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The Role of Hot Plate and General Environmental Stimuli in Morphine Analgesic Tolerance

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BARDO, M. T., P. J. WELLMAN AND R. A. HUGHES. *The role of hot plate and general environmental stimuli in morphine analgesic tolerance.* PHARMAC. BIOCHEM. BEHAV. **14**(5) 757–760, 1981.—Rats given one injection of morphine (5 mg/kg) paired with a hot plate test displayed greater analgesic tolerance than rats given nine injections of morphine paired with a distinct room in which the hot plate apparatus was located. Hot plate stimuli, rather than general environmental stimuli, are prepotent in the acquisition of morphine analgesic tolerance assessed by the hot plate procedure.

Morphine analgesia Morphine tolerance Hot plate Rat

MORPHINE analgesic tolerance has been interpreted to reflect the acquisition of a classically conditioned hyperalgesic response [9, 10, 11, 12]. According to this interpretation, when morphine is repeatedly and reliably paired with distinct environmental cues, these cues come to serve as conditioned stimuli which elicit a conditioned hyperalgesic response. The conditioned hyperalgesic response is thought to be opposite in direction to and summative with the unconditioned morphine-induced analgesic effect to produce tolerance.

Assessments of morphine analgesic tolerance generally involve the administration of morphine in the presence of complex environmental stimuli, including those provided by the injection-test room and analgesiometric apparatus. Although morphine analgesic tolerance is obtained in rats given morphine paired with either general test room stimuli [12] or hot plate apparatus stimuli [1, 3, 4, 6], it is not clear which of these stimuli is prepotent in producing tolerance. The present experiment therefore directly examined the relative contributions of general test room and hot plate stimuli in producing morphine analgesic tolerance.

METHOD

Animals

The animals were fifty-one adult male Long-Evans hooded rats (Blue Spruce Farms, NY). They were maintained in a temperature and humidity controlled colony room under a 12:12 hr light:dark cycle in individual metal cages with Teklad pellets and water freely available.

Apparatus

The hot plate apparatus consisted of a slide warming tray (Chicago Surgical and Electrical Co., 26020) with its temperature control dial set to remain constantly on at the highest temperature. Temperature was controlled and maintained at a relatively constant 50°C by a Variac (Standard Electrical Co., 300 BU). A 30×15×35 cm clear plastic chamber with a hinged top and open bottom was placed on the hot plate surface. The chamber was covered with brown adhesive paper except for the top and a 30×7 cm window along the bottom of the front wall through which animals could be observed. The hot plate apparatus was placed in a 100×30×90 cm wooden wall cabinet with the doors and shelves removed. A 15 W white light was mounted on the back wall of the cabinet interior and illuminated the hot plate apparatus. Response latencies were recorded to the nearest 0.1 sec by a hand-operated electronic timer. The apparatus was located in a test room isolated from the colony room environment. The test room was illuminated by a 15 W white light mounted on a table where the animals were injected, and was supplied with 70-db white noise background (Grason-Stadler noise generator, 1724).

Procedure

Animals were randomly assigned to one of three main treatment groups: One group received morphine paired with test room stimuli (Morph-Room group); one group received morphine paired with colony room stimuli (Morph-Colony group); and one group received saline (Sal group). On the first day of the experiment (Day 1), all animals were transported in their home cages to the test room. Upon arrival, Morph-Room animals were injected SC with 5 mg/ml/kg morphine sulfate, while Morph-Colony and Sal animals were

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TABLE 1
SUMMARY OF TREATMENT GROUPS

Group	Subgroup	N	Injection in Room	Injection in Colony	Injection on Tests 1–3	Injection on Test 4
Morph-Room	Morph-Test	8	Morph	Sal	Morph	Morph
	Sal-Test	8	Morph	Sal	Sal	Morph
Morph-Colony	Morph-Test	9	Sal	Morph	Morph	Morph
	Sal-Test	7	Sal	Morph	Sal	Morph
Sal	Morph-Test	9	Sal	Sal	Morph	Morph
	Sal-Test	8	Sal	Sal	Sal	Morph

injected with an equivalent volume of 0.9% saline. All animals were returned to the colony room 120 min after injection. This procedure was repeated on Days 3 and 5. On Days 2, 4, and 6, all animals were kept in the colony room for injection. On these days, Morph-Colony animals were injected SC with 5 mg/ml/kg morphine sulfate, while Morph-Room and Sal animals were injected with saline.

On Day 7, animals from each main treatment group were randomly assigned to one of two subgroups: One group received morphine and a hot plate test (Morph-Test), and the other group received saline and a hot plate test (Sal-Test). On this day (Test 1), all animals were transported to the test room, where one half from each main treatment group were injected with 5 mg/ml/kg morphine and the other half were injected with saline. Thirty min later, each animal was placed on the hot plate surface (50°C) and latency to perform a paw-lick response to a front or hind paw was recorded to the nearest 0.1 sec as paw-lick latency (PLL) by an observer unaware of each animal's treatment. If a paw-lick response was not observed within 60 sec, the test was terminated and PLL recorded as 60 sec. All animals were returned to the colony room following hot plate tests.

The injection regimen on Days 1–6 and the injection-test regimen on Day 7 (Test 1) was repeated for two more consecutive weeks. Days 14 and 21 were Tests 2 and 3 respectively. On Day 22, all animals were administered morphine in the test room and assessed for pain responsivity again (Test 4). Table 1 summarizes the different treatment groups.

Data Analysis

Split-plot analyses of variance with tests for simple main effects [7] were performed on PLLs obtained from Tests 1–3 and Test 4.

RESULTS

Figure 1 summarizes the PLLs obtained from each treatment group on Tests 1–3. Morphine-induced analgesia is evi-

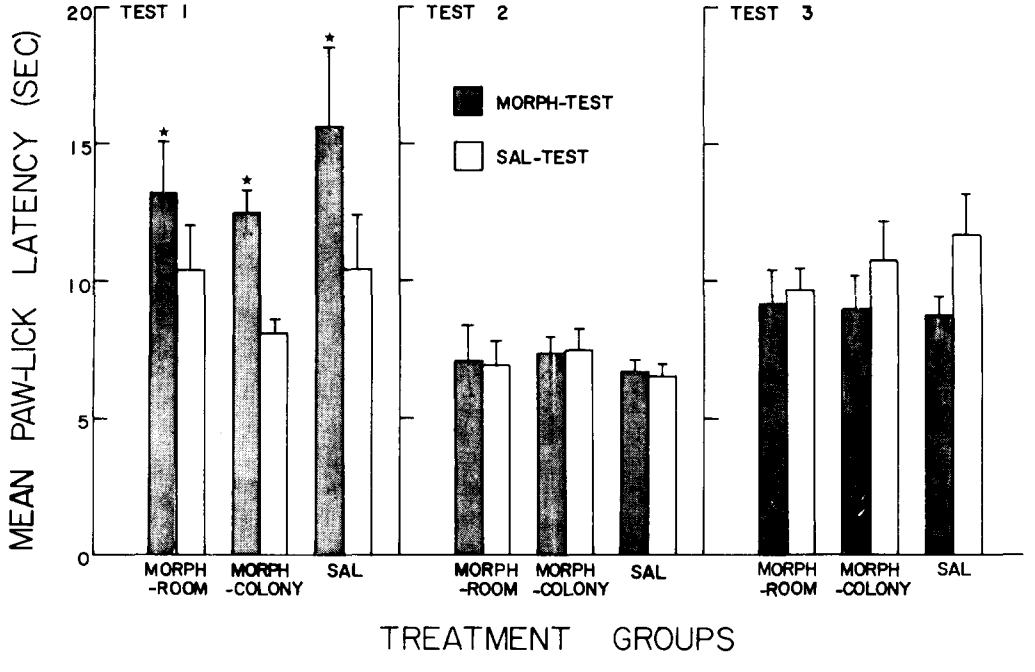

FIG. 1. Mean paw-lick latencies from the three main treatment groups given either morphine (black bars) or saline (white bars) on Tests 1–3. The lines above each bar represent standard errors of the means and the stars represent a significant difference from Sal-Test animals, $p<0.001$.

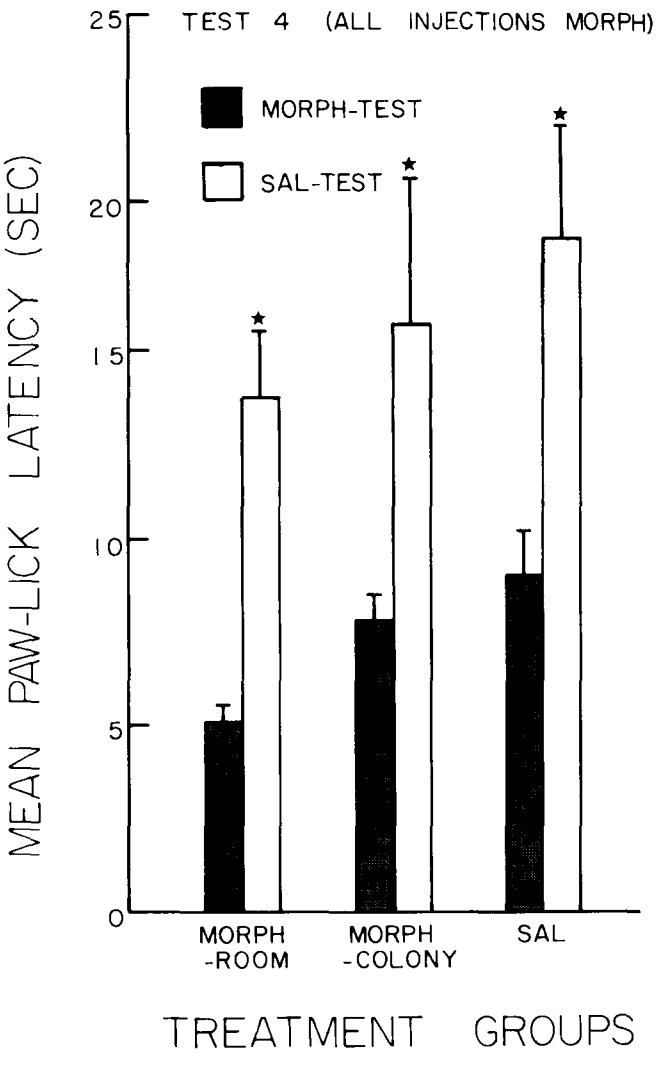

FIG. 2. Mean paw-lick latencies from the three main treatment groups given either morphine (black bars) or saline (white bars) on Tests 1–3, and then given morphine on Test 4. The lines above each bar represent standard errors of the means and the stars represent a significant difference from Morph-Test animals, $p<0.001$.

dent on Test 1, as Morph-Test animals displayed significantly longer PLLs than Sal-Test animals, $F(1,64)=13.73$, $p<0.001$. Tolerance is evident on Tests 2 and 3, as there was no significant difference in PLLs between Morph-Test and Sal-Test animals on these days. There was a significant decline in PLLs from Test 1 to Test 3 in Morph-Test animals, $q(86)=6.67$, $p<0.05$, but not in Sal-Test animals. Moreover, the split-plot analysis of variance revealed no significant differences between Morph-Room, Morph-Colony, and Sal groups on Tests 1–3. While it appears that Morph-Room animals showed an attenuated analgesic response to morphine compared to Sal animals on Test 1, one-tailed t-tests performed post hoc failed to substantiate this trend. Thus, the statistical analyses indicate that the rate of tolerance development was not influenced by morphine injections paired with either test or colony room environments without the hot plate test.

Figure 2 summarizes the PLLs obtained from each treatment group on Test 4, when all animals received morphine. On this test, Morph-Test animals received a fourth test injection of morphine, whereas Sal-Test animals received a first test injection of morphine. Tolerance is clearly evident in Morph-Test groups, as they displayed significantly shorter PLLs than Sal-Test animals, $F(1,43)=27.29$, $p<0.001$. However, there were no significant differences in PLLs between Morph-Room, Morph-Colony, and Sal groups on Test 4, indicating that the morphine injections paired with either test or colony room environments failed to contribute to the tolerance observed here.

DISCUSSION

The present results demonstrate that morphine paired with hot plate test stimuli, rather than general room stimuli, is prepotent in the acquisition of morphine analgesic tolerance. Rats given a single morphine injection paired with a single hot plate test displayed "complete" tolerance one week later (cf. Sal groups on Test 1 vs Sal groups on Test 2 in Fig. 1). This tolerance cannot reflect a purely pharmacological effect, as animals given as many as nine morphine injections failed to display significant tolerance (cf. Sal-Test groups in Fig. 2). Furthermore, this tolerance cannot reflect a purely behavioral effect, as there is no significant decline in PLLs in saline-injected animals across as many as three hot plate tests (cf. Sal-Test groups across Tests 1–3 in Fig. 1). Thus, while tolerance has been demonstrated to result from exposure to morphine or to the hot plate per se [2,6], these results support the notion that the most profound tolerance is acquired from an interaction of morphine and hot plate testing [1].

The drug-test interaction effect observed here may reflect the acquisition of a classically conditioned hyperalgesic response. Although considerable evidence supports the classical conditioning interpretation of morphine analgesic tolerance [9, 10, 11, 12], cogent evidence also indicates that acquisition of a hyperalgesic response is neither necessary nor sufficient for tolerance development [5,8]. It remains to be determined whether the difference in tolerance produced by pairing morphine with either test room or hot plate test stimuli in the present experiment reflects a difference in acquisition of conditioned hyperalgesia.

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