Pharmacology Biochemistry & Behavior, Vol. 13, pp. 45–50. Printed in the U.S.A.

Ontogenesis of Morphine-Induced Behavior in the Rat

PATRICIA A. CAZA AND LINDA PATIA SPEAR

Department of Psychology
and
Center for Neurobehavioral Sciences, State University of New York at Binghamton, Binghamton, NY 13901

Received 29 February 1980

CAZA, P. A. AND L. P. SPEAR. *Ontogenesis of morphine-induced behavior in the rat.* PHARMAC. BIOCHEM. BEHAV. 13(1) 45–50, 1980.—Ten, seventeen and twenty-four day old rats were observed using a behavioral-time sampling procedure following injection of saline, 0.1, 0.5, 1.0 or 5.0 mg/kg/5cc morphine sulphate. At Day 10, the predominant response to morphine was a depression of locomotor activity. At the 5 mg/kg dose, catalepsy was also seen. In animals of this age, 0.1 mg/kg morphine, which was not sufficient to depress activity, also had no effect on stereotyped gnawing/mouthing behavior, nor did it produce any increase in locomotor activity. A morphine-induced increase in locomotion was first seen on Day 17 (after 0.5 mg/kg morphine). As with Day 10 animals, Day 17 animals given 5 mg/kg morphine showed a depression of locomotion and catalepsy. Stereotypic gnawing/mouthing behavior was first seen in Day 24 animals (after 5 mg/kg morphine), although no dose of morphine produced significant differences in activity at this age. Possible mechanisms resulting in these marked alterations in behavioral response patterns to morphine during this two week period of ontogeny are discussed.

Developmental psychopharmacology Morphine Locomotor activity Stereotyped gnawing/mouthing Catalepsy

EARLY reports indicating that neonates exhibit greater sensitivity to morphine than their adult counterparts [8,10] have prompted recent developmental studies of morphine toxicity and analgesic potency. Toxicity to the intraperitoneally administered opiate is high for the first 12 days postpartum in the rat (when the lethal dose in 50% of the animals (LD_{50}) is 45 mg/kg), after which it steadily decreases to reach an LD_{50} of 220 mg/kg by 32 days postnatally [14]. An increase in the analgesic effects in rats following subcutaneous injection of 1.0 mg/kg morphine was found to occur between Day 5 and Day 15, after which time the analgesic effect decreased until stabilizing at Day 30 [4]. Twenty-day old rats exhibit approximately 10 times greater sensitivity to morphine analgesia than adults, while 26-day old rats exhibit 3 times the sensitivity [12]. With respect to morphine tolerance, it has been reported that 21-day old rats display a larger increase in pain threshold after the first drug injection compared to adults, while subsequent injections produce a sharper decline in this threshold for the younger animals [16].

From such studies measuring toxicity of and analgesia to morphine, it appears that preweanling and weanling rats are markedly more sensitive to the effects of morphine than older animals. These ontogenic differences in the physiological consequences of morphine may be related to age-dependent alterations in brain morphine concentrations. For example, the concentration of morphine in the brains of 20-day old rats following subcutaneous injection is twice that found for 26-day old rats. Twenty-six day old rats have higher brain concentrations than 42 day-olds, and the levels of morphine in 42-day olds do not differ from the adult [12]. These findings have coincided with others reporting that the blood/brain ratio of morphine concentration increases between 15 and 30 days of age in rats [4]. Moreover, conjugation and blood clearance rates for morphine are also radically different between 16- and 32-day old rats, with the older animals having a much higher rate for both [14].

Although brain permeability to morphine appears to play an important role in the early responsiveness to morphine in the rat, another factor should also be considered—the ontogenetic development of the opiate receptor. Although opiate receptors are present in fetal rat brain [4,9], the greatest increases in receptor binding to ^{3}H-naltrexone and ^{3}H-naloxone occurs during the preweaning period. This increase in receptor binding is characterized by an increase in the number of receptors with no change in their affinity [9]. This increase in the number of opiate receptors might be related to the increase in morphine's analgesic effect in rats between postnatal Day 5 and 15 [4].

Whereas the ontogenetic patterns of morphine's physiological consequences are well established, studies examining the ontogeny of morphine-induced behavior are notably in absence. Although, in a recent study, morphine was reported to decrease spontaneous activity of the 18-day rat fetus [13], there has been a dearth of information on the behavioral response to morphine in the early postnatal period. While it is known that in adult animals, morphine can induce dose-dependent hyperactivity, hypoactivity, stereotyped behavior or catalepsy, the ontogeny of these behaviors in response to morphine has not been systematically examined. Low doses of morphine (1.0 and 2.0 mg/kg) increase locomotor activity in adult rats [5,11]. Higher doses of mor-

phine (3–6 mg/kg or greater) administered to adult rats have been reported to stimulate self-directed oral behavior in the form of gnawing or mouthing the fore- and hindlimbs [7,11]. Doses of morphine greater than 5–10 mg/kg produce hypomobility in adult rats [11,21], and doses greater than approximately 20 mg/kg induce catalepsy with muscular rigidity [11]. The purpose of the present experiment was to examine the ontogeny of these behavioral effects of morphine in the rat as a function of morphine dose.

METHOD

Subjects

Sprague-Dawley derived albino rats, aged 10, 17 or 24 days, born in the laboratory from established mating pairs were used as subjects in this experiment. All pups were housed with their parents and conspecifics in standard maternity cages with food and water ad lib. Litters were maintained on a 16-hr light/8-hr dark illumination cycle, with light onset at 1800 hours. All testing was conducted in red light between 1200 and 1500 hours as morphine-induced stereotypic behavior has been reported to be increased during the dark cycle [7].

Procedure

All testing was conducted in 30×15×19.5 cm glass-sided boxes with shields placed between each box to prevent subjects from observing one another. The floors were marked off into 7.5×7.5 cm squares for the 10- and 17-day olds and 15×7.5 cm squares for the 24-day olds. Red lights were suspended above the boxes; for the 10- and 17-day old pups, heating pads underneath the apparati maintained the box temperature at 32° ± 1°C.

On each test day, five pups from a litter (8–10 pups/litter) were removed from their home and randomly assigned to the five drug-dosage conditions. Saline, 0.1, 0.5, 1.0 or 5.0 mg/kg/5 cc morphine sulfate (Merck Chemical Company) in a 0.9% saline solution was administered IP in the testing room. In addition, some 24-day old animals were also given a 10.0 mg/kg/5 cc dose. In this case, since animals were tested in groups of 5, not all of the 6 dosage conditions could be represented on a given test day. After injection, subjects were placed into individual holding cages containing clean pine shavings (11.5×10.5×17.5 cm, heated at 32 ± 1°C for the 10-and 17-day olds) for 15 minutes, after which time they were transferred to the observation boxes. After each testing session, the floors of the observation boxes were washed. Six to eight animals were tested under each drug condition at each age with an approximately equal number of male and female pups being placed into each treatment group at each age.

Behavioral observations were recorded using a time sampling procedure (see [18]). Essentially, the behavior of each pup was observed for 5 sec and recorded at 60 sec intervals for a 60 min testing period; activity in terms of squares crossed was monitored continuously. Behavioral categories included locomotor activity, stationary behavior, rearing, sniffing, self-directed gnawing (defined as mouthing or chewing limbs in a matter not resembling grooming), mouthing, and grooming (which included washing and scratching). At the end of the hour observation period, immobile pups were given a catalepsy test; this involved placing subject's forearms over a slightly elevated rod for 60 sec followed by placing the pup on its back and noting a righting response. A positive catalepsy score was recorded if the subject remained elevated on the rod for at least 30 sec and exhibited a righting response.

RESULTS

The frequency of each of the measures observed during the 1 hour test (matrix crossings), mobility, stationary behavior, rearing, sniffing, grooming, self-directed gnawing and mouthing) was partitioned into four blocks of 15 minutes each. An examination of the test results indicated no reliable sex-related difference in any of the behaviors at any age. However, baseline frequencies of the behaviors varied with age in the saline control groups. For example, as can be seen in Fig. 1 (note the scale differences on the y-axes), Day 17 control animals maintained high levels of matrix crossing activity throughout the hour test; however, Days 10 and 24 control animals were somewhat active during the first time block with very low levels of activity thereafter. The enhanced and persistently elevated matrix crossing activity of the Day 17 as compared with Days 10 and 24 control animals is consistent with reports of a similar inverted U-shaped function in stabilimeter cage activity with age [6]. Moreover, the relatively low matrix crossing activity seen in the Day 10 animals may be related to immaturity of hind limb motor coordination which is important for forward locomotion [1]. Consequently, as seen in this example, since baseline frequencies of the measures varied with age in the saline control groups, each measure was independently analyzed at each testing age using a two-way (Drug Doses×Time Blocks) analysis of variance (ANOVA) with one repeated measure (Time Blocks). Posthoc individual comparisons using an ANOVA test were calculated in the case of a significant main effect or interaction effect. Levels of significance were determined at the 95% confidence interval ($p<0.05$).

On Day 10, all but the lowest dose of morphine (0.1 mg/kg) produced a depression in matrix crossings, $F(4,28)=4.49$, $p<0.01$. In addition, the highest morphine dose (5.0 mg/kg) elicited catatonic behavior in pups at this age. As seen in Fig. 1a, the number of matrix crossings declined with time in all subjects at Day 10, $F(3,84)=7.61$, $p<0.001$. Although control levels of self-directed gnawing/mouthing behavior were very low at Day 10, morphine administration at this age decreased the occurrance of this behavior when compared to that seen in saline treated pups ($mean_{saline}=0.72$, $mean_{0.1}=0.08$, $mean_{0.5}=0.57$, $mean_{1.0}=0.25$, $mean_{5.0}=0.03$). The drug did not significantly affect grooming, sniffing or rearing behaviors at this age.

In Day 17 rat pups, the 0.5 mg/kg morphine dose elicited a marked increase in the number of matrix crossing, $F(1,25)=4.76$, $p\leq0.05$, whereas the 5.0 mg/kg dose again produced a decrease in matrix crossings and catatonic behavior. In pups of this age, the number of matrix crossings was not significantly affected by 0.1 mg/kg or 1.0 mg/kg morphine administration. These data are represented in Fig. 1b. The number of matrix crossings was not altered across time in the saline, 0.1 mg/kg or 5.0 mg/kg groups. However, the number of matrix crossings gradually increased over the time blocks in the 0.5 mg/kg and 1.0 mg/kg groups, $F(3,25)=4.88$, $p\leq0.01$; $F(3,25)=7.29$, $p\leq0.01$, respectively. Thus the hyperactivity observed in 17-day old rats following morphine injection was not evident until the middle of the observation period.

Sniffing, rearing and gnawing/mouthing behaviors were not significantly influenced by morphine at Day 17. The pat-

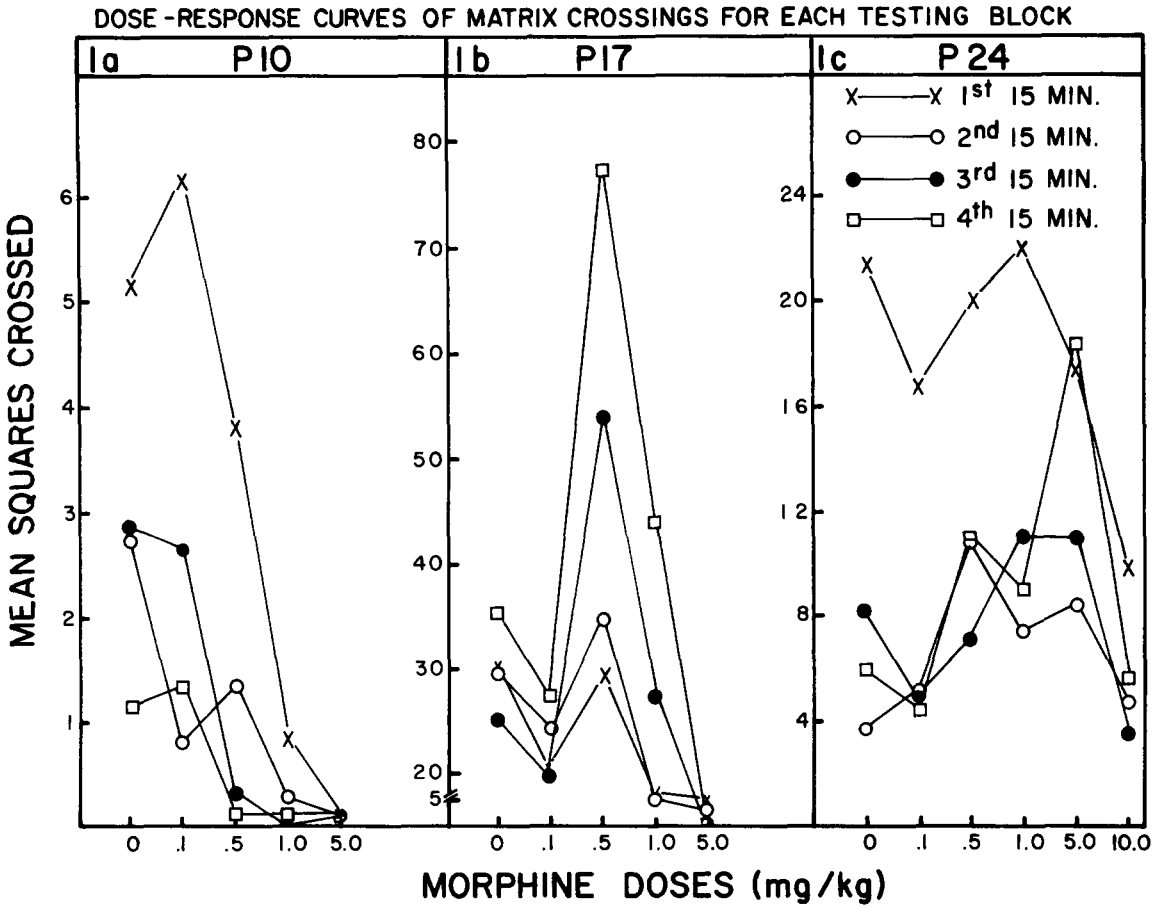

FIG. 1. Mean number of matrix crossings per 15 min time block for each drug condition. Panel 1a represents the dose response curve for the 10-day old pups; Panel 1b, the 17-day old pups; and Panel 1c, the 24-day old pups. "P" refers to postnatal day.

tern of grooming behavior across the four 15 minute blocks, however, was influenced by morphine in 17-day old pups in a dose-dependent fashion, $F(12,75)=2.32$, $p\leq 0.05$. Figure 2a illustrates this interesting pattern. While saline treated animals increased grooming activity from the first time block to reach approximately equivalent amounts of grooming over the second through fourth time blocks, grooming activity peaked during the second time block for subjects given 0.1 mg/kg morphine, and during the last time block for subjects given 1.0 mg/kg. Although there was a trend for subjects receiving 0.5 mg/kg morphine to display increased grooming during the third time block, this trend was insignificant. Subjects given 5.0 mg/kg showed little grooming behavior after the first time block as they became less active and catatonic.

Although the number of squares crossed was not altered by morphine administration in 24-day old rats, matrix crossing activity decreased across the 60 min observation period in all subjects, $F(3,114)=11.74$, $p\leq 0.01$ (See Fig. 1c). Grooming, sniffing and rearing behaviors were also not significantly affected by morphine administration in Day 24 subjects. Stereotypic behavior in the form of self-directed gnawing was elicited in 24-day old pups after the 5.0 mg/kg dose of morphine, $F(5,38)=12.23$, $p\leq 0.001$. Figure 2b demonstrates that the frequency of this behavior declined over the four time blocks of this group, $F(3,114)=2.90$, $p\leq 0.05$. It is interesting that the 10 mg/kg dose did not elicit any stereotypic behavior in 24-day old rats. These animals were not cataleptic or hypoactive, thus the high dose depression of stereotypic behavior does not appear to be a result of a general drug-induced depression of behavior. This pattern is consistent with that observed in adolescent and adult male rats where self-directed gnawing is also seen in rats injected with 5 mg/kg but not 10 mg/kg morphine (Spear, Lipovsky & Horowitz, in preparation).

For all ages, the measures of mobility and stationary behavior reflected patterns of drug-dose interactions similar to that of the number of squares crossed, and are thus not discussed in detail here.

DISCUSSION

Behavioral response patterns to morphine showed marked alterations during the two-week period of ontogeny from postnatal Days 10 to 24. At Day 10, the predominant response to morphine was a depression of locomotor activity. At the highest (5 mg/kg) dose, catalepsy was also seen. In animals of this age, the low dose of morphine (0.1 mg/kg), which was insufficient to depress activity, also had no effect on stereotyped gnawing/mouthing behavior, nor did it produce any increase in locomotor activity. A morphine-induced increase in locomotion was first seen on Day 17 (at the 0.5 mg/kg dose). As with the Day 10 animals, Day 17 animals given 5 mg/kg morphine showed a depression of

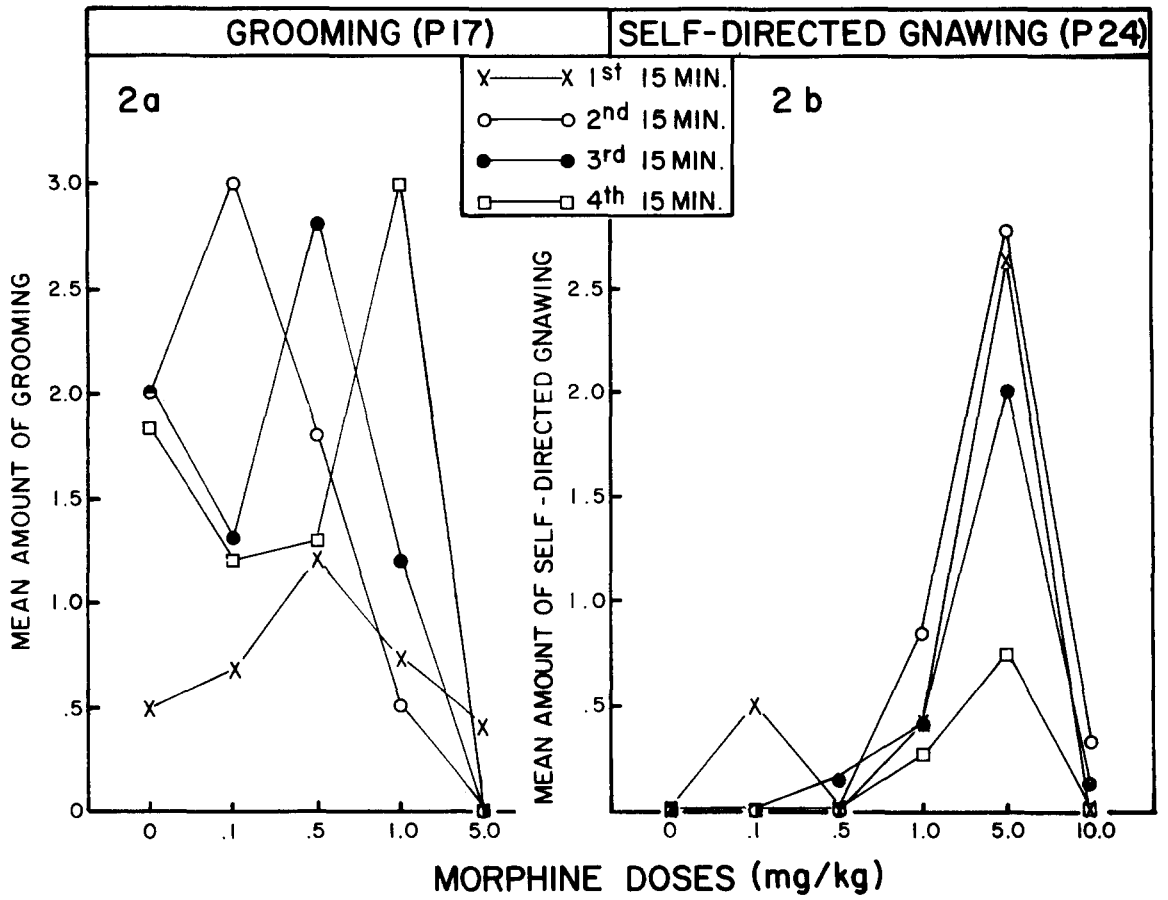

FIG. 2. (a) Morphine dose response curve per 15 min time block for grooming behavior in 17-day old rats. "P" refers to postnatal day. (b) Morphine dose response curve per 15 min time block for stereotypic self-directed gnawing behavior in 24-day old rats. "P" refers to postnatal day.

TABLE 1

Ages	Morphine doses (mg/kg) 0.1	0.5	1.0	5.0	10.0	
P10	—	↓ Locomotion	↓ Locomotion	↓ Locomotion & catalepsy		
P17	—	↑ Locomotion	—	↓ Locomotion & catalepsy		
P24	—	—	—	Stereotypic behavior	—	
Adults		— [11]	↑ Locomotion [5,11]	↓ Locomotion [11]	↓ Locomotion [11]	↓ Locomotion & catalepsy (>20 mg/kg; [11])
				Stereotypic behavior (6 mg/kg & 9 mg/kg, [7])		

locomotion and cataleptic behavior. Stereotypic gnawing/mouthing behavior was first seen in the Day 24 animals (at the 5 mg/kg dose). However, no dose of morphine produced any significant differences in activity at this age. Table 1 summarizes the different behavioral response patterns to morphine as a function of dose and age. The letter "P" preceding the various ages refers to postnatal day. A comparison of these results with previously reported behavioral response patterns in adult rats is included in this table.

The marked depression of locomotor activity after morphine administration to the youngest animals in this study correlates well with reports concerning the increased sensitivity to morphine-induced toxicity and analgesia in young rats. At day 10, subjects showed a depression of locomotor behavior at doses much lower than that required to produce hypoactivity in adult animals. Moreover, the cataleptic response to 5 mg/kg morphine in 10- and 17-day old rats is a characteristic reaction to very high levels of morphine in adult animals [15]. The absence of any morphine-related differences in activity at 24 days of age may be a result of lower brain concentration of morphine following injection in animals of this age when compared with the younger animals. It has been reported that between 20 and 26 days of age in rats, a decrease in the amount of morphine entering the brain occurs [12].

In contrast to the early appearance of a morphine-induced depression of locomotor activity, morphine-induced hyperactivity was first seen at postnatal Day 17, while stereotypic behavior first appeared as a response to morphine at 24 days. The differential appearance of these behaviors may be related to a gradual increase in the amount or specific type of opiate receptors. As previously discussed, during the first three weeks of life in the rat, there is a rapid proliferation in opiate receptor binding and number of opiate receptors in the brain [9]. Not only are opiate receptors widely distributed throughout the brain in adults (e.g. [2,3]), but there is also evidence to suggest that there may be different subpopulations of brain opiate receptors (e.g. [19,20]) which might subserve different behavioral functions. Perhaps the age-dependent appearance of these different behavioral responses to morphine are related to the maturation of a specific opiate receptor sub-type or to the development of opiate receptor populations in specific brain regions.

The behavioral psychopharmacological results of the present study have added further information beyond that available from previous studies of the physiological consequences of morphine administration. The marked sensitivity of developing animals to morphine seen by other laboratories when measuring analgesic potency and toxicity were also seen in this study in Day 10 and 17 animals using hypomobility and catalepsy as behavioral measures for morphine responsivity. However, morphine-induced hyperactivity and stereotyped self-directed gnawing behavior were not seen until postnatal Days 17 and 24, respectively. These results illustrate the importance of using a variety of behavioral response measures when examining the ontogeny of psychopharmacological responsivity. The ontogenetic pattern of drug responsivity for a psychopharmacological agent may vary depending upon the response measure used (see [17] for a discussion). Future work in the area of developmental psychopharmacology may benefit from the use of a variety of behavioral and physiological response measures when assessing the ontogenetic pattern of drug responsivity.

ACKNOWLEDGEMENTS

This research was supported in part by Grants 0144-03-240-780 from the National Foundation of the State University of New York and R01MH33215-01 from the National Institute of Mental Health to L. P. Spear and Grant BNS78-02360 from the National Science Foundation to N. E. Spear.

Request for reprints should be sent to Linda Patia Spear, Department of Psychology, State University of New York, Binghamton, New York 13901.

REFERENCES

1. Altman, J. and K. Sudarshan. Postnatal development of locomotion in the laboratory rat. *Anim. Behav.* **23:** 896–920, 1975.
2. Atweh, S. F. and M. J. Kuhar. Autoradiographic localization of opiate receptors in rat brain. II. The brain stem. *Brain Res.* **129:** 1–12, 1977.
3. Atweh, S. F. and M. J. Kuhar. Autoradiographic localization of opiate receptors in rat brain. III. The telencephalon. *Brain Res.* **134:** 393–405, 1976.
4. Auguy-Valette, A., J. Cros, C. Gouarderes, R. Gout and G. Pontonnier. Morphine analgesia and cerebral opiate receptors: A developmental study. *Br. J. Pharmac.* **63:** 303–308, 1978.
5. Ayhan, I. H. and A. Randrup. Behavioral and pharmacological studies on morphine-induced excitation of rats. Possible relation to brain catecholamines. *Psychopharmacology* **29:** 317–328, 1973.
6. Campbell, B. A. and P. D. Mabry. Ontogeny of behavioral arousal: A comparative study. *J. comp. physiol. Psychol.* **81:** 371–379, 1972.
7. Charness, M. E., Z. Amit and M. Taylor. Morphine-induced stereotypic behavior in rats. *Behav. Biol.* **13:** 71–80, 1975.
8. Chen, K. K. and E. B. Robbins. Age of animals and drug action. *J. Am. pharm. Ass.* **33:** 80–82, 1944.
9. Clendeninn, N. J., M. Petraitis and E. J. Simon. Ontological development of opiate receptors in rodent brain. *Brain Res.* **118:** 157–160, 1976.
10. Eddy, N. B. Studies of morphine, codeine and their derivatives. The variation with age in the toxic effects of morphine, codeine and some of their derivatives. *J. Pharm. exp. Ther.* **66:** 182–201, 1939.
11. Fog, R. Behavioral effects in rats of morphine and amphetamine and of a combination of the two drugs. *Psychopharmacology* **16:** 305–312, 1970.
12. Johannesson, T. and B. A. Becker. Morphine analgesia in rats at various ages. *Acta pharmac. tox.* **33:** 429–441, 1973.
13. Kirby, M. L. Effects of morphine on spontaneous activity of 18-Day rat fetus. *Devl Neurosci.* **2:** 238–244, 1979.
14. Kupferberg, H. J. and E. L. Way. Pharmacologic basis for the increased sensitivity of the newborn rat to morphine. *J. Pharmac. exp. Ther.* **141:** 105–112, 1963.
15. Lal, H., G. Gianutsos and S. K. Puri. A comparison of narcotic analgesics with neuroleptics on behavioral measures of dopaminergic activity. In: *The Opiate Narcotics: Neurochemical Mechanisms in Analgesia and Dependence,* edited by A. Goldstein. New York: Pergamon Press, 1975, pp. 183–188.

16. Nicak, A. and A. Kohut. Development of tolerance to morphine and pethidine in rats is dependent on age. *Activities nerv. sup.* **20:** 231–235, 1978.
17. Spear, L. P. The use of psychopharmacological procedures to analyze the ontogeny of learning and retention: Issues and concerns. In: *Ontogeny of Learning and Retention,* edited by N. E. Spear and B. A. Campbell. Hillsdale, New Jersey: Lawrence Erlbaum, 1979, pp. 135–156.
18. Spear, L. P. and J. Brick. Cocaine-induced behavior in the developing rat. *Behav. Neural Biol.* **26:** 401–415, 1979.
19. Squires, R. F. and C. Braestrup. Characteristics and regional distributions for two distinct [^{3}H] naloxone binding sites in the rat brain. *J. Neurochem.* **30:** 231–236, 1978.
20. Terenius, L. Opiate peptides and opiates differ in receptor selectivity. *Psychoneurendocrinology* **2:** 53–58, 1977.
21. Vasko, M. R. and E. F. Domino. Tolerance development to the biphasic effects of morphine on locomotor activity and brain acetylcholine in the rat. *J. Pharmac. exp. Ther.* **207:** 848–858, 1978.