tral neural mechanisms underlying drug tolerance. In several instances, the behavioral effects of prenatal exposure to alcohol tended to dissipate with age (*15*). The long-lasting effects reported here and previously by our laboratory with regard to learning deficits (*5*) suggest that permanent functional changes in the central nervous system result from in utero exposure to alcohol.

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Morphine-Induced Attenuation of Morphine Tolerance

Abstract. *Rats experienced both morphine and an environmental cue, but the cue always signaled a drug-free period. They were subsequently administered morphine in the presence of the cue, and the development of analgesic tolerance was assessed. The prior experience retarded such tolerance. The finding that a procedure of opiate administration can retard opiate tolerance suggests that an association between cues preceding the drug and the drug itself contributes to tolerance.*

Several investigators have suggested that learning contributes to opiate tolerance, and recent analyses of tolerance emphasize the principles of Pavlovian conditioning (*1*). Pavlov (*2*) suggested that the administration of a drug normally constitutes a conditioning trial. The conditional stimulus (CS) consists of environmental cues uniquely present at the time of drug administration, and the unconditional stimulus (UCS) consists of the systemic effects of the drug. The development of an association between the environmental and pharmacological stimuli is revealed if the subject is administered a placebo. Drug conditional responses (CR's) are often opposite in direction to the unconditional effects of the drug (*1, 3*). These anticipatory responses, antagonistic to the effects of the drug, should attenuate the effect of the drug and may contribute to tolerance.

The conditioning analysis of tolerance is supported by demonstrations that a variety of nonpharmacological manipulations similarly affect CR formation and morphine tolerance (*1*). Thus, if placebo injections are presented either before, interspersed among, or after morphine injections, the acquisition of morphine tolerance is attenuated, much as presentations of the CS before, during, or after paired CS-UCS presentations attenuate CR strength [so-called "latent inhibition," partial reinforcement, and extinction effects, respectively (*4, 5*)]. Such results demonstrate that tolerance can be diminished by presenting without the drug the cues that normally signal the drug. The conditioning account of tolerance suggests an even more dramatic and counterintuitive demonstration of the contribution of learning to tolerance—morphine tolerance should be retarded by the administration of the drug itself in the absence of environmental cues.

Consider the situation in which the analgesic effect of morphine is tested, in subjects experienced with the drug, in the context of a distinctive CS. Subjects who, before the test, receive morphine paired with the CS (paired morphine group, PM) should, on the basis of the conditioning model, display tolerance—that is, the effect of the drug should be partially canceled by the drug-compensatory CR. In contrast, subjects with the same exposure before the test to morphine and the cue, but in an explicitly unpaired manner (explicitly unpaired morphine group, EUM) should be retarded in the subsequent acquisition of tolerance when the distinctive cue is paired with morphine. This prediction is based on evidence from a variety of classical conditioning studies indicating that an explicitly unpaired procedure imbues the CS with inhibitory properties (*6*). Such inhibition is evidenced by retarded learning when the explicitly unpaired cue is subsequently paired with the UCS. If Pavlovian conditioning contributes to morphine tolerance, such tolerance should be subject to inhibitory learning. The results of our experiment demonstrate that an initial series of explicitly unpaired presentations of environmental CS and morphine retard the analgesic tolerance subsequently developed when the CS is arranged to signal the drug.

Throughout the experiment, Wistar-derived rats (90 to 110 days old) were each housed in a translucent cage located in one drawer of a filing cabinet. There was no illumination in the drawers, and a ventilation fan at the rear of each drawer provided 70 dB of background noise. Subjects were placed in the drawers and left undisturbed for 5 days before the first experimental session. The purpose of housing the subjects in this manner was to allow the presentation of a conveniently manipulated environmental CS, which consisted of a 1-hour period during which the filing cabinet drawers were opened, exposing the rats to illumination provided by the overhead room lights, and the ventilation fans were turned off, reducing background noise.

During the initial phase of the experiment, which consisted of 15 daily sessions, five groups of rats differed in their experience with the CS, morphine, or both. Group PM ($N = 12$) received explicit pairings of the CS and morphine: Morphine was injected 15 minutes after the drawers were opened. The drawers then remained open for 45 minutes, after which time the cabinet was closed and the fans turned on (*7*). Group EUM ($N = 12$) received the same experience with the CS and morphine, but the two were explicitly unpaired: morphine was injected 4 hours after each presentation of the CS. These explicitly unpaired morphine injections were given while a dim red light provided the only illumination, and the ventilation fans remained on (the drawer was opened, the animal injected, and the drawer immediately closed). A third group received daily

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exposure to the 60-minute CS but received no drug (CS-alone, $N = 12$). A fourth group received daily injections of morphine but received no exposure to the CS (morphine-alone, $N = 12$). The fifth group received neither the CS nor the drug during this initial phase of the experiment (group N, $N = 11$). All morphine sulfate injections were subcutaneous at a dose of 5 mg per kilogram of body weight (5 mg/ml solution).

The second phase of the experiment was tolerance testing. Each rat's response to nociceptive stimulation was assessed once per day for 3 days after being injected with morphine paired with the environmental CS. For each test session, the ventilation fans were turned off and the drawer was opened. Each rat was injected with morphine 15 minutes later. Forty-five minutes after opiate administration (corresponding to the termination of the cue during phase 1 of the experiment), each rat's analgesic level was assessed, and the drawer was closed. Analgesia was measured with the "hot-plate" procedure (*8*): The rat was placed on a 54°C (± 0.2°C) surface for 60 seconds, and the time elapsing until the rat licked a paw was recorded.

Prior to the tolerance test phase, rats had no experience with the hot-plate testing apparatus; thus, any differences in response latencies between groups during testing cannot be attributed to differential practice. Furthermore, before the test, PM and EUM rats had the same handling experience and the same experience with both morphine and the CS; thus, any differences in the tolerance test performance of these two groups cannot be attributed to nonassociative factors [for example, stress or novelty (*9*)].

All groups displayed increasing analgesic tolerance (shorter response latencies) over the course of the test sessions, but they differed in the rate tolerance developed (Fig. 1). A mixed-design analysis of variance indicated a statistically significant group by sessions interaction [$F(8, 108) = 5.68, P < .001$]. Subsequent analyses of the mean response latency of each group during each of the three tolerance test sessions indicated that, for all three sessions, there was a significant groups effect [$F(4, 54) = 18.86$, 6.27, and 7.02, respectively; all P's $< .001$]. The source of the interaction was assessed with multiple group comparisons for each session (Newman-Keuls' test, two-tailed).

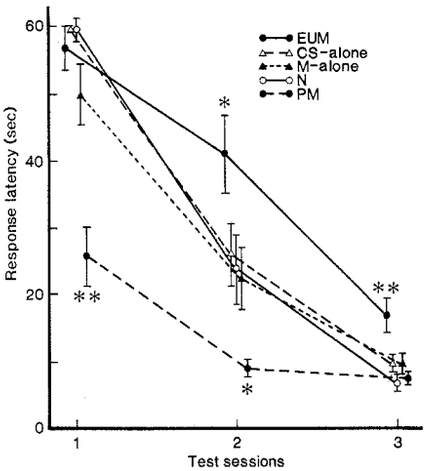

Fig. 1. Mean response latencies (± 1 standard error of the mean) for each of the three tolerance test days (*10*). Differences between means were assessed by the Newman-Keuls' test. $*P < .05$. $**P < .01$. *M*, morphine.

The results of this experiment replicate previous findings (*7*) demonstrating that rats with a history of morphine administration, each injection paired with a distinctive CS, display tolerance when the drug is subsequently administered in conjunction with the CS. Rats in group PM were tolerant to the analgesic effect of morphine from the outset of testing. However, the tolerance displayed by group PM rats cannot be attributed simply to repeated drug stimulation. Rats in group morphine-alone, which before the test had the same morphine experience as rats in group PM, were not tolerant to the analgesic effect of morphine during the first test session. The difference between groups PM and morphine-alone demonstrates the importance of the organism's experience with the drug administration environment, as well as the drug, in the display of tolerance.

An even more dramatic demonstration of the crucial role of the organism's experience with the drug administration environment in determining tolerance is provided by group EUM. Those rats, which had 15 morphine injections before the test, became tolerant more slowly than rats in groups CS-alone or N, both of which had not been exposed to morphine before the test. The retarded acquisition of morphine tolerance produced by the explicitly unpaired procedure suggests that the acquisition of morphine tolerance is subject to inhibitory learning. The finding that tolerance may be retarded by morphine injections is not readily interpretable by theories that emphasize only the neurochemical consequences of repeated drug stimulation, but is consistent with the hypothesis that learning contributes to tolerance.

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