body Temperature-Conditioning Days.

The group mean rectal temperatures obtained on the seven conditioning days for each of the two groups are shown in Figure 10. As can be seen, Group Pimozide-Morphine was hyperthermic relative to Group Pimozide-Sham throughout. Both groups showed some fluctuation from day to day. A repeated measures analysis of variance conducted on these data revealed significant effects of groups [$F(1,9)=55.55$, $p<.001$] and days [$F(6,54)=2.70$, $p<.025$].

Body Temperature-Conditioning Test.

Figure 11 shows the group mean rectal temperatures obtained on the conditioning test day for each of the two groups. Again, there is no evidence of conditioning. A T-test for independent samples conducted on these data revealed that there was no significant difference in body temperature between the two groups on this test day ($T=0.26$, $p>.05$).

Discussion

The results of this experiment demonstrate that pretreatment with pimozide effectively blocks the increases in locomotor activity (as previously shown by Joyce and Iversen, 1979). The data from the conditioning test day revealed that when the

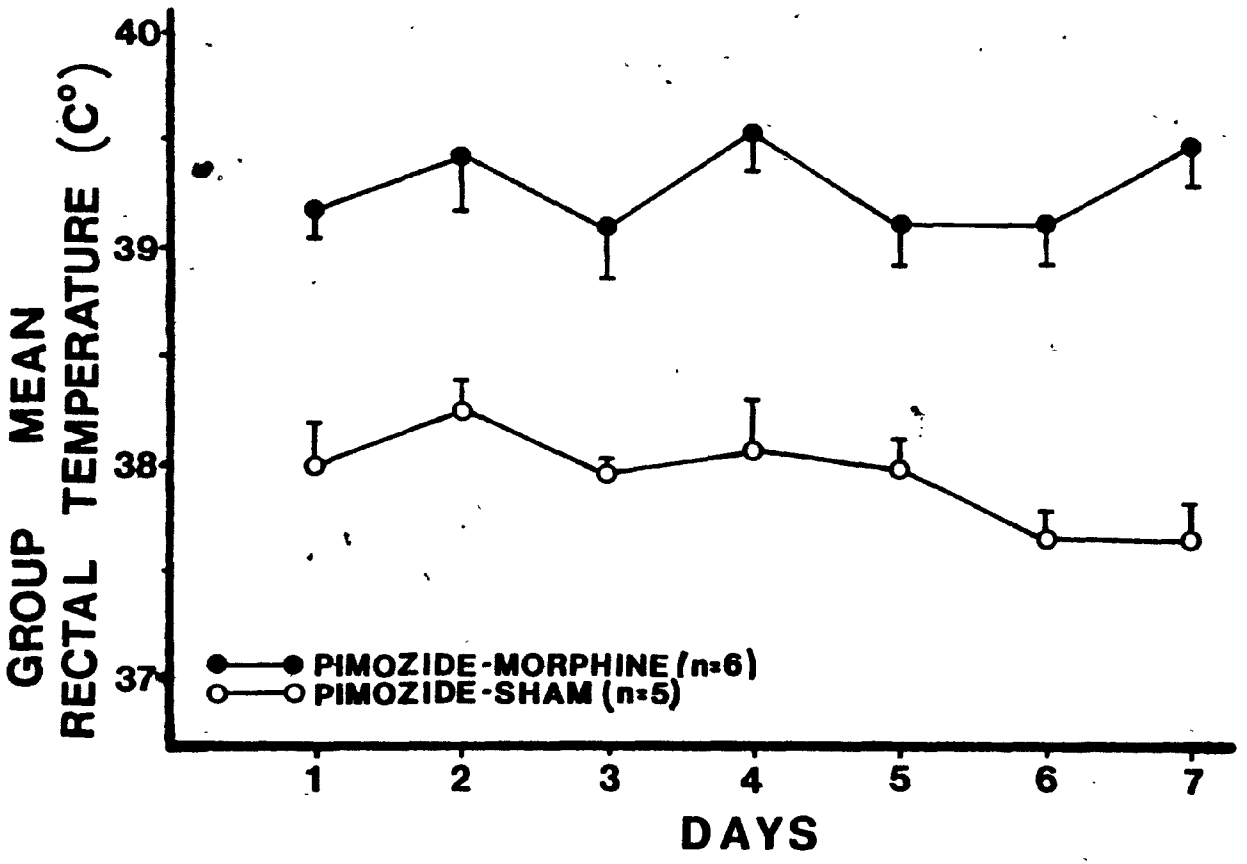

Figure 10. Group mean rectal temperatures (± 1 S.E.M.) obtained on the seven conditioning days for each of the two groups in Experiment 2.

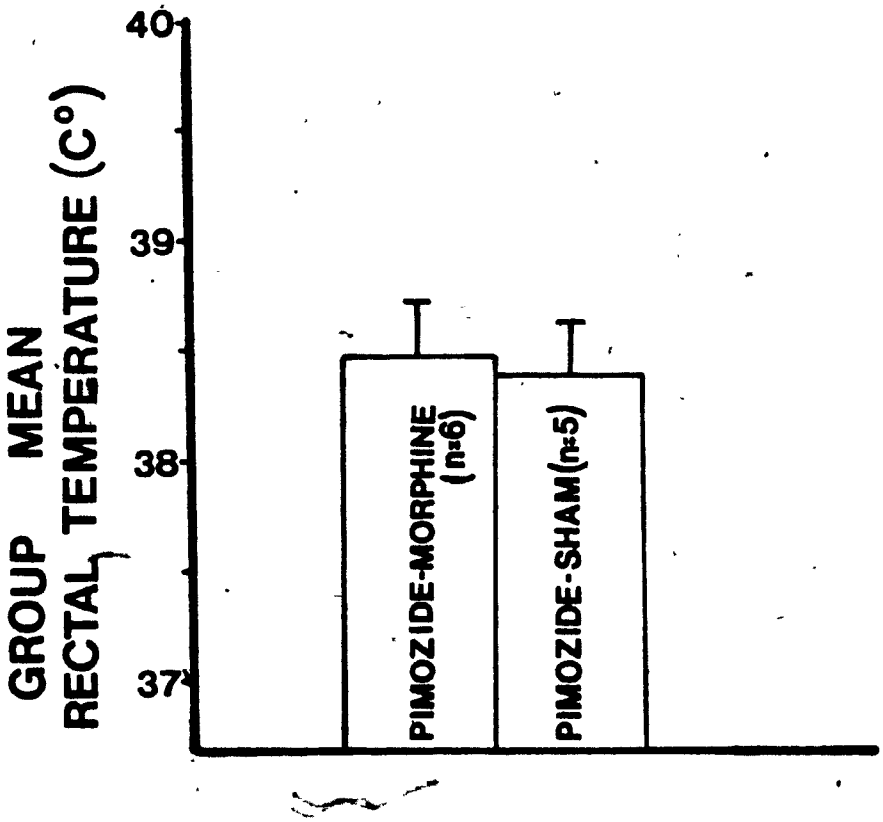

Figure 11. Group mean rectal temperatures (+ 1 S.E.M.) obtained on the conditioning test day for each of the two groups in Experiment 2.

morphine-induced locomotor activity was blocked by pimozide, conditioning did not take place. This finding suggests that it is the action of DA post-synaptic to the mesolimbic DA neuron terminals that acts as the UCS when morphine is administered into the VTA.

It might be argued that conditioning did not occur because fewer pairings were given than in Experiment 1. Another possibility is that because both groups in this experiment received pimozide throughout, evidence for conditioning may have been masked by aftereffects of extended neuroleptic treatment such as dopaminergic supersensitivity. These two possibilities were investigated in Experiment 3, however, and the results support the conclusions drawn from the present experiment.

The finding that pimozide did not block the hyperthermia induced by morphine administration into the VTA is interesting in two respects. It suggests that this morphine-induced hyperthermia is not DA mediated and that it is not due to heat production occuring as a result of increased muscle activity in active animals as suggested by Lotti et al. (1965).

EXPERIMENT 3

This experiment was designed to investigate the possibility that the sensitization to the effect of morphine on locomotor activity reported by Joyce and Iversen (1979) could be a conditioned phenomenon specific to the injection environment. Such a possibility is suggested in part by recent reports of environment-specific or conditioned sensitization to the behavioral effects of cocaine (Hinson and Poulos, 1981; Post, Lockfeld, Squillace, and Contel, 1981). Furthermore, it has been suggested that when the CR mimics the UCR, these two responses can summate to produce a greater observed effect (Eikelboom and Stewart, 1982). It will be recalled that in Experiment 1, the CR obtained did indeed mimic the unconditioned increases in locomotor activity induced by morphine administration into the VTA. Because Joyce and Iversen (1979) repeatedly tested their animals in the same environment, it is possible that the progressively increasing responses they obtained were specific to this environment and reflected the development of an association between it and morphine.

Another purpose of the present experiment was to investigate the effect of pimozide on the development of the conditioned sensitization of the locomotor activity increases induced by morphine administration into the VTA. In Experiment 2, it was found that pimozide pretreatment effectively blocked the development of conditioning. The present experiment provided an opportunity to extend this finding to conditioned sensitization.

There have been some reports that although pretreatment with pimozide may block the development of conditioning, it does not block the expression of already established conditioning (Beninger and Hahn, 1983; Franklin and McCoy, 1979). This possibility was also tested in this experiment. Animals were first given repeated pairings between morphine and a distinctive environment. On a subsequent test day, animals were pretreated with pimozide and their responses to morphine administration in the distinctive environment were compared to those of similarly treated animals but which had not received the explicit morphine-distinctive environment pairings.

Finally, the present experiment also provided an opportunity to attempt to replicate the conditioning of the morphine-induced increases in locomotor activity obtained in Experiment 1. In addition, body temperature was also measured. It was thus possible to also verify the body temperature results obtained in the first two experiments.

Methods

Subjects

Forty-one male Wistar rats, obtained from Charles River Canada Inc. (St. Constant, Quebec) and weighing 275-300 g on arrival, were used. One to three weeks after arrival, they were stereotaxically implanted with chronic bilateral guide cannulae aimed at the VTA. Surgery and housing details were as specified in Experiment 1.

Apparatus

Locomotor activity was measured in the same activity boxes

described in Experiment 1. However, the technique used to record activity counts was changed. In this experiment, animals in the AB's were filmed with a red light-sensitive television camera mounted in the AB room. The audio-visual monitor and recording equipment were situated in an adjacent room.

Two strips of tape were mounted on the front Plexiglas door of each AB so as to divide each box into three equal spaces. These spaces corresponded to the left, center, and right thirds of the steel grid floor described in Experiment 1.

Five activity scorers were hired to view the films and instructed to register one count each time an animal moved at least one limb across one of the strips (i.e., line crossings). For rearing, the scorers were instructed to register one count each time an animal placed a limb on the steel plates mounted on the inside walls of the AB's. Thus, as in Experiments 1 and 2, both horizontal locomotion and rearing were measured.

The apparatus used to measure body temperature was as specified in Experiment 1. The sham, morphine, and pimozide administration procedures and doses were as specified in Experiments 1 and 2.

Design and Procedure

Animals were randomly assigned to one of five groups:
Group Morphine-AB/Sham-HC (n=8),
Group Sham-AB/Morphine-HC (n=8),
Group Sham-AB/Sham-HC (n=8),
Group PimMorphine-AB/T.A.Sham-HC (n=8), and
Group PimSham-AB/T.A.Sham-HC (n=9).

Animals in the two Pim groups were injected in their home cages with pimozide (.5 mg/kg) four hours prior to their AB administration and tartaric acid four hours prior to their HC administration.

The experimental design involved giving animals their respective AB administration on one day and their respective HC administration on the following day. (Because only one administration was experienced each day, there was no need to control for order effects as in Experiment 1.) On the third day, animals remained in their home cages and received no administrations. This sequence of sessions was repeated five times and represents the conditioning phase of the experiment. The AB and HC administration and data recording procedures were as specified in Experiment 1. In order to provide suitable sham control data for the conditioning test, the sequence of sessions experienced in conditioning was repeated a sixth time for animals in Group Sham-AB/Sham-HC.

On the first day following the conditioning phase, all animals were given a morphine administration in the AB's. This session constituted the test for conditioned sensitization. Four hours prior to the session, animals in the two Pim groups were injected in their home cages with tartaric acid.

Three days following this test, animals in Group Morphine-AB/Sham-HC and Group Sham-AB/Morphine-HC were returned to the AB's and given sham administrations. This session constituted the conditioning test. The data obtained on the sixth conditioning AB administration day for Group Sham-AB/Sham-HC were used as the control group data for this test.

Finally, three days following the conditioning test, animals in Group Morphine-AB/Sham-HC and Group Sham-AB/Morphine-HC were injected with pimozide (.5 mg/kg) in their home cages. Four hours later, they were given morphine administrations in the AB's. This session tested for the effects of pimozide on established conditioned sensitization.

On days between tests, animals remained in their HC's and received no administrations.

Results

Locomotor Activity-Conditioning Days.

Figure 12 shows the group mean activity counts obtained on the five conditioning days for each of the five groups. It can be seen that Group Morphine-AB/Sham-HC was consistently more active than all four other groups. Furthermore, the activity shown by this group increased substantially over days. There was no evidence for sensitization in any of the other groups. Their activity levels were slightly higher at the beginning of the sessions and diminished with time.

A repeated measures analysis of variance conducted on the day total activity counts revealed significant effects of groups [$F(4,36)=167.52$, $p<.001$], days [$F(4,144)=4.62$, $p<.001$] and groups x days interaction [$F(16,144)=9.36$, $p<.001$]. Multiple comparisons between groups revealed that Group Morphine-AB/Sham-HC was significantly more active overall than the remaining four groups combined [$F(4,36)=161.71$, $p<.001$]. The only other significant effect was between Group Sham-AB/Morphine-HC and Group Sham-AB/Sham-HC combined and the two Pim groups combined

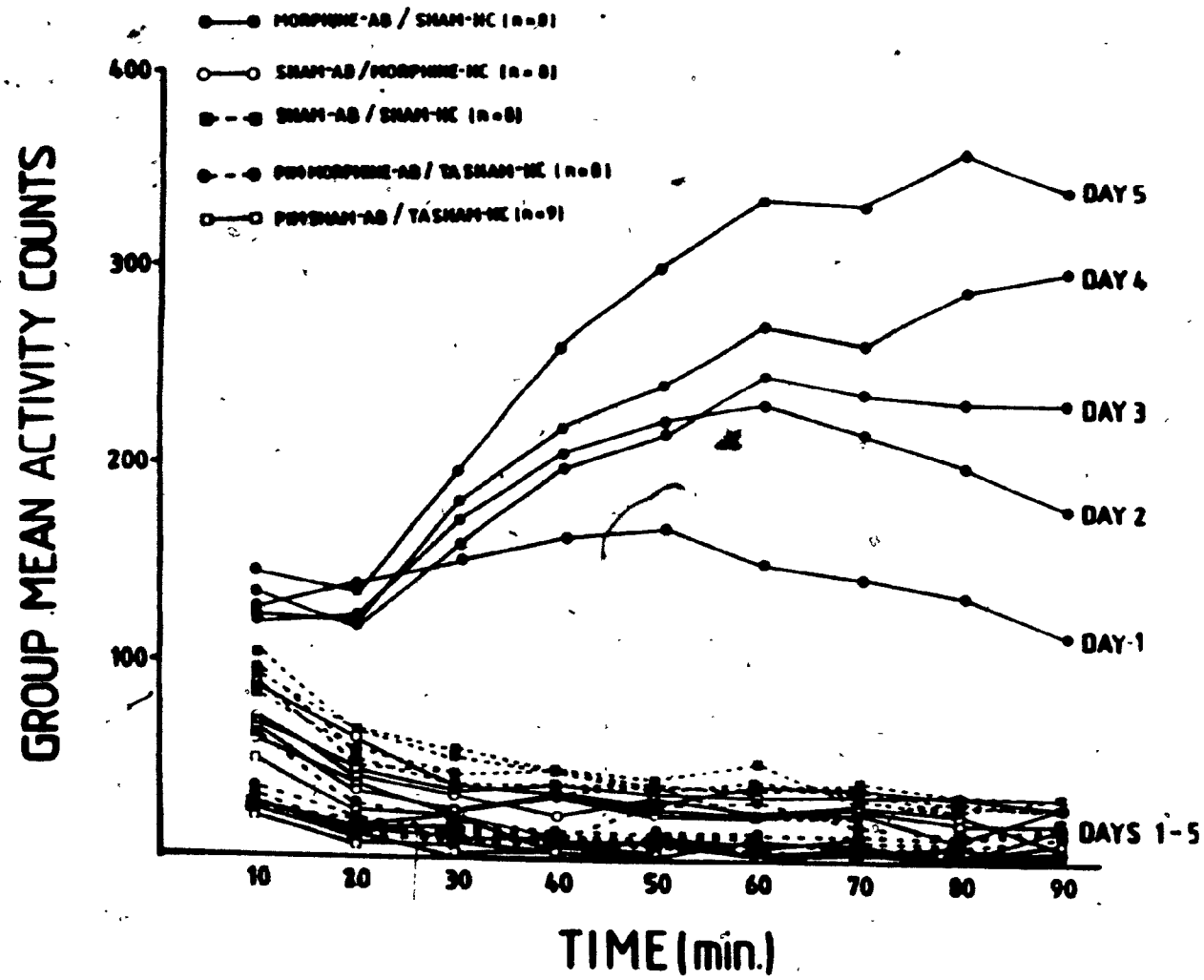

Figure 12. Group mean activity counts obtained on the five conditioning days for each of the five groups in Experiment 3.

[F(4,36)=5.12, p<.005]. Thus, the dose of pimozide used not only blocked the morphine-induced increases in locomotor activity, but also appreciably reduced levels of locomotor activity.

Group Morphine-AB/Sham-HC showed a pronounced and significant increase in activity from Day 1 to Day 5 [F(4,144)=35.66, p<.001]. The activity of the other four groups combined did not differ significantly on these two days [F(4,144)=1.25, p>.05].

Body Temperature-Conditioning Days.

The group mean rectal temperatures obtained on the five conditioning days for each of the five groups are shown in Figure 13. Both Group Morphine-AB/Sham-HC and Group PimMorphine-AB/T.A.Sham-HC were consistently hyperthermic relative to the three remaining groups and there was no evidence for a change in the effectiveness of morphine over days. The body temperatures of the latter three groups, with the possible exception of Group Sham-AB/Morphine-HC, showed a slight decrease with days.

A repeated measures analysis of variance conducted on these data revealed a significant effect of groups only [F(4,36)=27.93, p<.001]. Multiple comparisons between means revealed that Group Morphine-AB/Sham-HC and Group PimMorphine-AB/T.A.Sham-HC did not differ significantly from each other [F(4,36)=.99, p>.05]. However, both these groups combined had significantly higher body temperatures than all three other groups combined [F(4,36)=24.71, p<.001]. The only other significant effect was that Group Sham-AB/Morphine-HC had significantly higher body temperatures than Group PimSham-AB/T.A.Sham-HC [F(4,36)=2.88, p<.05].

Locomotor Activity-Conditioned Sensitization Test.

Figure 14 shows the group mean activity counts obtained on

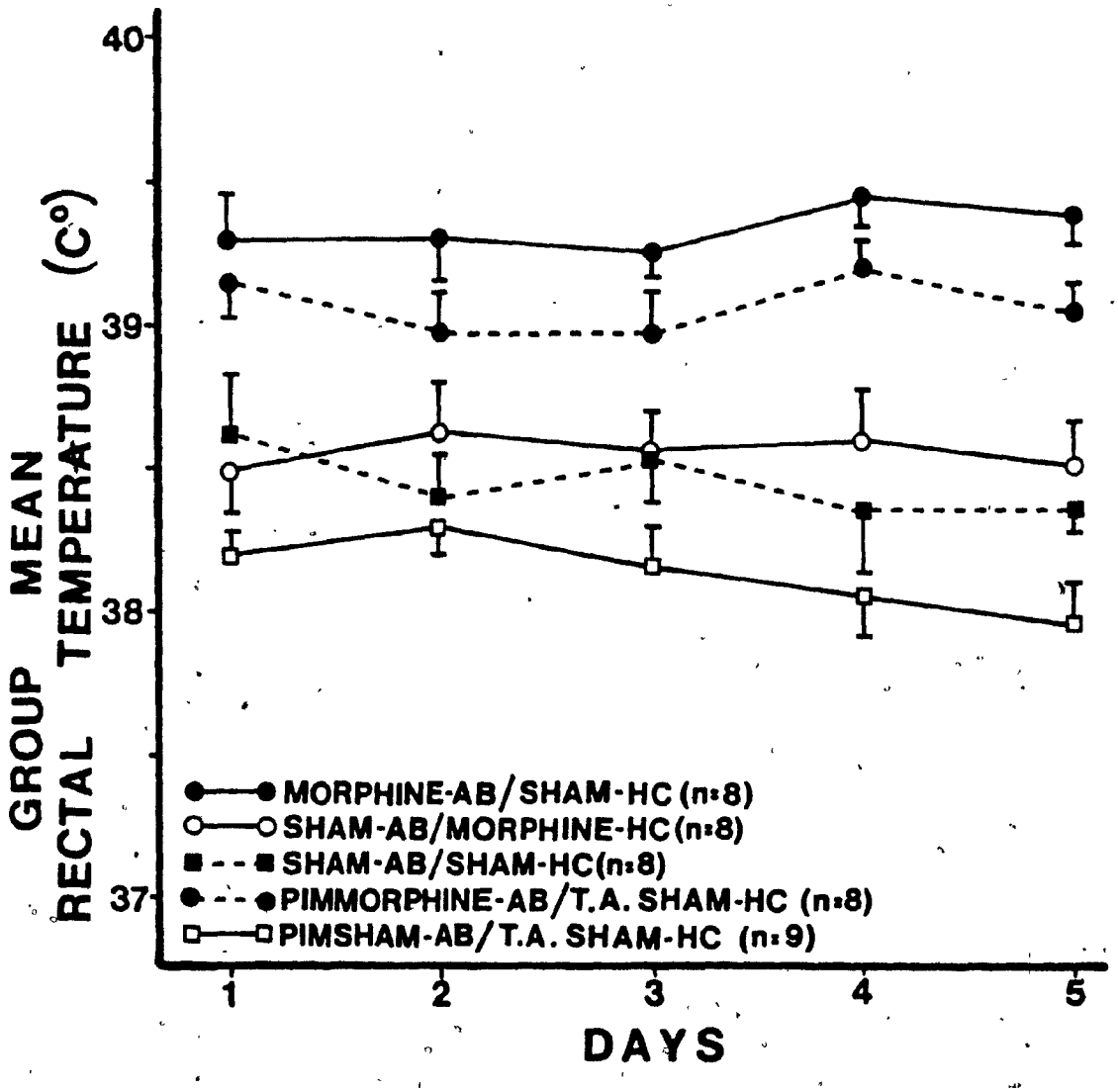

Figure 13. Group mean rectal temperatures (± 1 S.E.M.) obtained on the five conditioning days for each of the five groups in Experiment 3.

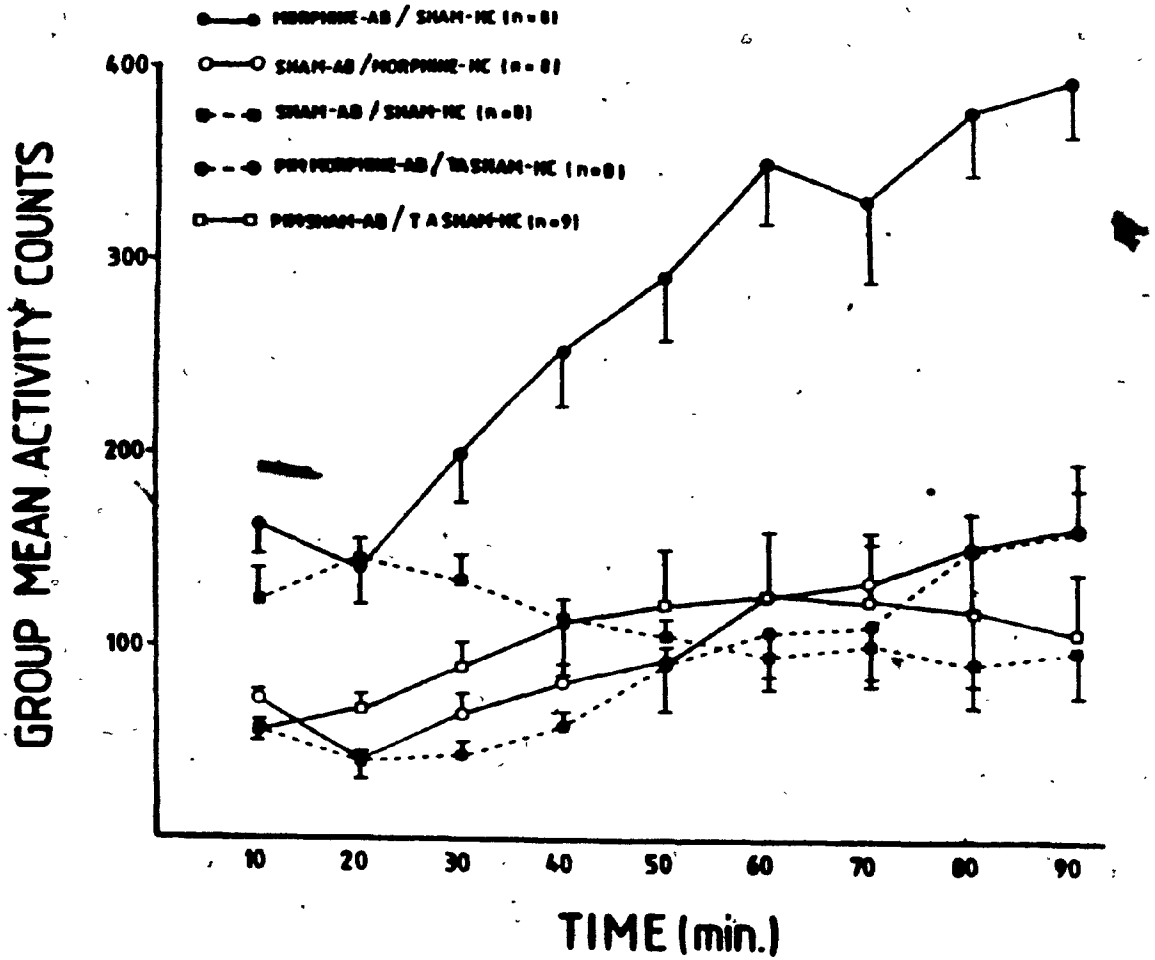

Figure 14. Group mean activity counts (± 1 S.E.M.) obtained on the conditioned sensitization test day for each of the five groups in Experiment 3. All animals received a morphine administration on this day. Animals in the two Pim groups received tartaric acid injections four hours prior to the test.

the conditioned sensitization test day for each of the five groups. As can be seen, Group Morphine-AB/Sham-HC was more active than all four other groups even though all animals had received the same morphine administration. In the remaining groups, with the exception of Group Sham-AB/Sham-HC, activity levels increased moderately over time. In Group Sham-AB/Sham-HC activity levels were somewhat higher at the beginning of the session and diminished gradually with time.

A repeated measures analysis of variance conducted on these data revealed significant effects of groups [$F(4,36)=16.94$, $p<.001$], time [$F(8,288)=30.60$, $p<.001$], and groups x time interaction [$F(32,288)=8.88$, $p<.001$]. Multiple comparisons between groups revealed that Group Morphine-AB/Sham-HC was significantly more active overall than all other groups combined [$F(4,36)=16.79$, $p<.001$]. The remaining four groups did not differ significantly overall from each other.

Although the significant groups x time interation was clearly due in large part to the steep increase in activity levels shown by Group Morphine-AB/Sham-HC relative to the other groups, Group Sham-AB/Sham-HC was also found to be significantly more active than the remaining three groups during the first 20 minutes of the session [$F(4,324)=4.21$, $p<.005$].

Body Temperature-Conditioned Sensitization Test.

Figure 15 shows the group mean rectal temperatures obtained on the conditioned sensitization test day for each of the five groups. Body temperature was high in all groups. A one-way analysis of variance conducted on these data revealed no group differences [$F(4,36)=.96$, $p>.05$].

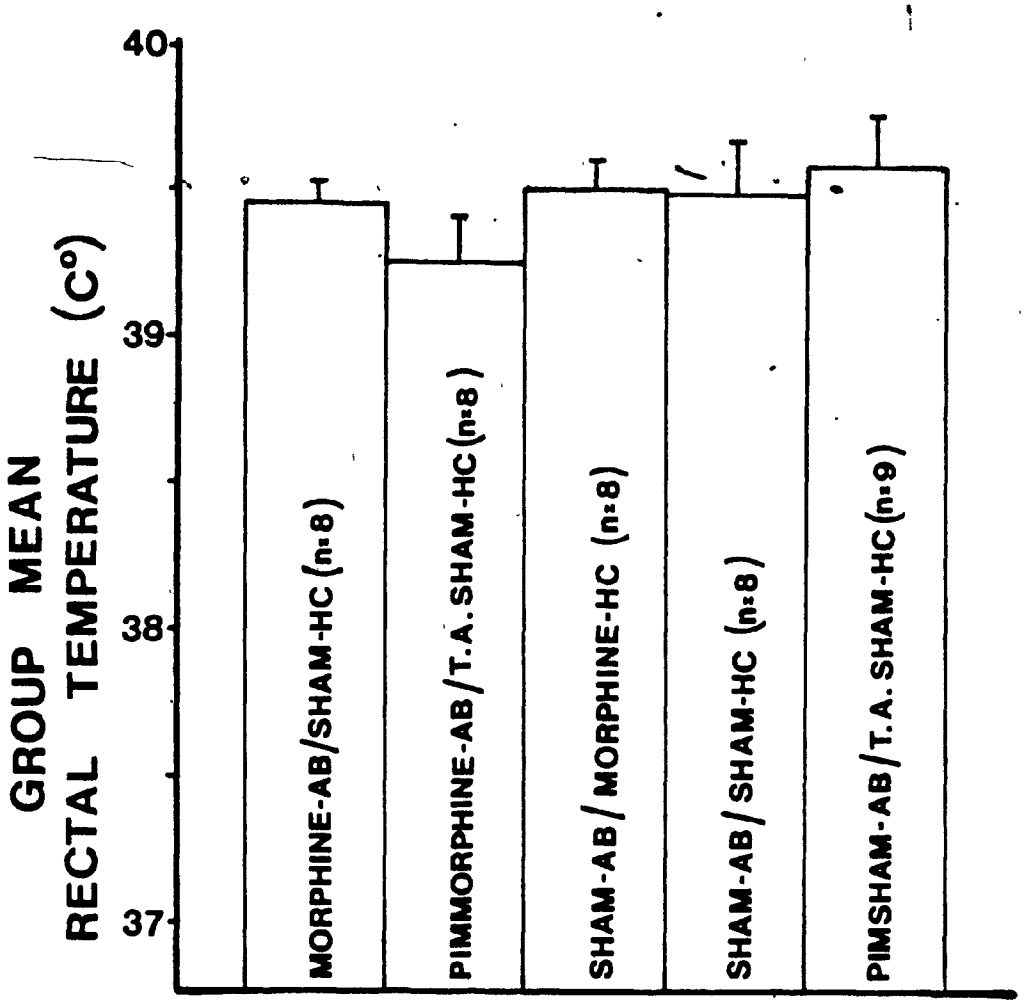

Figure 15. Group mean rectal temperatures (+ 1 S.E.M.) obtained on the conditioned sensitization test day for each of the five groups in Experiment 3. All animals received a morphine administration on this day. Animals in the two Pim groups received tartaric acid injections four hours prior to the test.

Locomotor Activity-Conditioning Test.

The group mean activity counts obtained on the conditioning test day for Group Morphine-AB/Sham-HC, Group Sham-AB/Morphine-HC, and Group Sham-AB/Sham-HC are shown in Figure 16. It will be recalled that the data for Group Sham-AB/Sham-HC used for this test were those obtained on its sixth AB administration day during conditioning. Furthermore, Group Sham-AB/Morphine-HC had received one morphine administration in the AB (on the conditioned sensitization test) prior to this test. Despite this, there was a difference between groups indicating that conditioning occurred. Group Morphine-AB/Sham-HC showed activity levels higher than those of the other two groups for at least two thirds of the session. These activity levels were high at the beginning of the session and diminished with time. The activity levels for the other two groups similarly diminished with time.

A repeated measures analysis of variance conducted on these data revealed significant effects of groups [$F(2,21)=8.36$, $p<.001$], time [$F(8,168)=29.20$, $p<.001$], and groups x time interaction [$F(16,168)=3.42$, $p<.001$]. Multiple comparisons between means revealed that Group Morphine-AB/Sham-HC was significantly more active overall than the two other groups combined [$F(2,21)=8.22$, $p<.005$]. These latter two groups did not differ significantly from each other. The activity levels of Group Morphine-AB/Sham-HC were significantly higher than that of these two groups during the first 50 minutes of the session [$F(2,189)=43.57$, $p<.001$] and diminished to nonsignificant levels for the remainder of the session [$F(2,189)=2.47$, $p>.05$].

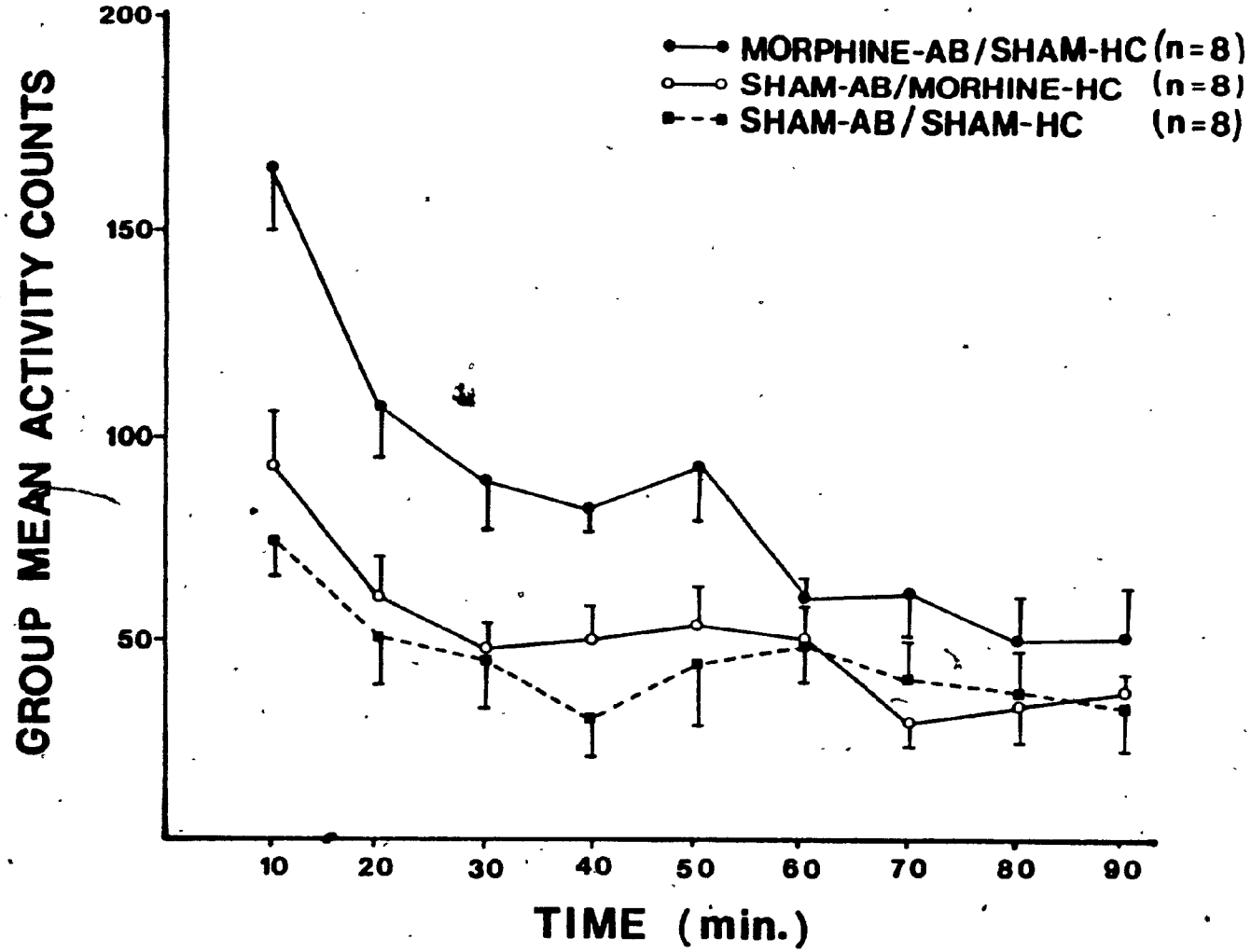

Figure 16. Group mean activity counts (± 1 S.E.M.) obtained on the conditioning test day for Group Morphine-AB/Sham-HC, Group Sham-AB /Morphine-HC, and Group Sham-AB/Sham-HC. All animals received a sham administration on this day.

Body Temperature-Conditioning Test.

Figure 17 shows the group mean rectal temperatures obtained on the conditioning test day for Group Morphine-AB/Sham-HC, Group Sham-AB/Morphine-HC, and Group Sham-AB/Sham-HC. It can be seen that the two morphine groups were slightly hyperthermic relative to the sham control group. A one-way analysis of variance conducted on these data, however, revealed a nonsignificant groups effect [$F(2,21)=1.56$, $p>.05$].

Locomotor Activity- Pimozide Challenge to Condtioned Sensitization.

Figure 18 shows the group mean activity counts obtained on this test day for Group Morphine-AB/Sham-HC and Group Sham-AB/Morphine-HC. As can be seen, both groups had low levels of activity throughout the session. Group Morphine-AB/Sham-HC had slightly, but consistently, higher activity levels relative to Group Sham-AB/Morphine-HC.

A repeated measures analysis of variance conducted on these data revealed only a significant effect of time[$F(8,112)=3.02$, $p<.005$] which was clearly due to the slight fluctuations in activity level in both groups. It should be noted, however, that the overall difference between the two groups very nearly approached significance [$F(1,14)=4.08$, $p<.063$]. Thus, although pimozide clearly blocked the morphine-induced increases in activity, this effect was somewhat less in Group Morphine-AB/Sham-HC.

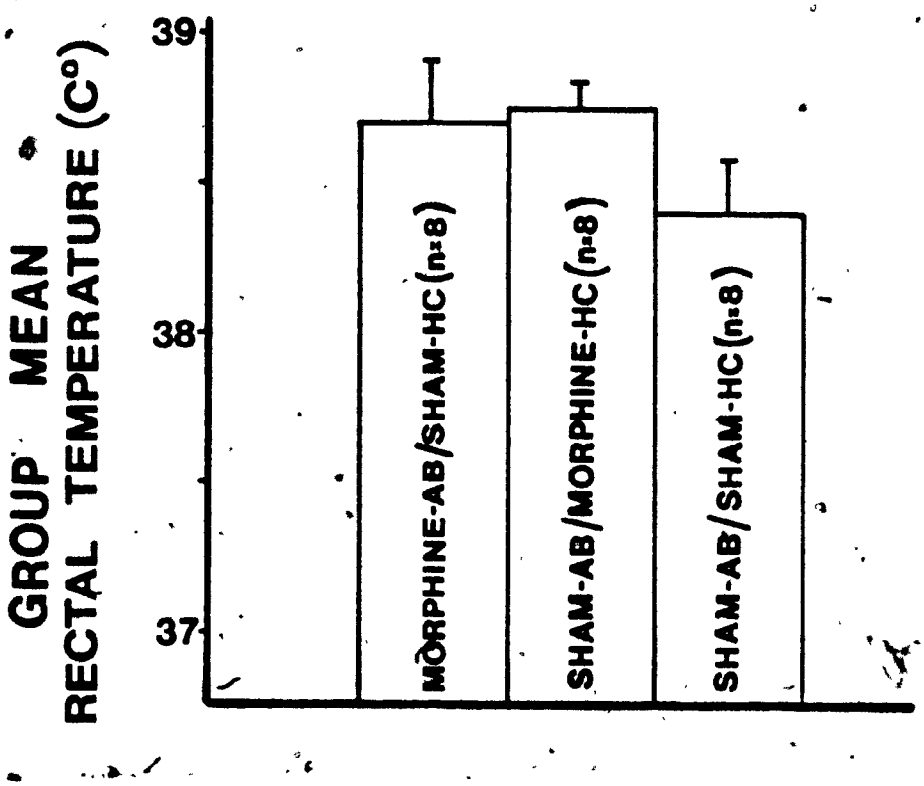

Figure 17. Group mean rectal temperatures (+ 1 S.E.M.) obtained on the conditioning test day for Group Morphine-AB/Sham-HC, Group Sham-AB/Morphine-HC, and Group Sham-AB/Sham-HC. All animals received a sham administration on this day.

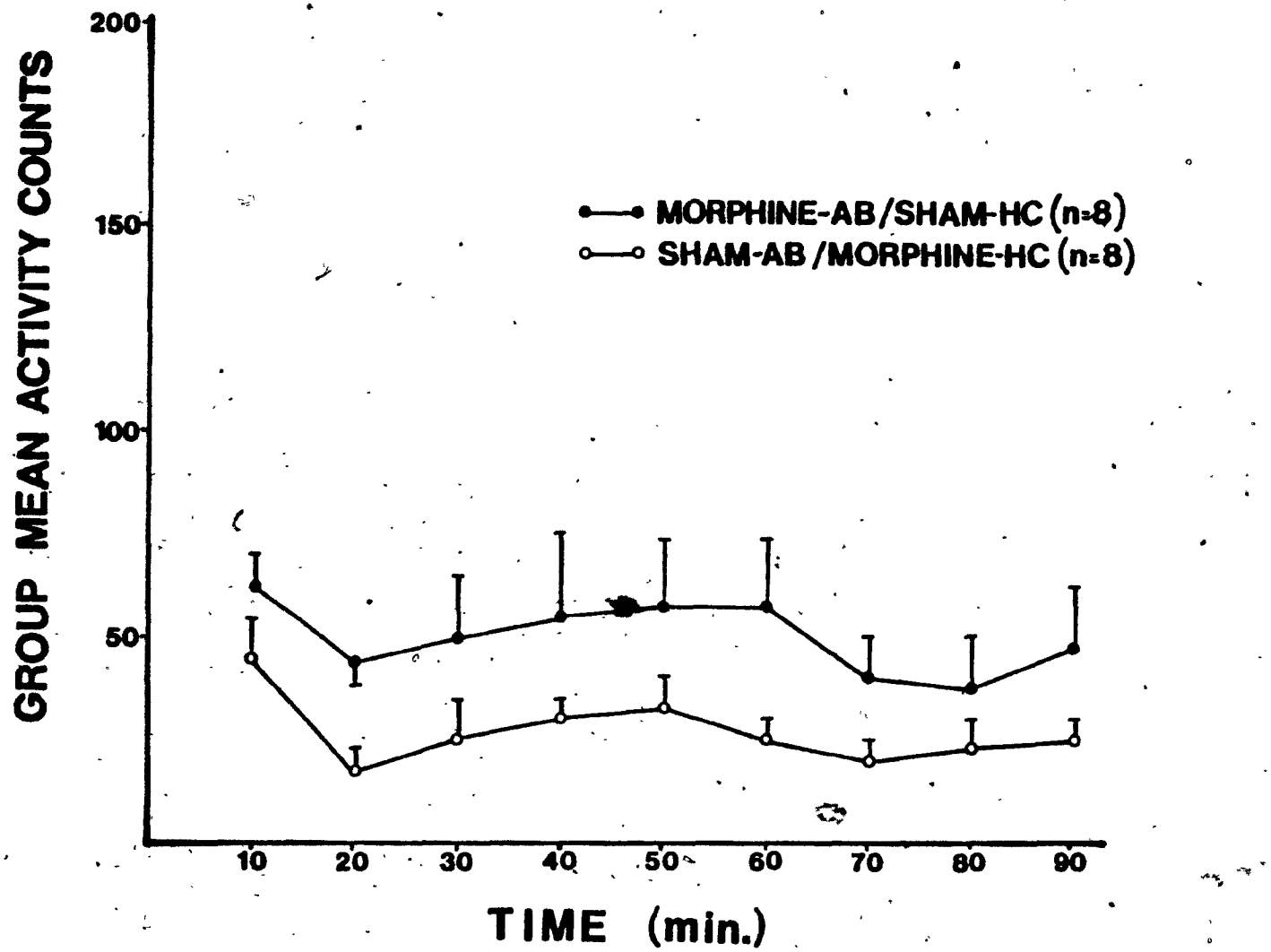

Figure 18. Pimozide challenge to conditioned sensitization. Group mean activity counts (± 1 S.E.M.) for Group Morphine-AB/Sham-HC and Group Sham-AB/Morphine-HC. All animals received a pimozide injection in their home cages four hours prior to the test and a morphine administration in the AB during the test.

Discussion

The results of the present experiment clearly demonstrated that, with repeated administration, sensitization occurred to the increased locomotor activity, but not to the hyperthermia, induced by morphine in the VTA (Figures 12 and 13). Furthermore, this sensitization was specific to the distinctive environment in which morphine was administered (Figure 14) suggesting that it was due to a learned association between morphine and the environment and not to the increased sensitivity of opiate receptors in the VTA as suggested by Joyce and Iversen (1979).

As in Experiment 2, pretreatment with pimozide was found to effectively block both the morphine-induced increases in activity (Figure 12) and the development of conditioning (Figure 14). This finding suggests again that the biochemical UCS for locomotor activity induced by morphine in the VTA is an effect postsynaptic to mesolimbic DA terminals. There was no evidence for any aftereffect of neuroleptic treatment such as dopaminergic supersensitivity in this experiment: Group Sham-AB/Morphine-HC, Group Sham-AB/Sham-HC, and the two Pim groups did not differ from each other on the conditioned sensitization test day (Figure 14). It might be noted, however, that Group Sham-AB/Sham-HC showed slightly higher activity levels during the first 20 minutes of this test relative to the three other groups. There is, at present, no explanation for this finding.

The results of the conditioning test day once again demonstrated that the morphine-induced increases in locomotor activity could come to be elicited by the environment in which morphine was repeatedly administered again suggesting that a

learned association developed between morphine and the distinctive AB environment. It should be noted that only five pairings between morphine and the AB environment were needed to obtain conditioning.

Although pimozide was found to effectively block the morphine-induced increases in locomotor activity and the development of conditioned sensitization of these activity increases, it did not completely block the manifestation of already established conditioned sensitization (Figure 18). This finding is in agreement with earlier reports that pimozide does not completely block the expression of already established conditioning (Beninger and Hahn, 1983; Franklin and McCoy, 1979).

Finally, the body temperature results obtained in this experiment confirmed those obtained in Experiments 1 and 2. Morphine administration into the VTA results in a significant hyperthermia which is not blocked by pretreatment with pimozide. There was no evidence in any of the tests for conditioning of this morphine-induced hyperthermia.

EXPERIMENT 4

This experiment was designed to verify one aspect of the anatomical specificity of the locomotor activity and body temperature effects of morphine administration into the VTA. It is well known that one problem with intracranial drug administration is that the drug may diffuse up the cannula shaft and produce an observed effect which is due to drug action at a site distal to the injector cannula tip (Routtenberg, 1972). To control for this possibility, animals were implanted with cannulae aimed at a site dorso-lateral to the VTA and subsequently administered morphine. Their locomotor activity and body temperature levels were then compared to those of animals that received either a morphine or a sham administration into the VTA.

Methods

Subjects

Five male Wistar rats, obtained from Charles River Canada Inc. (St. Constant, Quebec) and weighing 275-300 g on arrival, were used. Housing details were as specified in Experiment 1. The VTA morphine and sham administration data were provided by the Day 1 group means for Group Morphine-AB/Sham-HC and Group Sham-AB/Sham-HC obtained in Experiment 3.

Surgery

One week after arrival, animals were stereotaxically implanted with chronic bilateral guide cannulae aimed at the VTA

and positioned 3 mm above the final injection site. Because the guide cannulae were implanted at 16 degrees to the vertical and the injector cannulae lowered 1 mm beyond the guide cannulae tips, the final injection site was 2 mm dorso-lateral to the VTA. The position of the injector cannulae tips for these animals relative to those of animals in Group Morphine-AB/Sham-HC and Group Sham-AB/Sham-HC are shown in a coronal section 3.8 mm posterior to bregma in Figure 19. For purposes of illustration, cannulae tip placements located in the rostral-caudal zone extending 2.8 to 3.8 mm posterior to bregma were all represented on this single coronal section. Histological verification of injector cannulae placements conducted for all the experiments in this thesis confirmed that all cannulae placements fell between these two boundaries. This rostral-caudal zone corresponds to the approximate location of the mesolimbic DA cell bodies and VTA morphine administrations into this area have been shown to produce place preference (Bozarth, 1982).

Procedure

Animals were given morphine administrations in the AB's and their locomotor activity and body temperature subsequently measured as specified in Experiment 3.

Results

Locomotor Activity.

In Figure 20, the activity counts obtained for the five individual animals with dorso-lateral placements can be compared to those obtained for Group Morphine-AB/Sham-HC and Group Sham-AB/Sham-HC on the first day of conditioning in Experiment 3. It

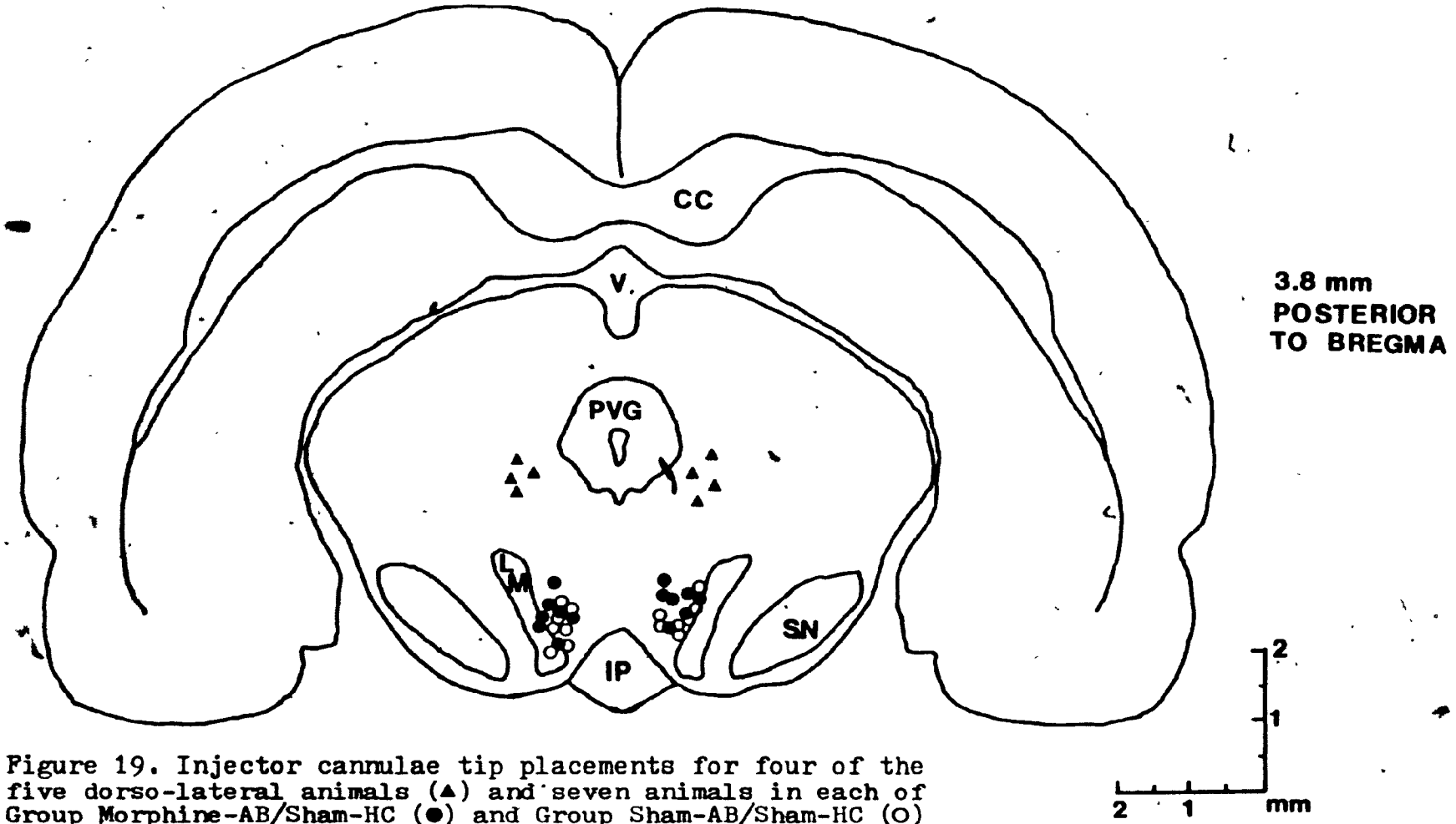

Figure 19. Injector cannulae tip placements for four of the five dorso-lateral animals (▲) and seven animals in each of Group Morphine-AB/Sham-HC (●) and Group Sham-AB/Sham-HC (O) in a coronal section 3.8 mm posterior to bregma. Omitted animals died before perfusion could be performed, precluding accurate histology. For purposes of illustration, cannulae tip placements located in the rostral-caudal zone extending 2.8 to 3.8 mm posterior to bregma were included in this section. The brain section was adapted from Pellegrino et al. (1979). Abbreviations: CC, corpus callosum; IP, interpeduncular nucleus; LM, medial lemniscus; PVG, periventricular gray substance; SN, substantia nigra; V, ventricle.

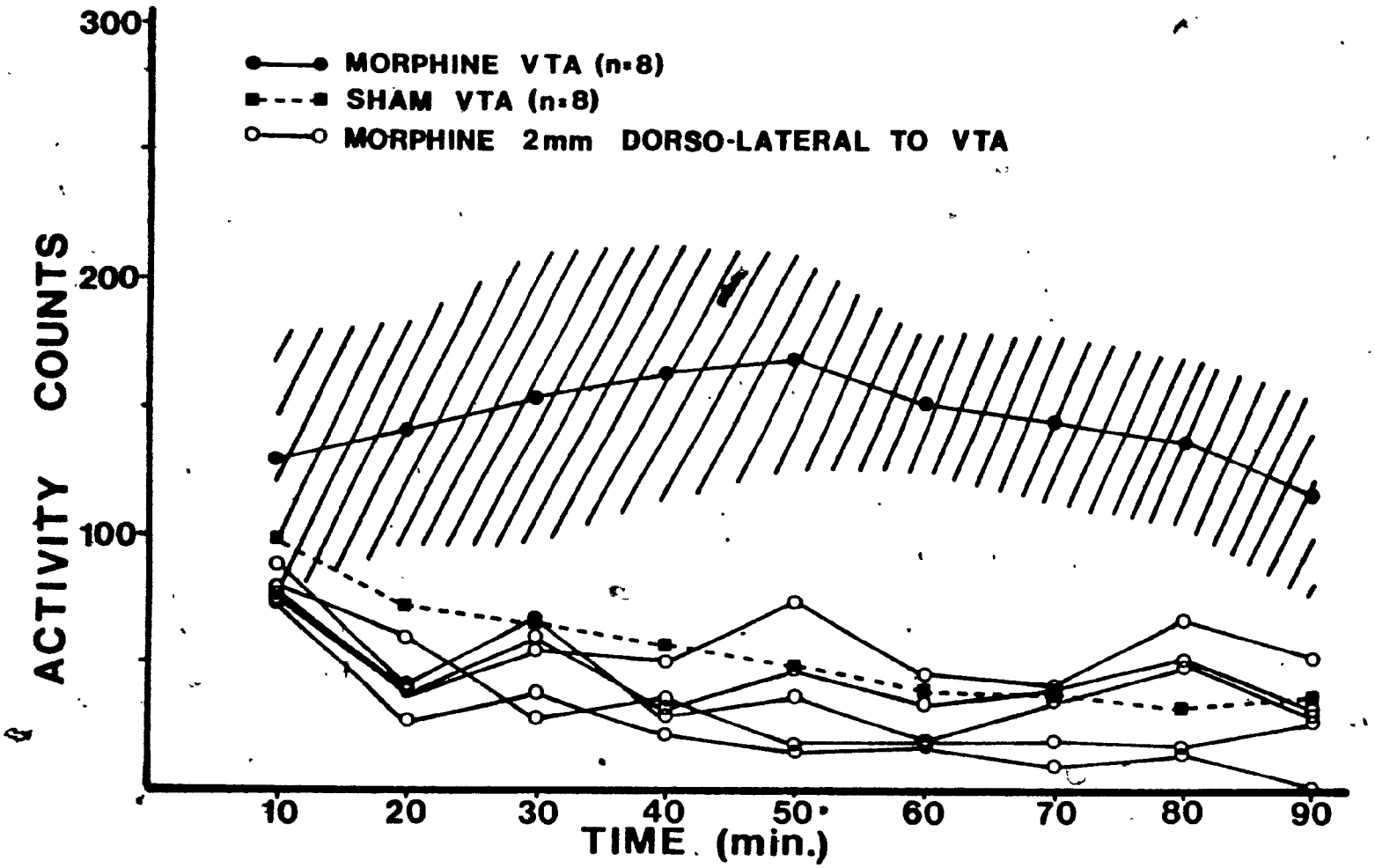

Figure 20. Activity counts obtained for five individual rats with injector cannulae tip placements 2 mm dorso-lateral to the VTA. These are compared to the group mean activity counts obtained on the first day of conditioning for Group Morphine-AB/Sham-HC (Morphine VTA) and Group Sham-AB/Sham-HC (Sham VTA) in Experiment 3. The cross-hatched area represents the 95% confidence interval for the means of Group Morphine-AB/Sham-HC.

can be seen that the activity counts for the five animals with dorso-lateral placements clustered around the group means for Group Sham-AB/Sham-HC. Furthermore, with the exception of activity counts obtained in the first 10 minutes of the session, these were all outside the 95% confidence interval for the means of Group Morphine-AB/Sham-HC. Thus, none of the dorso-lateral animals showed levels of locomotor activity that approached those of Group Morphine-AB/Sham-HC.

Body Temperature.

The rectal temperatures obtained for these five animals are shown in Figure 21. Again, these can be compared to the mean rectal temperatures obtained for Group Morphine-AB/Sham-HC and Group Sham-AB/Sham-HC on the first day of conditioning in Experiment 3. It can be seen that all five animals were hyperthermic relative to Group Sham-AB/Sham-HC. The rectal temperatures obtained for all five animals fell outside the 95% confidence interval for the mean of this group.

Discussion

The results of this experiment demonstrated that the morphine-induced increases in locomotor activity obtained in Experiments 1 and 3 were due to an action of morphine in the VTA and not to drug action at a site dorso-lateral to the injector cannulae tips. The body temperature data, on the other hand, are more difficult to interpret. The results of this experiment suggest that the area where morphine is acting to induce hyperthermia extends well outside the VTA.

Because body temperature was measured 90 min after morphine

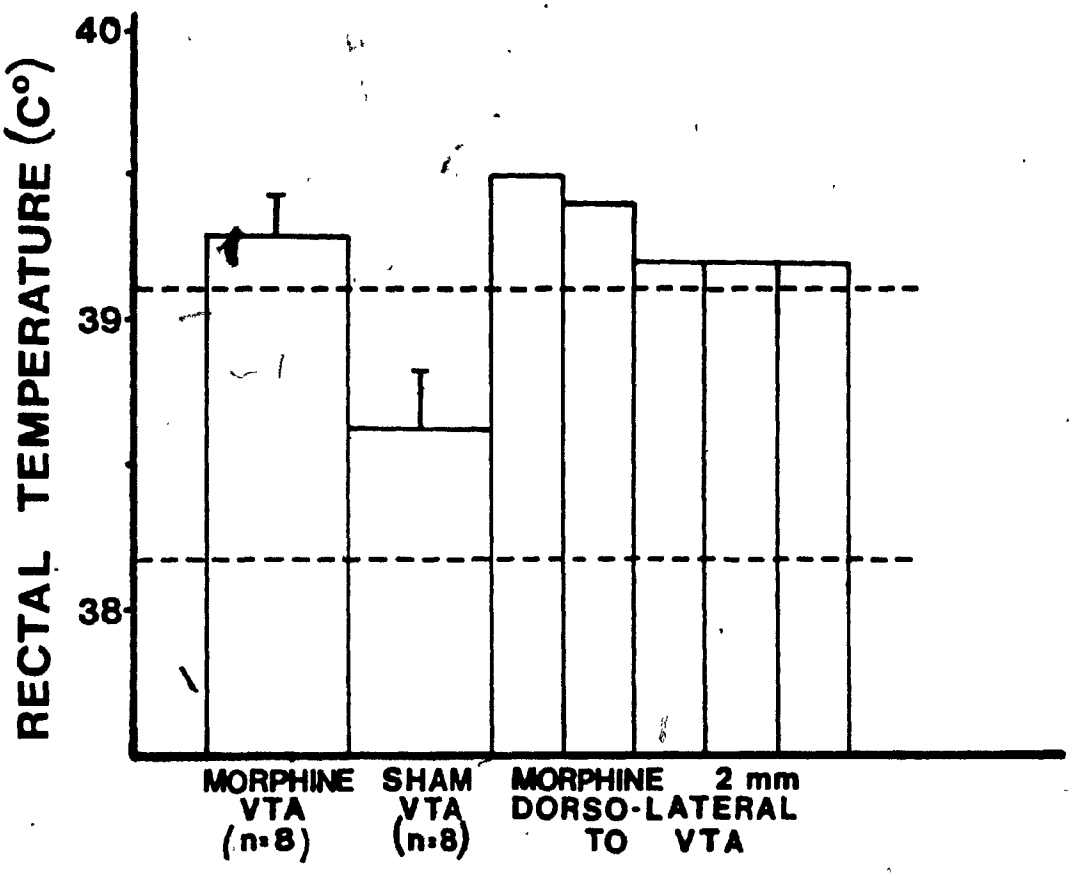

Figure 21. Rectal temperatures obtained for five individual rats with injector cannulae tip placements 2 mm dorso-lateral to the VTA. These are compared to the group mean rectal temperatures obtained on the first day of conditioning for Group Morphine-AB/Sham-HC (Morphine VTA) and Group Sham-AB/Sham-HC (Sham VTA) in Experiment 3. The dashed lines represent the upper and lower limits of the 95% confidence interval for the mean of Group Sham-AB/Sham-HC.

administration, it is possible that drug may have diffused sufficiently to reach the VTA by the time body temperature was measured. This possibility is unlikely, however, since the animals did not show any signs of increased locomotor activity in this period.

EXPERIMENT 5

This experiment was designed to verify the pharmacological specificity of the locomotor activity effects of morphine administration into the VTA. This was achieved by giving animals morphine administrations into the VTA and subsequently injecting them systemically with the opiate receptor blocker naloxone. If this pharmacological challenge reversed the effect of morphine on locomotor activity, it could be safely concluded that the effect was due to the action of morphine at opiate receptors in the VTA and not to changes in cell membrane osmolarity, calcium chelation, or other nonspecific factors associated with the intracranial administrations (Bozarth, 1983).

Methods

Subjects

Three days following the termination of Experiment 3, seven rats in Group Morphine-AB/Sham-HC were used as subjects in this experiment.

Procedure

Once in the AB room, all animals were given intraperitoneal injections of isotonic saline (1 ml/kg) and administered morphine intracranially. They were then placed in the AB's and their locomotor activity measured. After one hour, animals were removed from the AB's, injected intraperitoneally with naloxone, and returned to the AB's where their locomotor activity was measured for another hour. Naloxone hydrochloride (Endo Laboratories) was

dissolved in an isotonic saline vehicle and injected at a dose of 2 mg/kg in a volume of 1 ml/kg.

Results

The mean activity counts obtained for these animals are shown in Figure 22. It is clear that naloxone effectively reversed the increases in locomotor activity induced by morphine administration into the VTA. During the first hour, activity levels increased progressively over 10 minute intervals. Immediately following the naloxone injection, however, activity levels fell sharply reaching their lowest point 20 minutes after the naloxone injection. After this time, activity levels showed a gradual increase apparently paralleling the dissipation of active naloxone at opiate receptors.

A T-test for related samples conducted on the pooled activity counts obtained 30 minutes before and 30 minutes after the naloxone injection revealed that activity levels following naloxone challenge were significantly lower than levels obtained before naloxone challenge [$T=14.58$, $p<.001$].

Discussion

The results of this experiment confirmed that the increases in locomotor activity induced by morphine administration into the VTA obtained in Experiments 1 and 3 were due to morphine action at opiate receptors and not to nonspecific factors associated with intracranial administrations.

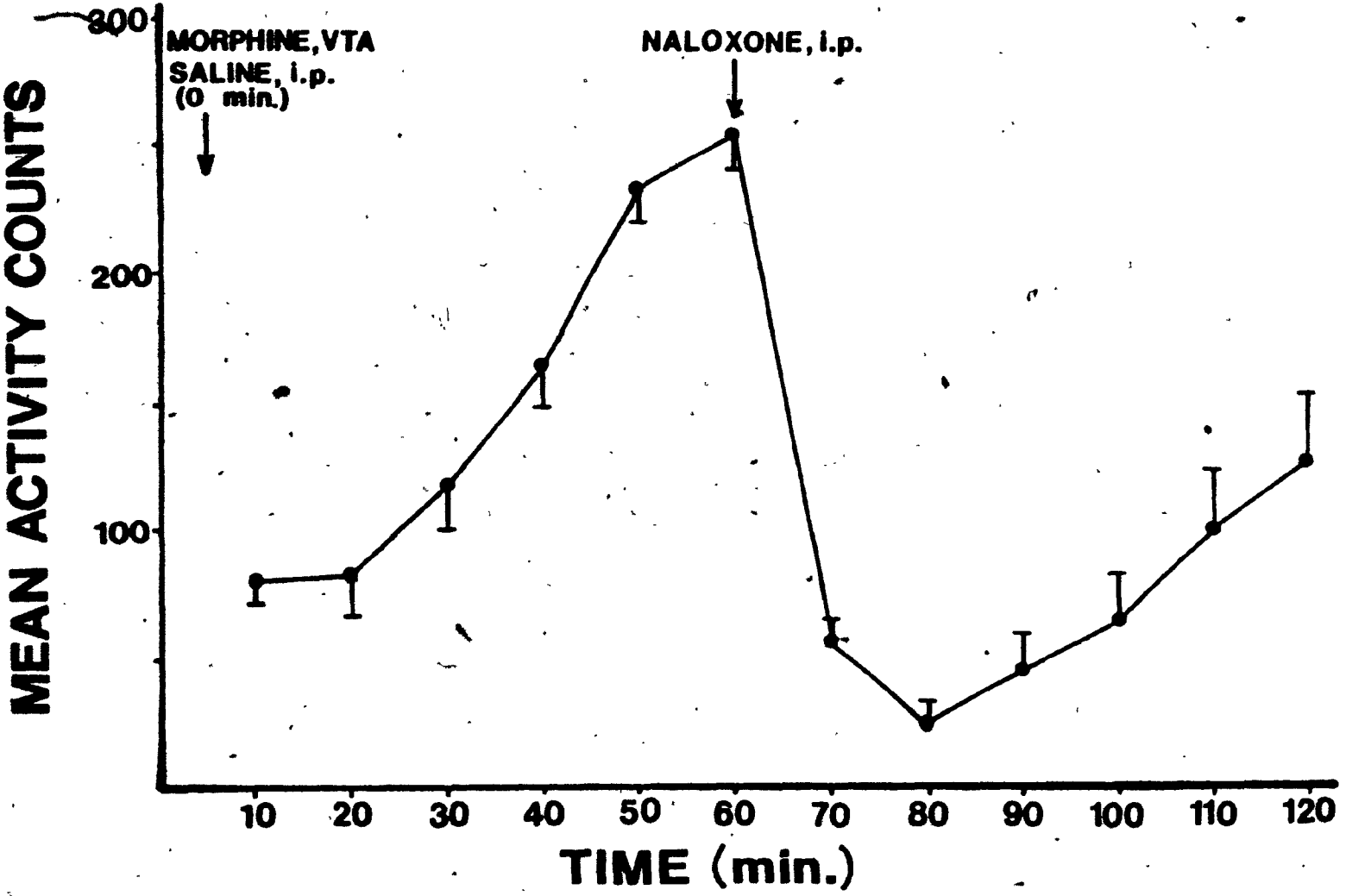

Figure 22. Mean activity counts (± 1 S.E.M.) before and after a challenge injection of naloxone (2 mg/kg, i.p.) obtained for animals given a morphine administration into the VTA at time 0. (n=7)

EXPERIMENT 6

This experiment was designed to verify the pharmacological specificity of the body temperature effects of morphine administration into the VTA. As in Experiment 5, animals were given morphine administrations into the VTA and were subsequently challenged by a systemic injection of naloxone.

Methods

Subjects

Three days following the termination of Experiment 5, six rats in Group Morphine-AB/Sham-HC and six in Group Sham-AB/Morphine-HC were used in this experiment. Both groups of animals were originally used in Experiment 3.

Procedure

Three drug administration conditions were studied: Morphine-Saline, Morphine-Naloxone, and Sham-Saline. In each condition, animals were given an intracranial administration into the VTA followed one hour later by a systemic injection of either naloxone or saline. Every animal experienced all conditions every second day. The order in which animals experienced each condition was counterbalanced.

Animals were given either a morphine or sham administration and placed in the AB's. After one hour, animals were removed from the AB's, injected intraperitoneally with either isotonic saline (1 ml/kg) or naloxone (2 mg/kg), and returned to the AB's. 10, 30, and 60 minutes after this challenging injection, animals were

removed from the AB's and their body temperature measured.

Results

Figure 23 shows the mean rectal temperatures obtained for each drug condition 10, 30, and 60 minutes after the systemic injection. As can be seen, in the Morphine-Naloxone condition, naloxone effectively reversed the morphine-induced hyperthermia 10 minutes following naloxone. At 30 minutes, the hyperthermia was still reversed, but by 60 minutes, body temperature had returned to hyperthermic levels. Body temperatures in the Morphine-Saline condition were consistently high, while those in the Sham-Saline condition were slightly elevated at 10 minutes, but subsequently decreased to remain low.

Separate repeated measures analysis of variance were conducted on the data obtained at each time after the systemic injection. All revealed significant drug condition effects [$F(2,22)=23.35$ at 10 min; 29.54 at 30 min; and 42.05 at 60 min; $p<.001$]. Multiple comparisons between means revealed that at 10 minutes post-injection, body temperatures in the Morphine-Naloxone condition were significantly lower than those in the Morphine-Saline condition [$F(2,22)=20.06$, $p<.001$] and not significantly different from those in the Sham-Saline condition [$F(2,22)=.44$, $p>.05$]. At 30 minutes post-injection, the Morphine-Naloxone condition continued to show body temperatures significantly lower than the Morphine-Saline condition [$F(2,22)=16.66$, $p<.001$] but not significantly different from the Sham-Saline condition [$F(2,22)=1.2$, $p>.05$]. At 60 minutes post-injection, body temperatures in the Morphine-Naloxone condition

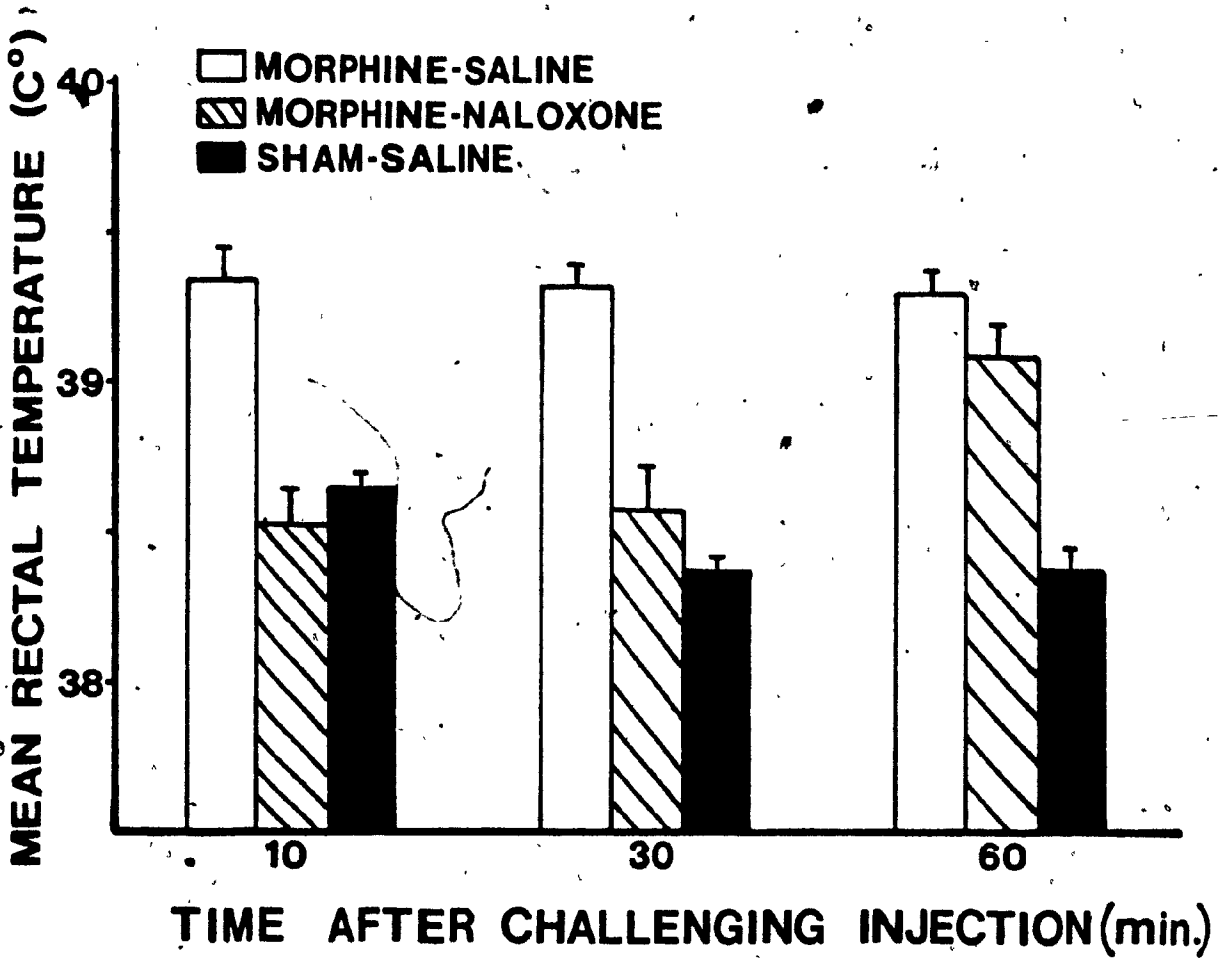

Figure 23. Mean rectal temperatures (+ 1 S.E.M.) obtained for each drug condition 10, 30, and 60 minutes after the challenging injection. (n=12)

were no longer significantly different from those in the Morphine-Saline condition [$F(2,22)=2.12$, $p>.05$], but were now significantly higher than those in the Sham-Saline condition [$F(2,22)=22.52$, $p<.001$].

Discussion

The results of this experiment demonstrated that the morphine-induced hyperthermia obtained in Experiments 1 to 3 was due to morphine action at opiate receptors and not to other nonspecific factors. In light of the dorso-lateral placement results obtained in Experiment 4, however, the site of these opiate receptors cannot be specified.

GENERAL DISCUSSION

These experiments have demonstrated that administration of morphine into the VTA induces both an increase in locomotor activity and hyperthermia. Both effects were found to be reversed by the opiate receptor blocker naloxone suggesting that they are dependent on morphine action at opiate receptors. Although the increases in locomotor activity seem to be due to morphine action at opiate receptors located in the VTA, the area where morphine is acting to induce hyperthermia seems to extend beyond the VTA. Furthermore, the DA receptor blocker pimozide blocked the morphine-induced increases in locomotor activity but did not affect the hyperthermia suggesting DA mediation of the former but not the latter.

The morphine-induced increase in locomotor activity became progressively enhanced with repeated morphine administration and this enhancement or sensitization was found to be specific to the environment in which morphine was administered. The conditioning tests also revealed that, in the absence of morphine, increased locomotor activity could come to be elicited by the administration environment. Pimozide effectively blocked the development of both conditioning and conditioned sensitization of the morphine-induced locomotor activity increases. It did not, however, completely block the expression of the already established conditioned sensitization. No evidence for conditioning of the morphine-induced hyperthermia was found in any of the conditioning tests conducted.

Conditioned Increases in Locomotor Activity

The results obtained in the present experiments make it clear that the conditioned increases in locomotor activity were due to the conditioning of the excitatory effects of morphine on locomotor activity. As reported by others (Broekkamp et al., 1979; Joyce and Iversen, 1979; Schwartz et al., 1981), the UCR to morphine administration into the VTA was an increase in locomotor activity. The CR reported here mimicked this UCR. Because morphine administration into the VTA at no time resulted in decreases in locomotor activity, it is unlikely that the conditioning obtained in the present experiments reflected the development of a conditioned compensatory increase in locomotor activity as suggested by Mucha et al. (1981) and Siegel (1975, 1977a, 1979).

Tolerance did not develop to the effect of morphine on locomotor activity obtained in the present experiments. On the contrary, with repeated administrations, the increases in locomotor activity became enhanced or showed sensitization. This sensitization was found to be specific to the environment in which morphine was repeatedly administered.

Such results may provide an explanation for the changes in locomotor activity seen following repeated systemic injections of morphine. For example, in the Mucha et al. (1981) experiments, conditioned increases in locomotor activity were obtained following repeated injections of both low and high doses of morphine. Because low systemic doses do not produce unconditioned decreases in locomotor activity, it would be difficult to explain their findings in terms of the development of conditioned

compensatory increases in locomotor activity. Rather, the explanation suggested by the present experiments is that the conditioned increases in locomotor activity obtained by Mucha et al. (1981) reflect the independent conditioning of the excitatory effects of morphine on locomotor activity.

Whether this view can also adequately explain the development of tolerance to the depressant effects on locomotor activity obtained from high systemic doses remains to be determined. While it is clear that the direct activation of mesolimbic DA neurons by morphine action in the cell body region and the sensitization of this activation can provide an explanation, alternative explanations cannot be ruled out. For example, tolerance to the depressant effects of morphine on locomotor activity may also arise from continued inhibition by morphine of DA release from terminals that subsequently initiates increased DA synthesis through negative feedback, or to other effects such as decreased affinity for morphine of opiate receptors at mesolimbic DA neuron terminals. If tolerance were shown to be situation specific, however, it is unlikely that this latter explanation would apply.

Conditioned Appetitive Motivational State

The main purpose of the experiments in this thesis was to obtain evidence to suggest whether or not a CS associated with morphine can acquire the ability to generate an appetitive motivational state similar to that generated by morphine itself. Because motivational states are hypothetical constructs, they must be inferred from the behavior of the organism. The approach taken in the present experiments was to study one of the

unconditioned effects of morphine administration into the VTA, namely, increased locomotor activity. If this response is assumed to be one manifestation of the motivational state generated by morphine, then the extent to which a CS can come to elicit this response reflects its ability to generate the same motivational state. In this sense, the finding that the morphine-induced increases in locomotor activity were elicited by the environment in which morphine was repeatedly administered (the CS) provides positive evidence for this view.

Several lines of evidence and recent theoretical formulations suggest that morphine-induced increases in locomotor activity may be inextricably linked to the appetitive motivational state generated by morphine. There is now condiderable evidence implicating morphine action in the VTA in the mediation of both the rewarding (Bozarth, 1983) and locomotor activity (present experiments; Broekkamp et al., 1979; Joyce and Iversen, 1979; Schwartz et al., 1981) effects of morphine. Furthermore, both of these effects seem to be dependent on the ascending mesolimbic DA system (Bozarth and Wise, 1981b; Joyce and Iversen, 1979; present experiments). Recently, Wise (1982, 1983) has concluded on the basis of these studies that the activity of this DA system is necessary for the subjective experience of pleasure that accompanies morphine administration and thus necessary for the generation of an appetitive motivational state by morphine. Because this same dopaminergic system is also highly implicated in the mediation of morphine-induced increases in locomotor activity, it follows that increased levels of locomotor activity may reflect the generation of an appetitive motivational state by

morphine (e.g., see Iversen, 1983).

Such an interrelationship between increases in locomotor activity and the generation of an appetitive motivational state by morphine sits well with incentive-motivational views of behavior. As discussed earlier, the generation of an appetitive motivational state is thought to enhance the salience of stimuli in the animal's environment and to increase the liklihood that the animal will approach and interact with them (Bindra, 1976, 1978). Indeed, the ability to elicit and direct locomotor approach is viewed as a key aspect of appetitive motivational or incentive stimuli (Bindra, 1968; see also Adams, 1979; Panksepp, 1982). The animal will engage in locomotion that tends to bring it in contact with the incentive object. If a CS is repeatedly paired with an incentive stimulus and then the latter removed, the animal will tend to approach and interact with the CS. When a drug like morphine is the incentive stimulus, it is clear that there is no external embodiment of the reward, other than the stimuli already present in the animal's environment. And, indeed, if an animal is administered morphine into the VTA, it will locomote towards these stimuli, that is,explore its environment. In the case when morphine is administered repeatedly, these stimuli are consistently accompanied by the generation of an appetitive motivational state. If the stimuli do, in fact, acquire the ability to generate this state, then they should produce behavioral consequences similar to those produced by morphine itself. This is what has been found in the present experiments. Interestingly, the results of conditioned place preference studies can be interpreted in a similar manner (cf.,

Rossi and Reid, 1976).

It is important to remember that the present analysis of the conditioning of morphine-induced locomotor activity is very different from one that would view this conditioning as due to the reinforcement by morphine of specific movements. This latter analysis would require that morphine administration be contingent on the prior occurrence of specific responses and it is unlikely that this was the case in the present experiments. First, because morphine was administered by the experimenter (i.e., passive administrations), no response was required by the animals in order to obtain morphine reward. Second, if it were the case that morphine reward accidentally reinforced specific momentary responses in the course of the sessions, then one would have expected different animals to subsequently exhibit different conditioned responses (e.g.: Skinner, 1948). This is not what was found in the present experiments. All experimental animals showed conditioned increases in locomotor activity and there was no evidence for individual idiosyncratic movements by any of these animals. Similar results were obtained by Bindra and Campbell (1967). In their experiment, a CS was paired with rewarding electrical brain stimulation (EBS) and it was explicitly ensured that this EBS was not contingent on the prior occurrence of any particular response. They found that the CS subsequently elicited increases in locomotor activity, a CR which mimicked the UCR to the EBS. Their conclusion, similar to the conclusion put forward here, was that the CS acquired the ability to generate an appetitive motivational state similar to that generated by the EBS itself. The only difference in the present experiments was

that the UCS was morphine rather than EBS.

Implications for Relapse to Drug Use

As discussed in the introduction, the traditional explanation of relapse to morphine use after long-term abstinence has been that it is due to the classical conditioning of the "drive-to-take-morphine" (Wikler, 1948). This, of course, is the drive-reduction view and is based on the tenet that drug-seeking behavior is maintained in order to reduce or avoid the trauma of withdrawal. Demonstrations of the classical conditioning of withdrawal reactions (Goldberg and Schuster, 1967; Irwin and Seevers, 1956; Trost, 1973; Wikler and Pescor, 1967) or of compensatory responses (Siegel, 1975, 1977a, 1979) have thus been interpreted as the classical conditioning of the "drive-to-take-morphine." Notwithstanding several attempts, however, there have to date been no successful and unequivocal demonstrations that these conditioned responses (i.e., conditioned "drive") are able to reinitiate drug-taking in animals (Thompson and Oslund, 1965; Wikler and Pescor, 1967).

An alternative explanation of relapse to drug use is proposed by the incentive-motivational view of drug taking. The central and distinguishing feature of this view is that it is the presence of the drug itself and not its absence that acts to create an appetitive motivational state that facilitates drug-seeking behavior (Stewart, in press; Stewart, deWit, and Eikelboom, Note 2). Support for this notion comes from experiments which have demonstrated that extinguished drug self-administration behavior can be reinstated by a noncontingent

"priming" administration of the previously self-administered drug. Such reinstatement of drug-taking behavior by a noncontingent "priming" drug administration has been demonstrated in animals previously trained to self-administer amphetamine (Gerber and Stretch, 1975), cocaine (deWit and Stewart, 1981; Gerber and Stretch, 1975), morphine (Davis and Smith, 1976), and heroin (deWit and Stewart, 1983). In another experiment, Stewart (1982) demonstrated that noncontingent morphine administrations into the VTA, but not into other brain sites, could reinitiate drug-taking behavior in rats previously trained to self-administer heroin intravenously.

These results suggest, therefore, that the generation of an appetitive motivational state by morphine (and by stimulants) enhances the salience of drug-related stimuli (e.g., the self-administration lever) in the animal's environment and increases the likelihood that the animal will approach and interact with them. If, therefore, a CS continually paired with morphine can come to generate an appetitive motivational state similar to that generated by morphine, as the results of the present experiments suggest, it would be expected that this CS could also enhance the salience of other drug-related stimuli and increase the likelihood that an animal would reinitiate drug-taking behavior.

Thus, the disagreement between the drive-reduction and incentive-motivational views of relapse centers on the particular association that must be learned. One view asserts that the CS must be paired with morphine-withdrawal while the other asserts that the CS must be paired with the appetitive motivational state generated by morphine. Clearly, the question no longer is whether

an animal can learn these associations but rather which association is critical for relapse to drug-use. Recent reports suggest that the incentive-motivational view might provide the proper approach.

In one experiment, deWit and Stewart (1981) trained two groups of animals to self-administer cocaine. During training, one group was exposed to a tone (the CS) during each rewarding drug infusion (correlated group). The other group was also exposed to the tone but not paired with drug infusions (uncorrelated group). On test sessions after a period of extinction, the CS alone was presented and the animals' self-administration behavior observed. They found that only animals in the correlated group reinitiated self-administration behavior upon presentation of the CS. These results suggest that the CS acquired the ability to generate an appetitive motivational state similar to that generated by cocaine and that like the drug itself (deWit and Stewart, 1981; Gerber and Stretch, 1975) was able to reinstate drug-taking behavior. Thus, the critical association for relapse in this experiment was that between the CS and cocaine reward.

Additional evidence which supports the incentive-motivational view of relapse comes from research concerned with the conditioning of eating. For many years, the traditional approach was to pair a CS (usually a distinctive chamber) with deprivation or, as it was called, "hunger drive." After a number of pairings, animals, now sated, were returned to the CS and presented with food. Such a paradigm has consistently failed to demonstrate conditioned eating or the inferred conditioned "hunger drive"

(see Cravens and Renner, 1970; D'Amato, 1974; Mineka, 1975). Recently, however, Weingarten (1983) approached the problem by pairing a CS with food. After a number of pairings, he found that presentation of the CS reliably elicited feeding in sated rats. Indeed, the size of the meals induced by the CS was large: approximately 20% of daily intake. These data, therefore, clearly demonstrate that the critical association that animals must learn is that between the CS and reward (be it food or a drug) and not the association between the CS and food deprivation or drug withdrawal.

Implications for a Biochemical Substrate

Perhaps one of the most baffling problems in neuroscience concerns the specification of the neurobiological basis of associative learning. Although the present experiments were not specifically designed to address this issue, the results obtained and the theoretical view proposed do bear on the question of a biochemical substrate for conditioned increases in locomotor activity induced by morphine administration into the VTA.

It should be clear from the onset that nonassociative changes such as increased sensitivity of opiate receptors in the VTA induced by repeated administration of morphine into this region (as suggested by Joyce and Iversen, 1979) cannot adequately explain the conditioned increases in locomotor activity obtained in the present experiments. In Experiments 1 and 3, a control group that received an equal number of morphine administrations as the experimental group, but never paired with the CS, did not show a CR upon presentation of the CS. Furthermore, another

control group that received morphine administration paired with the CS, but was pretreated with pimozide, also did not show a CR upon presentation of the CS. For similar reasons, other nonassociative changes, such as increased DA receptor density in mesolimbic DA terminal areas, could not account for the conditioned increases in locomotor activity found in the present experiments (see also Carlson and Seeger, 1981). Rather, these results suggest that the neural changes that underly the behavioral manifestations of conditioning must be associative in nature. Thus, in the present case, the question is: What is the neural substrate of the CS-UCS association?

The approach to this issue taken in the present experiments was first to attempt to identify the biochemical basis of the UCS when morphine is administered into the VTA. For example, in Experiments 2 and 3 it was found that pimozide blocked the development of conditioning and this was interpreted to mean that the biochemical basis of the UCS was an action of DA postsynaptic to mesolimbic DA neuron terminals. To the extent that the action of released DA in the area of the nucleus accumbens has been strongly implicated in the mediation of increased locomotor activity (Costall and Naylor, 1976; Pijnenburg et al., 1976; Wachtel et al., 1979) and that this DA synapse may be important in the generation of an appetitive motivational state by morphine (Wise, 1980), the critical biochemical UCS for increased locomotor activity when morphine is administered into the VTA may be the action of released DA in the nucleus accumbens.

If the CS, during conditioning, acquires the ability to

generate an appetitive motivational state similar to that generated by morphine and, with it, some of the same behavioral consequences, as suggested by incentive-motivation theory, it might be expected that the biochemical basis of the CS may resemble that of the UCS. Thus, presentation of a CS may also result in the action of released DA in the nucleus accumbens. Support for this possibility comes from experiments in which DA turnover was measured subsequent to presentation of a CS. For example, Perez-Cruet (1976) reported that the increase in DA turnover in rat striatum brought about by repeated injections of morphine could subsequently be elicited by a CS. Similarly, Schiff (1982) demonstrated that a CS repeatedly paired with d-amphetamine injections could subsequently elicit increases in DA turnover in mesolimbic areas and that these accompanied conditioned increases in locomotor activity. In a different kind of experiment, Miller, Sanghera, and German (1981) monitored the single-unit activity of mesolimbic DA cells. They found that these cells increased their firing rates following presentation of a CS that had previously been paired with a gustatory UCS. These results are consistent with those obtained in the present experiments. They suggest that the conditioned increases in locomotor activity obtained were elicited by the CS-induced action of released DA in the nucleus accumbens, and lendsupport to the proposed notion that the biochemical substrate of the CS mimics that of the UCS.

Little is known of the mechanism that may mediate this learning. Recently, however, Beninger (in press) proposed a model which may provide some insight. The model is an extension of a

model originally proposed by Libet, Kobayashi, and Tanaka (1975) and is based on studies of the rabbit superior cervical ganglion. This ganglion contains cholinergic afferents and DA interneurons both of which synapse on efferent neurons. Libet et al. (1975) demonstrated that a brief superfusion of the ganglion with DA resulted in a long-lasting enhancement of the efferent neurons' responses to acetylcholine. Furthermore, this enhancement or change seemed to be mediated intracellularly by the activation of DA-receptor-linked adenylate cyclase in the efferent neurons. Similar models (although not without some possibly important differences) have also stemmed from invertebrate research (e.g.: Hawkins, Abrams, Carew, and Kandel, 1983; Walters and Byrne, 1983).

Beninger (in press) makes a convincing argument that because "all the elements of the peripheral heterosynaptic mechanism demonstrating a role for DA in learning [as proposed by Libet et al. (1975)] may be found in the CNS," the same mechanism may mediate incentive learning such as the conditioning demonstrated in the present experiments. Specifically, cholinergic "sensory" interneurons and mesolimbic DA afferents synapse on common efferent "motor" neurons in the nucleus accumbens. When a neutral cholinergic sensory discharge (CS) is paired with an incentive stimulus-induced DA discharge (UCS), the result is synaptic change, a DA-receptor-linked adenylate cyclase-mediated change at the cholinergic synapse most recently active. As a result, only those stimuli that activate cholinergic interneurons with modified synapses will subsequently elicit CR's.

What it is important to note about this model as well as the

invertebrate models is that DA (or serotonin in invertebrates) is viewed primarily as a neuromodulator which; through pre- or post-synaptic facilitation, enhances the synaptic efficiency between incoming "sensory" neurons and outgoing "motor" neurons. The activity of this neuromodulator does not seem to be subject to plasticity. The turnover and single-unit recording results reviewed above, however, suggest that this may not be the case. Although an important role of DA may very well be that of neuromodulation, it is not clear how the heterosynaptic facilitation models can account for either conditioned changes in DA turnover or CS-elicited changes in firing rates of mesolimbic DA neurons. One possibility, of course, is that synaptic plasticity in the nucleus accumbens can influence mesolimbic DA neuron activity via a descending projection from the nucleus accumbens to the VTA (Yim and Mogenson, 1980). This possibility remains to be explored.

One problem with the notion that a CS may result in the action of released DA in the nucleus accumbens is that pimozide does not completely block the behavioral expression of the CR (Experiment 3; Beninger and Hahn, 1983; Franklin and McCoy, 1979). These findings suggest that a mechanism other than DA action in the nucleus accumbens must elicit the CR during initial DA receptor blockade. This problem is, of course, circumvented by the heterosynaptic facilitation models in which DA is viewed primarily as a modulator which is not necessary for eliciting the CR.

As with all biochemical substrate of learning models in existence today, it is clear that neither of the two above views

is completely satisfactory. Recent studies, however, may provide directions for future studies. For example, it has been suggested that A10 DA projections to the frontal cortex may exert an inhibitory influence on locomotor behavior (Tassin, Stinus, Simon, Blanc, Thierry, LeMoal, Cardo, and Glowinski, 1978). The possibility arises, therefore, that a dynamic interaction between DA action in the nucleus accumbens and fronto-cortical DA terminal areas may somehow influence learning. Interestingly, Sakurai and Hirano (1983) have recently suggested a close functional relationship between neurons in the VTA and prefrontal cortex (as well as the dorsomedial thalamus) in mediation of the learning of different contingencies in operant conditioning. They found that neurons in the VTA and dorsomedial thalamus progressively increased their firing rates during positive reinforcement training. However, during subsequent omission training, cells in the prefrontal cortex now showed increased firing rates. Although the biochemical nature of these conditioning-linked changes in neural activity is not known, such results nonetheless suggest that other brain regions may be involved in learning. Whether or not such changes are related to synaptic plasticity in other regions (such as the nucleus accumbens) remains to be elucidated.

Body Temperature

The brain site most implicated to date in the thermoregulatory effects of morphine is the preoptic-anterior hypothalamic region (PO/AH; Ary and Lomax, 1979; Clark, 1981). Injections of opiate into this area have been found to induce

dose-dependent changes in body temperature with high doses producing hypothermia, low doses producing hyperthermia, and intermediate doses producing a biphasic response of initial hypothermia followed by hyperthermia (Cox et al., 1976; Lotti et al., 1965; Teasdale et al., 1981; Tseng et al., 1980).

The results obtained in the present experiments suggest that another central site may be involved in the thermoregulatory effects of morphine: administration of morphine into a region ranging from the VTA to a site 2 mm dorso-lateral to the VTA induced a significant hyperthermia. This hyperthermia was not due to increased muscle use in active animals: pretreatment with pimozide, which completely blocked the morphine-induced increases in locomotor activity, did not block the hyperthermia. This lack of effect of pimozide is in accordance with the finding, from other experiments, that the DA receptor blocker haloperidol was ineffective in blocking both the hypothermia (Burks and Rosenfeld, 1977) and the hyperthermia (Glick, 1975) induced by systemic injections of morphine.

Whether the hyperthermia obtained in the present experiments is due to the action of morphine on thermoregulatory cells in the VTA and the area dorso-lateral to it or whether it is due to an ultimate action at the PO/AH via ascending projections from these sites remains to be determined. The sparing of the hyperthermia by pimozide, in the present experiments, effectively ruled out the possibility that morphine administration into the VTA may be exerting its hyperthermic effect via an ascending dopaminergic projection to the PO/AH. This possibility was also contraindicated by the ability of morphine to induce

hyperthermia, but not locomotor activity, at a site 2 mm dorso-lateral to the mesolimbic DA cell bodies in the VTA. Given the density of the noradrenergic and serotonergic fibers that ascend both through the VTA and the area dorso-lateral to it (Lane, Smith, and Fagg, 1983), it is possible that the hyperthermia was mediated by noradrenergic and/or serotonergic projections to the PO/AH region. Both noradrenaline and serotonin have been implicated in the thermoregulatory effects of morphine (Burks and Rosenfeld, 1977).

Furthermore, given the dose-depenedent nature of the temperature effects obtained from morphine infusions into the PO/AH, it must be determined whether the temperature effects obtained from morphine administrations into the VTA and the area dorso-lateral to it are also dose-dependent. Investigation of this possibility was precluded in the present experiments by the use of the crystaline mode of administration (Routtenberg, 1972).

Finally, no explanation for the failure to find conditioning of the hyperthermia induced by morphine administration into the VTA is apparent. It should be noted, however, that conditioned hyperthermia is only one of two possible CR's. Indeed, because there exists no independent evidence about where in relation to the thermoregulatory integrator morphine action in the VTA is having its effect, there is no way of predicting a priori whether the expected CR in the present experiments should have been hyperthermia or hypothermia (Eikelboom and Stewart, 1982).

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