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# Conditioned Flavor Aversions for Assessing Precipitated Morphine Abstinence in Rats[1]

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PILCHER, C. W. T. AND I. P. STOLERMAN. *Conditioned flavor aversions for assessing precipitated morphine abstinence in rats.* PHARMAC. BIOCHEM. BEHAV. 4(2) 159–163, 1976. – Rats receiving twice-daily morphine injections acquired aversions to a saccharin solution which had been presented for 1 hr immediately prior to injections of naloxone. The degree of aversion was related to the maintenance dosage of morphine. Rats maintained on a regimen of daily saline injections did not show significant aversion to saccharin paired with naloxone, even at doses as high as 40 mg/kg. The sensitivity of the technique was such that significant aversions could be demonstrated in rats receiving doses of morphine as low as 1 mg/kg twice daily. It is suggested that conditioned flavor aversions provide a useful method for assessing the aversive quality of abstinence precipitated from low doses of morphine.

Morphine dependence Naloxone Conditioned taste aversion

DEPENDENCE on opioid drugs is often accompanied by a complex set of symptoms called the narcotic abstinence syndrome. Both the natural and the antagonist-precipitated abstinence syndromes have been much studied, at least in part because escape from, or avoidance of, the abstinence syndrome may reinforce opioid self-administration behavior [12,23]. The present experiments have been carried out to examine a possible method for assessing directly the aversive property of precipitated abstinence in rats repeatedly treated with morphine.

Recently, evidence has been reviewed that rats reduce their intake of a distinctly-flavored but innocuous solution, if its consumption on earlier occasions was followed by radiation or poison-induced "illness" [16,17]. The radiation or poison was, therefore, defined empirically as an aversive stimulus, since it reduced the frequency of behavior occurring shortly before its presentation. Parker and Radow [14] have shown that rats reduced their intake of a flavored solution when it was presented during natural withdrawal from moderately large doses of morphine. An aversive stimulus property of the narcotic antagonist naloxone has been demonstrated in morphine dependent monkeys [7]. Conditioned flavor aversions have now been used to examine this property further and to determine whether abstinence could be precipitated from low doses of morphine in rats. A preliminary account of this work has appeared previously [15].

## GENERAL METHOD

One hundred and twenty experimentally naive, male hooded rats were used. They were of a randomly bred strain maintained in the animal laboratory of the Department of Psychology. Their ages were 96–136 days and their weights 214–393 g, but within each experiment the control and experimental groups were matched for age and weight. The animals were housed individually and adapted to a 12 hr light-dark cycle, the dark phase of which began at 13.00 hr. Distilled water was made available from 13.00–14.00 hr every day from calibrated tubes. No fluid was available at other times but food was always obtainable. Drug injections began after this deprivation regime had been in effect for 8 days, and continued for the duration of the experiment.

Morphine hydrochloride (May and Baker) and naloxone hydrochloride (Endo Laboratories) were dissolved in physiological saline and injected in volumes of 1 ml/kg. The route of administration was intraperitoneal throughout. Morphine was given twice daily to experimental groups (09.30 and 17.30 hr) and saline to control rats. The procedures for aversive conditioning were modified from earlier work with psychoactive drugs [3]. The first pairing of saccharin with naloxone took place on the eleventh day of injections. A solution of saccharin or sodium saccharin in distilled water was presented instead of distilled water;

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immediately after the 1 hr drinking session, the rats were weighed and injected with either a dose of naloxone or saline. After a further 1 hr period, the rats were reweighed and defecation during that preceding 1 hr was assessed by counting the number of feces deposited. On the two days following the saccharin presentation, distilled water was given as before. On the next, and on every third day thereafter until the end of the experiment, a saccharin solution was presented and the procedures of the eleventh day were repeated. Details of the procedures, and doses of drugs are given in the relevant parts of the text.

The results were analysed statistically by two-factor analyses of variance with repeated measures, one-way analyses of variance and by *t*-tests [24].

## EXPERIMENT 1

### *Method*

Sixteen rats were assigned to 2 groups by a randomisation method (n = 8). The experimental group received morphine (10 mg/kg) twice daily and the controls saline only. On the eleventh day of injections, saccharin solution (0.1% w/v) was presented for 1 hr. Immediately after removal of the drinking tubes, the rats were weighed and injected with naloxone (10 mg/kg). One hour later, the rats were reweighed and defecation was assessed. This procedure was repeated every third day until saccharin had been presented on 4 occasions.

### *Results*

The mean intakes of saccharin solution on successive presentations are shown in Fig. 1. In the control rats receiving saline daily, 3 pairings of naloxone with saccharin solution failed to reduce the mean intake of saccharin to less than its initial value. In marked contrast, a single pairing of naloxone with saccharin in the experimental rats reduced the mean intake of saccharin from 11.0 ml to 3.0 ml. After 2 and 3 pairings, saccharin intake was barely detectable in most of the experimental rats, since it averaged less than 1 ml. The overall intake of water was slightly but significantly higher for the experimental rats on the days between saccharin presentations, $F(1,14) = 5.23$, $p<0.05$, possibly as compensation for the reduced fluid intake when only saccharin was available.

For 3–10 min following naloxone the rats were observed in their home cages. The experimental animals were seen to adopt unusual postures, lying with the hind legs outstretched, and to exhibit ptosis and abdominal writhing. These changes, which were not observed in control rats, are all typical signs of mild, precipitated abstinence [1,2]. One hour after naloxone, the experimental rats had excreted quantities of unusually soft feces. The mean loss of weight for the experimental rats during the hour after the first injection of naloxone was 7.3 g as compared with 2.0 g for the controls ($t = 6.25$; $df = 14$; $p<0.001$).

## EXPERIMENT 2

### *Method*

In this experiment, the dose of morphine was reduced to 5 mg/kg and additional control groups were included to make possible direct comparisons of saccharin-saline pairings with saccharin-naloxone pairings. Sodium saccharin

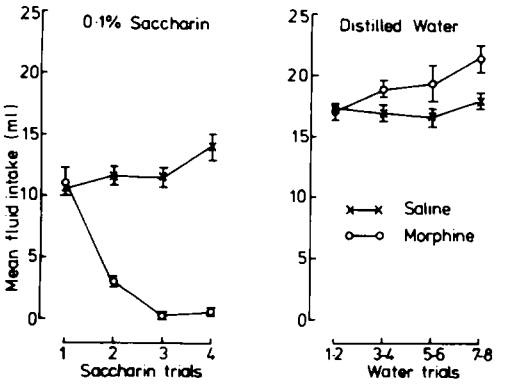


FIG. 1. Aversion to saccharin after saccharin-naloxone pairings in morphine-treated rats (n = 8). Prior to naloxone administration, the intake of saccharin solution or distilled water was essentially unchanged by morphine (10 mg/kg IP twice daily) given to the experimental rats. Naloxone (10 mg/kg IP) was administered to all rats immediately after each saccharin presentation.

was used instead of saccharin in this and subsequent experiments. Twenty-four rats were allocated to 4 groups by a random method (n = 6). Two groups received morphine (5.0 mg/kg twice daily) and 2 groups received saline. On the eleventh day of injections, a solution of sodium saccharin (0.1% w/v) was presented to all rats. At the end of the 1 hr drinking session, naloxone (10 mg/kg) was administered to 1 of the groups receiving morphine twice daily and to 1 of the saline groups. The remaining 2 groups received saline. This procedure was repeated every third day until saccharin had been presented on 5 occasions, except for one variation; after the third presentation of saccharin, naloxone (10 mg/kg) was administered to the group which was receiving morphine twice daily and which had received saline after the first 2 presentations of saccharin. This was the only occasion on which that group received naloxone.

### *Results*

The control rats receiving saline only progressively increased their mean intake of saccharin (Fig. 2). The markedly greater intake of flavored solution in this experiment, as compared with that in Experiment 1, may be due to the use of the sodium salt of saccharin.

When naloxone was administered after the first presentation of saccharin to rats receiving morphine daily, the mean intake of saccharin fell from 22.0 to 9.0 ml ($t = 8.28$; $df = 5$; $p<0.001$). This suppressed intake remained substantially below that for the controls across all 5 presentations of saccharin, and was associated with a significant overall difference between the groups, $F(3,20) = 17.1$, $p<0.001$. The results with the remaining two groups showed that neither morphine alone, nor naloxone alone, was sufficient to suppress saccharin intake. However, a single administration of naloxone to morphine-treated rats immediately after the third presentation of saccharin reduced intake upon the fourth presentation of the flavor ($t = 4.71$; $df = 5$; $p<0.05$). The reduced aversion when naloxone was administered after the third presentation of saccharin only, may be an example of the latent inhibition occurring when an illness is paired with a flavor to which the rats have been familiarised by prior, unpunished, presentations [16].

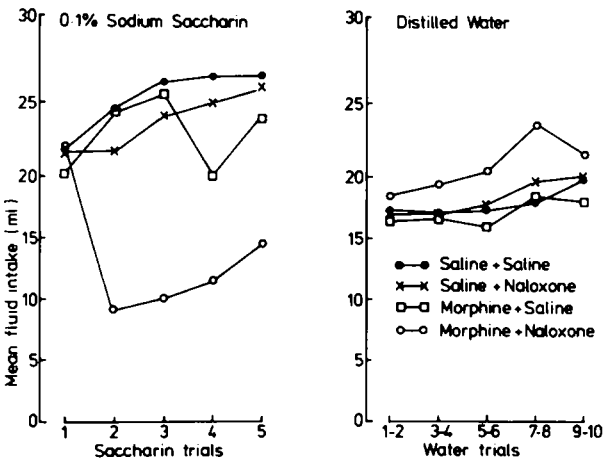


FIG. 2. Aversions to saccharin after saccharin-naloxone pairings in morphine-treated rats (n = 6). Naloxone (10 mg/kg) was administered immediately after saccharin to rats receiving morphine (5 mg/kg) or saline twice daily. A single administration of naloxone after the third saccharin presentation reduced the subsequent saccharin intake in morphine treated rats (saccharin trial 4).

The intake of distilled water on the days preceding and between saccharin presentations is also shown in Fig. 2. Differences between the groups were small but significant, $F(3,20) = 5.13$, $p<0.01$. This overall difference was associated with the increased intake of distilled water by the rats receiving morphine twice daily and naloxone after each saccharin presentation, $F(1,20) = 8.55$, $p<0.01$. The remaining groups did not differ significantly from the group receiving saline only, $F(1,20)<1$, in each case.

Observations of the rats in their home cages after the administration of naloxone revealed unusual posturing in animals also receiving morphine twice daily. Morphine-treated rats receiving naloxone after the first presentation of saccharin lost an average of 6.7 g weight during the following hour, as compared with 1.8 g for the saline controls which had not received morphine ($t = 3.21$; $df = 20$; $p<0.01$). Weight loss in the rats receiving naloxone but not morphine averaged 0.5 g, which was not significantly different from the control value. The mean number of feces was also increased from 2.3 to 5.6 after administering naloxone but in the morphine treated rats only ($t = 5.29$; $df = 20$; $p<0.001$).

## EXPERIMENT 3

### *Method*

In this experiment the dose of naloxone was held constant and the dose of morphine varied. Twenty-eight rats were assigned to 4 groups by a randomisation method (n = 7). The groups were injected twice daily with either saline or a dose of morphine (0.25, 1.0 or 4.0 mg/kg). On the eleventh day of injections, sodium saccharin solution (0.1% w/v) was presented instead of distilled water and immediately after the 1 hr drinking session, naloxone (10 mg/kg) was administered to all rats. This procedure was repeated every third day until saccharin had been presented 4 times. Since it was not possible to house 28 rats at the same time, the experiment was run in 2 parts; all treatments were represented equally in the first part and in the replication, which yielded similar results.

### *Results*

Figure 3 shows that throughout the experiment, the mean intake of saccharin was similar for the groups receiving saline or the lowest dose of morphine (0.25 mg/kg). Rats receiving morphine at a dose of 1.0 mg/kg showed a lower mean intake of saccharin upon its fourth presentation, but this did not differ significantly from the intake of the saline controls ($t = 2.03$; $df = 23$; $p<0.1$). However, there was a very clear and progressive reduction in saccharin intake of rats receiving the highest dose of morphine (4.0 mg/kg). This was associated with a significant overall difference between the groups, $F(3,24) = 29.62$, $p<0.001$, and a significant groups x trials interaction $F(9,70) = 4.27$, $p<0.001$. There were no significant differences between the groups with respect to water intake on the days preceding or between the presentations of saccharin, $F(3,24)< 1$.

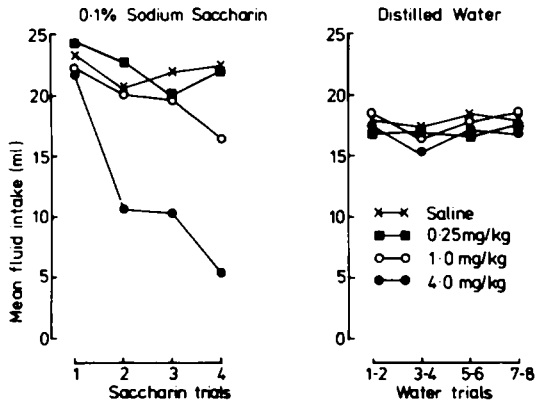


FIG. 3. Aversion to saccharin after saccharin-naloxone pairings in morphine-treated rats (n = 7). The doses of morphine shown were given twice daily. Naloxone (10 mg/kg) was administered to all rats.

Following naloxone, unusual posturing was observed in some of the rats receiving the highest dose of morphine (4.0 mg/kg). These changes were also seen in 2 of the 7 rats receiving morphine at a dose of 1.0 mg/kg. Rats receiving morphine at the highest dose lost an average of 8.1 g weight 1 hr after the first administration of naloxone, which was significantly greater than the loss of 3.7 g for the controls ($t = 3.24$; $df = 24$; $p<0.01$). Rats receiving the smaller amounts of morphine did not differ significantly from the controls with respect to weight loss.

## EXPERIMENT 4

### *Method*

Experiment 3 suggested that the reliability of the method for detecting conditioned aversions was at threshold when morphine was given in a dose of 1.0 mg/kg twice daily. Since the dose of naloxone needed to precipitate abstinence signs increases as the degree of dependence is reduced [22], naloxone was administered in larger doses in an attempt to develop reliable aversions to saccharin in rats receiving morphine at the low dose of 1.0 mg/kg.

Twenty-seven rats were assigned to 4 groups by a randomization method (n = 6–7). Rats in all groups were given morphine (1.0 mg/kg) twice daily throughout the experiment, and sodium saccharin solution (0.1% w/v) was

presented on the eleventh day of injections. After the 1 hr drinking session, the rats were injected with saline or a dose naloxone (10, 20 or 40 mg/kg). This procedure was repeated every third day until the end of the experiment, which was run in 2 parts. All treatments were represented in both parts. Some rats receiving naloxone (10 mg/kg) were common to Experiments 3 and 4 and are, therefore, included in both sets of data.

*Results*

Figure 4 shows that the rats receiving naloxone after drinking saccharin solution reduced their intake relative to the rats receiving saline. The overall difference between the mean intakes of saccharin was statistically reliable, $F(3,23) = 13.7$, $p<0.001$ and remained so when each dose of naloxone was compared separately to saline, $F(1,23) = 26.3$, 10.0 and 36.7, $p<0.01$, in each case. There was no clear relationship between the dose of naloxone and the reduction in intake of saccharin, although the changes were marginally greater at the highest dose, 40 mg/kg. The groups did not differ with respect to water intake on the days intervening between presentations of saccharin, $F(3,23)<1$.

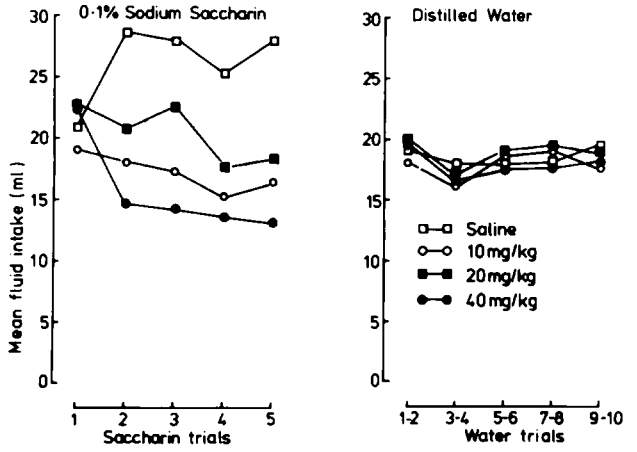


FIG. 4. Aversion to saccharin after saccharin-naloxone pairings in rats treated with morphine. All animals received morphine at 1.0 mg/kg twice daily (n = 6–7). The doses of naloxone shown were administered after each presentation of saccharin.

Observations of the rats after naloxone failed to reveal any obvious changes in their gross behavior. The saline controls lost an average weight of 3.6 g during the hour after the first saccharin presentation, as compared with 2.9, 3.8 and 4.4 g for the groups receiving naloxone in doses of 10, 20 and 40 mg/kg respectively, $F(3,23)<1$. However there was a distinct change in defecation after naloxone; the feces remained firm, but were smaller and increased in number, from an average of 1.2 for the controls, to 2.8, 3.0 and 2.4 after the 3 doses of naloxone, $F(3,23) = 3.55$, $p<0.05$.

In an additional experiment, the same doses of naloxone were compared with saline in rats not receiving morphine; there were no significant differences between the groups (n = 6) with respect to their intakes of saccharin or distilled water. After 3 pairings of naloxone with saccharin, the mean saccharin intakes were 22.8, 23.0 and 19.2 ml after 10, 20 and 40 mg/kg naloxone respectively, as compared with 24.0 ml after 3 saccharin-saline pairings, $F(3,20) = 1.38$.

## DISCUSSION

Pairing the presentation of saccharin solutions with doses of naloxone reduced the subsequent consumption of saccharin by rats treated chronically with low doses of morphine. Naloxone was therefore considered to serve as an aversive stimulus, since it suppressed a discriminative behavioral response occurring shortly before its administration. Evidence suggestive of the ability of naloxone to serve as an aversive stimulus has been reported previously [25]; response-contingent naloxone produced a prolonged suppression of bar-pressing behavior in rhesus monkeys with a history of morphine self-administration. The present results extend the finding with a quite different method for assessing the aversive properties of stimuli. It is also known that monkeys with a history of morphine self- and programmed administration can learn to press keys to delay or terminate infusions of naloxone, thus showing naloxone to serve as an aversive stimulus through negative reinforcement [7]. Naloxone was not an aversive stimulus for monkeys without a history of morphine administration [8].

Naloxone suppressed saccharin intake after saccharin-naloxone pairings in rats receiving doses of morphine ranging from 1.0 to 10.0 mg/kg twice daily. The suppression appeared specific to the saccharin stimulus since the intake of distilled water on the days between saccharin presentations was either unchanged (Experiments 3 and 4) or even tended to increase (Experiments 1 and 2) but generalization to other novel stimuli was not examined. The results appear analogous to those of experiments in which the intake of a saccharin or otherwise flavored solution was suppressed by pairings with illness induced by radiation or toxins [16]. Under the conditions of the present experiments, there was virtually no evidence that saccharin intake was suppressed in rats receiving the same doses of naloxone, but no morphine. Parker and Radow [14] have found that the intake of saccharin solution is reduced when it is presented during natural abstinence from moderately large (40–160 mg/kg) doses of morphine, a finding interpreted as either a conditioned aversion or an unconditioned change in saccharin intake. Rats have been shown not to avoid entering a visually distinctive area previously paired with natural abstinence [10]. The positive results with flavor aversion techniques may suggest a greater "preparedness" [19] of rats to associate the abstinent state with gustatory than with visual cues.

The assessments of weight loss and gross behavior after naloxone help to relate the conditioned aversions to the precipitated abstinence syndrome. In rats pretreated with large doses of morphine, narcotic antagonists can induce weight loss, diarrhea, jumping, writhing, abnormal posturing, tooth chattering and other signs [1,2]. In our experiments, weight loss, abnormal posturing and diarrhea were easily detectable in rats receiving morphine at 10 mg/kg twice daily. As the dose of morphine was progressively reduced in Experiments 2, 3 and 4, the losses in weight and the gross signs became smaller or less frequent, and could not be detected consistently. The size and clarity of the conditioned aversions approximately paralleled the losses in weight and gross signs. At a morphine dose of 1.0

mg/kg, weight loss was not significant, abnormal posturing occurred in only a small proportion of rats, and there was a just detectable increase in the number of feces after naloxone. However, highly significant aversions were conditioned to saccharin with doses of morphine as low as 1.0 mg/kg (Experiment 4); this seems to represent the approximate limit to the sensitivity of the present technique.

At one time it was throught necessary to administer large doses of morphine to produce abstinence signs in rats [18]. With the traditional methods for assessing abstinence, experimenters have frequently administered doses of 80–400 mg/kg/day in order to produce measurable, reliable signs [11, 13, 21]. However, it has recently been reported that, using such methods, morphine abstinence is detectable in rats receiving morphine at 10 mg/kg for 3 days [6]. The aversions conditioned to saccharin indicate that precipitated abstinence can be detected at doses around the minimum frequently used to assess the acute effects of morphine in nontolerant rats (e.g. [5, 9, 20]).

In conclusion, it has been suggested previously that escape from or avoidance of the opioid abstinence syndrome may reinforce opioid self-administration behavior [12,23]. A necessary step in the experimental verification of this hypothesis involves demonstrating that abstinence can control behavior through punishment or negative reinforcement. The experiments on conditioned flavor aversions confirm and extend earlier reports of an aversive property of naloxone, apparently mediated through precipitated morphine abstinence.

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