Pharmacology Biochemistry and Behavior, Vol. 42, pp. 535–539, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

RAPID COMMUNICATION

Morphine Hyperalgesic Effects on Developmental Changes in Thermal Nociception and Respiration in Domestic Fowl (*Gallus gallus*)

RICHARD A. HUGHES,[1] MARK BOWES AND KENNETH J. SUFKA

Department of Psychology, Iowa State University, Ames, IA 50011-3180

Received 19 February 1992

HUGHES, R. A., M. BOWES AND K. J. SUFKA. *Morphine hyperalgesic effects on developmental changes in thermal nociception and respiration in domestic fowl* (Gallus gallus). PHARMACOL BIOCHEM BEHAV 42(3) 535–539, 1992.— Domestic fowl tested at 3, 5, and 7 days posthatch jumped from a heated grid more rapidly than animals tested at 14 days posthatch. Morphine (2.5 mg/kg) decreased jump latency in 14-day-old chicks but did not significantly affect jump latency in younger chicks. Respiration was lower in 3-day-old chicks than in the older groups but morphine depressed respiration at each age. In a second experiment morphine significantly decreased jump response latency in 5-day-old chicks when thermal stimulus intensity was lowered and morphine dose increased (5 mg/kg). Posttest respiration rate was depressed by morphine. Morphine hyperalgesia and respiratory depression were reversed by naloxone (5 mg/kg). However, naloxone alone increased jump response latency. Young domestic fowl are more sensitive and/or reactive to a noxious thermal stimulus and are less sensitive to morphine than 14-day-old chicks but morphine hyperalgesia was evident in both 5- and 14-day-old chicks. These hyperalgesic chicks may be tolerant at birth to morphine hypoalgesic effects on nociception.

Morphine Hyperalgesia Nociception Respiration Naloxone Hot-plate Ontogeny Developmental Domestic fowl Chicken Cockerel Avian Opiate

ACUTE administration of morphine produces hypoalgesia against various measures of nociception in most animal models (1,13). This antinociceptive morphine effect is attenuated by the opioid antagonist naloxone and appears to be mediated primarily by μ and κ opioid receptors (31). In striking contrast to the typical hypoalgesic effects of morphine, acute morphine administration in domestic fowl produces hyperalgesia on tests of thermal and chemical nociception (9,10,12). This morphine hyperalgesic effect is strain dependent, naloxone sensitive, and like morphine hypoalgesia in other species, appears to be mediated primarily by μ and κ opioid receptors at central nervous system loci (9,27). Morphine-induced hyperalgesic effects have been observed in rodents after chronic morphine administration (14) and at long intervals after acute morphine administration (7), but these morphine hyperalgesic effects are preceded by hypoalgesia. Morphine hyperalgesia in domestic fowl are atypical; they occur on first exposure to morphine and are not preceded by hypoalgesia (12,24). This unusual morphine effect may be unique to nociception in fowl, however, because the characteristic respiratory depression induced by morphine in other animals also occurs in hyperalgesic fowl (12,27).

The data on morphine hyperalgesia in domestic fowl were obtained with chicks 14–17 days old. Unpublished observations from our laboratory show similar morphine effects in fowl 21–28 days of age. Although we have evaluated thermal nociception in younger fowl (11) we have not systematically evaluated morphine effects on nociception in chicks younger than 14 days of age. Substantial changes in the substrates of nociception and the behavioral manifestation of nociception occur within this early postnatal period both in rat pups (5,6,22) and in chicks (2,11). The present study was, therefore, designed to examine the ontogeny of morphine effects on thermal nociception in domestic fowl at 3, 5, 7, and 14 days after

[1] To whom requests for reprints should be addressed.

hatching. Respiration was also monitored to provide a second index of morphine effects.

EXPERIMENT 1

METHOD

Subjects

Eighty cockerels (Welp-Line 542 commercial stock; Welp Inc., Bancroft, IA) were obtained 1 day after hatching. Chicks were housed in pairs in enclosures (described below) that provided physical separation without visual or auditory isolation from other chick pairs. Animals were maintained under 24-h overhead fluorescent illumination at a room temperature of 32.0 ± 1.0°C for the first week and 29.0 ± 1.0°C thereafter. Food (Wayne pullet starter, St. Charles, MO) and water were continuously available in the home cage.

Apparatus

Four separate 125 × 56 × 30 cm housing enclosures were constructed of hardware cloth. Each enclosure was divided into 25 × 28 cm compartments (10 compartments per enclosure) with outer walls surrounded by white cotton cloth fabric. The test apparatus consisted of a 16 × 29 × 30 cm Plexiglas chamber with hinged lid. The walls of the chamber were covered with white paper except for a 5-cm window near the chamber floor for observation of the apparatus interior. The floor was composed of eight glass tubes 1-cm diam spaced 2 cm center to center and mounted in holes drilled through the walls of the Plexiglas chamber. The tubes were heated by a nichrome wire heating element (Glocoil Incubator, Eagle Manufacturing Company, Long Island City, NY), threaded through the glass tubing of the test chamber floor. Temperature was regulated by a 7.5 Å variable transformer (Standard Electric Co., Dayton, OH, model 300BU) and monitored by a digital thermometer. One end of the test chamber was occupied by a 12.5 × 15.5 × 7 cm wooden platform that rested on the grid floor. Response latencies were measured to the nearest 0.1 s with an electronic timer.

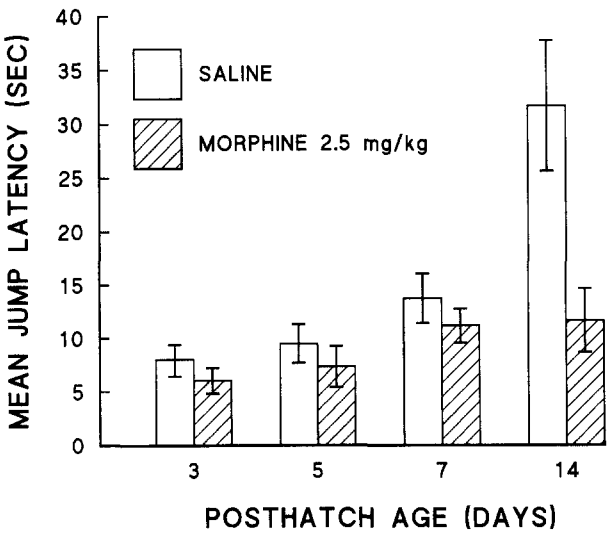

FIG. 1. Mean latency to jump from a heated grid as a function of posthatch age in domestic fowl given saline or morphine (vertical lines = SEM).

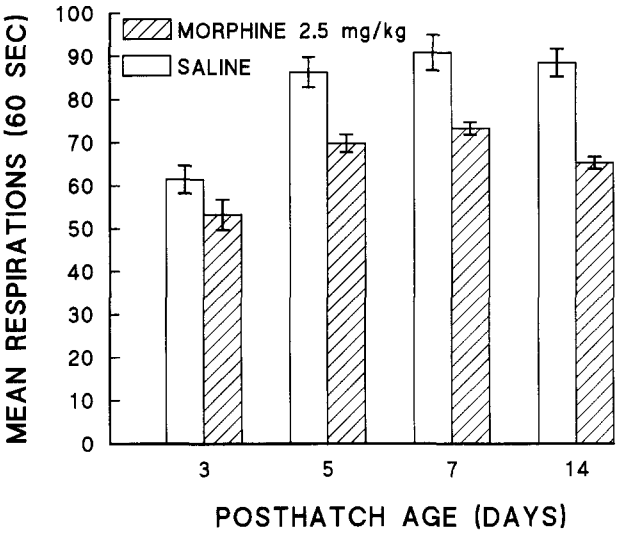

FIG. 2. Mean number of posttest respirations as a function of posthatch age in domestic fowl given saline or morphine (vertical lines = SEM).

Procedure

The experimental design was a 2 × 4 factorial (n = 10 per cell) which combined two levels of drug (saline vs. morphine) with 4 age levels (3, 5, 7, 14 days after hatching). Each housing unit was randomly assigned to one age level and within each unit chicks were randomly assigned to one of the two drug levels. At the appropriate test age a chick was removed from the housing unit, weighed to the nearest 0.1 g, and injected with morphine sulfate (2.5 mg/kg, IM) or the 0.9% saline vehicle (volumes = 1.0 ml/kg). Chicks were color coded under one wing with a felt tip marker pen for group identification and returned to the home cage.

Testing for thermal nociception occurred 30 min after injection. A subject was taken from the housing unit, placed in an opaque cylindrical plastic container (14 cm diam × 19 cm high) which was covered with a vented lid, and carried to an adjacent room where behavioral testing was conducted. Chicks were placed on the glass grid (temperature = 80 ± 0.3°C monitored by a thermistor placed against the interior surface of the glass tubing) facing away from the raised platform. An electronic timer was started as the chick's feet touched the grid. The apparatus lid was lowered quietly and timing continued until the animal jumped (both feet completely off the grid), stepped onto the wooden platform, or 70 s elapsed. The chick was then immediately removed from the apparatus and the number of respirations (rhythmic chest movements) was counted for 60 s after which the chick was returned to its home cage.

RESULTS

Inspection of the jump latency (JLAT) data, summarized in Fig. 1, shows an age-related increase in latency that is attenuated by morphine. In support of the visually apparent effects displayed in Fig. 1, analysis of variance (ANOVA) indicated that JLAT increased significantly with age [$F(3, 72) = 10.84$, $p < 0.0001$] and decreased with morphine treatment [$F(1, 72) = 11.16$, $p = 0.0013$]. These main effects were qualified by a significant age × drug interaction [$F(3, 72) = 4.95$,

$p = 0.0035$]. Simple main effects analysis (17) for drug at each age level indicated that morphine decreased JLAT significantly only in the oldest age group [$F(1, 72) = 25.07$, $p < 0.01$] and that age was significant only at the saline level [$F(3, 72) = 14.83$, $p < 0.01$]. Post-hoc analysis of the saline groups by power adjusted t-tests (17) demonstrated that the mean JLAT for the 14-day-old chicks was significantly greater than the mean JLATs displayed by the other three ages ($p^s < 0.01$); the 3, 5, and 7 day age comparisons were not significantly different. In brief summary, JLAT showed a consistent increase as a function of posthatch age and although morphine attenuated JLAT at each age, these effects were significant only for the relevant 14-day age group comparisons.

The respiration data are summarized in Fig. 2. In general, respiration rate increased with increased age and morphine attenuated respiration at each age. An ANOVA demonstrated a significant main effect for age [$F(3, 72) = 27.7$, $p < 0.0001$] and drug [$F(1, 72) = 60.49$, $p < 0.0001$]. The age $\times$ drug interaction was not significant. For both morphine and saline treatment mean respiration for 3-day-old chicks was significantly lower than that of all older same treatment age groups ($p^s < 0.05$). Comparisons among the older treatment groups were not significant for same treatment comparisons across ages. Furthermore, power-adjusted t-tests demonstrated that morphine significantly decreased respiration at each age ($p < 0.05$ at age 3; <0.01 for each of the remaining age comparisons).

EXPERIMENT 2

The results of Experiment 1 suggest that chicks 3, 5, and 7 days of age may be less sensitive to morphine effects on thermal nociception than 14-day-olds. Alternatively, response latency displayed by the younger chicks may have been nearly asymptotic and therefore not able to reflect morphine hyperalgesic effects. Experiment 2 was designed to examine this possibility by evaluating morphine effects in 5-day-old chicks at a lower thermal stimulus intensity than used in Experiment 1. This manipulation should result in longer response latencies and increase the likelihood of obtaining morphine hyperalgesia. Morphine effects on respiration were also evaluated in this experiment and morphine interactions at opioid receptors were evaluated by the use of the opioid receptor antagonist naloxone.

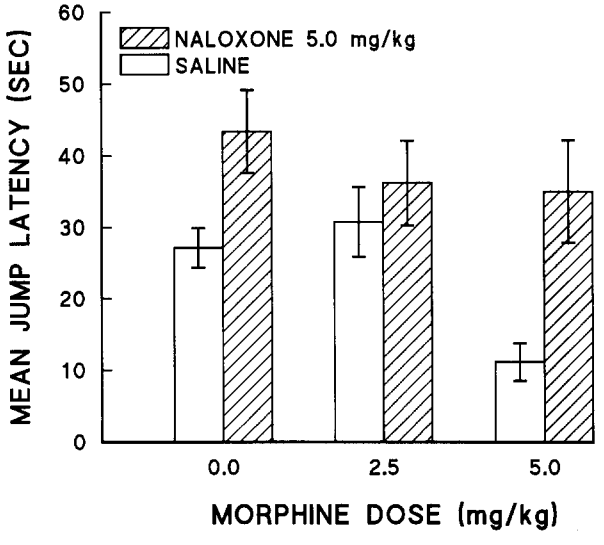

FIG. 3. Mean latency to jump from a heated grid as a function of morphine dose in 5-day-old chicks pretreated with saline or naloxone (vertical lines = SEM).

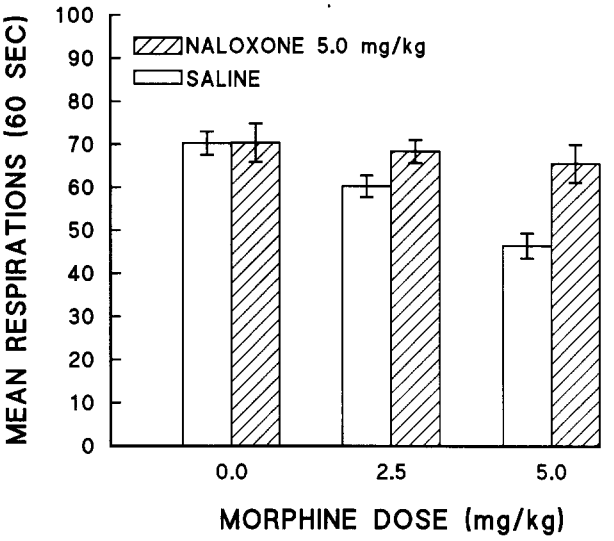

FIG. 4. Mean number of posttest respirations as a function of morphine dose in 5-day-old chicks pretreated with saline or naloxone (vertical lines = SEM).

METHOD

Subjects

Sixty cockerels (Welp-Line 542 commercial stock) were obtained one day after hatching and were housed and maintained as described in Experiment 1. Apparatus and procedure were generally as described in Experiment 1 except that animals received two IM injections one immediately after the other and the grid temperature for thermal nociception was lowered to 74 ± 0.3°C. The design of this experiment was a 2 × 3 factorial which combined two levels of naloxone hydrochloride (0.0 vs. 5.0 mg/kg) with three levels of morphine sulfate (0.0, 2.5, or 5.0 mg/kg). The drugs were dissolved in sterile 0.9% saline and administered at 1.0 ml/kg. At 5 days of age animals were randomly assigned to receive one of the six drug injection combinations and tested 30 min later for treatment effects on thermal nociception and respiration using procedures described in Experiment 1.

RESULTS

Jump latency (JLAT) data are summarized in Fig. 3. These data show that 5.0 mg/kg morphine decreased JLAT and this decrease was reversed by naloxone. Moreover, naloxone increased JLAT at the 0.0 morphine dose. In support of these trends, an ANOVA demonstrated a significant main effect of naloxone dose [$F(1, 54) = 13.17$, $p < 0.0006$] and morphine dose [$F(2, 54) = 3.31$, $p = 0.044$]. The naloxone $\times$ morphine interaction was not significant. Pairwise comparisons for saline vs. naloxone at each morphine dose demonstrated significant differences at the 0.0 ($p < 0.05$) and 5.0 ($p < 0.01$) mg/kg morphine doses. The saline group at the 5.0 mg/kg morphine dose differed significantly from saline groups at

the two lower morphine doses ($p < 0.05$ at 2.5 mg/kg; <0.05 at 0.0 mg/kg). There were no significant differences in JLAT among the naloxone groups.

Respiration data are summarized in Fig. 4. Morphine reduced respiration and naloxone reversed this morphine effect. The main effect of morphine dose was significant [$F(2, 54) = 9.01, p = 0.0004$] as was the main effect of treatment with naloxone or saline [$F(1, 54) = 10.85, p = 0.0017$]; the interaction term, however, was also significant [$F(2, 54) = 3.99, p = 0.02$]. Comparison across morphine dose for saline and naloxone treatments indicated that respiration declined significantly for saline [$F(2, 54) = 12.5, p < 0.0001$] but not for naloxone. Comparison of saline and naloxone treatments at each morphine dose revealed a significant difference only at the 5.0 mg/kg morphine dose [$F(1, 54) = 15.99, p = 0.0002$]. Pairwise comparisons among the saline treatment groups demonstrated significant differences between the 5.0 morphine dose and the two lower doses ($p < .05$ at 2.5 mg/kg; $p < 0.01$ at 0.0 mg/kg).

DISCUSSION

The present study was designed to examine morphine effects on thermal nociception in young fowl at 3, 5, 7, and 14 days of age. Consistent with earlier research (11), the results of Experiment 1 demonstrated an age-related increase in jump latency. Animals tested at 14 days of age displayed longer jump response latencies than the younger animals. Also consistent with earlier research (9,24,27) the jump latency at 14 days posthatch was significantly faster in animals given 2.5 mg/kg morphine sulfate. This morphine dose did not significantly affect jump response latency of 3-, 5-, or 7-day-old chicks. These young animals were not insensitive to morphine effects, however, because respiration was depressed by morphine at each test age. It appeared likely that the rapid response latencies displayed by the youngest chicks in the saline condition reduced the likelihood that morphine could produce a further decrease in response latency. This possibility was examined in Experiment 2.

In Experiment 2, 5-day-old chicks given saline injections displayed relatively long jump latencies from a grid maintained at a lower temperature than used in Experiment 1. Morphine at 2.5 mg/kg did not significantly affect jump latency but a 5.0 mg/kg dose did. These results indicate that 5-day-old chicks are less sensitive to morphine hyperalgesic effects than their 14-day-old counterparts in which 2.5 mg/kg morphine produced hyperalgesia. As suggested by the results of Experiment 1, 5-day-old animals are not generally insensitive to 2.5 mg/kg morphine because this dose, and the higher 5.0 mg/kg dose, significantly depressed respiration. Although young chicks may be less sensitive to morphine hyperalgesic effects than their 14-day-old counterparts, they nevertheless display hyperalgesia in response to their first exposure to morphine. Morphine hyperalgesic effects are atypical on first exposure to morphine but have been observed to occur in morphine tolerant animals (14). Morphine hyperalgesia in domestic fowl is strain dependent (9,26) and this suggests the possibility that selective breeding may have produced a model that is morphine tolerant at birth. Monoamines play a significant role in morphine hypoalgesic effects and we have recently shown that hyperalgesic fowl differ from hypoalgesic fowl in brain but not spinal monoaminergic response to morphine (26). Thus, one interpretation of morphine hyperalgesia in domestic fowl is that it reflects tolerance to morphine produced by genetically determined changes in the profile of monoaminergic response to morphine.

The age-related increase in jump latency observed in Experiment 1 corresponds well with the emergence and age-dependent increase in fear indicators in this precocial avian species (4,8,11,20,21). This increase in jump latency may, therefore, reflect a stress-induced analgesic effect (15,28) produced by social separation (25) and by the predatory component of an initial encounter with the experimenter (23). We have demonstrated a robust hypoalgesic effect produced by experimenter contact and brief social separation in 7-day-old domestic fowl. This effect can be attenuated by handling and by the presence of social companions (25). Animals in the present study were not handled before morphine administration and they were separated briefly from their social companions for the nociceptive test session. It is, therefore, very likely that the response latencies displayed by animals in the present study reflect a stress-induced hypoalgesic component that increases within the first 2 weeks after hatching as a concomitant of increased fear.

Although contrary evidence exists (30), substantial evidence demonstrates that morphine can decrease and naloxone can increase some indices of stress elicited by social separation (16,19). Therefore, a plausible explanation for the present results is that naloxone produced hypoalgesia by increasing stress, morphine produced hyperalgesia by decreasing stress, and when administered together these competitive opiates interacted, presumably at opioid receptors, to produce intermediate effects (see Fig. 3). This interpretation necessarily assumes that, in the present avian model, morphine and naloxone act on systems that subserve social comfort (19) and that the stress of social separation elicits a nonopioid hypoalgesia (3,29). Without this assumption morphine and naloxone would be expected to increase and decrease, respectively, the index of nociception (16). This interpretation is consistent with the earlier suggestion that the avian model used in the present study is born tolerant to morphine effects on nociception.

ACKNOWLEDGEMENTS

An abbreviated version of these data was presented at the November 1991 meeting of the Psychonomic Society, San Francisco, CA. The authors thank Karin Rettig, Tammy McCormick, and James Borland for their assistance in data collection.

REFERENCES

1. Abbott, F. V.; Melzack, R.; Samuel, C. Morphine analgesia in the tail-flick and formalin pain tests is mediated by different neural systems. Exp. Neurol. 75:644–651; 1982.
2. Bardo, M. T.; Bhatnagar, R. K.; Gebhart, G. F.; Hughes, R. A. Opiate receptor development in midbrain and forebrain of posthatch chicks. Dev. Brain Res. 3:668–673; 1982.
3. Bodnar, R. J. Types of stress-inducing analgesia. In: Tricklebank, M. D.; Curzon, G., eds. Stress-induced analgesia. New York: John Wiley; 1984:19–32.
4. Colias, N. E. The development of social behavior in birds. Auk 69:127–159; 1952.
5. Fanselow, M. S.; Cramer, C. P. The ontogeny of opiate tolerance and withdrawal in infant rats. Pharmacol. Biochem. Behav. 31: 431–438; 1988.

6. Giordano, J.; Barr, G. Morphine- and ketocyclazocine-induced analgesia in the developing rat: Differences due to type of noxious stimulus and body topography. Dev. Brain Res. 32:247–253; 1987.
7. Hendrie, C. A. Naloxone sensitive hyperalgesia induced by morphine and environmental stimulation. Pharmacol. Biochem. Behav. 32:961–966; 1989.
8. Hughes, R. A. Shock-potentiated tonic immobility in chickens as a function of posthatch age. Anim. Behav. 27:782–785; 1979.
9. Hughes, R. A. Strain-dependent morphine-induced analgesic and hyperalgesic effects on thermal nociception in domestic fowl (*Gallus gallus*). Behav. Neurosci. 104:619–624; 1990.
10. Hughes, R. A. Codeine analgesic and morphine hyperalgesic effects on thermal nociception in domestic fowl. Pharmacol. Biochem. Behav. 35:567–570; 1990.
11. Hughes, R. A.; Sufka, K. J. The ontogeny of thermal nociception in domestic fowl: Thermal stimulus intensity and isolation effects. Dev. Psychobiol. 23:129–139; 1990.
12. Hughes, R. A.; Sufka, K. J. Morphine hyperalgesic effects on the formalin test in domestic fowl (*Gallus gallus*). Pharmacol. Biochem. Behav. 38:247–251; 1991.
13. Jaffe, J. H.; Martin, W. R. Opioid analgesics and antagonists. In: Gilman, A. G. S.; Goodman, L. S.; Rall, T. W.; Murad, T., eds. The pharmacological basis of therapeutics. New York: Macmillan Press; 1985:491–531.
14. Kayan, S.; Woods, L. A.; Mitchell, C. L. Morphine-induced hyperalgesia in rats tested on the hot plate. J. Pharmacol. Exp. Ther. 177:509–513; 1971.
15. Kelly, D. D., ed. Stress induced analgesia. Ann. NY Acad. Sci. 467:1–449; 1986.
16. Kehoe, P.; Blass, E. M. Opioid mediation of separation distress in 10-day old rats: Reversal of stress with maternal stimuli. Dev. Psychobiol. 19:385–398; 1986.
17. Kirk, R. E. Experimental design: Procedures for the behavioral sciences. Monterey, CA: Brooks/Cole; 1982.
18. Martin, W. R. Pharmacology of opioids. Pharmacol. Rev. 35: 283–323; 1984.
19. Panksepp, J.; Siviy, S.; Normansell, L. Brain opioids and social emotions. In: Reite, M.; Field, T., eds. The psychobiology of attachment and separation. New York: Academic Press; 1985:3–49.
20. Ratner, S. C.; Thompson, R. W. Immobility reactions (fear) in domestic fowl as a function of age and experience. Anim. Behav. 8:186–191; 1960.
21. Salzen, E. A. Imprinting and the immobility reactions of domestic fowl. Anim. Behav. 11:67–71; 1963.
22. Spain, J. W.; Roth, B. L.; Coscia, C. J. Differential ontogeny of multiple opioid receptors (μ, δ, and κ). J. Neurosci. 5:584–588; 1985.
23. Suarez, S. D.; Gallup, G. G., Jr. Open field behavior in chickens: The experimenter is a predator. J. Comp. Physiol. Psychol. 96: 432–439; 1982.
24. Sufka, K. J.; Hughes, R. A. Dose and temporal parameters of morphine-induced hyperalgesia in domestic fowl. Physiol. Behav. 47:385–387; 1990.
25. Sufka, K. J.; Hughes, R. A. Differential effects of handling on isolation-induced vocalizations, hypoalgesia, and hypothermia in domestic fowl. Physiol. Behav. 50:129–133; 1991.
26. Sufka, K. J.; Hoganson, D. A.; Hughes, R. A. Central monoaminergic changes induced by morphine in hypoalgesic and hyperalgesic strains of domestic fowl. Pharmacol. Biochem. Behav. (in press).
27. Sufka, K. J.; Hughes, R. A.; Giordano, J. Opioid-receptor mediation of morphine hyperalgesia in domestic fowl. Pharmacol. Biochem. Behav. 38:49–54; 1991.
28. Tricklebank, M. D.; Curzon, G., eds. Stress-induced analgesia. New York: John Wiley & Sons; 1984.
29. Watkins, L. R.; Mayer, D. J. Organization of endogenous opiate and nonopiate pain control systems. Science 216:1185–1192; 1982.
30. Winslow, J. T.; Insel, T. R. Endogenous opioids: Do they modulate the rat pup's response to social isolation? Behav. Neurosci. 105:253–263; 1991.
31. Wood, P. L.; Iyengar, S. Central actions of opiates and opioid peptides: In vivo evidence for opioid receptor multiplicity. In: Pasternak, G. W., ed. The opiate receptors. Clifton, NJ: The Humana Press; 1988:307–355.