Pharmacology Biochemistry & Behavior, Vol. 15, pp. 773–778, 1981. Printed in the U.S.A.

Development of Opiate Tolerance in the Chick Embyro

M. B. NEWBY-SCHMIDT AND STATA NORTON

Department of Pharmacology and Ralph L. Smith Mental Retardation Center
University of Kansas, College of Health Sciences and Hospital, Kansas City, KS 66103

Received 30 March, 1981

NEWBY-SCHMIDT, M. B. AND S. NORTON. *Development of opiate tolerance in the chick embryo.* PHARMAC. BIOCHEM. BEHAV. 15(5)773–778, 1981.—Tolerance to morphine was produced in the chick embryo. Eggs were injected with morphine sulfate (MS) (20 mg/kg egg) or H_2O daily starting on incubation day 12. On day 16, embryo activities were recorded and eggs were injected with either MS or naloxone. Activity of H_2O-pretreated controls decreased after both MS and naloxone. Embryos treated with MS from incubation days 12–15 showed no activity change after morphine and responded to naloxone with increases in activity. Baseline rates of distress vocalizations (DV) of 1–2 day old chicks were not affected by MS pretreatment during incubation days 12–19. However, 1 mg/kg MS decreased the rate of DV of control chicks by 90% whereas MS-pretreated chicks were unaffected. At age 4–5 days, the baseline rate of DV and rate after MS were higher in MS-pretreated chicks. However, all chicks showed significant decreases in rate of DV after MS injection. Naloxone increased the rate of DV of paired 1–2 day old chicks, but response of MS-pretreated chicks was significantly greater than controls.

Chick Chick embryo Morphine Morphine tolerance

THE problems associated with maternal abuse of narcotics during pregnancy have been recognized in the literature for many years [3]. Risks to the exposed fetus involve direct effects of the narcotics on the fetus but also include indirect effects via maternal nutrition and hygiene. Laboratory studies involving methadone administration to the rat during gestation reveal increased maternal death, decreased litter size and increased perinatal mortality [2,5]. The contribution of maternal toxicity in these studies is not known. The chick is useful in studies of developmental toxicity from exogenous agents because there are no effects arising due to maternal toxicity.

The chick brain contains opiate receptors with properties and at concentrations similar to the receptors found in mammalian brain [16]. However, when analgesia has been tested in chicks using the toe-pinch method, very high doses of morphine are required to demonstrate any analgesic effect [19]. It would appear that either the endogenous opiate receptors perform some function other than analgesia in the chick or that the toe-pinch is not the correct test to use for opiate analgesia in chicks. Other investigators have found that morphine acts to potentiate tonic immobility [18] and increases the threshold of shock for eliciting the flight response in chickens [1]. However, neither of these techniques has been used with very young chicks.

Panksepp and co-workers have carried out extensive experimentation demonstrating that endogenous opiate systems function to mediate social attachment in the young of several species [4, 12, 13] including chicks [11, 13–15]. Their work has shown that very low doses of morphine or other opiate agonists suppress the rate of distress vocalization (DV) in young chicks, guinea pigs and puppies when they are put into social isolation. This test should be adaptable for monitoring the development of opiate tolerance and withdrawal in the chick. If the rate of DV is normally suppressed by low doses of opiate agonists, then a chick that is tolerant to morphine should show less of this "conforting" effect than a nontolerant chick. Described herein is the use of DV counting as a means of detecting opiate tolerance and abstinence in the young chick. In addition a simple, noninvasive method for measuring chick embryo activity is employed to monitor the effects of morphine on the chick embryo. Part of this report has appeared elsewhere in abstract form [8]. A separate report has described alterations of walking ability of chicks treated with MS as embryos [9].

METHOD

Animals

Fertile White Leghorn chicken eggs (obtained from Larson Laboratories, Gowrie, IA) were incubated at 38°C in a forced-draft incubator and automatically turned once every hour. The eggs were segregated by treatment group shortly before hatching (incubation day 21). After hatching, the chicks were placed in a heated brooder with food (Purina Chick Starteena) and water available ad lib.

Injections

All injections of eggs were made into the air space of the egg. One day prior to the initial injection, the egg was candled and the air space was delineated. A hole was drilled through the shell above the air space. To do this, a scratch was first made using a diamond pen followed by insertion of

a 20 ga needle which was rotated to make a round hold. The hole was sealed with a drop of melted paraffin. All injections were made through this hole which was sealed with melted paraffin after each injection.

Morphine sulfate (MS) was injected once daily at a dose of 20 mg/kg egg beginning on incubation day 12 and continuing up to and including incubation day 19. The drug was dissolved in sterile water and the solution was sterilized using a Millipore filter apparatus. Volume of injection was 0.2 ml/100 g egg or approximately 0.1 ml/egg. Controls received equivalent volumes of sterile water. Naloxone (Narcan®, Endo Laboratories Inc., Garden City, NY) was obtained as a sterile solution of 0.4 mg/ml in saline. It was injected at a volume of 0.2 ml/100 g egg, with a resultant dose of 0.8 mg/kg egg.

Injections of young chicks after hatching were performed IP at a volume of 0.5 ml/100 g body weight. Morphine sulfate was dissolved in saline (0.9 per cent NaCl) and was administered at a dose of 1.0 mg/kg. Naloxone solution was diluted 1:4 with saline and was administered at a dose of 0.5 mg/kg. Care was taken not to inject the drugs into the residual yolk of the very young chicks as this would impede the absorption of the drug into the bloodstream.

Embryo Activity

The apparatus for measuring embryo activity is depicted in Fig. 1. It consisted of a large plastic beaker within a heated water bath. Two eggs were set into nylon mesh hammocks suspended from rods within the beaker. One line for each egg was run from the hammock to connect with a strain gauge which was connected with a dynograph and recorder (Beckman/Offner Type R, Beckman Instruments Inc., Schiller Park, IL). The beaker was covered with a Plexiglas hood that held a thermometer at the level of the eggs. A section of the beaker was removed and two slits in the hood were made to allow passage of the lines to the transducers. Temperature within the hood stabilized at 36 to 37°C when the water in the water bath was held at 46°C.

Embryo movements were detectable in all viable eggs by day 16 of incubation. Eggs were removed from the incubator and hung up in pairs, one from each treatment group. Lines were connected to the strain gauges and adjusted so that there was a slight tension. Within reasonable limits the amount of tension on the line did not affect the recording of activity. The hood was lowered and the eggs were allowed to stand until the temperature reached 34.5 to 35°C. This required from 3 to 5 min. Then the recorder was turned on and the activity was measured for 10 min. Movements were recorded by ink on Beckman chart paper at a chart speed of 2.5 mm/sec. An embryo movement was considered to be any deviation of the pen that was twice the size of the background pen movement (of 60 Hz). The number of movements/10 min was counted in a blind manner after the experiment was finished. All eggs were injected immediately after obtaining the baseline activity. Those eggs receiving MS were returned to the incubator and activity was recorded again 30 min later. Those eggs receiving naloxone were immediately replaced into the apparatus and activity was recorded again 5 min after injection.

Distress Vocalization

Chicks were socially isolated by removing them from the incubator or (in the case of chicks more than 2 days old) from the brooder containing the other chicks and placing them one at a time into a box (32×32×30 cm high) in a separate room. The box was then covered and after one minute, the number of vocalizations was counted in the first 10 sec of the next 9 min. Thus the results are expressed as number of DV's/90 sec obtained over a 10 min period. When the DV's of more than one chick were being counted at one time, a tape recording was made to facilitate counting after the experiment was over. Immediately after obtaining the baseline rate of

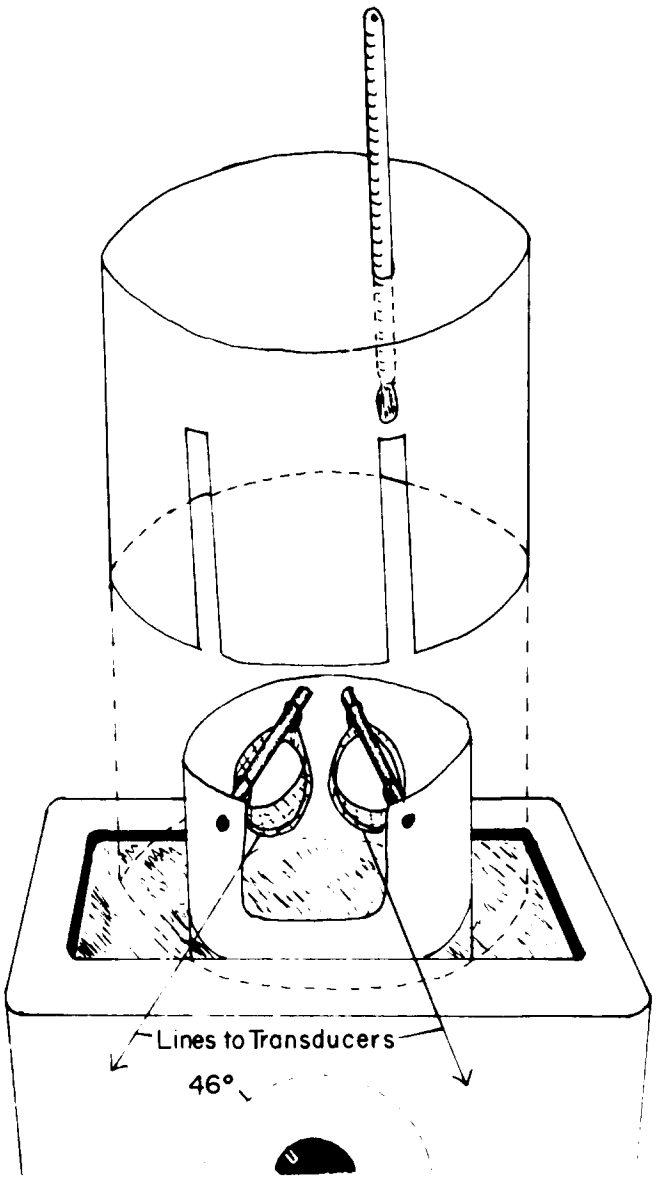

FIG. 1. Apparatus for measuring activity of chick embryo during the last week of incubation. Eggs are suspended in hammocks above a heated water bath. A cylindrical Plexiglas hood maintains proper temperature and humidity during activity recording. Embyro movements are recorded via pressure transducers connected to each egg. (See text for detailed description of the procedure).

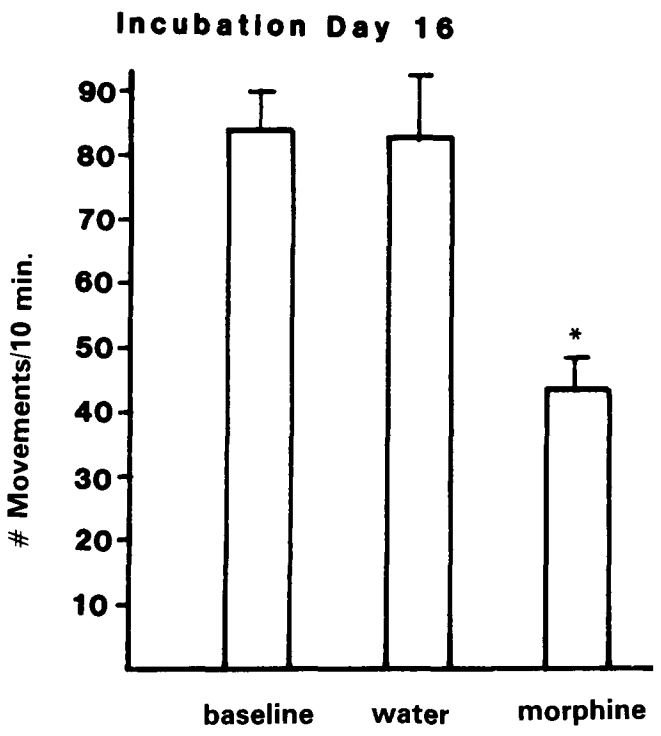

FIG. 2. Response of day 16 chick embryos to morphine or water injection. Activity of naive chick embryos was determined on incubation day 16 for 10 min. Immediately after obtaining the baseline rate of activity, the eggs were injected with either 20 mg MS/kg or H_2O and were returned to the incubator. After 30 min activity was recorded again. Values represent mean±S.E.. n=26 for baseline, 13 each for water and morphine. *Significantly different from both baseline and water control using an unpaired Student's t-test for comparison with respective baseline, $t=6.28$, $p<0.001$, and an unpaired t-test for comparison with water control, $t=3.70$, $p<0.01$.

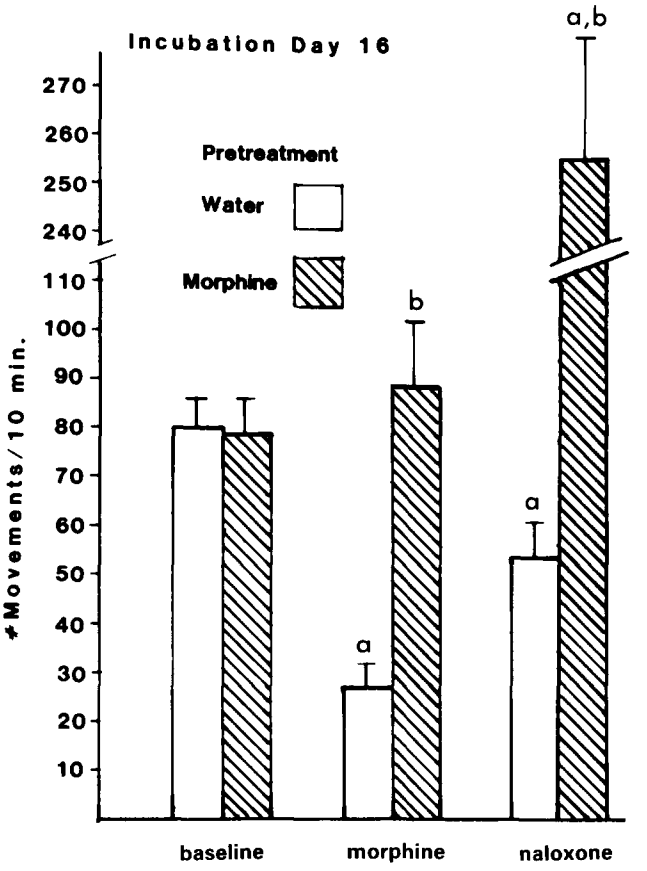

FIG. 3. Chick embyro activity after challenge with morphine or naloxone. Chick embryos were injected from incubation day 12 daily with either 20 mg MS/kg or H_2O. Embryo activity was measured prior to injection on incubation day 16 (baseline). Immediately after obtaining the baseline rate of activity, the eggs were injected with either 20 mg MS/kg or 0.8 mg naloxone/kg egg. The eggs that received MS were returned to the incubator and activity was measured again 30 min later. The eggs that received naloxone were replaced into the apparatus and activity was measured again 5 min after injection. Values represent mean±S.E. n=10 for baseline, 6 were challenged with naloxone, 4 with MS. [a]Significantly different from respective baseline using a paired Student's t-test (H_2O pretreated, MS challenge, $t=6.61$, $p<0.01$; H_2O pretreated, naloxone challenge, $t=7.59$, $p<0.001$; MS pretreated naloxone challenge, $t=11.54$, $p<0.001$). [b]Significantly different from respective H_2O-pretreated control using an unpaired Student's t-test (MS challenge, $t=4.31$, $p<0.01$; naloxone challenge, $t=7.77$, $p<0.001$).

DV, the chicks were injected and returned to the company of the other chicks. Distress vocalizations were counted again 20 min after morphine injection or 5 min after naloxone.

Statistics

Statistical significance of differences in rate of DV or embryo activity were determined using either paired or unpaired Student's t-tests as indicated [20].

RESULTS

Embryo Activity

In order to assess development of tolerance to morphine in the chick, it was necessary to know what effects were produced in the embryo by morphine injection. Embryo activity was measured in previously uninjected eggs on day 16 of incubation. Figure 2 shows the effect of MS (20 mg/kg) or H_2O injection on chick embryo activity. Morphine injection produced a significant decrease in embryo activity whereas injection with H_2O did not produce any alteration of embryo activity.

The next experiment examined the development of tolerance to morphine in the chick embryo on incubation day 16. Results are shown on Fig. 3. The chick embryos were injected with either MS or H_2O once daily starting on incubation day 12. Baseline activity was recorded prior to injection on day 16. Baseline activity rates of the MS and H_2O pretreated embryos did not differ from each other. Some of the eggs were then injected with MS (20 mg/kg). As shown in Fig. 3, the morphine injection caused a significant decrease in the activity of the control eggs, similar to the decrease

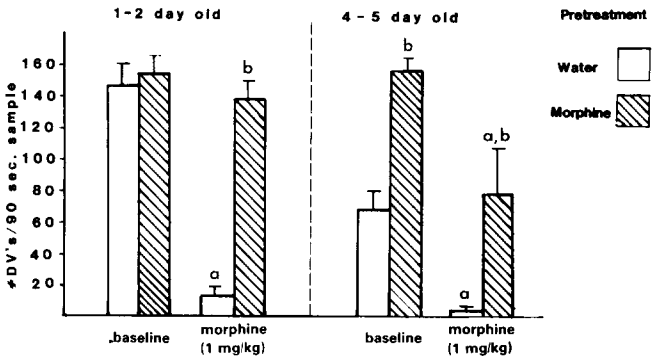

FIG. 4. Effect of morphine on DV's of young chicks. Chicks were injected from incubation days 12 to 19 daily with either 20 mg MS/kg or H_2O and were allowed to hatch. At the indicated ages, chicks were socially isolated for one minute and the number of DV's was counted during the first 10 sec of each of the next 9 min. Immediately after obtaining the baseline rate of DV, the chicks were injected with 1 mg MS/kg. Rate of DV was determined again 20 min later. Values represent mean±S.E. See Table 1 for more information concerning these chicks. [a]Significantly different from respective baseline using a paired Student's t-test (1–2 day old, $t=9.23$, $p<0.001$; 4–5 day old, H_2O pretreated, $t=2.70$, $p<0.05$; 4–5 day old, MS pretreated, $t=2.88$, $p<0.05$). [b]Significantly different from respective H_2O-pretreated control using an unpaired Student's t-test (1–2 day old, $t=8.39$, $p<0.001$); 4–5 day old, baseline, $t=3.75$, $p<0.01$; 4–5 day old, MS challenge, $t=2.39$, $p<0.05$).

produced in naive eggs shown on Fig. 2. The eggs that had received daily injections of MS from incubation day 12 for a total of 4 injections did not show any change from baseline activity in response to morphine challenge. The remaining eggs were injected with naloxone. Control embryos responded with a significant decrease in activity after naloxone injection. The MS-pretreated embryos responded to naloxone injection with a very large increase in activity.It should be pointed out that in Fig. 3, the baseline activities represented all eggs of each pretreatment group, whether they were subsequently challenged with morphine or naloxone. Statistical analysis was done using paired t-test, comparing baseline activity with the activity after challenge, since each egg was used as it own control. Therefore, the MS and naloxone challenged eggs were compared only with the baseline activity obtained from the eggs of that particular challenge group. Since the eggs were randomly assigned to treatment groups, there were no differences in baseline activity that were dependent on the challenge drug used.

Posthatching Behavior

The distress vocalizations of socially isolated chicks were quantified at the ages of 1–2 days and 4–5 days (age range of 1 day due to variable hatch time). Results for both ages are shown on Fig. 4. At 1–2 days of age, the baseline rate of DV was the same for the chicks treated with H_2O or MS from incubation day 12 to 19. Injections of 1 mg MS/kg greatly decreased the rate of DV of the control chicks whereas the MS-pretreated chicks continued to vocalize at a high rate. At 4–5 days of age, the baseline rate of DV of control chicks was decreased relative to the baseline rate of 1–2 days of age. In

TABLE 1

CHANGE IN RATE OF DV PRODUCED BY MORPHINE IN CHICKS

Age	Treatment	No. Chicks	Body Weights	Change in DV's
1–2 days	H_2O	8	38.4 ± 1.1	−132 ± 14
	MS	11	37.1 ± 0.8	− 16 ± 11*
4–5 days	H_2O	6	49.6 ± 1.1	− 64 ± 24
	MS	7	42.3 ± 1.3*	− 76 ± 27

See Fig. 4 for description of experimental design. Change in DV was calculated for each chick by subtracting the baseline DV rate from the rate after morphine injection.

*Significantly different from respective control using unpaired Student's t-test (Body weights, $t=4.21$, $p<0.01$; DV's, $t=6.51$, $p<0.001$).

contrast, the baseline rate of DV of the 4–5 day old MS-pretreated chicks was comparable to that of the 1–2 day old chicks and was significantly elevated over the baseline rate of the 4–5 day old controls.Both the H_2O-pretreated and MS-pretreated chicks responded to MS injection with significant decrease in rate of DV at the age of 4–5 days. However, the rate of DV of the MS-pretreated chicks after morphine was elevated over the rate of DV of controls after morphine.

Table 1 contains additional data concerning the groups of chicks that were used in the experiments depicted on Fig. 4. At 1–2 days of age, the body weights of the two groups of chicks were not significantly different. At 4–5 days of age, the MS-pretreated birds had significantly lower body weights than the controls. The change in DV's represented the response to morphine injection and was calculated for each chick by subtracting the baseline rate of DV from the rate after receiving 1 mg MS/kg. At 1–2 days of age, the response of the MS-pretreated chicks to morphine was less than the response of the H_2O-pretreated chicks to morphine. At 4–5 days of age, however, the change in DV's was not different between the MS- and H_2O-pretreated groups of chicks.

Naloxone was administered in order to elicit signs of abstinence as evidence of tolerance in the MS-pretreated birds. The effect of naloxone on the rate of DV of 1–2 day old chicks is shown in Fig. 5. In this experiment, the DV's were recorded from pairs of chicks, instead of single chicks. Naloxone injection (0.5 mg/kg) increased the DV's in both treatment groups when compared to respective baseline rate. However, the rate of DV of the MS pretreated birds after naloxone was significantly elevated over the naloxone-injected controls.

DISCUSSION

This report confirms earlier findings [7,8] of the production of tolerance to morphine in the chick embryo. Using the technique of chick embryo activity, the chick has been shown to be capable of displaying tolerance to morphine on day 16 of incubation. The tolerance persists for several days after receiving the last injection of morphine. In addition, signs of abstinence are detectable after injection of the chick embryos with naloxone.

The experiments utilizing chick embryos were performed to determine at what age the chick develops tolerance to

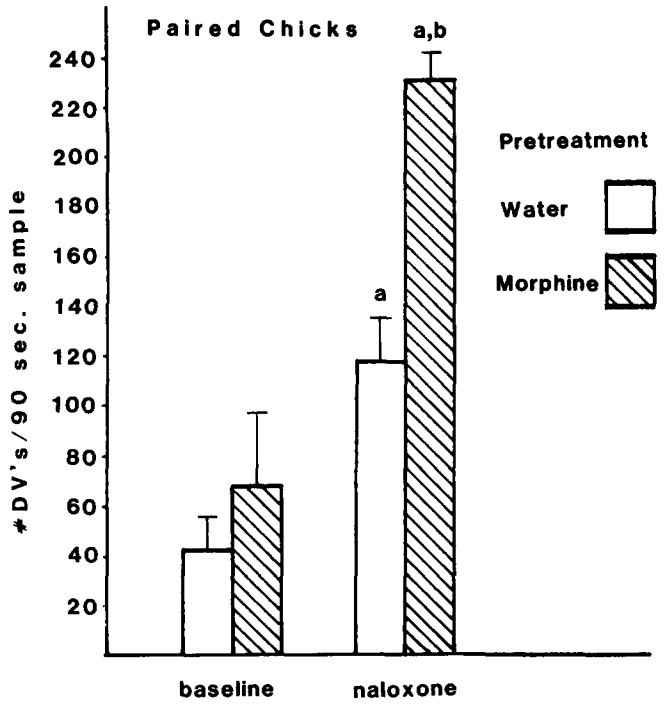

FIG. 5. Naloxone-induced increase in rate of DV of paired chicks. Chicks were injected from incubation days 12 to 19 daily with either 20 mg MS/kg or H_2O and were allowed to hatch. When the chicks were 1–2 days old, pairs of chicks were removed from the company of the other chicks. After 1 min the number if DV's was counted during the first 10 sec of the next 9 min. Immediately after obtaining the baseline rate of DV, the chicks were injected with 0.5 mg naloxone/kg. Rate of DV was determined again 5 min after injection. Values represent mean±S.E. n=5 pairs of chicks per group. [a]Significantly different from respective baseline using a paired Student's t-test (H_2O pretreated, $t=4.47$, $p<0.02$; MS pretreated, $t=5.31$, $p<0.01$). [b]Significantly different from respective H_2O-pretreated control using an unpaired Student's t-test, $t=5.10$, $p<0.01$.

morphine. Incubation day 16 was about the earliest age at which embryo movements could be reliably quantified using our method and this limits detection of tolerance earlier.

The lack of response of the MS-pretreated embryos to morphine challenge on incubation day 16 indicates that the embryos were tolerant to morphine at this time (Fig. 3). Further evidence for production of tolerance and dependence in these embryos is provided by the large increase in embryo activity after challenge with naloxone (Fig. 3). The MS-pretreated embryos were observed to be in almost constant motion during the recording of activity following naloxone challenge. This response of the MS-pretreated embryos to naloxone, when contrasted to the slight decrease in activity produced by naloxone in the controls points to the production of an abstinence syndrome in the tolerant chicks.

It has been reported [3] that narcotic-addicted pregnant women may experience excessive fetal activity when deprived of narcotics. This is interesting in light of the increased embryo activity during precipitated abstinence in this report.

Counting distress vocalizations proved to be an easy and effective means of detecting morphine tolerance. The socially-isolated birds will normally vocalize at a high rate, but the rate will dramatically decrease upon administration of low doses of morphine or other opiates. The findings described in this report concerning the control chicks are in agreement with Panksepp's work [15].

The distress vocalizations of the 1–2 day old control (H_2O-pretreated) chicks were greatly decreased by morphine (Fig. 4 and Table 1). In contrast, the MS-pretreated chicks continued DV's at a high rate after MS injections; i.e. they were tolerant to the conforting or silencing effect of morphine. These birds received the last embryonic dose of morphine on incubation day 19, three days before testing. The presence of complete tolerance to this effect of morphine would suggest that they continued to receive morphine (possibly stored in the spare yolk) beyond the final injection. Another possibility is that the morphine is metabolized at a very slow rate in the chick embryo.

The findings in the 4–5 day old chicks stand in contrast to the findings in the 1–2 day old chicks. The decrease in baseline rate of DV in control chicks from day 1 to day 4 is in agreement with findings of Panksepp (personal communications) who has stated that the highest rate of DV is obtained shortly after hatching. The baseline rate of the MS-pretreated chicks was essentially the same at both ages. The rate of DV of the 4–5 day old MS-pretreated chicks was higher than the control rate for both baseline and after MS injection. But, as shown in Table 1, the actual reponse to morphine was not different between the two pretreatment groups. This suggests that the MS-pretreated chicks are not significantly tolerant to morphine at the age of 4–5 days, whereas tolerance is present at the age of 1–2 days. The depressed body weights of the MS-pretreated chicks at 4–5 days (Table 1) and the occurrence of diarrhea in these birds indicate that the birds are undergoing morphine withdrawal. It is possible that the discomfort due to withdrawal is responsible for the increased DV's at both the baseline test and after morphine challenge.

The injections of naloxone, a narcotic antagonist, were done to precipitate withdrawal and hence to demonstrate the presence of tolerance and dependence to morphine in the MS-pretreated chicks. Since MS is known to decrease the rate of DV one would expect that naloxone would have the opposite effect of increasing the rate of DV. However, it is difficult to increase the rate of DV of isolated chicks [11]. Since the rate of vocalization decreases in the company of other chicks, pairs of chicks were used in this experiment. The depressed baseline produced by pairing the chicks allowed for the detection of an increased rate of DV in response to naloxone in both the control and MS-pretreated chicks. However, the rate of DV of the MS-pretreated chicks after naloxone was nearly twice that of the controls (232±11 versus 118±19 as shown in Fig. 5). This is evidence of the production of abstinence and hence the development of morphine dependence in the MS-pretreated chicks. The increase in rate of DV produced by naloxone in the control pairs of chicks agrees with the hypothesized role of endogenous opiate systems in social attachments [13].

Studies using rats have demonstrated tolerance to the analgesic effect of morphine (using a hotplate) in offsrping that were treated with morphine *in utero* [6,10]. Others have found that methadone administration produced alterations in brain RNA and protein content plus persistent behavioral deficits [17]. The developing chick offers some advantage

over mammalian species for study of developmental toxicity. Since the chick can be reared in the laboratory apart from the hen and substances can be administered directly to the egg, maternal influences on development are eliminated. We have described the production of tolerance and dependence to MS in the chick embryo and some behavioral manifestations of abstinence in the chick embryo and developing chick.

In a recent report, activity of day 19 embryos was measured after a single injection of a long acting opioid agonist on incubation day 3 [7]. In agreement with our findings, naloxone challenge caused increased embryo activity in the opiate-pretreated embryos. However, in contrast to the results presented here, the baseline activities of the pretreated embryos were lower than the activity of the controls. In addition, the activity after naloxone challenge (1.7 mg/kg) was the same for the two treatment groups, i.e. naloxone caused the depressed activity of the pretreated embryos to become comparable with the control baseline activity. The authors present this as evidence of opiate tolerance and withdrawal, but since they did not measure the response of the embryos to opiate agonist challenge, tolerance was not measured [7]. In fact the depressed activity of the pretreated embryos at day 19 would suggest that tolerance was not present. The magnitude of the embryo's responses to naloxone was not as great as that reported here, in spite of the fact that the lowest dose of naloxone used was more than twice the dose used in these studies (1.7 vs 0.8 mg/kg). Since there is evidence that the effects of naloxone are not completely specific for antagonism of opioid effects [21], the nature of the behavioral response to naloxone in this report [7] is questionable.

The present studies present evidence of the production of tolerance to and dependence on MS in chick embryos and describe some behavioral manifestations of abstinence in the chick embryo and developing chick.

REFERENCES

1. Bardo, M. T. and R. A. Hughes. Shock-elicited flight response in chickens as an index of morphine analgesia. *Pharmac. Biochem. Behav.* **9:** 147–149, 1978.
2. Buchenauer, D., M. Turnbow and M. A. Peters. Effect of chronic methadone administration on pregnant rats and their offspring. *J. Pharmac. exp. Ther.* **189:** 66–71, 1974.
3. Cobrinik, R. W., R. T. Hood and E. Chusid. Review: The effect of maternal narcotic addiction on the newborn infant. *Pediatrics* **24:** 288–304, 1959.
4. Herman, B. H. and J. Panksepp. Effects of morphine and naloxone on separation distress and approach attachment: Evidence for opiate mediation of social affect. *Pharmac. Biochem. Behav.* **9:** 213–220, 1978.
5. Hutchings, D. E., H. F. Hunt, J. P. Towey, T. S. Rosen and H. S. Gorinson. Methadone during pregnancy in the rat: Dose level effects on maternal and perinatal mortality and growth in the offspring. *J. Pharmac. exp. Ther.* **197:** 171–179, 1976.
6. Johannesson, T. and B. A. Becker. The effects of maternally-administered morphine on rat foetal development and resultant tolerance to the analgesic effect of morphine. *Acta pharmac. tox.* **31:** 305–313, 1972.
7. Kuwahara, M. D. and S. B. Sparber. Prenatal withdrawal from opiates interferes with hatching of otherwise viable chick fetuses. *Science* **212:** 945–947, 1981.
8. Newby-Schmidt, M. B. and S. Norton. Behavioral effects of repeated morphine injection on the developing chick. *Toxicologist* **1:** Abstr. 106, 1981.
9. Newby-Schmidt, M. B. and S. Norton. Alterations of chick locomotion produced by morphine treatment *in ovo*. *Neurotoxicology*, accepted for publication.
10. O'Callaghan, J. P. and S. G. Holtzman. Prenatal administration of morphine to the rat: Tolerance to the analgesic effect of morphine in the offspring. *J. Pharmac. exp. Ther.* **197:** 533–544, 1976.
11. Panksepp, J., N. J. Bean, P. Bishop, T. Vilberg and T. L. Sahley. Opioid blockade and social comfort in chicks. *Pharmac. Biochem. Behav.* **13:** 673–683, 1980.
12. Panksepp, J., B. Herman, R. Conner, P. Bishop and J. P. Scott. The biology of social attachments: Opiates alleviate separation distress. *Biol. Psychiat.* **13:** 607–618, 1978.
13. Panksepp, J., B. H. Herman, T. Vilberg, P. Bishop and F. G. DeEskinazi. Endogenous opioids and social behavior. *Neurosci. Biobehav. Rev.* **4:** 473–487, 1978.
14. Panksepp, J., R. Meeker and N. J. Bean. The neurochemical control of crying. *Pharmac. Biochem. Behav.* **12:** 437–443, 1980.
15. Panksepp, J., T. Vilberg, N. J. Bean, D. H. Coy and A. J. Kastin. Reduction of distress vocalizations in chicks by opiate-like peptides. *Brain Res. Bull.* **3:** 663–667, 1978.
16. Pert, C. B., D. Aposhian and S. H. Snyder. Phylogenetic distribution of opiate receptor binding. *Brain Res.* **75:** 356–361, 1974.
17. Peters, M. A. The effect of maternally administered methadone on brain development in the offspring. *J. Pharmac. exp. Ther.* **203:** 340–346, 1977.
18. Peters, R. H. and R. A. Hughes. Naloxone interactions with morphine-and shock-potentiated tonic immobility in chickens. *Pharmac. Biochem. Behav.* **9:** 153–156, 1978.
19. Schneider, C. Effects of morphine-like drugs in chicks. *Nature* **191:** 607–608, 1961.
20. Steel, R. G. D. and J. H. Torrie. *Principles and Procedures of Statistics*. New York: McGraw-Hill Book Co., 1960, pp. 67–80.
21. Zuluzny, S. G., G. B. Chesher, D. M. Jackson and R. Mator. The attenuation by Δ^9-tetrahydrocannabinol and morphine of the quasi-morphine withdrawal syndrome in rats. *Psychopharmacology* **61:** 207–216, 1979.