Persistence of Relapse of Morphine-Seeking Behavior in Rats: the Relative Role of Certain Biological Variables*

ARTHUR S. SCHWARTZ** and PATRICIA L. MARCHOK
Division of Neurobiology, Barrow Neurological Institute of St. Joseph's Hospital & Medical Center, Phoenix, Az. 85013, U.S.A.

Abstract. The theory that narcotic-induced protracted biological changes are responsible for relapse of opiate-reinforced behavior was examined in the rat. Groups of rats were conditioned to prefer a distinctive environment by pairing it with morphine doses from 1–200 mg/kg, and were retested for persistence of this preference after a 3-week abstinence period. They were then observed for protracted signs such as sensitivity to naloxone, tolerance to morphine analgesia, hyperaggression, or changes in endocrine activity. Acquisition and relapse of the preference, as well as long-term tolerance, were dose related. None of the purported protracted signs showed any consistent relationship to the tendency to relapse. However, relapse correlated significantly with original acquisition scores in all relapsing groups. The results suggest that original conditioning factors, rather than protracted changes, are responsible for the observed relapse.

Key words: Morphine – Relapse – Protracted tolerance – Dose-response.

INTRODUCTION
The incidence of relapse, or resumption of narcotic-seeking behavior after a period of abstinence, remains one of the most important problems in treatment of addiction, yet there has been little systematic study of its etiology. Current thinking concerning the sources of motivation for relapse may be divided into four categories: 1. “Euphoric” or positive reinforcement effects learned during the addiction phase (Bejerot, 1972; McAuliffe and Gordon, 1974); 2. Negative reinforcement due to escape from abstinence, also learned during the addiction phase (Beach, 1957; Lindesmith, 1968); 3. Long-lasting physiological and behavioral changes induced by previous narcotic administration (Wikler, 1965; Dole, 1972); and 4. Hyperresponsivity to stress in association with the above-mentioned changes (Martin, 1972; Martin et al., 1974).

Most authors agree that more than one of the above factors may influence the tendency to relapse, but differ in their attribution of primacy. For example, the persistent biological and behavioral changes are considered by Dole (1972) to generate a drug drive which leads to relapse, while these changes are considered only adjunctive to narcotic-reinforced conditioned responses by others (Wikler, 1971, 1973; Goldberg, 1970). It would appear most useful from a practical and theoretical point of view to examine the relationship between relapse and certain aspects of the purported long-term biological changes. The literature contains reports that several months after complete abstinence: 1. reactions resembling precipitated withdrawal symptoms may occur after nalorephine injections in monkeys (Goldberg and Schuster, 1969); 2. rats show tolerance to the analgesic effects of morphine (Cochin and Kornetsky, 1964); 3. adrenal glands hypertrophy in rats (Sloan and Eisenman, 1968); 4. rats manifest increased metabolic rates (Martin et al., 1963) and 5. increased aggression (Gianutsos et al., 1974). Because these long-term phenomena are quantifiable, and because a demonstration of their involvement or lack of it in relapse would be important in designing future etiological research, we examined their association with relapse in the rat using the Beach (1957) model of morphine-seeking behavior. Our results support the view that the reinforcement effects of morphine experienced...
during initial conditioning are more important factors in relapse than protracted biological changes.

**METHOD**

**Subjects.** Male albino rats from the Arizona State University Animal Resource Center, derived from the Sprague-Dawley strain, were used for this study. Their body weights ranged from 200–250 g at the start of the experiment, and they were housed singly with food and water ad libitum at 23–25°C, humidity about 40%, and a 12-h artificial light-dark cycle. Number of rats in each group: Saline = 24, 1 mg = 22; 5 mg = 23; 20 mg = 23; 60 mg = 12; 100 = 11; 200 = 12.

**Apparatus.** A Y-maze with two contrasting goal boxes differed in shape, construction materials, and interior design decorations as well as in position. Guillotine doors allowed blocking off either alley or confinement of the rat in either goal box.

Analgesia tests were administered with a hot plate with a 15 cm² aluminum platform which was maintained at 55°C ± 0.5°C. A plexiglass chimney was placed over the rat during the test to prevent escape. For the precipitated abstinence and the aggression tests, the rats were placed in wire-mesh cages 18 x 34 x 18 cm.

**Training Procedure.** After one week of handling and adaptation to the Y-maze, the rats were tested for pre-training goal-box preferences with 16 free-choice trials, all barriers open. They were then made passively dependent on morphine sulfate by gradually increasing, twice-daily injections, i.p., until the reinforcement dose level was reached, over a 7-day period. Control rats received only saline. Training began on the 8th day with a saline injection in their home cages and an immediate forced-run to their preferred goal box. The controls (t = 2.23, 3.64, 3.79; P's < 0.05, < 0.01, < 0.001 respectively). Relapse dropped markedly after training with 100 and 200 mg morphine (Fig. 1); rats injected with these high doses became markedly cataleptic during the training session even at the end of which time they were given 16 free-choice tests again over a period of two days (i.e., relapse tests). No forced choices or injections were presented at this time.

**Tests for Protracted, Altered Biological States.** Protracted dependence, as described by Goldberg and Schuster in the monkey (1969), was examined by administration of a large dose of naloxone (10 mg/ kg i.p.) one day after the relapse tests (i.e., 23 days after the last morphine injection). Body weights and temperatures were recorded immediately before naloxone challenge, and the rats were monitored by two observers (one of whom was unaware of the rats’ drug history) for chattering, abnormal body posture, wet-dog shakes, piaos, and penis licking by noting the presence or absence of each of these signs in each 1-min interval of the 10-min observation period. The number of fecal boli and the presence of diarrhea was also recorded. Twenty minutes after the naloxone, body temperatures were taken again followed 5 h later by recording of body weight. Two days after the naloxone test the rats were examined for long-term tolerance on the hot plate. Thirty minutes after injection of 15 mg/kg morphine, they were dropped through the chimney onto the hot plate and scored for analgesia by noting the latency for paw-licking or escape movements. Tolerance was defined as a decreased analgesic effect (i.e., shorter hot plate latency) by the morphine injection in the drug-trained groups as compared to its effect on the saline-trained (i.e., morphine naive) control group. Two days later the rats were paired randomly and observed for aggressive behavior—i.e., the number of aggressive rears, attacks/bites, and vocalizations for each rat during a 30-min period.

The dependence, tolerance and aggression tests were omitted in the groups trained with 1 and with 100 mg/kg morphine. Two additional groups of rats, trained with saline or with 20 mg/ kg morphine exactly as the above animals, were sacrificed 24 h after relapse testing for measurement of adrenal weights and blood plasma levels of thyroxin and corticosterone. Radioimmunoassay determinations of the hormone levels were performed by Endocrine Sciences Laboratories, Tarzana, California.

Statistical evaluations of the data were carried out by analysis of variance, followed by the Dunnett’s test for multiple comparisons, and by Pearson product-moment correlations and the t-test, two-tailed. P values less than 0.05 were considered statistically significant.

**RESULTS**

**Acquisition and Relapse.** A graphical representation of acquisition and relapse performance as a function of training dose is shown in the upper portion of Figure 1. All the morphine training doses proved to be effective reinforcers as indicated by significant acquisition of morphine-box preferences by each drug-treated group (df = 6, 120; F = 15.45; P < 0.001). Individual comparisons with the saline control group yielded P-values less than 0.001 except for the 1 mg/kg group (P < 0.05). Acquisition was positively dose-related as indicated by a correlation coefficient of 0.468 between these two variables (df = 125, t = 5.92; P < 0.001).

The groups also differed significantly in relapse performance (df = 6, 120; F = 4.49; P < 0.001), but only the 5, 20, and 60 mg groups differed from the controls (t's = 2.23, 3.64, 3.79; P's < 0.05, < 0.01, < 0.001 respectively). Relapse dropped markedly after training with 100 and 200 mg morphine (Fig. 1); rats injected with these high doses became markedly cataleptic during the training session even at the end of the two week period, indicating incomplete tolerance to the narcotic’s depressing effects. Considering only the dose range up to 60 mg, relapse was positively correlated with dose (r = 0.398; df = 102; t = 4.32; P < 0.001). Finally, we asked whether individual acquisition scores were positively correlated with individual relapse scores among the groups showing significant relapse; the result was affirmative (r = 0.28; df = 56; t = 2.12; P < 0.05).

**Protracted Changes.** None of the signs usually associated with acute, precipitated abstinence were significantly increased in the post-addict animals by naloxone, as compared to its effect in the control