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Precipitated Morphine Withdrawal Induces a Conditioned Aversion in the Preweaning Rat

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BARR, G. A. AND G. A. GOODWIN. *Precipitated morphine withdrawal induces a conditioned aversion in the preweaning rat.* PHARMACOL BIOCHEM BEHAV **57**(4) 779–783, 1997.—Opiate abstinence in the adult of many species, including humans, alters autonomic function and motor behavior, and induces a negative affective state. The neurobehavioral bases of each consequence of opiate withdrawal differs. Little attention has been paid to the issue of drug withdrawal in infants, although it is a common consequence of the maternal use of illegal and legal drugs. Infant rats as young as 7 days of age that experience opiate withdrawal show an abstinence syndrome consisting of developmentally appropriate behaviors that differ from those of the adult rat, including fewer autonomic signs. Unlike the adult, there are no data in the infant on whether or not opiate withdrawal induces a negative affective state. We treated infant rats twice daily for seven days with either morphine or saline. Pups were injected with naltrexone or saline and exposed to a novel odor. After conditioning, pups were given the option of spending time with the conditioned odor or in a neutral environment. Fourteen day old pups, but not 7 day old animals, chronically treated for 7 days with morphine and conditioned with naltrexone, showed a significant avoidance of the conditioned odor. This suggests that a conditioned aversion had formed, a result similar to that shown for adult animals. © 1997 Elsevier Science Inc.

Abstinence Development Learned aversion Morphine Naloxone Opiates

DRUGS of abuse produce a variety of behavioral and physiological actions that include psychological changes such as euphoria associated with drug taking, and dysphoria with drug withdrawal (10,11,20,43). For opiate drugs, withdrawal in adult animals of a variety of species, including humans, results in overt somatic signs (4,7,42), physiological alterations (5), and a negative affective state (13,25,27,28,30,34,35). No single withdrawal sign characterizes opiate abstinence; rather it consists of a collection of behavioral and physiological changes that taken together define withdrawal.

Human and rat infants display somatic and physiological signs of opiate withdrawal, although the specific behaviors elicited by withdrawal differ by age. The withdrawal syndrome in the human infant is well described, although confounding factors such as multi-drug use, nutritional deficits, and other differences in the quality of prenatal care make analysis of the unique effects of opiate withdrawal difficult. In human infants, neonatal abstinence includes respiratory and gastrointestinal dysfunction, yawning, sneezing and fever. Behaviorally, opiate abstinent babies frantically suck their thumbs, display incessant and inconsolable high pitched crying, and are restless and irritable (15). In non-human animals, withdrawal of the fetus is associated with increased mortality and morbidity (22,23); during opiate withdrawal, infant rats increase their overall activity, express specific behaviors such as head swaying and rolling, and emit high levels of ultrasonic vocalizations (8,16,19,22). The behaviors expressed during precipitated opiate withdrawal in the infant likely define an

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opiate abstinence syndrome in the rat pup, and can occur as early as the late fetal period (17). Furthermore, classic withdrawal symptoms seen in the adult animal, such as tremor, wet dog shakes, and diarrhea, do not typify opiate withdrawal in the young rat since they are not seen prior to weaning during opiate withdrawal (16,44).

Opiate withdrawal is also dysphoric, and in the human adult, cues associated with withdrawal are responded to as noxious conditioned stimuli. Similar affective change has been shown experimentally using animal models. Adult rats suppress drinking fluids and learn taste aversions (13,25,28,33) decrease intracranial electrical self-stimulation (12,34,35), avoid entering places (27,30,35), and bury small objects that had been paired with precipitated withdrawal, when exposed to cues associated with opiate abstinence (29). Thus opiate abstinence engenders a strong negative affective state in the adult, and, on a number of parameters, is uncorrelated with the somatic signs and autonomic discharge also seen during morphine abstinence (27). Although a withdrawal syndrome exists in the rat fetus and infant (16,44), it is not known whether, opiate withdrawal in the infant also induces negative affective states. The purpose of this experiment therefore was to determine if odors paired with precipitated morphine withdrawal would induce a conditioned odor aversion in 7 and 14 day old pups. If so it might be concluded that opiate withdrawal is aversive in the infant, as it is in the adult.

METHODS

Subjects

The subjects were the pups of Long Evans Hooded rats that were mated in our animal facility. All rats were housed in plastic tubs measuring $40 \times 20 \times 24$ cm and the environmental temperature was maintained at a constant 22 ± 1°C. Parent animals were fed Purina Lab Chow and water ad lib. Cages were checked daily at approximately 1000 and 1800 h. Pups found on that day at either time were termed 0 days of age. Following parturition, litters were culled to ten pups, without regard for the ratio of males to females.

Chronic Injection Regimen

We injected the infant rat twice daily from the first to the 6th day, or from the 8th to the 13th day of postnatal life. Each litter received a single treatment only. Morphine sulfate or isotonic saline was given SC in a volume of 1 ml/100 g. The morphine dose was 10.0 mg/kg (salt weight). The injections were given twice daily at approximately 1000 and 1800 h. Controls were injected with saline. Twenty-one litters were used. The last injection was given on the morning of the 7th day. Body weight data were collected daily prior to the first injection each day.

Conditioning

Several methods were tried to induce conditioned place aversions. Preliminary findings suggested that two consecutive days of conditioning were most effective in producing a conditioned aversion. Conditioning took place on postnatal days 6 and 7 or 13 and 14, the last 2 days of chronic drug treatment. On the first day of conditioning, subjects were injected with morphine or saline, kept together as a litter, but separate from the dam. Pups were injected 1 h later with saline or one of two doses of naltrexone (0.3, 1.0 mg/kg SC). They were then put individually into round plastic containers (11 cm diameter) that were placed into a conditioning chamber that contained two cotton pads scented with lemon oil (0.3 ml, Humco). The temperature of the conditioning chamber was (32 ± 2°C). After 1 h of exposure to the lemon odor, pups were returned to their home cage, littermates and dam. On the 7th or 14th day, pups were given their final injection of morphine or saline in the morning followed by the second pairing of precipitated withdrawal and the lemon odor as described above. The acute injections (naltrexone or saline) and doses were the same for the animals on both days. Naltrexone was used rather than naloxone to be consistent with our prior studies on withdrawal (16). On the test day, subjects were not returned to the home cage, but rather were kept together in an incubator at (32 ± 2°C) for 6 h until testing.

Testing

The test chamber consisted of a wire mesh floor measuring 9×27 cm, covered with a small plexiglass box and maintained at 25°C. The floor was divided into three areas: Cotton gauze scented with lemon oil (20 μl) was placed under the mesh at the end of one side of the test chamber (S^+); in the center was a neutral zone, 3 cm wide. The opposite side (S^-) and neutral zone were bare. The wire mesh on the side over the lemon oil was also covered by cotton gauze to enhance the similarity of that side to the training chamber. The test procedure consisted of 4 one min discrete trials in which the amount of time the pup spent in each of the 3 areas was recorded. To begin each trial, the pup was placed into the neutral zone, facing one end of the chamber. The orientation of the subject was alternated on each trial. The number of times each pup crossed into the S^+ and S^- was also recorded. A cross was defined as when all 4 paws of the subject crossed the line dividing either side from the neutral zone and a pup was considered in a zone when its nose was in that area. The time spent in the neutral zone and the number of crossings were used as rough measures of activity.

Statistics

The time in each zone and the number of crosses were summed over the 4 trials. The dependent measures used to assess a preference or aversion to the conditioned side were the difference between the time spent on the 2 test sides (S^+–S^-), the time spent in each of the 2 sides, or the ratio of time spent in the 2 sides:

$$\left(\frac{s^+ - s^-}{s^+ + s^-}\right).$$

All measures gave virtually identical results; the time spent in the S^+ and the difference between the time spent in the S^+ compared to the S^-, (S^+ –S^-), are presented here. When multiple pups were tested under the same conditions in a litter, the data from individual animals was pooled to form a litter mean for that condition. Thus each litter provided three data points, one for saline and two for the doses of naltrexone. The data were analyzed by a factorial analysis of variance; the chronic treatment (saline or morphine) and age of the pups (7 or 14 days) were between subjects variables, and the dose of naltrexone was a within litter variable, treated as repeated measures in the ANOVA. As measures of activity, both the number of crosses and the time spent in the neutral zone were analyzed by a similar design of the ANOVA.

RESULTS

The major finding was that the 7 day old pups spent more time in the conditioned side regardless of treatment, but the 14 day old morphine treated pups that had been administered naltrexone avoided the conditioned side. This was shown by the significant three way interaction among age, chronic treatment and acute treatment for both dependent measures (Difference score: $F(2, 34) = 3.52$, $p = 0.04$; S^+ time: $F(2, 34) = 3.58$, $p = 0.04$). The results are depicted in Fig. 1. For the data from the chronic morphine treated pups, posthoc tests (Tukey's HSD) showed that for the 14 day old pups given chronic morphine, both doses of naltrexone produced a significantly greater aversion than did the saline treatment. Both doses of naltrexone produces a significant aversion compared to the vehicle controls for the S^+ and the difference score (for both dependent measures: $p < 0.05$ and 0.001 for the 0.3 and 1.0 mg/kg doses respectively). There were no differences nor trends towards differences among these injection groups (saline and naltrexone) in the chronic saline treated pups at either age.

In both activity measures, the 14 day old pups were significantly more active than 7 day old pups regardless of acute or chronic treatment, as would be expected (Fig. 2). There was no significant acute by chronic treatment interaction for either activity measure (Time in neutral zone: Acute × Chronic × Age–$F(2, 34) < 1.0$, Acute × Chronic–$F(2, 34) = 1.25$; Number of Crosses: Acute × Chronic × Age–$F(2, 34 < 1.0$; Acute × Chronic–$F(2, 34) < 1.0$).

DISCUSSION

The purpose of this experiment was to study the negative affective component of opiate abstinence in a paradigm that parallels that used in adult animals, and to make more definitive statements about the developmental continuities and discontinuities of the behavioral outcomes of opiate abstinence. Our results demonstrate that the "affective" aspect of withdrawal also exists early in development, although not until after the first week of life. In the adult animal the unconditioned somatic and aversive components of precipitated withdrawal are mediated by different mechanisms (27). The results of this experiment provide additional evidence for the differentiation of the physical signs and negative affective components of opiate withdrawal, since they show different developmental time courses. The somatic signs of withdrawal are seen during the first postnatal week (16,44) and may appear in a slightly altered form in the fetus (17), whereas the aversive component appears sometime after the first week of postnatal life. The reasons for this developmental difference is not known.

There are several alternative explanations, other than a learned aversion, that could account for these results. First, morphine dependent pups given saline could form a preference to the odor following the pairing of morphine with the odor. Naltrexone would block this preference. If this were the case, we would expect that the chronic morphine treated animals given saline would show a preference to the odor compared to the chronic saline treated pups given saline. This did not occur and thus it is an unlikely explanation for the data.

Second, because isolation is aversive to infant rats (36), chronic morphine treatment in animals given saline might block that learned aversion. If this were the case, it would be expected that the chronic saline treated pups would all exhibit an aversion to the odor relative to the chronic morphine treated animals. This also did not occur. Thus the most parsimonious explanation and likely cause of the avoidance of the side paired with precipitated withdrawal is that the odor became a conditioned aversive stimulus that was later avoided.

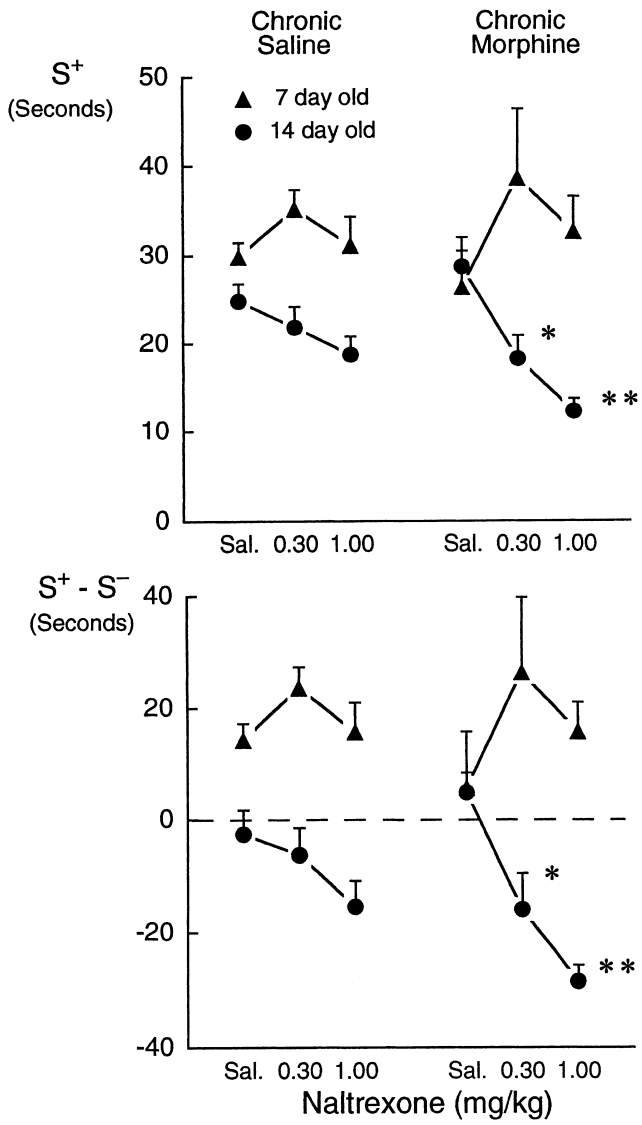

FIG. 1. Top: The number of seconds spent on the S^+ for 7 and 14 day old pups. The major significant difference as determined by post hoc analysis of the significant interaction effect was that, for the 14 day old animals that were chronically administered morphine, either dose of naltrexone reduced the time spent on the paired side (S^+) compared to the acute saline treated animals. This indicates an aversion induced by naltrexone (precipitated withdrawal) in the older pups. Bottom: The difference between the time spent on the S^+ (lemon scented) and the S^- (unscented) sides. As above, the only significant difference on posthoc analysis was a significant aversion induced by naloxone in the 14 day old morphine exposed group. For both panels, *indicates $p < 0.05$, **indicates $p < 0.001$ for naltrexone compared to the acute saline control.

Third, it is possible that there was differential tolerance to morphine at the two ages. However, our injection schedule was quite effective in inducing physical withdrawal in both 7 and 14 days of age with no apparent difference in the severity of withdrawal (16), and available data on the development of

tolerance suggests that the younger pups may become less tolerant to some of the effects of chronic morphine (44). Thus it is not likely that the differences between 7 and 14 day old pups seen here are due to differential development of tolerance between the two ages.

The somatic and physiological signs of opiate withdrawal in the adult include jumping, "wet dog" shakes, paw tremors, piloerection, teeth chattering, chewing, writhing, ejaculation, ptosis, yawning, chromodacryorrhea, cardiovascular pressor responses, hypothermia, and hyperreactivity as evidenced by squealing and biting on touch, (3–7,9,26,42). Abstinent rats also emit both low and high frequency ultrasounds (40). The infant and fetal rat demonstrate physical signs of opiate withdrawal and the infant increased ultrasonic vocalizations (1,16,17,44). Here we demonstrate that another component, conditioned aversion to cues associated with opiate abstinence, are also present in the infant rat, although at a later age than the somatic signs and vocalizations. The inability to learn an aversion at 7 days of age is not a limit of the capacity of the infant. Odor learning plays a important role in the initial attachment of the newborn pup to the dam immediately after birth (31), and rat pups are capable of learning both conditioned odor preferences and aversions at early ages, under a variety of conditions (2,31,32,37,38). For opiates, preferences for odors associated with met-enkephalin injection have been shown in utero (38), and 3 day old pups are capable of learning aversions to the κ opiate U50,488 (2). Thus, that the 7 day old pups were unable to learn the aversion is not likely due to a limit in their ability to form learned associations with odors. Whether or not they also show sympathetic discharge at this age is not known, although there is no evidence of diarrhea or weight loss during precipitated opiate abstinence at these ages (16). Cardiorespiratory changes also remain to be studied.

Infant rats undergo opiate withdrawal, but the abstinence syndrome is different from that of the adult. Furthermore, there is no reason to assume that the underlying neurophysiological causes of the opiate withdrawal syndromes in the infant and adult are the same. In the adult, the brain regions involved in opiate abstinence include the anterior hypothalamus (wet dog shakes), periaqueductal gray (rearing and locomotion), locus coeruleus (jumping, rearing, locomotion), substantia nigra (irritability, teeth chattering, wet dog shakes) and nucleus raphe magnus (wet dog shakes) (24). Injection of methylnaltrexonium into the PAG or locus coeruleus can also elicit behavioral withdrawal in the 7 day old pup (Jones, unpublished data). The nucleus accumbens and the amygdala, in contrast, have been shown to be important neural sites mediating aversive affect (21,39,41). Whether there is a similar organization for the aversive component of opiate withdrawal in the infant is not known.

If cues associated with abstinence can be conditioned, the infant may experience conditioned withdrawal; if the conditioned response is persistent, as it is in adults, this may have adverse consequences for the normal development of addicted neonates. Human infants who are in abstinence from opiates bond poorly with their mothers and are deficient in their capacity to respond to attention and other social cues (14,18). Although the reasons for these social deficits are unknown, it is tempting to hypothesize that the infant undergoing opiate withdrawal may associate normal maternal cues, both in utero and ex utero, with the physiological and psychological symptoms of withdrawal. Subsequent exposure to those cues (e.g. maternal odor, cuddling and so forth) may reinstate that negative state and alter the normally positive affect associated with those stimuli. Our data suggest that a negative affective state may be conditioned to cues associated with precipitated withdrawal, at least in the infant rat, although only sometime after the first postnatal week. Whether or not similar conditioned aversions can be learned by the human infant, and if so, at what age, remains to be studied.

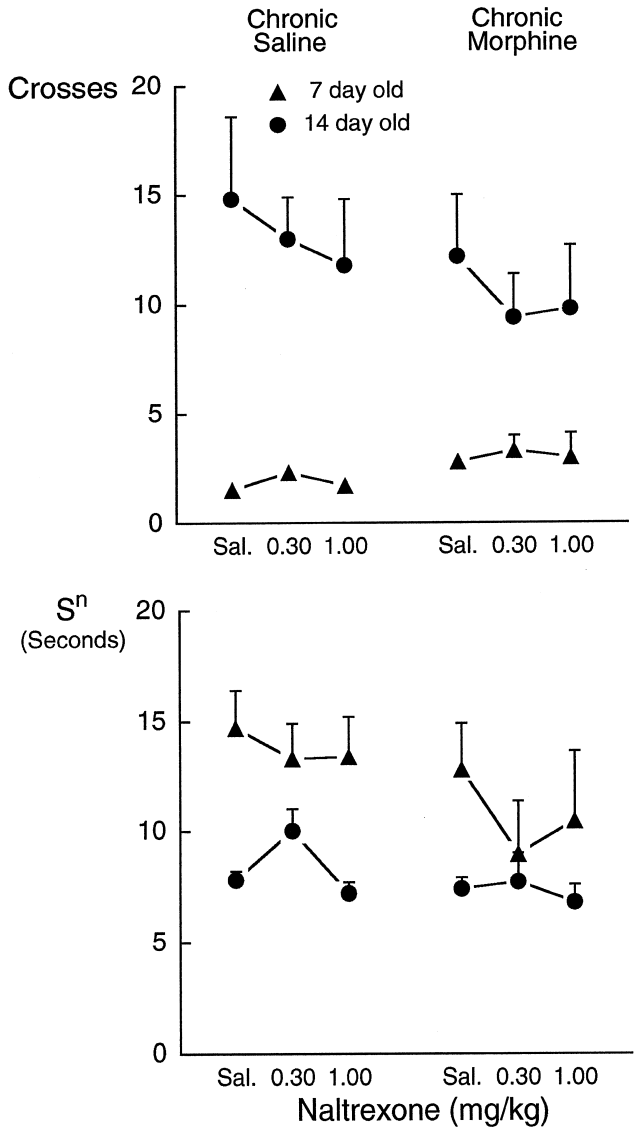

FIG. 2. Top: Number of crosses. There were no drug induced changes but the older pups were significantly more active than were the younger pups. Bottom: Time spent in the neutral zone. There were no differences due to naltrexone injection nor due to chronic morphine treatment. As might be expected, the older pups spent significantly less time in the neutral zone, indicating greater activity.

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