Evidence from Rats That Morphine Tolerance Is a Learned Response

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It is proposed that the direct analgesic effect of morphine becomes attenuated over the course of successive administrations of the narcotic by a conditioned, compensatory, hyperalgesic response elicited by the administration procedure, the net result being analgesic tolerance. Using the “hot plate” analgesia assessment situation with rats, this conditioning view of tolerance is supported by several findings: (a) It is necessary to have reliable environmental cues predicting the systemic effects of morphine if tolerance is to be observed, (b) a hyperalgesic conditioned response may be observed in morphine-tolerant subjects when drug administration cues are followed by a placebo, and (c) merely by repeatedly presenting environmental cues previously associated with morphine (but now presented with a placebo), morphine tolerance can be extinguished.

Tolerance is said to have developed when, after repeated administrations, the effect of a given dose of a drug is less than it was originally. Tolerance to many of the effects of narcotics (especially opiates), such as analgesia, develops rapidly and reliably, and numerous hypotheses have been presented to account for the phenomenon. In summary, (a) the relevant effect of early experience with the drug may be to alter the organism’s metabolism such that the drug is subsequently more efficiently metabolized (Mulé & Woods, 1969); (b) after the drug molecules exert their action on central receptor sites, they may continue to occupy these sites thereby decreasing the population of receptor sites that can be stimulated by the same drug on a later occasion (Schmidt & Livingston, 1933); (c) the initial drug administrations may induce the formation of “silent receptors,” which functionally reduce the effects of later drug administrations by serving as “dead spot” receptors for drug molecules that would otherwise stimulate active “pharmacological receptors” (Collier, 1965); or (d) narcotics may be conceived of as antigens, with tolerance reflecting an immunitylike process (Cochin & Kornetsky, 1968). All these theories of tolerance, hereinafter grouped together as physiological theories (for a review, see Cochin, 1970), postulate some systemic change within the organism as a result of the initial drug experience that decreases receptor sensitivity to the drug, allows the drug to be metabolized more quickly, or serves to bind the drug before it can exert its action.

An alternative approach, proposed here, might be termed a conditioning theory of tolerance. According to this view, narcotic tolerance is the result of the learning of an association between the systemic effects of the drug and those environmental cues that reliably precede these systemic effects. Pavlov (1927, pp. 35ff) suggested that the administration of a drug could be viewed as a conditioning trial, with the actual pharmacological assault constituting the unconditioned stimulus (UCS) and the immediately antecedent environmental cues serving as the conditioned stimulus (CS). The development of the association between these stimuli may be revealed if the subject, after a history of administration of the drug, is presented with the drug administration procedure not followed by the systemic ef-
fecteds of the drug—rather, for such a conditioned response (CR) test session, a placebo is administered.

There has been a considerable amount of interest in the theoretical and practical importance of conditioned pharmacological responses (Gantt, 1957; Loucks, 1937; Siegel & Nettleton, 1970). Although many forms of drug CRs can be conceptualized and have been reported, conditioned drug responses are commonly opposite in direction to the unconditioned effects of the drug. Thus, it has been reported that in animals with a history of administration of an anticholinergic drug, such as atropine or Ditran, which induces antisialosis, the administration procedure without the drug leads to excessive salivation (Lang, Brown, Gershon, & Korol, 1966; Wikler, 1948); in animals in which tachycardia has been repeatedly induced by injections of epinephrine, the injection procedure alone causes a decrease in heart rate (Subkov & Zilov, 1937); subjects repeatedly made to evidence allergic reactions by allergen injections evidence immune reactions when confronted with the injection procedure (for a review, see Hull, 1934, pp. 413–416); in animals with a history of administration of a hyperglycemic agent, such as glucose or epinephrine, the administration procedure alone leads to a decrease in blood sugar (Deutsch, 1974; Mityushov, 1954; Russek & Piña, 1962); in organisms repeatedly experiencing the hypoglycemic effects of injected insulin, the injection procedure alone leads to an elevation in blood sugar (Lichko, 1959; Siegel, 1972, 1975). These anticipatory responses, being compensatory in nature, should serve to attenuate the drug-induced unconditioned response (UCR), therefore the net effect of the drug should decrease over successive drug administrations. Such a decreased response to a drug as a function of successive experiences with the drug defines tolerance.

According to the present conditioning theory, tolerance to the analgesic effects of morphine results because environmental cues regularly paired with the administration of the drug come to elicit a compensatory CR, hyperalgesia, which algebraically summates with the stable, unconditioned analgesic effects of the narcotic. Thus, environmental cues consistently predicting the systemic effects of the drug should be crucial to the development of tolerance since they enable the subject to make timely compensatory CRs in anticipation of the analgesic UCR. Several experiments by Mitchell and his colleagues (Adams, Yeh, Woods, & Mitchell, 1969; Kayan, Woods, & Mitchell, 1969) indicate that the rate of development of analgesic tolerance to morphine is highly dependent upon the availability of environmental cues uniquely present at the time of drug administration. Using the standard “hot plate” assessment situation (Johansson & Woods, 1964), in which pain sensitivity in the rat is assessed by observing its latency to lick a paw when placed on a warm surface, these investigators reported that analgesic tolerance to morphine developed much more rapidly when subjects were confronted with the distinctive analgesia assessment environment on each of the five occasions that the drug was administered (even if the nociceptive stimulation was not applied until the fifth occasion) than if they were introduced into this environment for the first time on the fifth occasion that the drug was administered. Experiments 1A and 1B were designed to assess the reliability of these reports of the importance of drug-associated environmental cues in the acquisition of tolerance.

**Experiment 1A**

**Method**

*Subjects and apparatus.* The subjects, 29 experimentally naive, male, 90-110 day-old, Wistar-derived rats (obtained from Quebec Breeding Farms, St. Constant, Quebec, Canada), were housed in individual cages with food and water freely available. Responsivity to pain was assessed using recent modifications of the hot plate technique (Eddy & Leimbach, 1953). Briefly, a 1,200-ml mixture consisting of equal parts by volume of acetone and ethyl formate was boiled in a rectangular copper container (19 × 19 × 15 cm). The container was completely enclosed with the ex-
ception of provision for a condenser coil to liquify the vapor and return it to the vessel. The temperature of the vapor and the top surface of the vessel were constantly monitored and remained at 54.2° C (±.2° C). Pain sensitivity was assessed by placing the rat on the surface of the container for 1 min and noting the number of seconds that elapsed until it first licked a paw (hereafter referred to as the paw-lick latency). Thus, analgesic responses are indicated by relatively long paw-lick latencies and hyperalgesic responses by relatively short paw-lick latencies.

Procedure. Three groups of rats received equivalent morphine injections on four occasions, the interval between injections being 48 hr. The fourth occasion was the test session, during which the pain sensitivity of all the rats was assessed on the hot plate in the test environment subsequent to the drug injection. The groups differed only with respect to the cues associated with the morphine administrations on the three prior sessions.

One morphine group was used to demonstrate the initial analgesic UCRT of the drug and the development of tolerance over the successive drug administrations. This group was treated identically on Sessions 1-3 and Session 4, i.e., morphine-induced analgesia was assessed on the hot plate apparatus in the test environment each time the drug was administered (Group M-HP, i.e., morphine-hot plate; n = 8). For each session, rats in this group were transported in their home cages from the colony room to a different room, which contained the hot plate apparatus, subcutaneously injected with a 5 mg/kg dose (of a 5 mg/cc solution) of morphine sulfate, and .5 hr later, placed on the hot plate apparatus.

A second morphine group was included to assess whether any apparent drug tolerance observed in Group M-HP, as revealed by decreasing paw-lick latencies across sessions, may be attributable to merely increasing practice in making the possibly pain-ameliorating, paw-licking response while narcotized rather than to any functional decrease in the narcotic’s analgesic properties. This group was treated like Group M-HP except that the hot plate apparatus was not turned on until the fourth test session; for Sessions 1-3, these rats were placed on the surface of the vessel when it was at room temperature (21.2°-22.2° C). For this second morphine group (Group M-CP, i.e., morphine-cold plate; n = 7), the environmental cues preceding the morphine effects and analgesia assessment were the same on Sessions 1-3 as on Session 4. However, these rats never practiced the paw-licking response on the hot surface until the test session; thus, relatively rapid reactions on Session 4 by Group M-CP rats would be attributable to drug tolerance rather than to acquired proficiency in paw-licking while narcotized.

A third group of rats suffered the same systemic effects of the morphine equally as often and at the same intervals as rats in Groups M-HP and M-CP except that a different set of cues was associated with the systemic effects of the drug on the first three sessions than on the fourth, hot plate session. For this group, for Sessions 1-3, the morphine was administered in the colony room simply by removing the rat from its cage, injecting the drug, and returning the rat to its cage. Thus, this group (Group M-CAGE; n = 8), like Group M-CP, had its first morphine-induced analgesia assessment on Session 4, and differed from Group M-CP only because the environmental cues surrounding the morphine administration were different on the three prior occasions that the drug was administered.

Finally, a fourth group also received four hot plate analgesia-assessment sessions in the test environment, but all the injections were physiological saline rather than morphine. This group (Group S, i.e., saline; n = 6) provided an undrugged baseline, indicating the effects of the repeated injections and hot plate experiences per se.

Results and Discussion

The mean paw-lick latency for each group on each session is shown in Figure 1 (for Groups M-CP and M-CAGE, of course, the response was assessed only on the last session). As indicated in Figure 1, the characteristic analgesic effect of morphine was observed on the first session—rats receiving the narcotic for the first time (Group M-HP) had significantly longer paw-lick latencies than rats receiving saline (U = 7.5, p < .02). As was also expected, the analgesic effects of morphine became less and less pronounced on the successive sessions in which the drug was administered; a Wilcoxon matched-pairs signed-rank test indicated that Group M-HP rats had significantly shorter paw-lick latencies on Session 4 than Session 1 (T = 0, p = .01). As is also apparent in Figure 1, on Session 4 Group M-CP rats responded to the hot plate as did Group M-HP rats, the small difference between the two groups not approaching statistical significance, that is, Group M-CP rats evidenced the short-latency paw-licking response indicative of morphine tolerance on the fourth occasion that they received the drug despite the fact that they never experienced the heat stimulation and did not practice the response on Sessions 1-3, indicating that such practice (cf. Kayan et al., 1969) or repeated experience with the morphine while nocepectively stressed (cf. Adams et al., 1969) is irrelevant to the demonstration of analgesia tolerance in this test situation.
Both groups of morphine-tolerant rats, M–HP and M–CP, evidenced Session 4 paw-lick latencies not significantly different from that of Group S rats, despite the fact that Groups M–HP and M–CP were receiving morphine and Group S physiological saline prior to analgesia assessment. As is obvious in Figure 1, Group M–CAGE, in marked contrast to Groups M–HP and M–CP, was not tolerant to the analgesic effects of morphine. The differences between Group M–CAGE and each of Groups M–HP and M–CP were statistically significant (U = 5.5, p < .002 and U = 12, p < .04, respectively). Indeed, the Session 4 response latency of Group M–CAGE was not significantly different from that which would be expected the very first time rats receive the drug and analgesia assessment (i.e., the Session 1 value for M–HP rats). Thus, these results confirm the earlier reports of the importance of drug-associated environmental cues in the development of tolerance (Adams et al., 1969; Kayan et al., 1969).

Prior to the test session, all three morphine groups suffered the same morphine-induced systemic effects equally as often and at the same intervals. The rats in these groups should have been subjected to the same metabolic, cellular, or immunifacient modifications presumed to be responsible for tolerance (see Cochin, 1970). It would be expected, according to any of the physiological theories of tolerance, that the three groups should be equally tolerant to the analgesic effects of the narcotic. That Group M–CAGE, the group that received the drug in a distinctly different environment for the pretest sessions, does not evidence any indication of tolerance to morphine on the test session suggests that reliable cues associated with the drug administration are important in affecting the development of tolerance.

Experiment 1B

Method

Subsequent to the test session, Group M–CAGE rats continued to be injected with morphine at the same 5 mg/kg dose and pain sensitivity was assessed for three additional sessions (the interval between sessions remaining at 48 hr) so that the course of the development of tolerance over four drug administrations in these animals could be compared with that previously displayed by Group M–HP rats.

Results and Discussion

The mean paw-lick latencies for the four sessions for Group M–CAGE rats (subsequent to the three sessions in which they received the narcotic in their home cages) and the mean latencies previously demonstrated by Group M–HP rats on four sessions are shown in Figure 2. As is clear in Figure 2, the development of tolerance in M–CAGE rats was not at all facilitated by their earlier experiences with morphine in their home cages, i.e., the previous experience with the drug did not lead to any "savings" in the acquisition of tolerance. Tolerance acquisition appears to depend upon a number of pairings of a distinct drug administration/assessment ritual with the direct effects of that drug rather than upon merely the frequency of drug insults. Such pairings, according to a conditioning theory of drug tolerance, are necessary for the organism to associate predrug cues with the physiological effects of the drug and to make the compensatory CR that functionally attenuates the drug UCR.

Experiment 2A

As can be seen in Figure 1, by comparing the Groups M–HP and S reaction times over

![Figure 1. Mean paw-lick latency on hot plate for four sessions for groups in Experiment 1A, (Abbreviations used in group names: S = saline; M = morphine; HP = hot plate; CP = cold plate.)](image-url)
FIGURE 2. Comparison of the acquisition of morphine-analgesia tolerance (decrease in mean paw-lick latency after successive administrations of the drug) for Groups M-HP and M-CAGE of Experiment IB. (Abbreviations: M = morphine; HP = hot plate.)

the four sessions, morphine-injected rats tend to respond increasingly like saline-injected control rats as they have more and more experience with the drug. Indeed, Group M–HP rats rapidly became so tolerant to the analgesic effects of the drug that they responded on the hot plate as rapidly as saline-injected animals. According to a conditioning theory of morphine tolerance, the morphine-tolerant, short-latency response of Group M–HP rats results from a preparatory hyperalgesic CR summing with the narcotic’s analgesic UCR, and it should be possible to observe this conditioned increased pain sensitivity in response to those cues that have been predictors of systemic morphine. Experiment 2A was designed to demonstrate this hyperalgesic CR directly.

Method

Following the usual 48-hr intersession interval, the morphine-tolerant Group M–HP rats of Experiment 1A received a fifth session which was conducted like the previous four sessions except the substance injected was physiological saline rather than morphine.

The Group M–HP placebo-elicited hot plate response was compared with the placebo-elicited responses of two control groups. One was provided by Group S, which simply received a further placebo session. Thus, on Session 5, Group S rats had the same amount of experience with the injection procedure and assessment situation as Group M–HP rats, but never received morphine. A second control group was included to assess whether any unusual placebo-elicited hot plate sensitivity of Group M–HP could be attributed to residual systemic effects of previously injected morphine or to withdrawal from dependence upon the drug (see Tilson, Rech, & Stolman, 1973). This group received four morphine injections prior to the placebo test, of the same dose and at the same time as Group M–HP, but always in the colony room. Thus, this second control group, hereinafter called Group M–CAGE:4 (n = 8), was treated in the same manner as Group M–CAGE of the earlier experiment, but received four, rather than three morphine injections in the colony room. Group M–CAGE:4 received its first experience with the hot plate environment and analgesia-assessment situation when it received a placebo on Session 5.

Results and Discussion

The mean Session 5 paw-lick latencies, after the placebo, for Groups S, M–CAGE:4, and M–HP were, respectively, 10.3 sec, 9.1 sec, and 4.4 sec. The reaction latency was significantly shorter for Group M–HP than for Groups S or M–CAGE:4 (both $Us = 2.5$, both $ps < .002$), and these latter two control groups did not differ significantly from each other. Thus, in response to a ritual that had been associated with morphine administration but now not followed by the central effects of the drug, morphine-tolerant Group M–HP rats displayed hyperalgesia. Rats with equivalent experience with the ritual without association with the narcotic (Group S) or with the narcotic without association with the ritual (Group M–CAGE:4) did not evidence such hypersensitivity to pain.

EXPERIMENT 2B

A further experiment was conducted in an attempt to demonstrate in a within-subject rather than between-subject design that hyperalgesia follows morphine administration cues in morphine-tolerant rats.

Method

A hot plate response-latency baseline was established for each of the six Group S rats by calculating their mean response latency for a total of seven consecutive sessions in which they received physiological saline prior to pain sensitivity assessment. Longer-than-baseline response laten-
cies would be evidence of analgesia, and shorter-than-baseline latencies evidence of hyperalgesia. For the four sessions following baseline determination, these rats were injected with morphine (5 mg/kg) rather than saline prior to each hot plate placement. By comparing the rats' paw-lick latencies following each morphine injection with their baseline, the initial analgesic response and the development of tolerance could be evaluated. These now-morphine-tolerant rats were left undisturbed in their home cages for 2 wk, when they again received four physiological saline-hot plate sessions. With the exception of this 2-wk delay between the last morphine session and the first placebo test session, the intersession interval was 48 hr.

Results and Discussion

As would be expected from earlier work (e.g., Cochin & Kornetsky, 1964), the paw-lick latency did not vary much over the course of the initial baseline sessions (when subjects all received physiological saline prior to analgesia assessment). The overall mean baseline paw-lick latency was 12.9 sec; it was 11.7 sec on the first baseline session and 14.5 sec on the last baseline session, there being no significant trend across baseline sessions.

Figure 3 presents the mean percent change in paw-lick latency from baseline levels following each of the four morphine injections and, 2 wk later, after four physiological saline injections. As can be seen in Figure 3, the analgesic effect of the initial injection of morphine is clear; paw-lick latency almost doubled from baseline levels, i.e., increased by 100%. Reaction time decreased following subsequent morphine injections until by the fourth injection of the narcotic these rats were responding on the hot plate with about the same latency as their pre-drug baseline levels. When tested on the hot plate 2 wk later (after any residual systemic effects of the drug had dissipated; Tilson et al., 1973) after a placebo, these morphine-tolerant animals were clearly hypersensitive to the heat. Their reaction times were almost 40% below their baseline levels, a significantly shorter response latency (Wilcoxon matched-pairs signed-rank test, $T = 1.0$, $p < .025$). As is also clear in Figure 3, as these subjects were successively presented with the placebo test sessions the magnitude of the hyperalgesic response tended to decrease, i.e., their response latency returned to baseline levels. It appears that as the drug administration procedure is successively presented without the systemic effects of the drug, the hyperalgesic response in morphine tolerant rats is subject to extinction, suggesting that it is indeed a CR. Inasmuch as it is proposed that it is this hyperalgesic CR that is responsible for observed analgesia tolerance, extinction should be an effective procedure for eliminating tolerance.

EXPERIMENT 3

If in the morphine-tolerant animal those environmental procedures associated with the central effects of the drug elicit a compensatory CR, presenting these environmental procedures unaccompanied by the central effects of the narcotic should serve to extinguish these learned responses and morphine tolerance. In other words, placebo test sessions should constitute an effective procedure for attenuating established tolerance. This prediction of a conditioning theory of tolerance was assessed in this experiment.

Method

Two groups, each containing six experimentally naive rats of the same sex, strain, and age as those used in the previous experiments, were each given
MORPHINE INJECTIONS
FIGURE 4. Mean paw-lick latencies after each of six daily morphine injections for groups receiving either nine placebo sessions (Group M-P-M) or a 9-day rest interval (Group M-REST-M) interpolated between morphine Sessions 3 and 4 (Experiment 3).

a total of six morphine-analgesia assessment sessions, using the same procedures as described earlier. The interval between sessions was 24 hr with the exception of the protracted interval between Sessions 3 and 4, which was 9 days. The two groups differed only with respect to their treatment between these third and fourth sessions. One group was simply left undisturbed in its home cage (Group M-REST-M). The second group received daily placebo test sessions, i.e., they were treated in the same manner as on morphine sessions except the substance injected was physiological saline rather than morphine (Group M-P-M).

Results and Discussion

The mean paw-lick latencies of both groups on each occasion that they received morphine are shown in Figure 4. Both groups evidenced tolerance to the analgesic effects of morphine over the course of the three initial administrations of the drug. Group M-REST-M continued to evidence morphine-tolerant, short-latency responses when again tested with the narcotic after the delay interval, as would be expected from previous work demonstrating that morphine tolerance dissipates little simply with the passage of time (Cochin & Kornetsky, 1964). However, when tested with morphine after the same delay interval, Group M-P-M evidenced a nontolerant, long-latency response. There was no overlap in the Session 4 paw-lick latencies of Groups M-REST-M and M-P-M (U = 0, p = .001). As can be seen in Figure 4, Group M-P-M rats had to be "retolerated" to morphine, despite the fact that they had suffered the systemic effects of the narcotic equally as often as Group M-REST-M rats.

The finding that mere repeated presentations of a drug administration procedure unaccompanied by the central effects of the drug effectively obliterates morphine-analgesia tolerance is a unique prediction of a conditioning theory of tolerance, and is not explicable by theories of tolerance that do not emphasize the role of drug-associated environmental cues in the development of tolerance.

General Discussion

It has been previously suggested that learning can influence responsivity to drugs (see Thompson & Pickins, 1971) and that "... a drug-test interaction occurs with morphine and can play a role in the development of tolerance to the analgesic effect of this drug" (Adams et al., 1969, p. 251). The present experiments were designed to assess a specific Pavlovian conditioning interpretation of the phenomenon of morphine tolerance. Based on earlier reports that the CR to a variety of pharmacological agents is compensatory in nature, it seems reasonable that the direct, unconditioned analgesic effect of morphine is normally modulated by a morphine-anticipatory hyperalgesic CR, the net result being reflected by the development of morphine tolerance. This conditioning analysis of morphine tolerance is supported by several findings: (a) It is necessary to have a consistent set of environmental cues reliably predicting the systemic effects of morphine if rapid tolerance is to be observed (Adams et al., 1969; Kayan et al., 1969; Experiment 1A of the present report); (b) experience with morphine in one environment does not facilitate the acquisition of morphine tolerance in another environment (Experiment 1B); (c) the compensatory hyperalgesic CR may be directly observed in morphine-tolerant animals when they are confronted by the drug administration ritual not followed by...
the central effects of the drug (Experiments 2A and 2B), this hyperalgesic CR being subject to experimental extinction (Experiment 2B); and (d) mere presentation of those environmental cues previously associated with the narcotic, when presented in conjunction with a placebo, is an effective procedure for extinguishing established morphine tolerance (Experiment 3). The conclusions concerning the mechanism of morphine tolerance in the rat are, of course, limited to the relatively small dose of the drug (5 mg/kg) and to the analgesia-evaluation situation used in these experiments (although the hot plate procedure is perhaps the most commonly used of the simple assessment techniques for pharmacologically induced analgesia; see Evans, 1964).

The present findings concerning the importance of the interaction between conditioned and unconditioned responses in contributing to the observed effect of a drug parallel Pavlov's (1910) discussion of the significance of his original "psychic secretion" observations, i.e., that digestive responses in anticipation of feeding make a significant contribution to normally observed patterns of digestive functioning. Subsequent research has demonstrated the importance of conditional responses in the normal and pathological functioning of a variety of physiological systems in many species including humans (e.g., Ádám, 1967; Bykov, 1959). This work on the interaction of learning and physiological processes, conducted mostly by Eastern European and Soviet physiologists, is the foundation of a "synthetic physiology", "... a science of the course of vital processes in an integral organism during its various natural relations with the surrounding medium" (Bykov, 1960, p. 25; emphasis added). It would appear that inasmuch as drug administration is almost invariably predicted by a set of cues (the administration procedure, or ritual), the response of an "integral organism" to a drug can be best understood as a combination of the direct reflexive effects of the drug as it acts on central receptor sites and the effects conditioned to the drug administration procedure.

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