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# Temporal Analysis of Naloxone Attenuation of Morphine-Induced Taste Aversion[1,2]

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VAN DER KOOY, D. AND A. G. PHILLIPS. *Temporal analysis of naloxone attenuation of morphine-induced taste aversion.* PHARMAC. BIOCHEM. BEHAV. 6(6) 637–641, 1977. – In a dose-response study, 7.5 mg/kg of naloxone produced maximal attenuation of conditioned taste aversion to saccharin induced by 10 mg/kg of morphine. Naloxone was administered immediately after the morphine in this study. In a second experiment, naloxone still caused a significant attenuation of taste aversions when administered with a 1 hr delay after morphine, but not after delays of 4 or 8 hr. These results suggest that behavioral consequences of morphine which peak during the first hr after injection (analgesia, catalepsy, and depression of intracranial self-stimulation) are not correlated with the aversive effect of morphine. Nor can the aversiveness of morphine be attributed to withdrawal effects. Only the facilitative actions of morphine occurring 1 to 4 hr after injection, including the facilitation of intracranial self-stimulation, are temporally correlated with the naloxone-sensitive aversive effect. Thus, a temporal analysis cannot be used to dissociate the paradoxical positive reinforcement and aversive effects of morphine. Rather, the temporal correlation between the two opposite motivational effects of morphine serves to emphasize the nature of this paradox.

Morphine Naloxone Conditioned taste aversion Reinforcement

MORPHINE appears to have both positive reinforcing and aversive effects in rats. Rats self-administer morphine intravenously [8,21] and also show a long term facilitation of intracranial self-stimulation [12,13] after injections of the drug. In contrast, the administration of morphine at similar doses, immediately following the consumption of a novel saccharin solution, has been shown to produce a conditioned taste aversion [5,6]. Recently, the paradoxical reinforcing effects of apomorphine have been demonstrated within a single paradigm [22]. In this elegant experiment rats learned to avoid the taste of saccharin which was associated with self-administered intravenous injections of apomorphine.

Many of morphine's behavioral effects may be separated temporally. At low doses morphine's cataleptic effect [2], analgesic effect [2,20], and depressive effect on intracranial self-stimulation [12] all peak within the first hour after injection. Between the first hour and the fourth hour post-injection, the peak facilitative effects on self-stimulation [12] and on locomotor activity [1,7] occur. Withdrawal effects occur some time after morphine's direct facilitative effect on locomotor activity [14,18]. Therefore, the seemingly paradoxical positive and negative reinforcing properties of morphine may in fact represent biphasic effects of the drug which occur at different times after administration.

Naloxone, a pure morphine antagonist [15], has previously been shown to attenuate morphine induced taste aversions [11] when given shortly after morphine injections. In this present study naloxone was administered at various time intervals after morphine in a taste aversion paradigm in order to differentially antagonize the various behavioral effects of morphine. By allowing specific behavioral effects to occur before blocking subsequent action of the drug, it was hoped that we could identify the property of morphine critical for induction of taste aversion.

## METHOD

### *Animals*

One hundred-ten male Wistar rats, each weighing approximately 300 g at the beginning of the experiment, were used. All were housed individually with free access to food and water prior to the introduction of experimental treatment.

### *Procedure*

*Conditioning paradigm.* Experimental groups consisted of 10 rats in which each animal was maintained on a schedule of limited access to water for at least 12 days prior to conditioning tirals. At the same time each day, access to

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water in a 50 ml drinking tube was permitted for a 15 min period in the home cage. Food was continuously available. Conditioning trials began 24 hr after the last day of adaptation to the restricted schedule of drinking. Each animal was permitted access to a 0.1% solution of sodium saccharin for 15 min. Not more than 1 min later each animal received its first drug injection. There were 6 conditioning trials, during which only the saccharin solution was available, spaced at intervals of 72 hr. Six conditioning trials were employed because the aversions induced by morphine increase over repeated saccharin-morphine pairings [5,11]. Each animal received the same drug treatment on each trial, except the final one on which no drug injections were given following the drinking period. On the 2 days intervening between conditioning trials, the animals continued to have access to water for a 15 min period.

*Selection and preparation of drug doses.* A dose of 10 mg/kg of morphine sulfate was chosen to induce taste aversions in the present experiment, because this dose has been used most frequently in behavioral tests. All drug injections were given intraperitoneally. Drug concentrations were adjusted to provide an injection volume of 0.2 ml/100 g. Drug solutions were prepared in a vehicle of psychological saline.

The maximally effective dose of naloxone hydrochloride for attenuating morphine-induced taste aversions was chosen as follows. Three different experimental groups received naloxone injections in doses of 5 mg/kg, 7.5 mg/kg, and 10 mg/kg. A fourth group received only the morphine injections. Conditioning trials were conducted as described above, and naloxone treatment was given within 30 sec of the morphine injections. The aversiveness of naloxone itself is a limiting factor in the attenuation of morphine-induced taste aversions [11]. In order to assess the aversive action of naloxone alone, 3 more groups received injections of the same doses of naloxone (5 mg/kg, 7.5 mg/kg, and 10 mg/kg), without previous morphine injections on the conditioning trials. The saccharin intakes of these 3 groups were compared to a fourth saline (vehicle control) group which also received no morphine injections.

*Temporal analysis of naloxone effect.* Having chosen an appropriate dose of naloxone to attenuate morphine-induced taste aversions, the major part of the experiment involved delaying the naloxone injections in order to antagonize specific time related effects of morphine. Three final experimental groups were given standard taste aversion conditioning trials and naloxone (7.5 mg/kg) was administered either 1 hr postmorphine (after the occurrence of the peak depressive effects), 4 hr postmorphine (after the occurrence of the peak facilitative effects), or 8 hr postmorphine (after the possible occurrence of some acute withdrawal effects). These three groups were compared with two of the groups described above, which received only the morphine injections in one case and morphine injections followed immediately by naloxone (7.5 mg/kg) injections in the other case.

## RESULTS

### *Appropriate Dose of Naloxone*

The effect of different doses of naloxone on taste aversions induced by 10 mg/kg of morphine are shown in Fig. 1. There was a significant effect of trials, $F(5,36) = 7.30$, $p<0.01$, as all groups showed aversions to saccharin which increased over successive trials. The main effect of naxolone dose was not significant; however, there was a significant naloxone dose × trials interaction, $F(3,36) = 2.89$, $p<0.05$. Tests of simple main effects indicated significant differences of naloxone dose only on Trial 4, $F(3,36) = 3.89$, $p<0.05$, and Trial 5, $F(3,36) = 3.11$, $p<0.05$. Newman-Keuls a posteriori test with a criterion of $p<0.10$ required for rejection of the null hypothesis, revealed that the 7.5 mg/kg naloxone group drank significantly more saccharin than the other three groups on Trials 4 and 5. Thus, only the 7.5 mg/kg dose of naloxone showed attenuation of the morphine taste aversions.

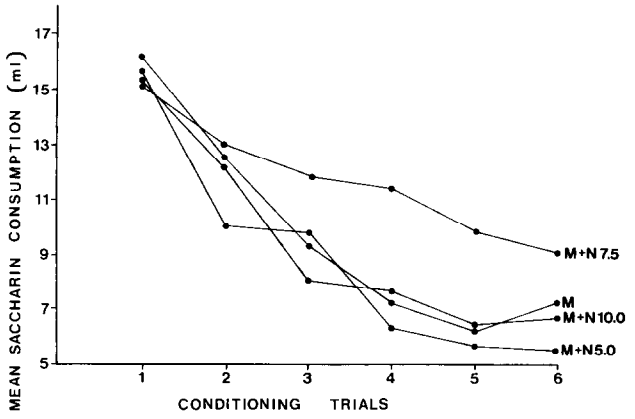


FIG. 1. Effects of various doses of naloxone on conditioned aversions induced by 10 mg/kg of morphine. M = morphine; N = naloxone. Numbers immediately following N refer to dose of naloxone (mg/kg) used. The standard errors of the mean for the data points in Figs. 1, 2, and 3 are shown in Table 1.

Taste aversions induced by the same doses of naloxone, but in this case not preceded by morphine injections, are shown in Fig. 2. The statistical analysis showed significant effects of trials, $F(5,36) = 13.18$, $p<0.01$, and naloxone dose, $F(3,36) = 3.50$, $p<0.05$, and a significant trials × naloxone dose interaction, $F(3,36) = 3.48$, $p<0.05$. Significant simple main effects were seen on Trials 3, 4, 5, and 6 ($p<0.05$). Newman-Keuls a posteriori tests revealed that the group receiving 10 mg/kg of naloxone drank significantly less saccharin than the saline control group on all of the final 4 trials and less than the 5 and 7.5 mg/kg groups on Trial 5. The group receiving 7.5 mg/kg of naloxone drank less than the saline group on Trial 3. Both the 7.5 mg/kg and 5 mg/kg naloxone groups drank less than the saline group on Trials 4 and 6. Thus, naloxone by itself induces dose dependent taste aversions, although the 5 mg/kg and 7.5 mg/kg groups do not appear substantially different from each other.

### *Temporal Analysis of Naloxone-Induced Attenuation*

Figure 3 shows the major result of the present experiment, the attenuation of morphine-induced taste aversions by naloxone after various delay periods. All 5 groups showed some degree of aversion, with the main effect of trials being significant, $F(5,45) = 79.31$, $p<0.01$. The main effect of groups was not significant, however, there was a significant groups × trials interaction, $F(4,45) = 2.63$, $p<0.05$. There were significant simple main effects on Trial 3, $F(4,45) = 2.19$, $p<0.10$, Trial 4, $F(4,455) = 2.67$, $p<0.05$, and Trial 5, $F(4,45) = 2.40$, $p<0.10$. Newman-

TABLE 1

GROUP MEAN AND STANDARD ERROR OF THE MEAN FOR EACH DATA POINT ILLUSTRATED IN FIG. 1(A); FIG. 2(B); FIG. 3(C)

| A. (Fig. 1) | Morphine | Morphine + naloxone (5.0 mg/kg) | Morphine + naloxone (7.5 mg/kg) | Morphine + naloxone (10 mg/kg) |
|---|---|---|---|---|
| Trial | | | | |
| 1 | 16.2 ± 1.2 | 15.7 ± 0.5 | 15.3 ± 1.0 | 15.6 ± 0.8 |
| 2 | 12.5 ± 1.0 | 12.1 ± 1.3 | 12.9 ± 0.9 | 10.1 ± 1.1 |
| 3 | 9.2 ± 1.0 | 8.0 ± 0.8 | 11.7 ± 1.2 | 9.8 ± 1.6 |
| 4 | 7.1 ± 1.5 | 7.6 ± 0.9 | 11.3 ± 0.9 | 6.3 ± 0.8 |
| 5 | 6.2 ± 1.3 | 6.2 ± 0.9 | 9.7 ± 1.0 | 5.2 ± 1.0 |
| 6 | 7.1 ± 1.6 | 6.6 ± 1.1 | 8.9 ± 0.8 | 5.1 ± 0.6 |

| B. (Fig.2) | Saline | Naloxone (5.0 mg/kg) | Naloxone (7.5 mg/kg) | Naloxone (10.0 mg/kg) |
|---|---|---|---|---|
| Trial | | | | |
| 1 | 13.9 ± 1.2 | 14.4 ± 1.3 | 14.3 ± 0.6 | 15.8 ± 1.0 |
| 2 | 15.8 ± 1.0 | 15.2 ± 0.9 | 12.9 ± 0.8 | 12.7 ± 1.0 |
| 3 | 15.6 ± 1.0 | 14.9 ± 1.3 | 12.7 ± 1.3 | 11.0 ± 0.9 |
| 4 | 16.0 ± 1.7 | 11.5 ± 1.0 | 13.0 ± 1.3 | 9.2 ± 1.2 |
| 5 | 13.8 ± 0.8 | 11.7 ± 1.4 | 13.0 ± 1.0 | 7.4 ± 0.9 |
| 6 | 13.4 ± 1.2 | 10.4 ± 1.2 | 10.8 ± 0.9 | 8.7 ± 1.2 |

| C. (Fig. 3) | Morphine | Morphine + naloxone (0 hr delay) | Morphine + naloxone (1 hr delay) | Morphine + naloxone (4 hr delay) | Morphine + naloxone (8 hr delay) |
|---|---|---|---|---|---|
| Trial | | | | | |
| 1 | 16.2 ± 1.2 | 15.3 ± 1.0 | 16.0 ± 0.6 | 15.5 ± 1.4 | 15.9 ± 1.2 |
| 2 | 12.5 ± 1.0 | 12.9 ± 0.9 | 13.1 ± 1.0 | 10.7 ± 1.2 | 10.7 ± 1.2 |
| 3 | 9.2 ± 1.0 | 11.7 ± 1.2 | 12.4 ± 1.4 | 8.9 ± 1.4 | 8.7 ± 1.0 |
| 4 | 7.1 ± 1.5 | 11.3 ± 0.9 | 12.2 ± 1.7 | 8.7 ± 1.6 | 8.1 ± 0.8 |
| 5 | 6.2 ± 1.3 | 9.7 ± 1.0 | 7.6 ± 1.5 | 5.4 ± 1.4 | 7.4 ± 1.1 |
| 6 | 7.1 ± 1.6 | 8.9 ± 0.8 | 7.4 ± 1.9 | 6.6 ± 1.8 | 6.1 ± 1.3 |

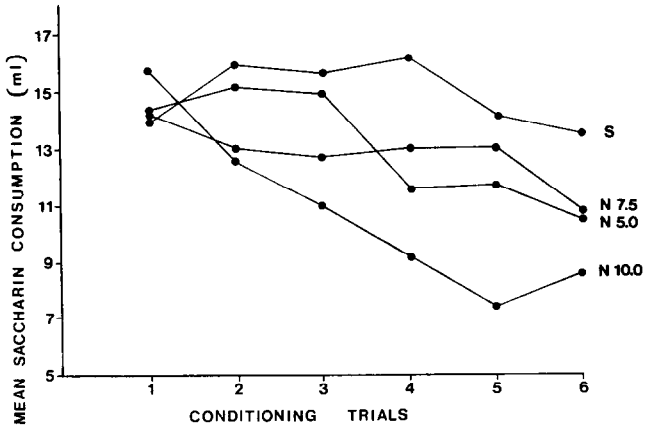


FIG. 2. Taste aversions induced by injections of naloxone alone. N = naloxone; S = saline. Numbers immediately following N refer to dose of naloxone (mg/kg) used.

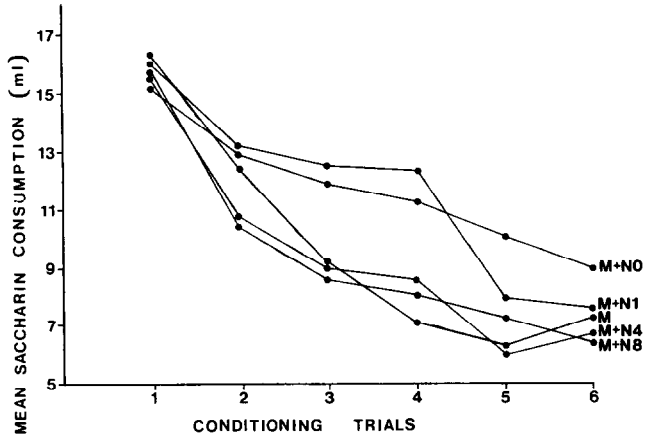


FIG. 3. Effects of naloxone (7.5 mg/kg) after various delays on morphine-induced (10 mg/kg) taste aversions. M = morphine; N = naloxone. Numbers immediately following N refer to temporal delay (hr) of naloxone injections after morphine injections on conditioning trials. M + NO signifies naloxone injections given with no delay after morphine injections.

Keuls tests (with a criterion of $p<0.10$) indicated that delaying naloxone injections by 4 or 8 hr resulted in aversions similar to those induced by morphine alone. Delaying naloxone by 1 hr significantly attenuates the aversion compared to the morphine alone, and naloxone 4 and 8 hr delay groups on both Trials 3 and 4. Injecting naloxone with no delay at all attenuates the aversion significantly on Trial 4 compared to the morphine alone and the 2 longest delay groups and on Trial 5 compared to the morphine alone and 4 hr delay group. These results suggest that delaying naloxone injections by 1 hr can still attenuate morphine-induced taste aversions. The attenuation is partial and resembles that seen when naloxone is given immediately after the morphine injections, although there is a slightly different time course for the antagonism in each case.

## DISCUSSION

In the present experiment, taste aversion induced by 10 mg/kg of morphine was attenuated only when followed immediately by 7.5 mg/kg of naloxone and not by the 5 or 10 mg/kg doses. A 10 mg/kg dose of naloxone by itself induced a taste aversion of a comparable magnitude to that induced by 10 mg/kg of morphine. Therefore, any antagonism of the morphine effect by the 10 mg/kg dose of naloxone was probably masked by the aversive effect of naloxone alone at that dose. Further attempts to attenuate the taste aversion induced by this dose of morphine with still higher doses of naloxone would most likely be ineffective. Le Blanc and Cappell [11] have reported a partial attenuation of the taste aversions induced by 20 mg/kg of morphine using a 9.6 mg/kg dose of naloxone. They suggest that different combinations of doses of naloxone and morphine might have yielded more complete antagonism of the taste aversions. However, in the present experiment several doses of naloxone were used but only a partial attenuation of aversions induced by 10 mg/kg of morphine was observed. Although more complete pharmacological antagonism of the morphine punishment effect may be masked by the punishing effect of naloxone itself as suggested above, another possibility must be considered. It is conceivable that naloxone cannot completely antagonize morphine-induced taste aversions in any situation, and that a portion of the punishing effect of morphine is mediated through a naloxone-insensitive mechanism. Further evidence supporting this possibility is provided by the relatively high dose (7.5 mg/kg) of naloxone necessary to achieve even partial attenuation of the aversion. Naloxone often blocks morphine's effects on locomotion [16] and analgesia [23] at doses of 2 mg/kg or less and the lack of attenuation seen with a 5 mg/kg dose in this study emphasizes the relative insensitivity of morphine-induced taste aversion to naloxone antagonism.

Interestingly, naloxone (7.5 mg/kg) still produced a significant attenuation of morphine-induced taste aversion, even when delayed 1 hr after the morphine injection. Slight differences were noted in the time course of the naloxone effect, as the attenuation produced by the 1 hr delay had waned by the fifth conditioning trial, a time at which attenuation was still present in the no delay group. Possibly the waning of the attenuation seen during the later trials in the 1 hr delay group can be explained by the development of tolerance. Tolerance occurs to the initial depressive effects of morphine over a series of 10 mg/kg morphine injections in rats. Following this, the facilitatory effects on intracranial self-stimulation [12] and locomotor activity 91] appear with a decreased latency after injections. Thus, over the latter conditioning trials in the present experiment, some of the facilitatory effects of morphine (which seem to be correlated with the aversive effect) may be occurring in the first hr after injection and therefore escape antagonism by naloxone given with a 1 hr delay.

In contrast to the inhibition of morphine-induced conditioned taste aversion after a 1 hr delay, no antagonistic effects were observed after delays of 4 and 8 hr. The effectiveness of naloxone may thus be attributed to antagonism of an aversive effect of morphine that occurs between 1 and 4 hr after injection. The peak facilitative effects of morphine on locomotor arousal [1,7] and intracranial self-stimulation [12] also occur between 1 and 4 hr after injection. The delayed onset of the peak facilitation of self-stimulation is even observed at anatomical placements where there is little or no early inhibition of self-stimulation behavior by morphine [4]. Therefore, the present experiment, rather than unravelling the paradoxical reinforcing effects of some psychoactive drugs [22], seems to provide strong confirmation of this paradox by demonstrating a temporal correlation between morphine's aversive effect and its facilitatory effect on intracranial reinforcement.

Although the paradoxical reinforcing effects of morphine have not been resolved by this experiment we can assume that the depressive behavioral effects of morphine seen during the first hr after injection are probably not involved in the aversive effect. Thus, none of the physiological events underlying the cataleptic effect [2], the analgesia effect [2,20], or the inhibitory effect on self-stimulation [12] appear to mediate the naloxone-sensitive aversive action of morphine. The depressive effect on intracranial self-stimulation might have seemed a particularly likely behavioral correlate of the taste aversions induced by morphine. However, the negative correlation observed in the present experiment is not unexpected in light of a recent study demonstrating that the depressive effect of morphine on self-stimulation is response dependent and primarily a performance rather than a motivational decrement (van der Kooy, Schiff and Steele, unpublished observations).

Finally we must consider the possible contribution of withdrawal stress to the phenomenon in question as taste aversions have been produced in morphine-dependent rats by pairing a novel taste with the onset of naloxone-induced withdrawal stress [17,19]. However, this interpretation appears unlikely, as morphine withdrawal occurs some time after the peak facilitative agonist effects of the drug [14,18], which are correlated with its aversive action. Moreover, the peak facilitative effects of morphine cannot be interpreted as initial signs of withdrawal as the two behavioral actions can be disassociated on the basis of tolerance. Tolerance to the facilitatory effect of small doses of morphine on intracranial self-stimulation can be demonstrated in rats [9]. In contrast, repeated administrations of morphine in low doses serve to intensify the development of physical dependence and withdrawal stress [3]. In addition, if withdrawal mediates the aversive effect of morphine, then intensifying the withdrawal by giving naloxone 1, 4 or 8 hr after morphine should have increased the avoidance of saccharin compared to the group receiving morphine alone. This was not the case in the present

experiment. Furthermore, behavioral withdrawal has not been unequivocally demonstrated after amphetamine treatments [10] and yet this drug possesses paradoxical reinforcing effects [22] similar to morphine.

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