PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 435-438 (1977)

Effects of Morphine Pellet Implantation in Neonatal Rats (39688)

THEO B. SONDEREGGER, BRUCE BROMLEY,* AND EMERY ZIMMERMANN*

*Department of Psychology, University of Nebraska-Lincoln Lincoln, Nebraska 68508 and *Department of Anatomy and Brain Research Institute, UCLA School of Medicine, Los Angeles, California 90024*

The newborn rat has been used successfully to study long-term behavioral and pituitary-adrenal effects of early exposure to narcotics (1, 2). Since early handling is known to exert profound influences on the development of behavioral and neuroendocrine functions in the rat (3), the effect of daily injections and of repeated handling may have obscured drug-induced changes in these earlier studies. To minimize the influence of manipulation (while avoiding problems associated with direct administration of the drug to the mother), morphine (M) was administered to rat pups by pellet implantation. This report describes developmental and lasting effects of this standardized treatment at different times during early postnatal life.

Materials and methods. The pellets used were prepared according to the method of Gibson and Tingstad (4). Each pellet contained 75 mg of morphine base. Placebo pellets (P) were prepared using the same weight of lactose.

Initial studies showed that subcutaneous implantation of one M pellet in 8-day-old pups resulted in death within a few hours, while all animals implanted with P pellets survived. In subsequent work, pellets were coated with beeswax and scored on each side with a needle: one scratch for animals under 1 week of age and two scratches for older animals. All pellets were implanted subcutaneously in the flank using brief ether anesthesia.

To study long-lasting effects of early M pellet implantation, newborn Sprague-Dawley female rats (Simonsen Farms, Gilroy, Calif.) were placed in litters of eight and maintained under standardized conditions of lighting (14-hr illumination alternating with 10-hr darkness) and temperature (26 ± 1°). Two pups from each litter received either one M or one P pellet on Day 5 or 11 of postnatal life. These times were chosen to coincide with times of daily injections of M in earlier studies (1, 2). Mortality was noted and surviving pups were subsequently handled only briefly at weekly intervals to measure body weight. All animals were weaned at 21 days of age and housed in pairs thereafter.

The effects of early implantation of M on resting pituitary-adrenal function were studied at 1600 hr on Day 43. Each animal was subjected to ether anesthesia and removal of a blood sample by jugular venipuncture as described previously (5). The plasma was separated and frozen and used subsequently for fluorometric determinations of corticosterone concentrations (6).

To determine whether early pellet implantation produced long-lasting tolerance to M, the analgesic response to a challenge dose of M was examined on Day 48 using the hot-plate test (7). After three control trials, in which response latencies (time to raise and lick a paw) were measured at 30-min intervals, each animal received a subcutaneous injection of M (10 mg/kg). Thirty minutes later each animal's response latency was again obtained. The mean control latency was calculated for each animal and subtracted from the value obtained 30 min after M injection to determine the change in response latency.

On Day 68, tolerance to M was again tested by challenging animals from each treatment group with a subcutaneous injection of 40 mg/kg of M. Thirty minutes later a blood sample was obtained by etherization and jugular venipuncture for determination of the plasma corticosterone response.

Since decreased survival of the offspring of female rats treated with M prior to mating has been reported (8, 9), a final test was performed to study fertility, litter size, and offspring mortality of rats addicted to M early in life. Five animals from each treatment group were bred with proven sires at approximately 120 days of age.

Statistical analyses were performed using

ISSN 0037-9727

Student's t test. A probability of 0.05 or less was considered significant.

Results. Mortality rates were 36 or 25% for animals implanted with M on Day 5 or 11, respectively, while only one P-implanted animal died (4%). The majority of deaths occurred within 24 hr after M pellet implantation, 64 and 52% of deaths among Day-5 and Day-11 animals, respectively.

As shown in Fig. 1, survivors of both M treatment groups showed immediate suppression of body weight gain compared to P-implanted controls, and these deficits remained significant ($P < 0.05$) throughout the 126 days of the experiment. Body weight depressions of the Day-5 M group appeared to occur more gradually than those of the Day-11 M group. Differences between the mean weights of the Day-5 M and P groups were 6.7 g on Day 12 ($P < 0.01$) and reached a maximum difference of 23.5 g on Day 63 ($P < 0.01$). Comparable differences in the Day-11 animals were 7.6 g ($P < 0.005$) on Day 12 and reached a maximum of 23.5 g ($P < 0.005$). Day-5 M-treated animals weighed less than ($P < 0.01$) Day-11 M-treated animals on Day 28 but thereafter their weights did not differ significantly. Mean body weights of the Day-5 and Day-11 P groups were similar throughout the experiment.

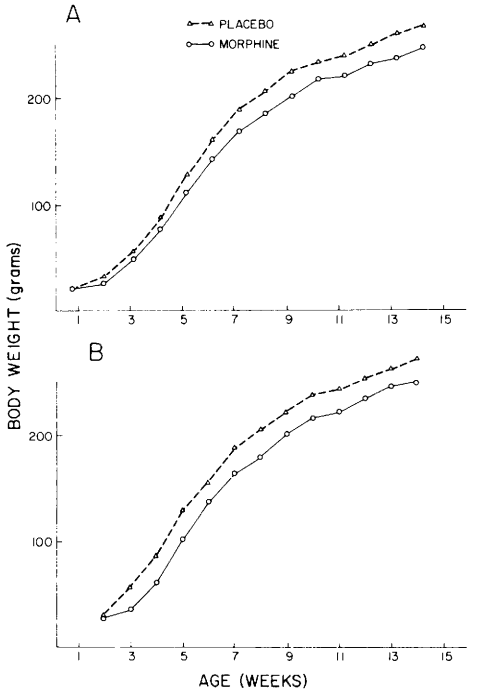

FIG. 1. Ponderal growth of female rats following neonatal implantation of morphine (○) or placebo (△) pellets at 5 (A) or 11 (B) days of age. Each point represents the mean weight of 10–12 rats.

Resting levels of plasma corticosterone on Day 43 were comparable in M- and P-implanted animals of the Day-5 and Day-11 treatment groups (approximately 20 μg/ml) and were in good agreement with levels observed in untreated female rats in our laboratories during the late afternoon (5).

As shown in Table I, animals implanted with M pellets on Day 11 showed impaired responses to the analgesic action of M when tested 30 min after injection of the opiate. In contrast, the analgesic action of M was not impaired in the Day-5 implanted animals. Both M- and P-implanted animals showed similar mean control response latencies prior to M injection.

The results obtained on Day 68 following injection of M (40 mg/kg) are presented in Table II. Control animals showed expected high levels of plasma corticosterone in re-

TABLE I. ANALGESIC RESPONSE (HOT-PLATE TEST) 30 MINUTES AFTER MORPHINE INJECTION (10 mg/kg sc) IN 48-DAY-OLD FEMALE RATS TREATED PREVIOUSLY WITH MORPHINE (M) OR PLACEBO (P) PELLETS AT 5 OR 11 DAYS OF AGE.

Neonatal treatment	No. of rats	Control response latency (sec)	Increase in response latency (sec)
Day 5			
P	9	7.6 ± 0.7[a]	4.6 ± 2.3
M	12	8.2 ± 1.0	6.4 ± 2.4
Day 11			
P	12	6.2 ± 0.6	4.2 ± 1.1
M	11	5.8 ± 0.4	0.8 ± 0.8[b]

[a] Mean ± SE.
[b] $P < 0.025$, compared to P control.

TABLE II. CORTICOSTERONE RESPONSE 30 MINUTES AFTER MORPHINE INJECTION (40 mg/kg sc) IN 68-DAY-OLD FEMALE RATS TREATED WITH MORPHINE OR PLACEBO PELLETS AT 5 OR 11 DAYS OF AGE.

Neonatal treatment	Corticosterone (μg/100 ml of plasma)	
	Placebo	Morphine
Day 5	51.0 ± 11.0 (10)[a]	64.5 ± 8.1 (12)
Day 11	50.8 ± 6.6 (11)	43.8 ± 7.9 (12)

[a] Mean ± SE. Numbers in parentheses indicate numbers of animals.

sponse to the M challenge (10), and those of the M-treated groups did not differ significantly.

In the breeding experiment, 19 of the 20 animals became pregnant, the exception being one of the Day-5 M-treated animals. Litter sizes of the four treatment groups ranged from 6 to 12; mean numbers of pups were 9.3, 9.8, 10.0, and 10.0 for Day-5 and 11 M and P groups, respectively, and mortality rates in these same groups were 61, 55, 67, and 52% by postnatal Day 11. It was necessary to terminate the experiment at this time, so the ability of the mothers to maintain survival of the pups for a longer period remains uncertain. Although the mortality rates in all groups were quite high, these results show that female rats exposed to neonatal implantation of M or P, as described in this experiment, can breed successfully and produce normal size litters.

Discussion. The results of these studies demonstrate the feasibility of producing in rats long-lasting suppression of growth and decreased sensitivity to M by implantation of a single M pellet early in life. The mortality (25–36%) associated with this method of M administration is comparable to that resulting from daily injections of M during the first or second week of postnatal life (2). The facts that cyanosis and hypothermia were observed in M-treated animals together with the low mortality rate of the P-treated groups, indicate that hypoxia due to M-induced respiratory depression, rather than ether anesthesia and surgery, was an important cause of death. Hypoxia also might have contributed to the protracted effects observed following neonatal M-implantation.

The persistent reduction of body weight observed following M pellet implantation resembles the somatic response of rats given M injections during the prenatal (9, 11, 12) or early postnatal periods (1, 2). We have observed similar suppression of body weight lasting at least 16 weeks in female rats implanted with a morphine pellet at 14 days of age (unpublished observation). The fact that a similar ponderal response follows M administration over a wide range of developmental stages suggests that a lack of critical timing is required for producing this opiate effect. It is not clear, however, that the same processes are responsible for weight depressions in the two M groups. Weights of Day-5 M-treated animals were depressed more gradually, reaching maximum depression on Day 63, than those of Day-11 M-treated rats, which reached a maximum depression 14 days after the implantation. This same general pattern of slower versus faster rates of body weight depression was observed in animals injected with M during the early (Days 3–12) vs the late (Days 12–21) preweaning period. The greater mortality of the Day-5 animals suggests caution in the interpretation of comparative differences among survivors and suggests that the younger animals may have suffered greater damage due to hypoxia. This possibility is consistent with the greater degree of cyanosis observed in the younger animals following M implantation. It is noteworthy that Bass and Lundborg (13) found greater depression of respiratory rates in younger (Day 5) compared to older (Day 30) rats.

In this as in previous injection studies (1, 2), rats showing M-induced suppression of body weight failed to evidence catch-up growth following cessation of drug administration. The reason for this is not clear but may relate to the duration of neonatal drug exposure since we have observed increased weight gain in growth-retarded rat pups following cessation of M injections for just a few days (unpublished observation). Growth inhibition appears to be a common effect of opiates since it has been observed in other rodents (14) and in human infants born to opiate-addicted mothers (15).

Tolerance to the analgesic action of M observed on Day 48 in animals implanted with M on Day 11 is consistent with results obtained previously (1, 2). Another advantage of the pellet implantation method is that it may not subject the pup to the stress of withdrawal. Since the stress of withdrawal may have lasting effects, it must be considered in studies wherein daily injections are terminated. Pellets implanted in this study were not removed, which possibly led to a gradual decrease in the amount of M released over a period of time. Although signs of M withdrawal were not seen in any rats during the periods of observation in this

study, verification of a lack of withdrawal effects will require additional study. Finally, it should be mentioned that pellet implantation is a rapid and convenient way to administer M. The present study showed that this method, like daily injections of M, is capable of producing protracted suppression of growth and sensitivity to M in rats.

Summary. A morphine pellet implantation method has been modified for use in very young rats. Compared to P-implanted controls, implantation of an M pellet on either Day 5 or 11 results in diminished body weight for periods at least up to 15 weeks. Such treatment on Day 11 is associated with reduced sensitivity to the analgesic action of morphine (10 mg/kg) when tested on Day 48 using the hot-plate technique. Resting pituitary–adrenal activity in animals implanted with morphine on Day 5 or 11 was within normal limits when examined on Day 43. Corticosterone responses to a challenge dose of morphine (40 mg/kg) on Day 68 failed to demonstrate tolerance unequivocally. These findings are consistent with those obtained earlier with daily injections of morphine and demonstrate that the long-lasting ponderal effect and tolerance to the analgesic actions of morphine may be produced without the confounding effects of daily handling and stress of abrupt withdrawal in neonatally addicted rats. This convenient method of prolonged early morphine exposure in young rats may be used as an experimental model for the study of long-range effects of neonatal exposure to narcotics.

We wish to thank Mr. Andrew Baldwin for his valuable technical assistance. This work was supported in part by USPHS Grant No. DA 826 and NIH Biomedical Research Support Grant No. RR-07055.

1. Zimmermann, E., Branch, B., Taylor, A. N., Young, J., and Pang, C. N., *in* "Narcotics and the Hypothalamus" (E. Zimmermann and R. George, eds.), p. 183. Raven Press, New York (1974).
2. Sonderegger, T., and Zimmermann, E., *in* "Tissue Responses to Addictive Drugs" (D. Ford and D. Clouet, eds.), p. 589. Spectrum, New York (1976).
3. Denenberg, V. H., and Zarrow, M. S., *in* "Early Childhood: The Development of Self-Regulatory Mechanisms," p. 39. (D. Walcher and D. Peters, eds.) Academic Press, New York (1971).
4. Gibson, R. D., and Tingstad, J. E., J. Pharm. Sci. **59,** 426 (1970).
5. Zimmermann, E., and Critchlow, V., Proc. Soc. Exp. Biol. Med. **125,** 658 (1967).
6. Glick, D., von Redlich, D. D., and Levine, S., Endocrinology **74,** 652 (1964).
7. Eddy, N. B., and Leimbach, D., J. Pharmacol. Exp. Ther. **107,** 385 (1953).
8. Smith, D., and Joffe, J., Nature (London) **253,** 202 (1975).
9. Friedler, G., and Cochin, J., Science **175,** 654 (1972).
10. Zimmermann, E., and Critchlow, V., Proc. Soc. Exp. Biol. Med. **143,** 1224 (1973).
11. Huidobro, J. P., and Huidobro, F., Psychopharmacology (Berlin) **28,** 27 (1973).
12. Steele, W. J., and Johannesson, T., Acta Pharmacol. Toxicol. **36,** 243 (1975).
13. Bass, N. H. and Lundborg, P., Biol. Neonate **23,** 456 (1973).
14. Crofford, M., and Smith, A. A., Science **181,** 947 (1973).
15. Wilson, G., *in* "Somatic Growth Effects of Perinatal Addiction" (R. D. Harbison, ed.), p. 333. Spectrum, New York (1975).

Received September 27, 1976. P.S.E.B.M. 1977, Vol. 154.