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# Conditioning and Place-Specific Sensitization of Increases in Activity Induced by Morphine in the VTA

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VEZINA, P AND J. STEWART *Conditioning and place-specific sensitization of increases in activity induced by morphine in the VTA* PHARMACOL BIOCHEM BEHAV 20(6) 925–934, 1984 —The conditionability of increases in locomotor activity induced by morphine administration into the ventral tegmental area was studied in rats Morphine produced a clear increase in locomotor activity that was reversed by the opiate receptor blocker, naloxone, and blocked by the neuroleptic, pimozide, suggesting the mediation of this effect by the ascending mesolimbic dopamine system The increase in locomotor activity showed sensitization with repeated morphine administrations 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 the conditioned sensitization These data demonstrate that a learned association developed between this excitatory action of morphine and the administration environment These results have important implications for the role of conditioning factors in relapse to drug use and may provide an explanation for conditioning data obtained when morphine is administered systemically

Conditioning Locomotor activity Morphine Dopamine Sensitization Ventral tegmental area Pimozide Rats

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 [1, 54, 60]. Low doses, on the other hand, result only in an increase in activity [1, 22, 60]. 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 [1, 36, 60].

The predominant explanation for these findings has been that morphine interacts with the mesolimbic dopamine (DA) system One view put forward by Pollard and co-workers [45,46] has been that morphine acts initially to inhibit DA release by acting on opiate receptors in the terminal region of mesolimbic DA neurons. The increase in DA synthesis reported to follow morphine administration is considered to be a response to this inhibition [2]. It is thought that this increase in synthesis acts to overcome the inhibitory effect and ultimately acts to bring about the increase in locomotor activity. Some support for this view comes from studies in which morphine is administered intracranially to the nucleus accumbens (one of the terminal regions of mesolimbic DA neurons). Although these findings have been given various interpretations, what generally results is an initial period of decreased locomotor activity followed two to four hours later by a period of increased activity [12, 13, 19]. 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 [14, 15, 44, 61].

At first glance, this explanation of the effects of morphine on locomotor activity sits quite well with the view that compensatory responses play a role in drug tolerance. According to this view, responses compensatory to a particular effect of a drug are partially responsible for tolerance to that effect. It has been suggested that the classical conditioning of these responses to the environment where they are elicited may account for the finding that tolerance is often specific to the environment in which a drug is administered [51, 52, 53]. Although the data which support this view of morphine tolerance are largely limited to those gathered from tests of analgesia, the extention of these findings to changes in locomotor activity has been suggested [38,53]. Here, morphine would be the unconditioned stimulus (UCS), the environment in which morphine is repeatedly administered the conditioning stimulus (CS), and the initial decrease in locomotor activity obtained from high systemic doses the unconditioned response (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 and co-workers [38]. 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 conditioned response (CR) was an increase in locomotor activity It was concluded, therefore, that tolerance to the initial activity de-

crease was probably due to the development of a conditioned compensatory increase in locomotor activity (the CR). The problem for this view is that conditioned increases in locomotor activity were also obtained when low doses of morphine were used 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 others [31,42]. In these cases, the CR mimicked the UCR to morphine rather than opposed it

Although it may be 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 [27, 39, 40, 60] Second, enkephalin terminals have been found in close relation to DA mesolimbic cell bodies and opiate receptors have been located either on or proximal to these cell bodies [21, 29, 49]. Noting this, Joyce and Iversen [30] 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 increases in activity have since been obtained following injections of enkephalin [11] and beta-endorphin [50] into the VTA. 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 a subpopulation of mesolimbic DA cells [25,37].

Thus, although the mechanism proposed initially [45,46] by which morphine might increase activity could possibly explain the effect of systemic injections of high doses of morphine, it cannot account for the increases in locomotor activity seen following either the administration of low systemic doses of opiates or the administration of opiates directly into the VTA Indeed, the Joyce and Iversen results [30] suggest that a direct action of morphine on the cell body region of mesolimbic DA neurons could be responsible not only for the increases in locomotor activity obtained with low doses of morphine, but also for the progressive enhancement of activity seen following repeated systemic injections of morphine. Because both low and high doses of morphine, when administered repeatedly in a distinctive environment, have been found to result in conditioned increases in locomotor activity [38], the possibility arises that these increases reflect the conditioning of independently elicited actions of morphine acting on opiate receptors in the VTA The UCR to morphine would be the increase in locomotor activity elicited directly by morphine at this site and not the decrease in activity as suggested by some [38] and required by Siegel's analysis [51, 52, 53].

The present experiments were designed, therefore, with three aims in mind. first, to determine whether the increase in locomotor activity induced by morphine administration into the VTA could become conditioned to the administration environment Second, given the possibility that the excitatory action of morphine on locomotor activity could be conditioned, it seemed likely that the sensitization of the activity increases reported by Joyce and Iversen [30] could also be a conditioned phenomenon specific to the administration environment. This possibility was investigated. And third, the effect of DA receptor blockade on the development and subsequent expression of this sensitization was investigated. Because neuroleptic pretreatment has been reported to block the unconditioned activity increase induced by morphine administration in the VTA [30], it was reasoned that such pretreatment may also interfere with the development of the conditioning of this increase in activity.

## GENERAL METHOD

### SUBJECTS

Male Wistar rats (Charles River Canada Inc.) weighing 275–300 g on arrival were used. They were housed individually in stainless steel cages (18×24×18 cm) located in a reverse cycle room (lit from 22:00 to 10:00 hr). Animals were always tested during their dark cycle. Food and water were available to the animals at all times

### SURGERY

One to two weeks after arrival, animals were anaesthetized with sodium pentobarbital (0.85 ml/kg Somnotol, M.T.C Pharmaceuticals Ltd.) and stereotaxically implanted with chronic bilateral guide cannulae (22 gauge, Plastic Products Company) aimed at the 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 [41] 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 gauge) and 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 [48]. The angled implants used in the present experiments, therefore, helped circumvent the problem of drug reaching the PVG and the cerebral ventrical and helped ensure the neuroanatomical specificity of the drug effect under study

Following the experiments, all animals were perfused transcardially with saline and formalin under deep anesthesia. Histological verification of cannulae tip placements was subsequently made on 40-$\mu$ thionin-stained coronal sections.

### APPARATUS

A bank of 12 activity boxes was used to measure locomotor activity. Each box (20×41×25 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 $\mu$A One count was registered when an animal completed any break in the circuit Thus, both horizontal locomotion and rearing were recorded and pooled together to give a locomotor activity count for each animal. Activity counts were totaled every 10

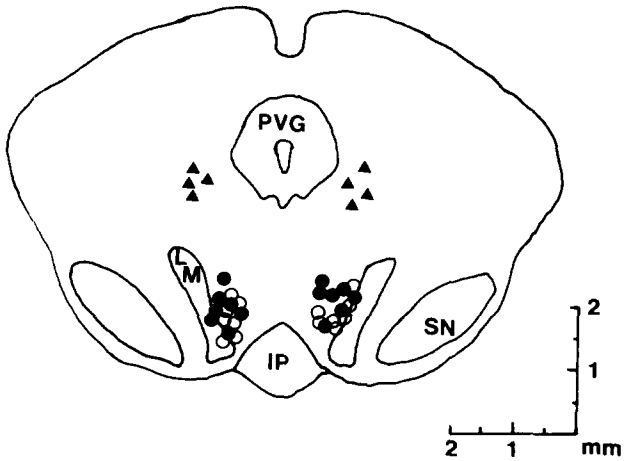


FIG 1. Injector cannulae tip placements of animals that received either morphine, n=7 (●), or sham, n=7 (○), administrations into the VTA and of four of five 2 mm dorsal control animals (▲) in a coronal section 3 8 mm posterior to bregma One of the dorsal control animals died before perfusion could be 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 This zone corresponds to the approximate location of the mesolimbic DA cell bodies. VTA morphine administrations into this area have been shown to produce conditioned place preference [3] and produced an increase in locomotor activity in all the animals tested in the present experiments. The brain section was adapted from Pellegrino, Pellegrino and Cushman [41] Aggreviations· IP (interpeduncular nucleus), LM (medial lemniscus), PVG (periventricular gray substance), SN (substantia nigra).

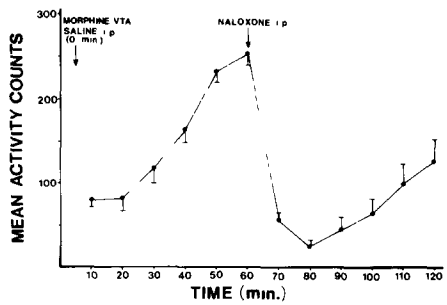


FIG 2. Mean activity counts (±SEM) before and after a challenge injection of naloxone (2 mg/kg, IP) obtained for animals given a morphine administration into the VTA at time 0. (n=7)

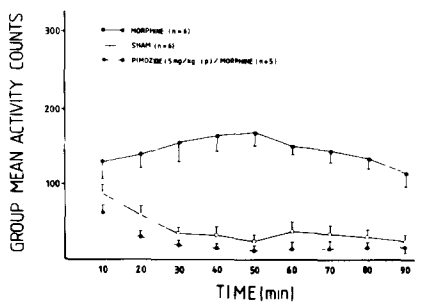


FIG 3. Mean activity counts (±SEM) obtained for three groups of animals given either a sham or a morphine administration into the VTA at time 0 Animals in Group Pimozide/Morphine were injected intraperitoneally with pimozide in the home cage four hours prior to a morphine administration in the AB.

min for each animal during the course of each experimental session. The activity boxes were kept in a room lit dimly with red light. White noise (75 dB) was continuously present to mask extraneous noise. The recording apparatus was situated in an adjacent room.

### INTRACRANIAL ADMINISTRATIONS

Morphine was administered in the form of morphine sulphate crystal (B. D. H. Chemicals, Toronto). The crystal (18±2 μg/injector cannula) was tapped into the tip of 28 gauge injector cannulae (10–15 taps) and these applied intracranially. 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 equally long 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 the duration of each experimental session.

### STATISTICS

Unless stated otherwise, the data were analyzed by analyses of variance. Analyses of simple main effects and post hoc Scheffé comparisons were made according to Kirk [32] The accepted level of significance was $p<0.05$ for all tests.

## PHARMACOLOGICAL AND ANATOMICAL SPECIFICITY OF THE MORPHINE ACTION

Figure 1 shows the injector cannulae tip placements of 14 animals representative of those with cannulae aimed at the VTA. All animals administered morphine to this site showed an increase in locomotor activity; sham administrations had no effect. In addition, the cannulae tip placements of four 2 mm dorsal control animals are shown. Because implants were angled, the administration site for these animals was 2 mm dorsolateral to the VTA. None of these animals (n=5), on two separate tests, showed increases in locomotor activity when administered morphine. These animals showed levels of activity similar to animals which received sham administrations into the VTA.

Three days following Experiment 1, animals in one group were injected with naloxone HCl (2 mg/kg, IP, Endo Laboratories Inc, Garden City, NY) 60 min after administration of morphine into the VTA (Fig. 2). Naloxone successfully reversed the increased locomotor activity response to morphine, $t(6)=14.58$, $p<0$ 001. Activity counts recorded 30 min before and 30 min after the naloxone injection were compared.

In a separate experiment, the effect of DA receptor blockade on the increased locomotor activity response to morphine in the VTA was examined. In one group, animals were injected with pimozide (0.5 mg/kg, IP) four hours prior to morphine administration. The pimozide solution was prepared by dissolving pimozide (Janssen Pharmaceuticals,

Beerse, Belgium) in a 3% solution of heated tartaric acid. Figure 3 shows that pretreatment with pimozide completely blocked the morphine-induced increase in locomotor activity, $F(2,14)=43.39$, $p<0.001$. Animals that were pretreated with pimozide did not differ significantly overall from animals that received a sham administration into the VTA, $F(2,14)=0.9$, $p>0.05$.

These data confirm previous findings [30] and suggest that morphine action at opiate receptors in the VTA increases the activity of ascending mesolimbic DA neurons which is reflected by increased levels of locomotor activity.

## EXPERIMENT 1

In this experiment, the conditionability of the increase in locomotor activity induced by morphine administration into the VTA was assessed Groups of animals were given daily morphine administrations either paired with a distinctive environment or explicitly unpaired with it. Conditioning was assessed by comparing the activity of these groups with that of sham treated control groups on a test day when all animals were returned to the distinctive environment without morphine administration

### METHOD

*Design and Procedure*

The experiment involved two phases (conditioning and testing) and four groups of animals In the first or conditioning phase, daily morphine administrations were given to one group of animals in a distinctive environment, the activity box (AB), and to another group in the home cage (HC). Each group also received daily sham administrations in the other environment. Sham administrations were given first followed three hours later by a morphine administration. As a result, one group experienced the AB administration first followed by the HC administration while the reverse was true for the other group. To control for a possible effect of the order of testing on locomotor activity, two control groups were included. Each received two daily sham administrations but in the opposite order. Animals were randomly assigned to one of the four following 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. Animals remained in their respective administration environments for 90 min. Between administrations, animals remained in their home cages

This procedure was maintained five days a week for three weeks. On Day 5 of each week, all animals received sham administrations in both environments (intermittent conditioning tests). 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 administrations in both environments (the final conditioning test)

### RESULTS AND DISCUSSION

Figure 4 shows the group mean activity counts obtained on Days 1, 6, and 11 of conditioning (the first day of each week) for each of the four groups. Morphine administration resulted in clear increases in locomotor activity. Statistical analysis of the data revealed a significant Groups effect,

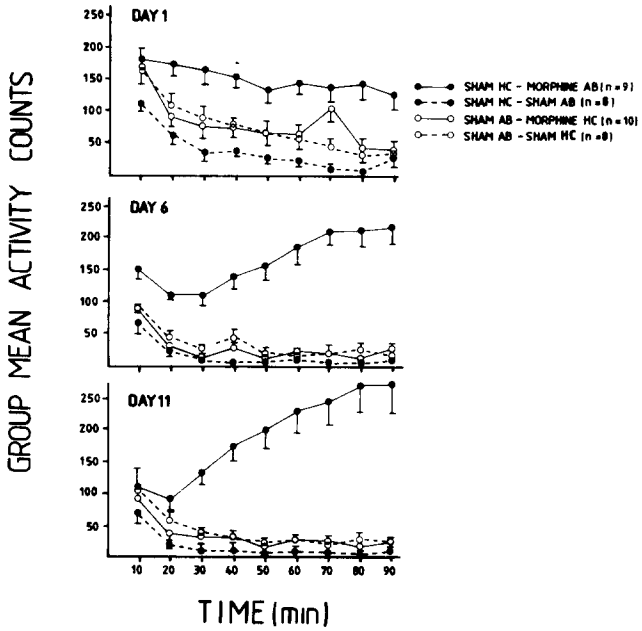


FIG. 4 Mean activity counts (±SEM) obtained on Days 1, 6 and 11 of conditioning for each of the four groups in Experiment 1

$F(3,31)=92.88$, $p<0.001$, and post hoc comparisons of day means indicated that Group Sham HC-Morphine AB was significantly more active than all three other groups on each day No significant differences between any of the three remaining groups were found when day means were compared. A significant Groups × Days interaction was found, $F(6,62)=3.34$, $p<0.01$. The simple days effect was significant only for Group Sham AB-Morphine HC, $F(2,62)=7.92$, $p<0.001$, and Group Sham AB-Sham HC, $F(2,62)=3.67$, $p<0.05$, both of which showed a decrease in locomotor activity with days On the other hand, the increased locomotor activity shown by Group Sham HC-Morphine AB clearly became enhanced with days but not significantly so. However, the Days × Time interaction was significant, $F(16,496)=11.09$, $p<0.001$, and post hoc comparisons indicated that activity levels for Group Sham HC-Morphine AB in the last 30 min of each session increased significantly from Day 1 to Day 11, $F(16,496)=13.99$, $p<0.001$. Finally, although no order effect was found when day means were compared, Group Sham AB-Sham HC was found to be significantly more active than Group Sham HC-Sham AB in the first 30 min of Day 1, $F(3,527)=3.94$, $p<0.001$, and Day 11, $F(3,527)=3\ 94$, $p<0.01$.

Figure 5 shows the group mean activity counts obtained on the final conditioning test day (following the 10 day procedure- and drug-free period) for each of the four groups. It is clear that conditioned locomotor activity was obtained. Statistical analysis of the data revealed a significant Groups effect, $F(3,31)=7.59$, $p<0.001$ Post hoc comparisons indicated that Group Sham HC-Morphine AB, which had received morphine administrations repeatedly in the AB, was significantly more active overall than its control, Group Sham HC-Sham AB, $F(3,31)=4.71$, $p<0.01$, 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 control, Group

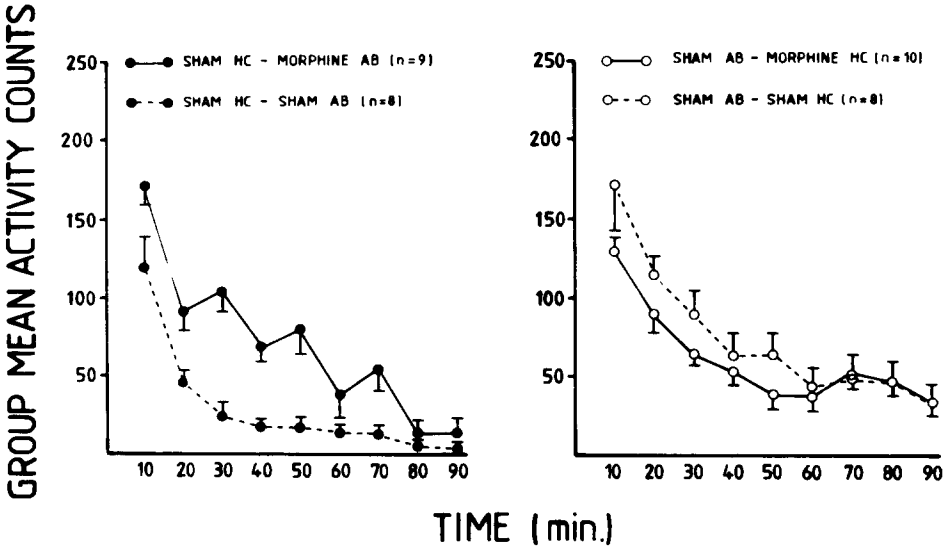


FIG 5 Mean activity counts (±SEM) obtained on the final conditioning test day for each of the four groups in Experiment 1 All animals received sham administrations in both environments on this day. The groups are separated according to the order in which they were tested in the AB

Sham AB-Sham HC, $F(3,31)=0.7$, $p>0.05$. These data show that the morphine induced increase 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). It should be noted that the CR mimicked this UCR to morphine and was not a compensatory CR that opposed the UCR.

Additional post hoc analyses of the conditioning test data revealed that Group Sham HC-Morphine AB was not significantly more active than Group Sham AB-Morphine HC. It should be noted, however, that the important comparison to make is that between Group Sham HC-Morphine AB and its sham control group, and, further, that Group Sham AB-Morphine HC did not differ significantly from its sham control group. 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, $F(3,31)=5.91$, $p<0.005$. An order effect was also manifest during conditioning. 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 conditioning test was due to classical conditioning, that is, to a learned association between morphine and the distinctive AB environment and not to pseudoconditioning [35].

One feature of the data obtained during the conditioning phase requires further comment. Although Group Sham HC-Morphine AB was significantly more active than the three other groups on all morphine treatment days throughout the conditioning phase, it showed large fluctuations in locomotor activity from day to day (not shown). These may have been due in part to the fact that morphine was administered daily during this period, not allowing sufficient time for recovery of tissue or receptors proximal to the injector cannulae tips. Regardless of the explanation, the regimen of daily morphine administrations was discontinued. In subsequent experiments, successive morphine administrations were separated by at least two drug-free days. These observations may be related to another finding that, although strong evidence for conditioned activity was found on the final conditioning test conducted ten days following the last morphine administration, there was no difference between groups on the intermittent conditioning tests made during the conditioning phase (not shown). The fact that these tests were made during the conditioning phase less than 24 hours after the last morphine administration suggests that their results may in part be an artifact of the regimen of daily administrations used in this experiment. The results of Experiment 2 would seem to confirm this interpretation.

## EXPERIMENT 2

The increase in locomotor activity induced by morphine administration into the VTA in Experiment 1 did not show tolerance. On the contrary, the activity became enhanced with repeated morphine administrations, a finding reported previously [30] In Experiment 2, we investigated the possibility that this sensitization to the effect of morphine on locomotor activity might reflect a conditioned phenomenon specific to the injection environment. Such a possibility was suggested in part by recent reports of environment-specific or conditioned sensitization to the behavioral effects of cocaine [26,47] and by the evidence for conditioned increases in activity seen in Experiment 1. It has been suggested that when the CR mimics the UCR, these two responses can summate to produce a greater observed effect [20]. Because animals were repeatedly tested in the same environment, it is possible that the progressively increasing responses obtained

were specific to this environment and reflected the development of an association between it and morphine. The investigation of this possibility provided a simultaneous test of the notion that the sensitization of this response to morphine is due to nonassociative changes such as increased sensitivity of opiate receptors in the VTA brought about by the repeated exposure of these receptors to morphine [30].

Another purpose of the present experiment was to investigate the effect of DA receptor blockade on the development of the conditioned sensitization of the locomotor activity increases induced by morphine administration into the VTA Pimozide blocks the activity produced by administration of morphine into the VTA and pilot data obtained in this laboratory suggested that pimozide pretreatment could effectively block the development of conditioning when morphine administration into the VTA was used to elicit the UCR. In the present experiment, some animals were pretreated with pimozide prior to pairings of morphine with the distinctive environment and were subsequently tested for conditioned sensitization It was reasoned that if pimozide blocked the development of conditioned sensitization, the action of morphine critical for the development of this conditioning would be an effect of the morphine-induced release of DA postsynaptic to mesolimbic DA neuron terminals.

Finally, the present experiment also provided an opportunity to replicate the conditioning of the morphine-induced increases in locomotor activity obtained in Experiment 1.

## METHOD

### *Apparatus*

Locomotor activity was measured in the same activity boxes described in the General Method section. 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 of each box. Five activity scorers were hired and trained to view and score the films. They were 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 Experiment 1, both horizontal locomotion and rearing were measured and pooled together to give a locomotor activity count for each animal.

### *Design and Procedure*

Animals were randomly assigned to one of five groups a conditioning group (Group Morphine AB-Sham HC), n=8, a pseudo-conditioning group (Group Sham AB-Morphine HC), n=8; a sham control group (Group Sham AB-Sham HC), n=8; and two pimozide pretreatment groups, Group Pimozide/Morphine AB-Tartaric Acid/Sham HC, n=8, and Group Pimozide/Sham AB-Tartaric Acid/Sham HC, n=9. Animals in the two Pimozide groups were injected in their home cages with pimozide (0.5 mg/kg, IP) four hours prior to their AB administration and the tartaric acid vehicle four hours prior to their HC administration The experimental

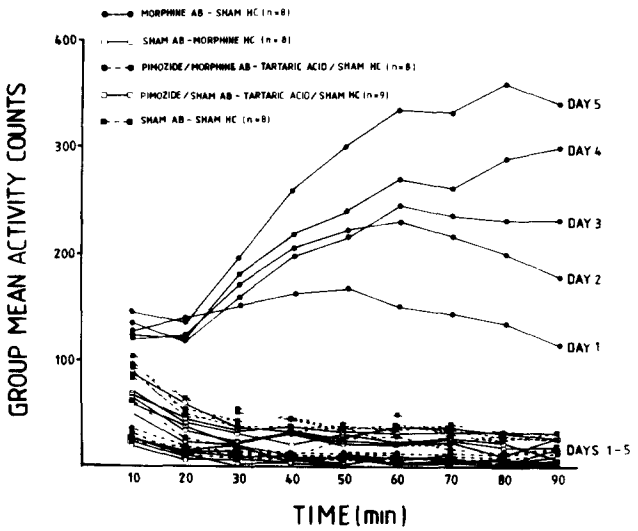


FIG 6 Mean activity counts obtained on the five conditioning days for each of the five groups in Experiment 2

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. 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 test day, three days following the last conditioning session, 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 Pimozide 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.

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

## RESULTS AND DISCUSSION

### *Conditioning Days*

The group mean activity counts obtained on the five conditioning days for each of the five groups are shown in Fig. 6 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. Statistical analysis of the day total activity counts revealed a significant Groups effect, $F(4,36)=167\ 52$, $p<0.001$, and post hoc comparisons confirmed that Group Morphine AB-Sham HC was

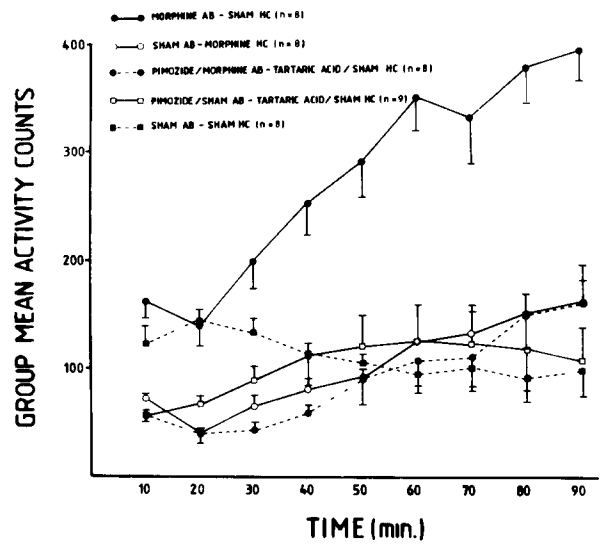


FIG 7 Mean activity counts (±SEM) obtained on the test for conditioned sensitization for each of the five groups in Experiment 2 All animals received a morphine administration on this test. Animals in the two Pimozide groups received tartaric acid injections four hours prior to the test

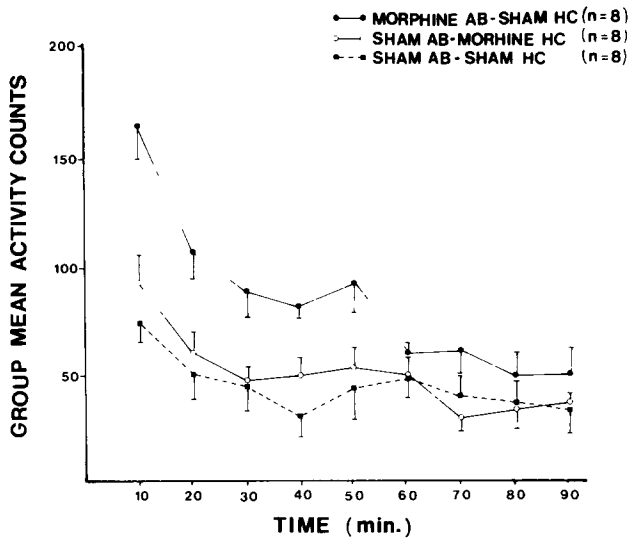


FIG 8. Group mean activity counts (±SEM) obtained on the conditioning test in Experiment 2 All animals received a sham administration on this test.

significantly more active than all four other groups on each day. A significant Groups × Days interaction was also found, $F(16,144)=9.36$, $p<0.001$. The simple days effect was significant only for Group Morphine AB-Sham HC, $F(4,144)=39.29$, $p<0.001$, which showed an increase in locomotor activity with days. The remaining groups maintained comparatively low levels of activity throughout conditioning. Additional post hoc comparisons revealed that while Groups Sham AB-Morphine HC and Sham AB-Sham HC did not differ significantly from each other, both combined were significantly more active than the combined Pimozide groups on each day. The latter two groups did not differ significantly from each other. Thus, the dose of pimozide used not only blocked the morphine-induced increases in locomotor activity but also slightly reduced levels of locomotor activity in this experiment.

*Conditioned Sensitization Test*

In this test, all animals were returned to the AB's and administered morphine. It will be recalled that the two Pimozide groups were pretreated with tartaric acid on this test. Figure 7 shows that Group Morphine AB-Sham HC was by far more active than all four other groups. A significant Groups effect, $F(4,36)=16.94$, $p<0.001$, was found and post hoc comparisons confirmed this observation. Additional post hoc comparisons revealed that the remaining four groups did not differ significantly from each other. Thus, Group Sham AB-Morphine HC, which had received an equal number of morphine administrations as Group Morphine AB-Sham HC but never paired with the AB, showed levels of activity similar to a group which received morphine for the first time on this test (Group Sham AB-Sham HC). That is, the sensitization of the morphine-induced increase in locomotor activity only appeared when animals were tested in the environment previously associated with morphine administrations. These data suggest, therefore, that the sensitization shown by Group Morphine AB-Sham HC is due to a learned association between morphine and the administration environment (the AB) and cannot be accounted for by changes brought about by the repeated exposure of opiate receptors in the VTA to morphine as previously suggested by some [30].

This interpretation is also supported by the activity levels shown by Group Pimozide/Morphine AB-Tartaric Acid/Sham HC. This group showed no evidence of having developed sensitization of the morphine-induced increase in locomotor activity even though it received an equal number of morphine administrations in the AB. Thus, pimozide not only blocked the unconditioned morphine-induced increase in activity but also blocked the development of the conditioned sensitization of this effect. These data suggest again that the critical action of morphine that becomes associated with the administration environment is not an effect at opiate receptors in the VTA but rather an effect of released DA postsynaptic to mesolimbic DA neuron terminals. No evidence for any after effect of neuroleptic treatment such as dopaminergic supersensitivity was found: Group Sham AB-Morphine HC, Group Sham AB-Sham HC, and the two Pimozide groups did not differ significantly from each other on this test.

Finally, a significant Groups × Time interaction was found, $F(32,288)=8.88$, $p<0.001$ Although this interaction 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 test, $F(4,324)=4.21$, $p<0.005$. There is, at present, no explanation for this finding.

*Conditioning Test*

The group mean activity counts obtained on this test for Groups Morphine AB-Sham HC, Sham AB-Morphine HC, and Sham AB-Sham HC are shown in Fig. 8. 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. A significant Groups effect was found, $F(2,21)=8.36$, $p<0.001$, and post hoc comparisons revealed that Group Morphine AB-Sham HC was significantly more active than Group Sham AB-Morphine HC, $F(2,21)=5.28$, $p<0.025$, and Group Sham AB-Sham HC, $F(2,21)=7.13$, $p<0.005$. These latter two groups did not differ significantly from each other. The significant Groups × Time interaction, $F(16,168)=3.42$, $p<0.001$, and additional post hoc comparisons indicated that the activity levels of Group Morphine AB-Sham HC were significantly higher than those of the remaining two groups during the first 50 minutes of the test and diminished to nonsignificant levels for the remainder of the test.

These data replicate those obtained in Experiment 1 and once again demonstrate that the morphine-induced increase 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.

## GENERAL DISCUSSION

The main findings of the present experiments are first, that morphine administration into the VTA induces an increase in locomotor activity which is reversed by naloxone and blocked by the DA receptor antagonist, pimozide. These results confirm previous findings [30] and suggest that morphine action at opiate receptors in the VTA increases the activity of ascending mesolimbic DA neurons which is reflected behaviorally by increased levels of locomotor activity. Second, the morphine-induced increase in locomotor activity could come to be elicited, in the absence of morphine, by the environment associated with morphine administrations. The CR in this case mimicked the UCR to morphine and was not a compensatory CR that opposed the UCR. Third, the morphine-induced increase in locomotor activity became progressively enhanced with repeated morphine administrations (again confirming previous findings [30]) and this sensitization was found to be specific to the administration environment. Finally, pimozide effectively blocked the development of conditioned sensitization of the morphine-induced locomotor activity increases.

These results 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 [11, 30, 50], 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 some [38, 51, 52, 53].

Tolerance did not develop to the effect of morphine on locomotor activity obtained in the present experiments. On the contrary, with repeated administrations, the increase in locomotor activity became enhanced or showed sensitization. The finding that this sensitization was specific to the environment in which morphine was repeatedly administered together with the finding that the morphine-induced increase in locomotor activity could be elicited, in the absence of morphine, by the administration environment suggests that a learned association developed between this excitatory effect of morphine and the administration environment.

It would be difficult to account for the conditioned sensitization in terms of nonassociative changes such as increased sensitivity of opiate receptors in the VTA brought about by repeated exposure of these receptors to morphine [30]. In Experiment 2, animals that received an equal number of morphine administrations as the conditioning group, but not paired with the distinctive AB environment, did not show a sensitized response when subsequently administered morphine in the AB. Furthermore, animals pretreated with pimozide prior to morphine administrations in the AB failed as well to show a sensitized response when subsequently administered morphine in the AB without pimozide pretreatment. Pimozide does not block the action of morphine at opiate receptors [33]. Rather, these data suggest that the critical action of morphine that becomes associated with the administration environment is not an effect at opiate receptors in the VTA but rather an effect of released DA postsynaptic to mesolimbic DA neuron terminals.

Such results may provide an explanation for the changes in locomotor activity seen following repeated systemic injections of morphine. It has been reported that conditioned increases in locomotor activity were obtained following repeated injections of both low and high doses of morphine [38]. Because low systemic doses do not produce unconditioned decreases in locomotor activity, it would be difficult to explain these 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 when low and high systemic doses of morphine are used as the UCS 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 effect 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 [2,45]. Since it seems to be the action of released DA postsynaptic to mesolimbic DA neuron terminals that is responsible for the sensitization obtained in the present experiments, however, such an explanation would be redundant. It, nonetheless, needs to be investigated. Another possibility is that tolerance to the depressant effect of morphine on locomotor activity may result from 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.

Finally, the present findings have important implications for the role of conditioning factors in relapse to opiate use after long-term abstinence. The traditional view of opiate use has been that it is maintained in order to avoid or reduce the trauma of withdrawal [34]. Demonstrations of the classical conditioning of withdrawal reactions [24, 28, 59, 63] or of

compensatory responses [51, 52, 53] have thus been interpreted to provide the acquired drive necessary for the reinforcing effects of the drug injection [62]. There have been to date, however, no successful and unequivocal demonstrations that these CR's are able to reinitiate self-administration of morphine in animals [58,63].

Alternatively, a number of reports have accumulated in recent years to suggest that morphine action in the VTA may be responsible for its rewarding properties [4, 5, 6, 9, 10, 43]. Furthermore, this site does not seem to be associated with the elicitation of withdrawal reactions upon termination of morphine administration [8] In addition, neuroleptic pretreatment has been found to block the conditioned place preference normally produced by systemic heroin suggesting, as with the morphine-induced increase in locomotor activity, DA mediation of morphine reward [7,64]. These findings taken together with the results of the present experiments suggest that morphine reward and morphine-induced increase in activity may share a common dopaminergic substrate. By extension of the present results, it would be expected that morphine reward could come to be elicited, in the absence of morphine, by the administration environment and thereby lead to reinitiation of self-administration behavior. Support for the notion that it is the presence of the drug (or stimuli associated with the drug) and not the absence of the drug that facilitates self-administration behavior comes from demonstrations that extinguished drug self-administration behavior can be reinstated by a noncontingent priming administration of the previously self-administered drug [16, 17, 18, 23, 55]. This view of drug taking and relapse has been discussed in more detail elsewhere [56,57] and its implications for relapse to morphine use after long-term abstinence are presently being investigated in this laboratory.

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