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Exposure to a Nonfunctional Hot Plate as a Factor in the Assessment of Morphine-Induced Analgesia and Analgesic Tolerance in Rats

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BARDO, M T AND R A HUGHES *Exposure to a nonfunctional hot plate as a factor in the assessment of morphine-induced analgesia and analgesic tolerance in rats* PHARMAC BIOCHEM BEHAV **10**(4) 481–485, 1979 —Rats not exposed to a hot plate with or without morphine and later tested on the functional hot plate with or without morphine, displayed increased paw lick latency relative to same-injected rats given pretest hot plate exposure This analgesic effect, was termed behavioral analgesia since it, unlike morphine-induced analgesia, was not reversed by naloxone (Experiment 2) Behavioral tolerance was evident in animals exposed to the nonfunctional hot plate regardless of drug treatment and was dissociated from pharmacological tolerance Behavioral analgesia and tolerance reported here may involve habituation to novel distractive stimuli associated with the hot plate test environment

Morphine-induced analgesia Behavioral analgesia Pharmacological tolerance Behavioral tolerance Morphine Naloxone Hot plate Rat

MORPHINE analgesic tolerance is reflected in a diminished analgesic response over repeated morphine injections Assessment of this effect involves both pharmacological and behavioral factors, each of which can contribute to the response diminution used to index tolerance. Pharmacological tolerance can be described as a diminished analgesic response brought about by morphine administration per se, whereas behavioral tolerance can be described as a diminished analgesic response brought about by experience with the analgesia assessment procedure and apparatus [3,5]

Behavioral tolerance related to morphine effects has been demonstrated under a variety of analgesia assessment conditions [1, 3, 5] In these instances, animals given exposure to a functional apparatus with or without morphine, and animals given exposure to a nonfunctional apparatus with morphine, subsequently displayed behavioral tolerance when tested with morphine relative to same-injected animals not given apparatus exposure

Various factors may contribute to behavioral tolerance Instrumental learning may be involved [4] but is not a necessary factor since morphine-injected animals given repeated exposure to a nonfunctional apparatus, and subsequently tested with morphine, display behavioral tolerance relative to same-injected animals not given apparatus exposure [1, 3, 9, 10] The basis of this nonfunctional apparatus exposure effect clearly cannot reflect instrumental learning, stress related to repeated noxious stimulus exposure, or a change in nociceptive receptor sensitivity, because the noxious stimulus is not present on pretest exposure sessions.

Nonstressful but distracting stimulation induces a concomitant analgesic effect [2]. Since this analgesic effect is nonpharmacological it can be termed behavioral analgesia It is possible that the behavioral tolerance evident in morphine-induced analgesia assessment situations reflects, in part, habituation to novel, and therefore, potentially distracting, apparatus stimuli According to this interpretation, animals given morphine for the first time and nonfunctional apparatus exposure for the first time would display analgesia (if tested) consisting of both a pharmacological (morphine) and behavioral (novel stimuli) component Pharmacological and behavioral tolerance would occur over repeated injection-exposure sessions as animals repeatedly experience morphine and, at the same time, habituate to novel environmental stimuli associated with the injection-apparatus exposure procedure. On a subsequent test of morphine-induced analgesic effects, in the now functional apparatus, these animals would display less analgesia than animals given similar repeated morphine injections but not given prior apparatus exposure This response difference would occur because, although all animals would have achieved pharmacological tolerance, only the exposed animals would have achieved behavioral tolerance This interpretation is consistent with observations that animals given nonfunctional apparatus exposure display greater

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tolerance than nonexposed animals when tested for morphine-induced analgesia [1, 3, 9, 10] If this nonfunctional apparatus exposure effect reflects habituation to novel stimuli which initially induce behavioral analgesia, then behavioral analgesia and behavioral tolerance ought to be dissociable from morphine-induced analgesia and pharmacological tolerance. There is little or no evidence to support this possibility

The first experiment to be presented here sought evidence of behavioral analgesia and behavioral tolerance, independent of morphine-induced analgesia and pharmacological tolerance, and at the same time sought to replicate previously reported observations of combined pharmacological and behavioral tolerance These factors were assessed within the context of nonfunctional apparatus exposure prior to analgesic test

EXPERIMENT 1

METHOD

Animals

The animals were sixty-four, 80-day-old male hooded rats (Blue Spruce Farms, New York) maintained in individual cages under a 12 hr light-dark cycle in a temperature and humidity controlled colony room where they were allowed free access to food and water

Apparatus

We used a low-temperature hot plate assessment procedure since this method has been reported to be more sensitive than high-temperature procedures in detecting subtle analgesic effects [8] The hot plate apparatus consisted of a slide warming tray (Chicago Surgical and Electric Co , Model 26020) covered with a 25×33×58 cm clear Plexiglas chamber with a hinged top The top and three sides of the chamber were vertically striped with 2 cm black tape spaced at 2 cm intervals. This apparatus was illuminated by a 7.5 W white light mounted 20 cm above the chamber top. The apparatus was placed on a table in a darkened experimental room isolated from the colony room A 23×33×25 cm wooden chamber with metal grid floor (grids spaced at 2 cm intervals) was enclosed within a larger 38×45×40 cm wooden chamber with a hinged top and was located at floor level next to the hot plate apparatus. A timer (Hunter, Model 120 A) which could be started and stopped with a foot-switch was placed between the hot plate and grid-floor apparatus.

Procedure

Each animal was randomly assigned to one of eight treatment groups combined as a balanced 2×2×2 factorial design (n=8 per group) Treatment consisted of administering a daily injection of either morphine or saline (pretest-drug factor) and post-injection exposure to either the nonfunctional hot plate or the grid-floor apparatus (pretest-exposure factor). These pretest treatments were given on four consecutive days at approximately 24 hr intervals Following these treatments, animals were injected with morphine or saline for either the first or the fifth time (test-drug factor) and were tested for pain responsivity in the functional hot plate apparatus

On the four consecutive pretest treatment days, each animal was individually transported in its home cage to the experimental room and injected SC with 1 cc/kg of either 5 mg/kg morphine sulfate or 0 9% saline Thirty minutes after each injection, one half of the morphine-injected and one half of the saline-injected animals were placed in the nonfunctional hot plate apparatus (plate surface at 25°C) for 120 sec. In order to provide similar handling experience, the remaining animals were each placed in the grid-floor apparatus for 120 sec All animals were caged individually without water in the experimental room during the 30 min injection-placement interval and were returned to the colony room following each hot plate or grid-floor apparatus placement

On the day following the last pretest treatment day, each animal was transported to the experimental room and injected with morphine or saline for either the first or the fifth time. Thirty minutes after injection, each animal was tested for pain responsivity in the functional hot plate apparatus (plate surface at 49.5°C). Pain responsivity was indexed by latency to perform a paw-lick response to the front or hind paws and was recorded to the nearest 0 1 sec as paw-lick latency (PLL) If a paw-lick response was not performed within 120 sec, the test was terminated and PLL recorded as 120 sec

The four pretest treatment groups were Morphine-injected, hot plate exposed (M-E Group), morphine-injected, not exposed (M-NE Group); saline-injected, hot plate exposed (S-E Group); and saline-injected, not exposed (S-NE Group) Within each pretest treatment group there were two subgroups, one was administered morphine and the other was administered saline on the test day. These groups and treatments are summarized in Table 1

TABLE 1
TREATMENT GROUPS EXPERIMENT 1*

Group	N	Test-Day Injection (Day 5)
S–E	8	Morphine
S–E	8	Saline
M–E	8	Morphine
M–E	8	Saline
S–NE	8	Morphine
S–NE	8	Saline
M–NE	8	Morphine
M–NE	8	Saline

*Group abbreviations refer to injections and treatment on Pretest Days 1–4 S, saline-injected, M, morphine-injected, E, exposed to ambient temperature hot plate, NE, not exposed to ambient temperature hot plate On the test day the hot plate was 49 5°C

Data Analysis

Paw-lick latency data were statistically analyzed using a 2×2×2 (pretest-drug×exposure×test drug) factorial analysis of variance and, when significant interactions occurred, tests of simple main effects [6]

RESULTS

Paw-lick latencies obtained from the eight treatment groups are summarized in Fig 1 Morphine-induced analgesia is clearly evident in significantly longer PLLs obtained from S-NE and S-E Groups administered morphine on

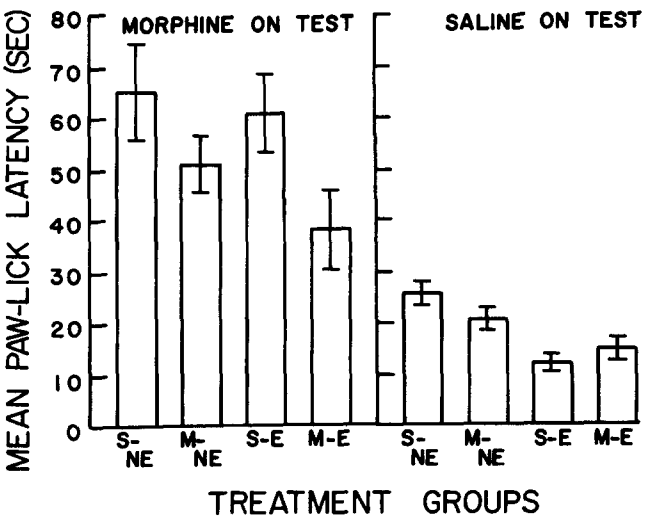

FIG 1 Mean paw-lick latencies from Experiment 1 for pretest treatment Groups S-NE (saline-injected, no pretest hot plate exposure), M-NE (morphine-injected, no pretest hot plate exposure), S-E (saline-injected, pretest hot plate exposure), and M-E (morphine-injected, pretest hot plate exposure) Within each pretest treatment group, subgroups were given either morphine or saline on the test day Standard error of the mean for each group is indicated by vertical lines, n=8 per treatment group

the test day relative to S-NE and S-E Groups administered saline on the test day, $F(1,56)=61.64$, $p<0.0001$. Analgesic tolerance to morphine effects is evident in significantly shorter PLLs of M-NE and M-E Groups administered morphine for the fifth time on the test day relative to S-NE and S-E Groups administered morphine for the first time on the test day, $F(1,56)=10.30$, $p<0.01$ This tolerance may be termed pharmacological since it is evident in both exposed and nonexposed chronic morphine-injected animals.

The data summarized in Fig 1 also demonstrate that exposure to the nonfunctional hot plate affected pain responsivity independent of morphine effects Nonexposed animals displayed analgesia relative to exposed animals as indicated by significantly longer PLLs across each of the pretest-drug by test-drug treatment combinations, $F(1,56)=5\ 98$, $p<0.05$ There was no significant interaction between the exposure factor and either the pretest-drug or the test-drug factor. This lack of interaction indicates that the analgesia inferred from long PLLs of nonexposed animals may be termed behavioral since it occurred independent of morphine effects

EXPERIMENT 2

Since analgesic manipulations are often characterized as opiate-specific if reversed or attenuated by opiate antagonists [7], this second experiment assessed the influence of the opiate antagonist naloxone on morphine-induced and on behavioral analgesia

METHOD

Animals

The animals were sixty-four, 85-day-old male hooded rats (Blue Spruce Farms, New York) maintained as described in Experiment 1

Apparatus

Apparatus consisted of the hot plate described in Experiment 1 and an open-field apparatus not previously described The open-field apparatus consisted of a 45×45×40 cm chamber with two clear Plexiglas walls, two opaque white walls and a floor made of metal grids spaced at 2 5 cm intervals The open-field apparatus was located in the colony room

Procedure

Each animal was randomly assigned to one of eight treatment groups combined as a balanced 2×2×2 factorial design (n=8 per group) Treatment consisted of administering each animal two saline injections (one SC and one IP) per day over four consecutive days, and post-injection exposure to either hot plate or open field (exposure factor) After this each animal received SC morphine or saline (morphine factor) followed by either IP naloxone or saline (naloxone factor) and was subsequently tested for pain responsivity in the functional hot plate apparatus

On the four consecutive pretest treatment days, one half of the animals were individually transported in their home cages to the experimental room. Each animal was given a 0 9% saline injection SC and, 30 min later, was given a second saline injection IP Ten minutes after the second saline injection, each animal was placed in the nonfunctional hot plate apparatus (plate surface at 25°C) for 120 sec (exposed group) and then was returned to the colony room. Each of the remaining animals was given similar injections except that these injections were administered in the colony room. Ten minutes after the second saline injection, these animals were placed in the open-field apparatus for 120 sec (not exposed group).

On the day following the last pretest treatment day, each animal was transported to the experimental room. Both hot plate exposed and not exposed animals were injected SC with 1 cc/kg of either 5 mg/kg morphine sulfate for the first time or 0 9% saline for the fifth time and, 30 min later, were injected IP with a similar volume of either 3 mg/kg naloxone hydrochloride for the first time or 0.9% saline for the fifth time Ten min after the second injection, each animal was tested for pain responsivity in the functional hot plate apparatus (plate surface at 49 5°C) as described in Experiment 1.

The groups formed by the treatments described above were Morphine-naloxone injected (M-N Group), morphine-saline injected (M-S Group), saline-naloxone injected (S-N Group), and saline-saline injected (S-S Group). Within each test-day treatment group there were two subgroups defined by pretest treatment; one was exposed and the other was not exposed to the nonfunctional hot plate. These groups and treatments are summarized in Table 2. Analyses of PLL data were as described for Experiment 1

RESULTS

Paw-lick latencies obtained from the eight treatment groups are summarized in Fig 2 These data demonstrate that naloxone, which clearly reversed morphine-induced analgesia, failed to reverse behavioral analgesia Behavioral analgesia is evident in significantly longer PLLs of groups not exposed to the hot plate apparatus relative to exposed groups across each of the morphine by naloxone treatment combinations, $F(1,56)=30\ 13$, $p<0\ 001$ Morphine-induced analgesia is evident in significantly longer PLLs of

TABLE 2
TREATMENT GROUPS EXPERIMENT 2*

Group	N	Pretest Exposure-Treatment (Days 1–4)
M–S	8	Exposed
M–S	8	Not Exposed
M–N	8	Exposed
M–N	8	Not Exposed
S–S	8	Exposed
S–S	8	Not Exposed
S–N	8	Exposed
S–N	8	Not Exposed

*On Day 1–4 each animal received two saline injections and was exposed or not exposed to the ambient temperature hot plate following the second injection Group abbreviations refer to test-day injections (Day 5) when animals received an injection of morphine (M) or saline (S) followed by an injection of naloxone (N) or saline

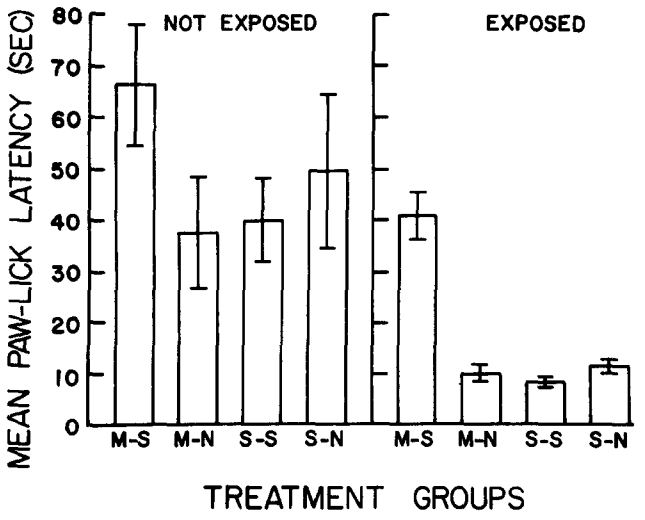

FIG 2 Mean paw-lick latencies from Experiment 2 for test-day treatment Groups M-S (morphine-saline injected), M-N (morphine-naloxone injected), S-S (saline-saline injected) and S-N (saline-naloxone injected) Within each test-day treatment group, subgroups were either previously exposed or not exposed to the nonfunctional hot plate following saline injections Standard error of the mean for each group is indicated by vertical lines, n`=8 per treatment group

M-S Groups relative to S-S Groups, $F(1,56)=13\ 95$, $p<0\ 01$ Naloxone reversal of morphine analgesia is evident in significantly shorter PLLs of M-N Groups relative to M-S Groups, $F(1,56)=14\ 14$, $p<0\ 01$ This reversal was complete since the difference in PLLs between M-N and S-S Groups was not significant In contrast, naloxone failed to reverse behavioral analgesia since the difference in PLLs between S-N and S-S Groups not exposed to the hot plate apparatus was not significant

DISCUSSION

The results of the present experiments demonstrate that animals given morphine for the first time, but not exposed to the hot plate apparatus prior to test, may display two dissociable sources of analgesic effect First, morphine-induced analgesia was evident in both exposed and nonexposed animals given morphine for the first time on test Second, a behavioral analgesia was evident in both morphine- and saline-injected animals not exposed to the hot plate apparatus prior to test The behavioral analgesia was evident as longer PLLs relative to same injected but hot plate exposed animals Behavioral analgesia was independent of morphine-induced analgesia since it occurred in animals not exposed to the apparatus regardless of drug treatment (Experiments 1 and 2), and was additive with morphine-induced analgesia since it, unlike morphine-induced analgesia, was not reversed by naloxone (Experiment 2)

The results of the present experiments also indicate that animals given repeated morphine injections and nonfunctional apparatus exposures may display both pharmacological and behavioral tolerance Pharmacological tolerance (morphine) developed with morphine administration per se and was evident in Experiment 1 in both exposed and not exposed animals given five morphine injections Behavioral tolerance (nonpharmacological) developed with exposure to the nonfunctional apparatus per se and was evident in Experiments 1 and 2 in animals exposed to the nonfunctional apparatus with or without morphine and tested with or without morphine Previous experiments demonstrated a combined effect of pharmacological and behavioral tolerance in animals given morphine on test [3,5] The present experiments are the first to demonstrate that behavioral tolerance can occur independent of pharmacological (morphine) effects

The diminished analgesic response evident in exposed animals, which defines behavioral tolerance in the present experiments, cannot reflect instrumental learning of a paw-lick response, stress related to repeated noxious stimulus exposure, or a change in nociceptive receptor sensitivity, because these animals were exposed to a nonfunctional apparatus Moreover, since in the present experiments animals exposed to the nonfunctional apparatus with saline displayed behavioral tolerance, this particular response diminution cannot reflect a classically conditioned hyperalgesic response which presumably requires pairing of morphine with environmental cues associated with the injection-exposure procedure [9, 10, 11, 12]

It is possible that behavioral tolerance observed here reflects habituation to stimuli that initially induce behavioral analgesia (e g , [2]) Animals not exposed to the hot plate apparatus were confronted with novel environmental stimuli when tested for pain responsivity These stimuli may have induced an increase in paw-lick latency which we have termed behavioral analgesia In contrast, exposed animals may have habituated to this novelty-induced effect over repeated exposures to the nonfunctional apparatus prior to test which may have induced decreased paw-lick latencies indicative of behavioral tolerance

If the above interpretation of behavioral analgesia and behavioral tolerance obtained in the present experiments has merit, then the stimuli that induce behavioral analgesia appear to be associated more specifically with the test apparatus than with the injection-handling procedure In Experiment 1, animals not exposed to the hot plate apparatus displayed behavioral analgesia relative to exposed animals, even though both groups were given similar injection-handling experience in the experimental room Stimuli associated with the experimental room may also contribute to this behavioral analgesia The greater degree of behavioral analgesia observed in Experiment 2 (cf Group S-NE given

saline on test in Experiment 1 and Group S-S not exposed to the hot plate in Experiment 2) may be a result of Group S-S animals being exposed to both the experimental room and hot plate apparatus for the first time on the test day.

Finally, although behavioral analgesia may be reversed by a naloxone dose different from the one used in Experiment 2 (3 mg/kg), the present failure to observe even a slight attenuation of effect with this opiate antagonist suggests that the exposure effect observed here involves a non-opiate substrate. Non-opiate analgesic mechanisms have been proposed to be mediated by the midbrain reticular formation [7]. This brain area may be involved in the exposure effect reported here.

REFERENCES

1. Adams, W. J., S. Y. Yeh, L. A. Woods and C. L. Mitchell. Drug-test interaction as a factor in the development of tolerance to the analgesic effect of morphine. *J. Pharmac. exp. Ther.* **168**: 251–257, 1969.
2. Gardner, W. J. and J. C. R. Licklider. Auditory analgesia in dental operations. *J. Am. dent. Ass.* **59**: 114–119, 1959.
3. Gebhart, G. F. and C. L. Mitchell. Further studies on the development of tolerance to the analgesic effect of morphine. The role played by the cylinder in the hot plate testing procedure. *Arch. int. Pharmacodyn.* **191**: 96–103, 1971.
4. Gebhart, G. F., A. D. Sherman and C. L. Mitchell. The influence of learning on morphine analgesia and tolerance development in rats tested on the hot plate. *Psychopharmacologia* **22**: 295–304, 1971.
5. Kayan, S., R. K. Ferguson and C. L. Mitchell. An investigation of pharmacologic and behavioral tolerance to morphine in rats. *J. Pharmac. exp. Ther.* **185**: 300–306, 1973.
6. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences*. Belmont, California: Wadsworth, 1968, p. 221.
7. Mayer, D. J. and D. D. Price. Central nervous system mechanisms of analgesia. *Pain* **2**: 379–404, 1976.
8. O'Callaghan, J. P. and S. G. Holtzman. Quantification of the analgesic activity of narcotic antagonists by a modified hot-plate procedure. *J. Pharmac. exp. Ther.* **192**: 497–505, 1975.
9. Siegel, S. Evidence from rats that morphine tolerance is a learned response. *J. comp. physiol. Psychol.* **89**: 498–506, 1975.
10. Siegel, S. Morphine analgesic tolerance: Its situation specificity supports a Pavlovian conditioning model. *Science* **193**: 323–325, 1976.
11. Siegel, S. Morphine tolerance acquisition as an associative process. *J. exp. Psychol.* **3**: 1–13, 1977.
12. Siegel, S., R. E. Hinson and M. D. Krank. The role of predrug signals in morphine analgesic tolerance: Support for a Pavlovian conditioning model of tolerance. *J. exp. Psychol.* **4**: 188–196, 1978.