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The Differential Effects of Naloxone Hydrochloride on the Acquisition and Maintenance of Schedule-Induced Polydipsia[1]

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RILEY, A. L. AND C. L. WETHERINGTON. *The differential effects of naloxone hydrochloride on the acquisition and maintenance of schedule-induced polydipsia.* PHARMACOL BIOCHEM BEHAV 26(4) 677–681, 1987.—Rats injected with the opiate antagonist, naloxone hydrochloride (10 mg/kg), 15 min prior to sessions in which they were given free food on a fixed time 75-sec schedule, displayed retarded acquisition of schedule-induced polydipsia relative to vehicle-injected subjects. Rats injected with naloxone after schedule-induced polydipsia had been acquired were unaffected, i.e., they continued to drink at control levels. Given that schedule-induced polydipsia has been considered non-opioid in nature, because of previous reports of its insensitivity to naloxone, the present report of differential effects of naloxone on the acquisition and maintenance of schedule-induced polydipsia suggests that some modification of this conclusion is necessary. Possible alternative mechanisms for these differential effects are discussed.

Schedule-induced polydipsia Naloxone Acquisition and maintenance Opiates and drinking

ALTHOUGH the opiate antagonist, naloxone hydrochloride, has been reported to suppress drinking under a wide variety of conditions (for reviews, see [7–9]), it fails to suppress drinking induced by the spaced delivery of food, i.e., schedule-induced polydipsia [1, 6, 20]. For example, in the initial report of the effects of naloxone on schedule-induced polydipsia (SIP) Brown and Holtzman [1] demonstrated that although doses as low as 0.1 mg/kg suppressed drinking in water-deprived rats, 10 mg/kg failed to affect either water intake or licking induced by scheduled food, i.e., SIP (see [1, 3, 6] for similar findings with cyclazocine, diprenorphine, naltrexone and pentazocine).

This failure of naloxone to suppress SIP has been interpreted to indicate that schedule-induced drinking is non-opioid in nature [1, 10, 20]. Although this conclusion follows directly from the ineffectiveness of naloxone on SIP, it should be noted that in each of the aforementioned reports the effects of naloxone were examined on SIP once it was well established. This point is important in view of research demonstrating that developing SIP and established SIP are differentially affected by a number of manipulations, with developing SIP generally more sensitive to disruption (see [4, 11–13, 22]; see also [15]). Yoburn and Glusman [22], for example, have demonstrated that a dose of amphetamine (1 mg/kg) ineffective in suppressing SIP at asymptote significantly suppressed its acquisition relative to vehicle-injected subjects. Because of such differential effects on developing and established SIP, in the following experiment the effects of naloxone on SIP were examined both during its acquisition and at its asymptote.

METHOD

Subjects

The subjects were 8 experimentally naive, female rats of Long-Evans descent approximately 120 days of age at the

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beginning of the experiment. They were individually housed in wire-mesh cages and maintained on a 12-hr-light/12-hr-dark cycle (lights on at 0800 hr) and at an ambient temperature of 23°C for the duration of the experiment. All subjects were maintained at 85% free-feeding weights by food restriction. Water was continuously available.

Apparatus

Four identical chambers (26.5×19.2×16.0 cm) were used throughout the experiment. The sides and the ceiling of each chamber were made of 0.6-cm clear Plexiglas, and the grid floor was constructed of 0.4-cm-diameter stainless steel rods spaced 2 cm apart. A 1×3 cm food hopper was centered on the front wall 3 cm above the grid floor. A graduated Nalgene drinking tube located outside the chamber was positioned such that the Girton metal drinking spout was flush with the outer wall 3 cm above the grid floor and 7 cm to the left of the food hopper. Licks were detected by a drinkometer (Lafayette Model 55008). A continuously illuminated 28-V houselight was centered on the front wall of each chamber 13.5 cm above the grid floor. All schedule events were programmed on and all lick responses were recorded by a TRS-80 Model III microcomputer interfaced to the chambers via an Alpha Interfacer 80. For a detailed description of both the hardware and software used in the conduct of this research, see [14].

Procedure

Phase I: Acquisition. On Day 1 of this phase, subjects were divided into two groups matched on body weight (n=4 per group). Subjects in Group *NW* were given an intraperitoneal (IP) injection of naloxone hydrochloride (10 mg/kg). Subjects in Group *WN* were given an equivolume injection of the distilled water vehicle. Fifteen min later, subjects in both groups were placed into the experimental chambers during which time they were presented with a single 45-mg Noyes pellet once every 75 sec on a fixed time (FT) 75-sec schedule until a total of 60 pellets had been delivered. Water was continuously available during the session via the graduated Nalgene tubes. At the termination of the session, total water intake for each rat was recorded and lick data were stored to disk. If necessary, the animals were supplementally fed Purina Rat Chow in the home cages to maintain their body weights at 85% of initial baseline values. This phase lasted 14 consecutive days.

Phase II: Maintenance. During this phase, all of the conditions described above were in effect except that the injection procedures were reversed for the two groups of animals, that is, subjects in Group *WN* were injected with naloxone (10 mg/kg) 15 min prior to being placed into the experimental chambers, while subjects in Group *NW* were injected with the distilled water vehicle. This procedure was maintained for 6 consecutive days. For each group, the first letter refers to the injection, water (W) or naloxone (N), given during acquisition and the second letter refers to the injection, water (W) or naloxone (N), given at asymptote.

RESULTS

All statistical comparisons were based on Mann-Whitney tests, $p<0.05$, two tailed.

Phase I: Acquisition

The left panel of Fig. 1 presents absolute water consumption for individual subjects in Group WN (top) and Group NW (bottom) over the 14 days of Phase I. During the first free-food session, all subjects in Group WN consumed water (range: 6.5 to 9.5 ml). Over days, consumption gradually increased for all subjects. By Day 14, consumption ranged from 19.5 to 33 ml. Not a single subject in Group NW consumed water on the first day of free-food presentations. Although Subjects 1 and 3 did not drink more than 0.5 ml on any specific day of this phase, Subjects 2 and 4 gradually increased water consumption over days, reaching levels of 16 and 6 ml, respectively, by Day 14. These between-group differences in water consumption were supported statistically in that Group WN drank significantly more water than Group NW during acquisition (U=0).

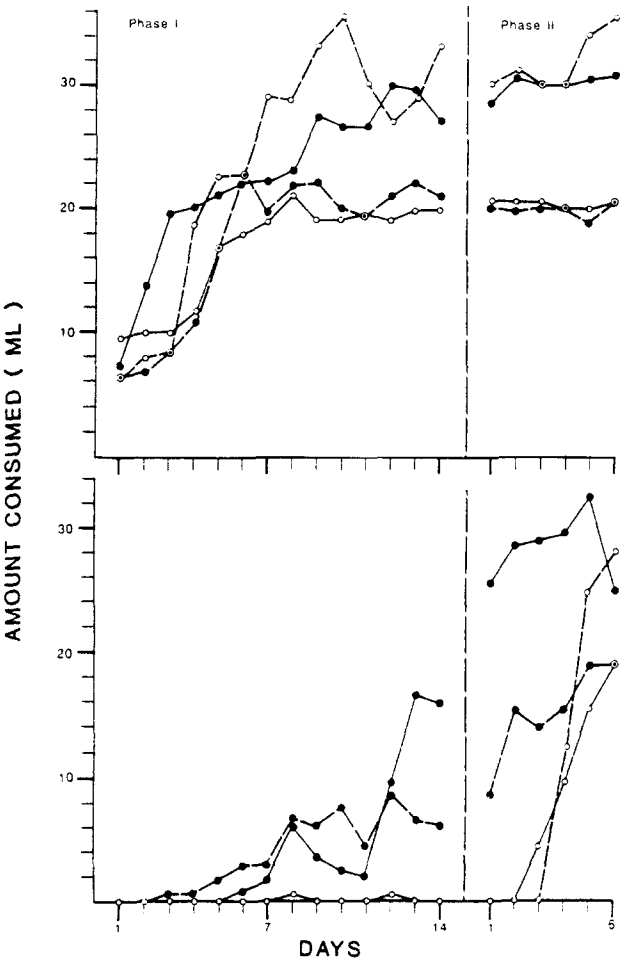

FIG. 1. The amount of water consumed for individual subjects in Group WN (top panel) and Group NW (bottom panel) over the 14 days of Phase I (left panel) and over the 6 days of Phase II (right panel). Individual subjects in each group are represented by ○— (Subject 1), ●— (Subject 2), ○— — (Subject 3) and ●— — (Subject 4). This legend also applies to the remaining figures.

The left panel of Fig. 2 presents the probability of post-pellet licking (i.e., the number of interpellet intervals containing at least one lick divided by 60 which is the total number of interpellet intervals within a session) for individual subjects in Group WN (top) and Group NW (bottom) over the 14 days of Phase I. Although the probability of

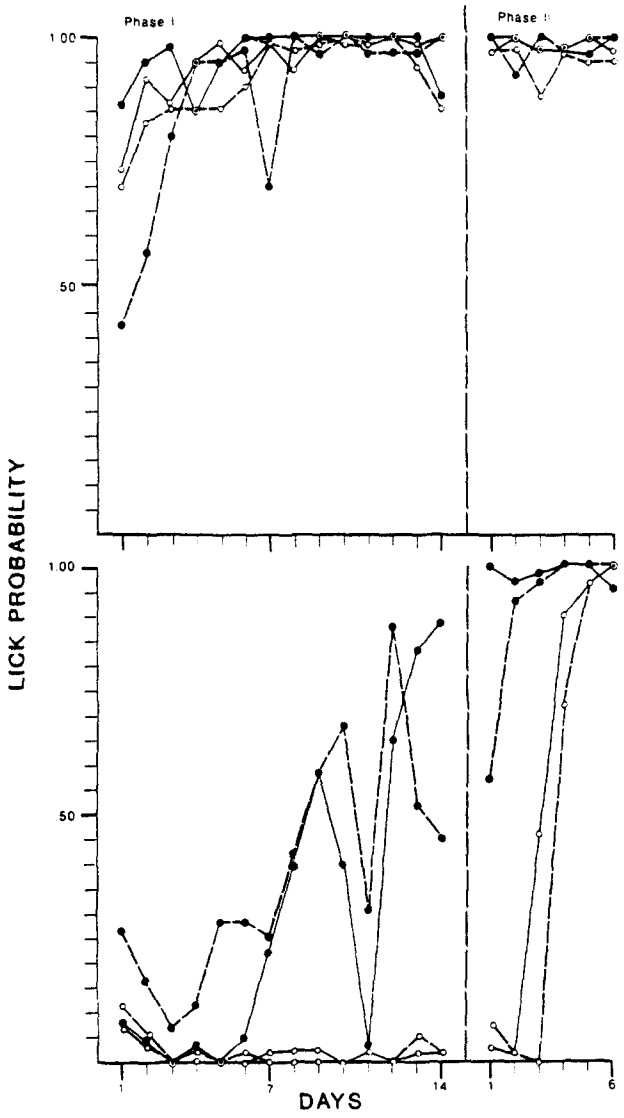

FIG. 2. The probability of post-pellet licking (the total number of interpellet intervals containing at least one lick divided by the total number of interpellet intervals within the session, i.e., 60) for individual subjects in Group WN (top panel) and Group NW (bottom panel) over the 14 days of Phase I (left panel) and over the 6 days of Phase II (right panel).

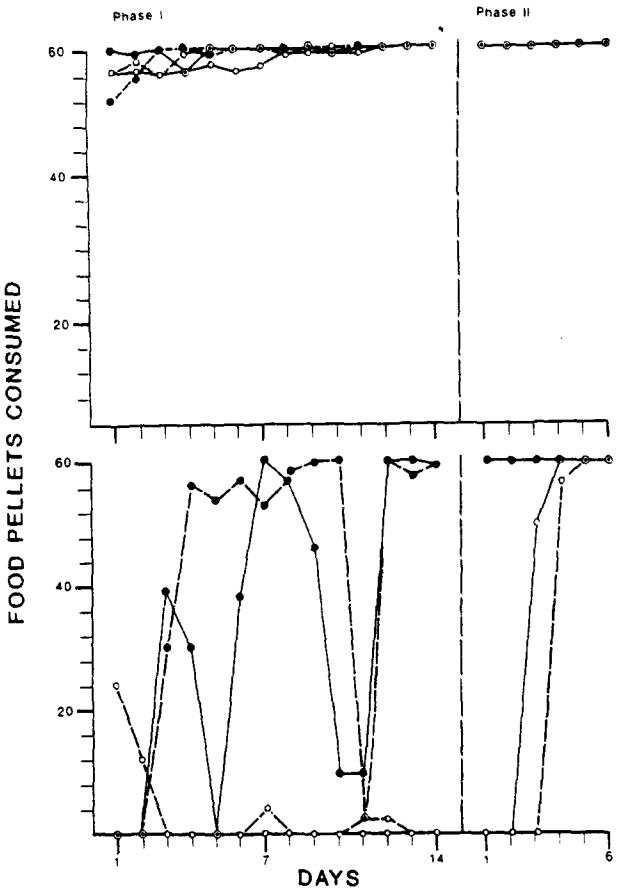

FIG. 3. The total number of pellets consumed for individual subjects in Group WN (top panel) and Group NW (bottom panel) over the 14 days of Phase I (left panel) and over the 6 days of Phase II (right panel).

post-pellet licking varied among individual subjects in Group WN, every subject in this group licked after some of the pellets on Day 1 (range: 43 to 87%). The probability of post-pellet licking rapidly increased for these subjects such that by Day 3 all subjects were licking after at least 80% of the pellets (range: 80 to 98%). This high probability of licking was maintained over sessions. By Day 14, the probability of post-pellet licking ranged from 85 to 100%. The probability of post-pellet licking was markedly different for subjects in Group NW. On Day 1, post-pellet licking ranged from 7 to 27%. Over the next two sessions, this probability decreased for all subjects. For Subjects 1 and 3, lick probability remained low throughout this phase (less than 5% on any specific session). For Subjects 2 and 4, lick probability gradually increased over sessions, reaching levels of 88 and 45% by Day 14, respectively. These between-group differences in lick probability were supported statistically in that Group WN had a significantly higher probability of post-pellet licking than Group NW during acquisition ($U=0$).

The left panel of Fig. 3 illustrates the total number of pellets consumed for individual subjects in Group WN (top) and Group NW (bottom) over the 14 sessions of Phase I. On the first free-food session, all subjects in Group WN consumed a majority of the 60 pellets delivered (range: 52 to 60). By Day 5, no more than two pellets were left in the hopper for any subject, and by the end of this phase all subjects were consuming each of the 60 pellets delivered in the session. On the other hand, only a single subject in Group NW ate any pellets on Day 1 of polydipsia training. This subject (Subject 3) ate 24 of the 60 delivered. Over sessions, this subject decreased pellet consumption, never consuming more than four pellets on any specific session for the remainder of this phase. Subject 1 never consumed a single pellet during this phase. Although Subjects 2 and 4 initially ate no pellets, they gradually increased the number of pellets consumed such that by Day 14 they were eating 59 of the 60 pellets delivered. These between-group differences in food consumption were supported statistically in that Group WN ate significantly more food pellets than Group NW during acquisition ($U=0$).

Phase II: Maintenance

As illustrated in the right panel in Fig. 1, subjects that received naloxone after SIP had been acquired (Group WN; top) continued to consume water at levels consumed in Phase I. There was no clear effect of naloxone on the overall level of consumption for any of these subjects at any point during this phase. When the naloxone vehicle was substituted for naloxone in Group NW, Subject 2 displayed an immediate increase in water consumption from 16 to 25.5 ml. A similar increase occurred in the remaining subjects, although these increases were not evident until Days 2, 3 and 4 of this phase for Subjects 4, 1 and 3, respectively. On the final day of vehicle injections, subjects in Group NW consumed an average of 22.5 ml (range: 19 to 28 ml). There were no significant differences in water consumption between groups during this phase ($U=2$).

As with overall amount consumed in Phase II, naloxone did not affect the probability of licking (top right panel, Fig. 2) or the overall number of pellets consumed (top right panel, Fig. 3). All subjects in Group WN continued to display post-pellet licking after a majority of the pellets and to eat every pellet delivered. Although only two subjects in Group NW were licking following pellet delivery (Fig. 2) and eating pellets (Fig. 3) on Day 14 of Phase I, within five days of receiving vehicle injections in Phase II all of the subjects in this group were licking after the majority of the pellets and consuming all of the pellets delivered. There were no significant differences in lick probability ($U=1$) or food consumption ($U=4$) between groups during this phase.

DISCUSSION

Similar to earlier work (see [1, 6, 20]), naloxone had no effect on established SIP. Neither amount consumed nor lick probability was affected by naloxone once SIP had developed. Although naloxone did not affect established SIP, it clearly affected its acquisition. Both overall water consumption and the probability of licking were markedly suppressed in subjects receiving naloxone during the acquisition of SIP. This suppression of the acquisition of SIP by naloxone is somewhat inconsistent with Tagi, Dantzer, Mormede and Le Moal's [19] recent report that 2 mg/kg naloxone only marginally suppressed developing SIP. Their relatively weaker effect (relative to the present data) may have been a function of the dose examined (2 vs. 10 mg/kg) and the delay between drug injection and onset of the session (30 vs. 15 min), variables each of which should reduce the effect of naloxone. Thus, it does appear that similar to the differential effects of amphetamine [22], conditioned taste aversions [13] and preloading [2, 4, 11, 12] on developing and established SIP, the acquisition and maintenance of SIP are differentially affected by naloxone.

Based on the earlier findings that SIP was unaffected by naloxone [1, 6, 20], it had been concluded that SIP is non-opioid in nature (see [10,20]). Given that the acquisition of SIP was affected by naloxone in the present study, however, this interpretation needs to be reevaluated. The interpretation could still be defended by arguing either that pre-asymptotic drinking is not induced or that it is *opioid* in nature. Although both arguments are consistent with the interpretation that naloxone's failure to affect SIP (at asymptote) is due to its non-opioid nature, there is simply no support for the position that developing SIP is not induced (see [5, 17, 18, 21]) or that the biochemical bases for acquisition and maintenance are different (other than the differential effects of naloxone).

The most parsimonious way to account for the differential effects of naloxone on the acquisition and maintenance of SIP and still assume that SIP is non-opioid in nature may be to consider the effects of naloxone on food consumption. As described, as early as Day 2 of Phase I all vehicle-injected subjects were eating over 90% of their pellets. However, only two of the four naloxone-injected subjects (Subjects 2 and 4) ate during this phase, and even for these two subjects pellets were not consistently eaten. Although naloxone suppressed food consumption during Phase I, it had no effect on feeding in Phase II, that is, subjects injected with naloxone during Phase II ate over 98% of their pellets, an amount similar to that eaten by these same subjects during Phase I when they received injections of the vehicle. According to this analysis, both developing and asymptotic SIP may be non-opioid in nature. The apparent naloxone-produced suppression of drinking during acquisition may simply be a spurious by-product of the naloxone-produced suppression of feeding. Consistent with this interpretation is the fact that the two naloxone-injected subjects that displayed SIP during Phase I (i.e., Subjects 2 and 4) were the only two subjects in Group NW that ate. This relationship between eating and SIP was significant, e.g., $r=.7272$, $p<0.025$ between number of pellets eaten and amount of water consumed; $r=.7826$, $p<0.025$ between number of pellets eaten and probability of licking. Further, when Subjects 1 and 3 in Group NW were given vehicle injections in Phase II, pellet consumption increased and SIP developed. The relationship between eating and SIP was significant in this phase as well, e.g., $r=.9629$, $p<0.025$ between number of pellets eaten and amount of water consumed; $r=.7779$, $p<0.05$ between number of pellets eaten and probability of licking. Although this analysis offers an explanation for the differential effects of naloxone on the acquisition and maintenance of SIP which is consistent with a non-opioid interpretation, it remains unknown why *feeding* was differentially affected by naloxone. Recent work by Sanger and Cooper [16] may provide some basis for this differential effect of naloxone on feeding. Sanger and Cooper reported that although naloxone reliably suppressed feeding (see also [7–9]), this suppression was significantly reduced in animals adapted to the feeding schedule. It is possible that in the present report the extended exposure to the daily feeding schedule for subjects given naloxone once SIP had been established attenuated the effects of naloxone on feeding and consequently its effects on SIP.

Independently of whether the above accounts can fully explain the differential effects of naloxone on the acquisition and maintenance of SIP, it is clear that a simple conclusion that SIP is unaffected by naloxone is premature. It remains to be seen whether these differential effects of naloxone on developing and established SIP reflect different types of drinking, e.g., noninduced and induced, different biochemical mediation, e.g., opioid and non-opioid, or different sensitivities of feeding to naloxone.

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