Pharmacology Biochemistry and Behavior, Vol. 2, pp. 613–618. ANKHO International Inc., 1974. Printed in the U.S.A.

Morphine-Like Physical Dependence: A Pharmacologic Method for Drug Assessment using the Rat[1]

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(Received 15 April 1974)

PARKER, L. F. AND B. L. RADOW. *Morphine-like physical dependence: a pharmacologic method for drug assessment using the rat.* PHARMAC. BIOCHEM. BEHAV. 2(5) 613–618, 1974. – Rats maintained on various dosage regimens of morphine showed dose related taste aversions to a saccharin solution offered to them upon withdrawal from the drug. Maximal saccharin aversions occurred between 72 and 96 hr after termination of morphine injections, and gradually returned to baseline preference levels after 14 days of morphine abstinence. The results were interpreted as suggesting that the morphine treated animals associated the aversive components of the morphine withdrawal syndrome with saccharin consumption, and manifested conditioned aversions to the saccharin solution which extinguished as the withdrawal syndrome subsided. The findings were further discussed with regard to the potential use of conditioned taste aversions in determining whether test compounds are capable of causing physical dependence in rats.

Physical dependence Morphine withdrawal Conditioned taste aversions

THE sociopathology associated with human drug abuse has stimulated an intense effort to develop laboratory techniques for the assessment of drugs in infrahuman species. Of primary interest has been the capacity of drugs to cause physical dependence; a biological adaptation to the presence of a drug such that it must be maintained at adequate levels in the body to deter gross homeostatic imbalances [2, 3, 28].

Since drug dependent animals are typically difficult to distinguish from normal animals, physical drug dependence is usually detected by closely observing drug abstinent animals for symptoms that may serve as indices of an "abstinence syndrome" [1, 2, 16]. The morphine abstinence syndrome in the rat, for example, has been variously characterized by hyperexcitability [1, 4, 8, 9, 21, 31, 32], sedation [12,17], increased fluid consumption [16], decreased fluid consumption [13], loss of body weight [1,16], and a host of other physiological or behavioral abnormalities. Although the reported indices of an abstinent syndrome are not always consistent, the duration and severity of such abstinence signs have been found to be reliable reflections of the extent to which physical dependence to a drug had developed [1, 2, 3, 15].

It has recently been established that if the rat is made ill within a matter of hours after consuming an unfamiliar substance, a conditioned aversion to that substance will subsequently be manifested. Furthermore, the magnitudes of such aversions have been found to be proportional to the severity of the postingestional illness [6, 23, 24, 26]. Several investigators have employed this associate ability of the rat as a means of behaviorally assessing noxious drug effects [5, 14, 19], and it has been suggested that such conditioned taste aversions might be utilized as a standardized technique for determining drug toxicity [5,19] and physical dependence capacity [22].

The latter was indicated by the finding that rats addicted to morphine showed aversions to a palatable solution offered to them during a bout of morphine abstinence [22]. Since abstinence from a compound that is capable of causing physical dependence is characteristically aversive [20], it was reasoned that determining the severity of an abstinence syndrome might be accomplished by offering drug treated rats an unfamiliar substance to consume at the onset of drug abstinence [22]. If the severity of an abstinence syndrome does reflect the extent to which physical drug dependence has developed [1, 2, 3], the magnitude of

[1] Supported in part by National Science Foundation Grant GB-8035, the University of Washington Alcoholism and Drug Abuse Institute, and PHS 5T01 GM 00666. The authors wish to thank Suzanne Clark for her assistance, and Lawrence Halpern and Moncrieff Smith for their helpful comments. L. Parker is a predoctoral fellow in the Physiology-Psychology Group.

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conditioned aversions shown to a substance consumed during abstinence should provide a measure of the degree to which the drug causes physical dependence [6, 22, 23].

Thus, the purpose of the present investigation was to determine whether rats maintained on a drug capable of causing physical dependence would show aversions to a test solution consumed during drug abstinence. Of major interest was the time course and magnitude characteristics of such aversions. Because the degree to which physical dependence develops to morphine is a dose related phenomenon [1, 3, 15, 16, 30], and the morphine abstinence syndrome in the rat has been studied extensively, morphine was employed in examining the relationship between physical drug dependence and preferences for a test solution consumed during drug abstinence.

METHOD

Animals

Sixty female albino rats of the Wistar strain were used. The experimentally naive animals were obtained from the colony maintained by the Department of Psychology at the University of Washington where they were raised in large colony cages (12 rats/cage). At the initiation of the experiment the animals were housed in individual stainless steel cages with ad lib Purina laboratory chow. The animals were approximately 120 days old and weighed between 265 and 320 g.

Initial Preference Test

Upon being individually housed all animals were given a 5 day preference test between water and a sweet 0.23% (w/v) Na saccharin solution. Two fluid bottles, one containing water and the other containing the saccharin solution, were fastened to the front of each cage such that their respective drinking spouts projected into the cages adjacent to each other. Daily consumption from each fluid bottle was recorded, afterwhich the left–right positions of the bottles were switched. Following the fifth daily recording only the water bottles were placed on the cages, and the animals were matched by their 5-day mean preference scores such that five groups (n = 12/group) showed approximately equal preferences for the saccharin solution (mean of 92% ± 1.5 of total fluid consumed as saccharin).

Drug Treatments

The morphine "staircase" drug regimens were designed after Nichols *et al.* [20]. Four of the 5 groups were randomly assigned to morphine maintenance dosages of either 20, 40, 80, or 160 mg/kg; and the remaining group served to control for the effects of the injection procedures. These dosages were employed because they represent the lower range of dosages normally used in investigations of the morphine abstinence syndrome in the rat. The dosage regimens for the 4 drug groups consisted of daily intraperitoneal injections of morphine sulfate (50 mg/ml) beginning at a dose of 20 mg/kg the day after the initial preference test; followed by daily dosage increases of either 0, 1, 3, or 7 mg/kg thereafter for 20 consecutive days. The group dosages of 20, 40, 80, and 160 mg/kg, attained on Day 21 of the injections, were maintained for an additional 7 days. The injection control group received daily intraperitoneal injections of isotonic saline in volumes equivalent to those administered to the 160 mg/kg drug group. Fluid consumption and body weights were recorded daily prior to the injections at 1900 hr.

Post-drug Preference Test

Twenty-four hr after their last injection on Day 28, all animals were again given access to the saccharin solution and a second two-bottle preference test between saccharin and water was carried out for 2 weeks. It should be noted that the drug treated animals had been habituated to daily cycles in systemic drug levels and, presumably, to daily cycles of morphine withdrawal [13]. The preference test was therefore initiated at the end of their last cycle (at the time of their usual injection) to pair the consumption of saccharin with the onset of the unfamiliar portion of the morphine withdrawal syndrome.

All animals had continuous access to saccharin, water, and food during the test. Every 12 hr their consumption of saccharin and water was recorded and the left–right positions of the two fluid bottles were switched. Consumption recordings were made under blind conditions, in that the experimenters had no knowledge of animal group assignments.

RESULTS

Prior to the daily injections, the drug treated animals could not be differentiated from control animals by observation of their homecage behavior, although animals in the 80 mg/kg and 160 mg/kg morphine groups showed signs of lacrimation and rhinorrhea [28,33]. Immediately following the injections, however, all animals injected with morphine manifested marked disturbances of ongoing behavior and remained still and rigid upon being returned to their home cages. As previously described [10,13], the animals remained fixed in a crouched position for about 1–2 hr after the injections. Immobility and rigidity were eventually supplanted by an excitatory phase which consisted of voracious food and water consumption and hyperactivity which continued for another 3–4 hr. During the early stages of the excitatory phase their consummatory behavior was stereotypic in nature. That is, the animals appeared to be compelled to gnaw on whatever was available and, if their food was removed, they would vigorously gnaw on their cages, on their own forepaws, or on paper towels if they were provided. After about one-half hr their consummatory behavior became more directed towards food and water.

Periodic inspections of collecting trays beneath their food containers indicated that, while the control animals consumed the bulk of their daily food during the night cycle from 2200 to 0800 hr, the morphine treated rats consumed the bulk of their daily food intake within 5 hr after the injections (before 0000 hr) and refrained from eating for the remainder of the night cycle.

Changes in body weight for the various groups during the drug treatments are shown in Fig. 1. As depicted, all of the morphine groups showed decrements in growth weight gains over the 4 week period. Previous investigators have reported similar weight losses after administering morphine intraperitoneally [13, 15, 16, 21, 30], whereas others have found that administering morphine to rats orally [29] or subcutaneously [1] resulted in no decrements in body weight gains. That intraperitoneal doses of morphine have been

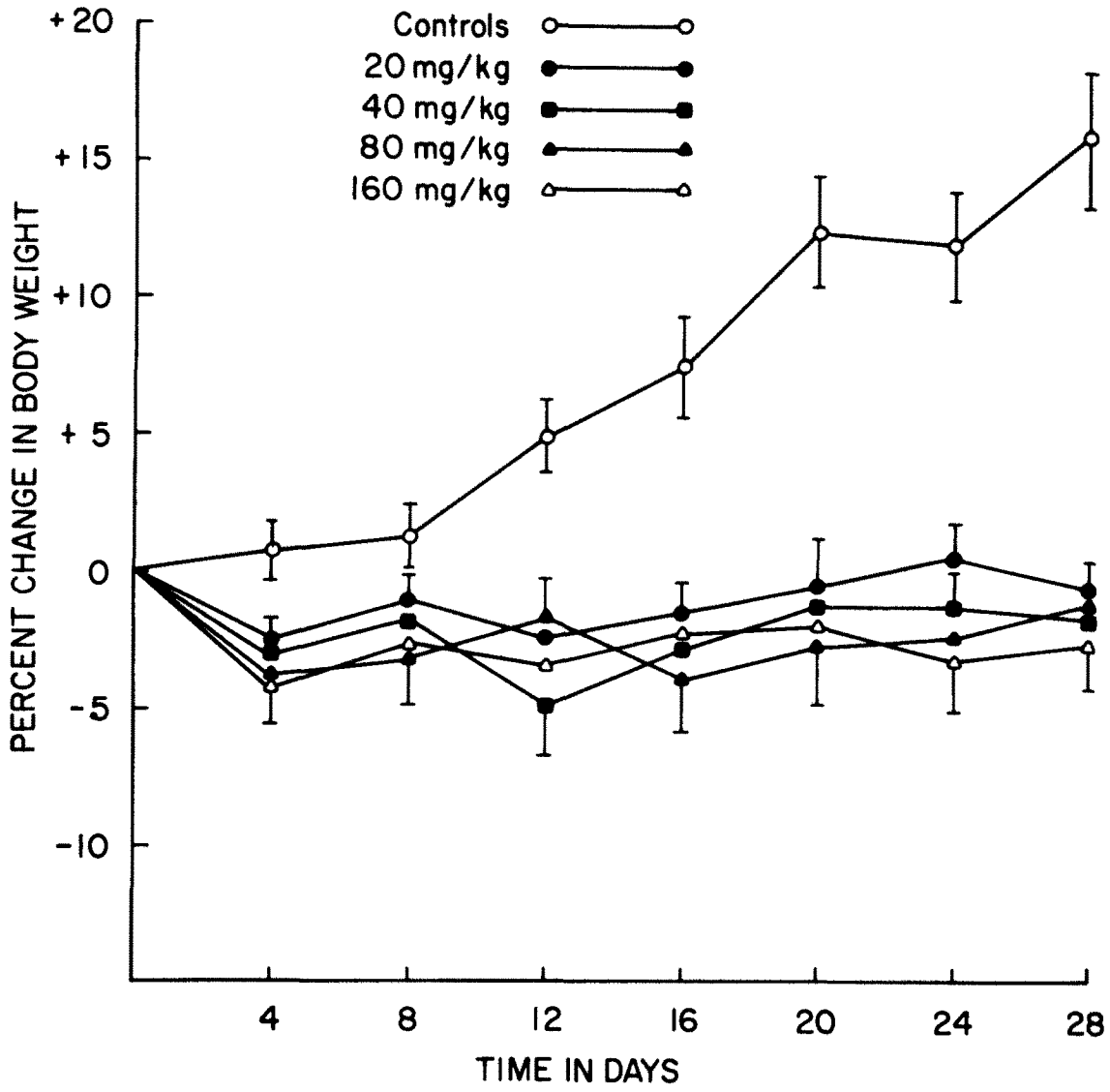

FIG. 1. Changes from initial body weights of the various groups during the drug treatment portion of the experiment. Vertical bars indicate standard errors of the means.

found to disrupt normal diurnal feeding patterns in rats both herein and elsewhere [13], while no such disruption of feeding was reported to occur after oral or subcutaneous doses of morphine [1,29], suggests that rapid increases in systemic morphine levels may cause disruption of food intake regulation and, hence, cause rats that are receiving morphine intraperitoneally to lose body weight.

The effects of morphine abstinence on fluid consumption during the second preference test between saccharin and water are shown in the upper portion of Fig. 2. An analysis of variance applied to the group consumption scores during the test revealed that there were significant differences in total fluid consumption among the 5 groups ($F = 14.65$). Subsequent pair-wise comparisons with Fisher's Significant Difference test indicated that animals in the 3 highest morphine dose groups consumed significantly less fluid than the control group (40 mg/kg vs Controls; $t = 3.12$; 80 mg/kg vs Controls; $t = 4.12$; 160 mg/kg vs Controls; $t = 5.16$), whereas the 20 mg/kg morphine group did not differ significantly from the control group ($t = 1.13$). As indicated in Fig. 2, the lesser fluid consumption shown by the 40 mg/kg, 80 mg/kg, and 160 mg/kg morphine groups was not a reduction in fluid intake below that consumed prior to the preference test, but rather a failure to manifest the typical polydipsic response to saccharin [7,11] as shown by the 20 mg/kg morphine and control groups, Inspection of the lower portion of Fig. 2, which shows the saccharin preference scores of the 5 groups during abstinence, suggests that their failure to show a polydipsic response to saccharin was due to the development of aversions to the saccharin solution as morphine abstinence proceeded. That is, the 20 mg/kg morphine and control groups consumed large quantities of saccharin during the preference test and their total daily fluid intake much surpassed their intake prior to the test. The 40 mg/kg, 80 mg/kg, and 160 mg/kg morphine groups, on the other hand,

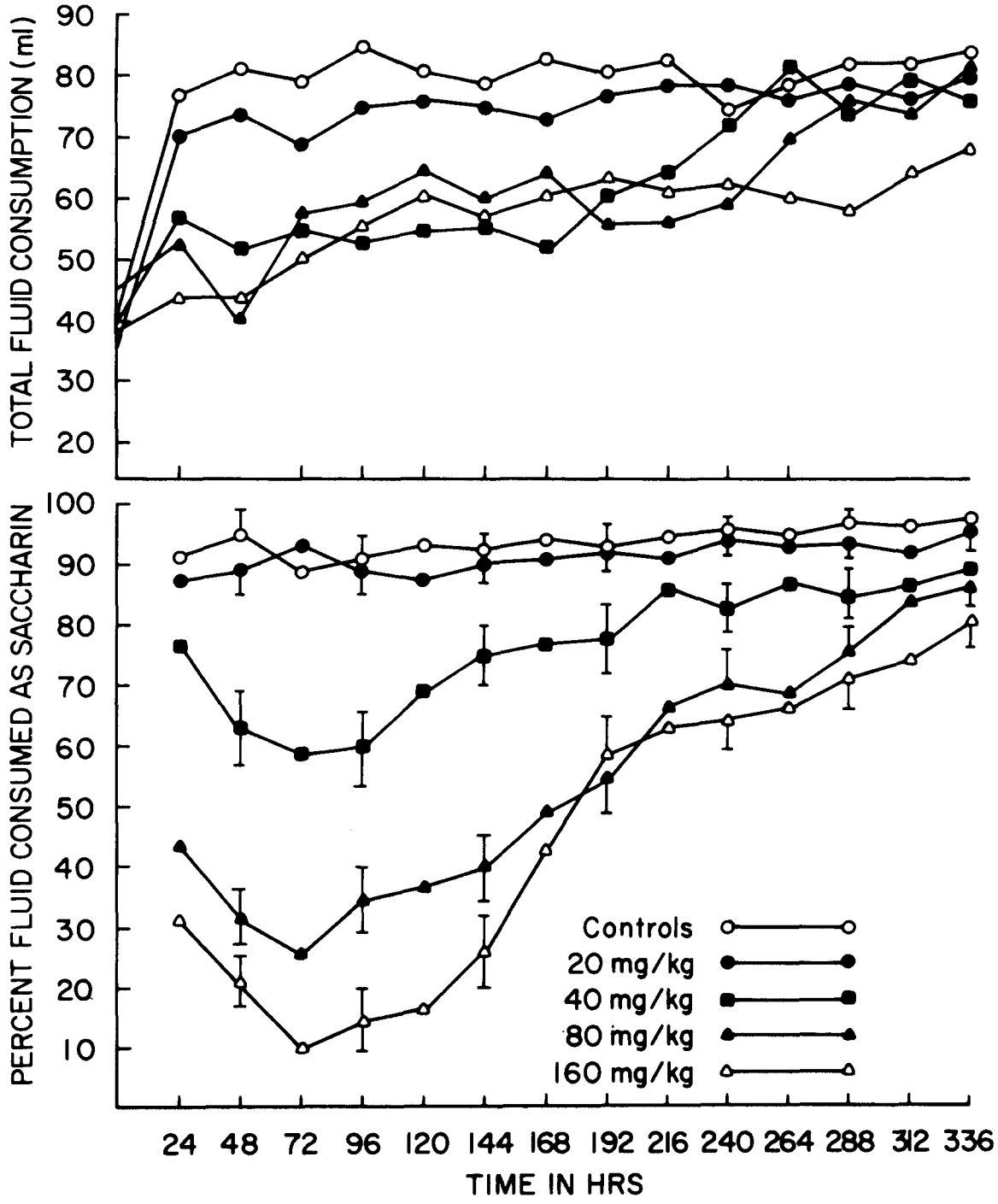

FIG. 2. Total daily fluid consumption (upper panel) and daily saccharin preferences of the various groups during the first two weeks of morphine abstinence in the saccharin vs water preference test. Scores were computed from the sums of two 12 hr consumption readings. Vertical bars indicate standard errors of the means.

showed aversions to the saccharin solution and their total daily fluid consumption did not much exceed that shown prior to the preference test until their aversions to saccharin began to dissipate.

As depicted in the lower portion of Fig. 2, the 40 mg/kg, 80 mg/kg, and 160 mg/kg morphine groups maximally avoided the saccharin solution after approximately 96 hr of morphine abstinence (72 hr into the preference test); afterwhich their saccharin avoidance eventually dissipated and approached the control group's saccharin preference. That the severity of the morphine abstinence syndrome in the rat has been shown to be most severe between 48 and 72 hr of morphine abstinence and to slowly subside thereafter [1, 16, 21, 30] suggests the animals maintained on 40 mg/kg,

80 mg/kg, and 160 mg/kg morphine associated the consumption of the saccharin solution with the onset of severe withdrawal symptoms and, hence, manifested conditioned aversions to saccharin that extinguished as the abstinence syndroms subsided (i.e., after 72 hr) [22].

Such an interpretation also encompasses the finding that the degree to which the morphine treated rats avoided saccharin was a monotonic function of their morphine maintenance dosages (see Fig. 2). That is, the magnitude of conditioned taste aversions in the rat have been found to increase with the severity of postingestional illness [6, 23, 24, 25], and the severity of the morphine abstinence syndrome in the rat has been found to increase with the morphine maintenance dosages administered prior to abstinence [1, 3, 15, 16, 30]. The apparent correlation between the development of the morphine abstinence syndrome and the saccharin aversions shown by the 40 mg/kg, 80 mg/kg, and 160 mg/kg morphine groups, and the finding that the magnitude of the aversions was dose-dependent, converge to support the notion that the animals in this study showed aversions to the saccharin solution because they associated its consumption with the onset of severe withdrawal symptoms. Accordingly, the failure of the 20 mg/kg morphine group to show an aversion to the saccharin solution during abstinence was due to their abstinence syndrome being too mild to produce such conditioning.

GENERAL DISCUSSION

In agreement with previous findings [22], rats given 40 mg/kg, 80 mg/kg, and 160 mg/kg morphine daily showed aversions to a relatively unfamiliar saccharin solution offered to them at the onset of abstinence from morphine. Furthermore, the development and magnitude of their aversions to saccharin corresponded closely with the reported parameters of development and magnitude of the morphine abstinence syndrome. That is, the animals showed their strongest saccharin avoidance after 96 hr of morphine abstinence, during the period that maximal withdrawal symptoms are reported to occur [1, 16, 21, 30], and their aversions diminished as the abstinence syndrome subsequently subsided. The overall magnitudes of their aversions to the saccharin solution were found to be a monotonic function of their preceding morphine maintenance dosages and, as the severity of the morphine abstinence syndrome is a function of the maintenance dosage regimen [1, 3, 5, 16, 30], the strength of their saccharin aversions were positively correlated to the presumed strength of their respective abstinence syndromes.

Since the rat can readily associate the consumption of an unfamiliar substance with the development of subsequent illnesses, even over delays of several hours, and will show conditioned aversions to such substances that are proportional in magnitude to the severity of postingestional illness [6, 23, 24, 26], it is suggested that animals maintained on morphine in this study may have associated the consumption of the relatively unfamiliar saccharin solution (i.e., less familiar than water or lab chow) with the contiguous development of their morphine abstinence syndromes and formed conditioned taste aversions to saccharin that extinguished as their abstinence symptoms subsided. Such an interpretation not only accounts for the animals in the 40 mg/kg, 80 mg/kg, and 160 mg/kg morphine groups showing aversions to the highly palatable saccharin solution, but moreover it encompasses the magnitude and time course characteristics of their aversions to saccharin. It should be noted, however, that their avoidance behavior may not have been directed specifically at saccharin. That is, wild rats manifest a strong reluctance to approach new food sources after poisoning [25,27], and recent evidence indicates that laboratory rats also show a "neophobic" avoidance of unfamiliar food sources [18]. Since the saccharin solution was relatively unfamiliar to the morphine treated animals (i.e., less familiar than water or lab chow), their saccharin avoidance during abstinence may have been due in part, at least, to a generalized neophobic avoidance of unfamiliar foods during a bout of illness. The relative roles of specific avoidance conditioning and such a generalized neophobic avoidance in determining the morphine treated animals' avoidance behavior cannot, unfortunately, be delineated.

That the strength of the aversions shown by the morphine treated rats developed in close correspondence with the severity of the morphine abstinence syndrome indicates that such aversions, whether due to conditioning or not, might be well utilized as a relatively sensitive laboratory method for determining a drug's capacity to cause physical dependence. At present, the only conclusive evidence for a drug's physical dependence capacity is the appearance of physiological and behavioral abnormalities during drug abstinence. The nature and strength of such indices of an abstinence syndrome have been found to vary considerably with the compound being tested, the dosage regimens employed, and the species used for the test [2,28]. Since the rat will manifest flavor aversions after experiencing any of a wide variety of experimentally induced physiological disturbances [5, 6, 14, 19, 23, 24, 26], the appearance of flavor aversions during drug abstinence should be fairly independent of a drug's specific spectrum of pharmacological activity, and a general index of physical drug dependence in the rat may be available. The dose related aversions shown by the animals in this study further indicate that such aversions might provide a relatively sensitive quantitative measure of a compound's capacity to cause physical dependence.

The general utility of such taste aversions in determining physical dependence capacity remains to be demonstrated, however. The findings reported herein show that the rat's preference for highly palatable saccharin solution during morphine abstinence is dependent upon the dosage of morphine on which the animal was previously maintained, and correlated with the development and subsequent subsidence of the morphine abstinence syndrome.

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