Morphine treatment at different infant ages: influence on later morphine effects in rats

Michael Thomas Bardo

Iowa State University

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Morphine Treatment at Different Infant Ages: Influence on Later Morphine Effects in Rats

Iowa State University  Ph.D.  1980

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Morphine treatment at different infant ages: Influence on later morphine effects in rats

by

Michael Thomas Bardo

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major: Psychology

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In Charge of Major Work

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For the Major Department

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For the Graduate College

Iowa State University

Ames, Iowa

1980
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Evidence suggests that opiate receptor development plays a role in morphine-induced analgesia. From birth to about 15 days of age in rats, there is a concomitant increase in number of opiate receptors and morphine-induced analgesic effect with virtually no change in blood-brain barrier maturation. The present two experiments examined possible age-related changes in tolerance to morphine-induced analgesic, hypoactive, and hyperthermic effects during this period.

In Experiment 1, rats were administered either morphine (1 mg/kg), saline, or no treatment at one day of age. At 26 days of age, one half of the animals from each infant treatment group received morphine (5 mg/kg) and the other half received saline. All animals were then assessed for pain responsivity, activity and body temperature. This injection-test procedure was repeated at 27 and 28 days of age. At 29 days of age, all animals received morphine and were similarly tested. Rats treated with morphine one day after birth subsequently displayed tolerance to morphine-induced analgesic and hypoactive effects, but not to hyperthermic effects. Infant saline-treated animals displayed less activity and higher body temperatures than infant untreated animals.

In Experiment 2, rats were administered either morphine (1 mg/kg) or saline at 1, 5, 9, or 13 days of age. At 26 days of age, one half of each infant treatment group received morphine (5 mg/kg) and the other half received saline. Injections and tests for pain responsivity, activity, and body temperature were similar to those of Experiment 1. Animals treated with morphine at 1 but not at 5, 9, or 13 days of age displayed tolerance
to morphine-induced analgesic and hypoactive effects. Animals treated with saline early in infancy displayed less pain responsivity and activity than animals treated with saline late in infancy.

These results indicate that single-dose tolerance to 1 mg/kg morphine in infants is age-dependent. Age-dependent changes in susceptibility to single-dose morphine tolerance may reflect a change in either blood-brain barrier or opiate receptor development. Furthermore, these results indicate that saline injection in infancy may produce stress-related changes in later pain responsivity, activity, and body temperature.
INTRODUCTION

A single systemic injection of morphine (5-15 mg/kg) can produce various transient behavioral and physiological effects in rats. These effects include: analgesia (Bonnet and Peterson, 1975; D'Amour and Smith, 1941; Evans, 1961; Johannesson and Woods, 1964; Saunders, Paolino, Bousquet, and Miya, 1974), hypoactivity (Collins, 1965; Kuschinsky and Hornykiewicz, 1974) and hyperthermia (Cox, Ary, Chesarek and Lomax, 1976; Martin, Pryzbylik, and Spector, 1977; Siegel, 1978). Each of these effects diminishes with repeated morphine injections at a constant dose and this diminished effect is termed tolerance. Although tolerance to the analgesic effect can be obtained following a single morphine injection (Cochin and Kornetsky, 1964; Kornetsky and Bain, 1968), tolerance to the hyperthermic effect requires a substantially greater number of injections (Fernandes, Kluwe and Coper, 1977). These results demonstrate that the rate of tolerance development depends on which morphine effect is assessed.

Recent evidence indicates that some morphine effects depend on the developmental age of the animal. In particular, two major developmental events are important determinants of morphine effects across the life-span. First, the blood-brain barrier (BBB) to morphine in rats is virtually non-existent before about 16-20 days of age. Although fetal rats are partially protected from morphine by a placental barrier (Johannesson, Steele, and Becker, 1972), neonates are extremely susceptible to morphine effects. This morphine susceptibility diminishes at about 16-20 days of age, as the BBB to morphine begins to develop, and susceptibility rapidly becomes adult-like by about 26-32 days of age as BBB development stabilizes (Johannesson...

Development of brain opiate receptors is a second major determinant of some morphine effects. Studies investigating stereospecific binding of the opiate antagonists naloxone and naltrexone in rats indicate that opiate receptors are first evident about 15 days into gestation, and that the number of receptors progressively increases throughout the remainder of gestation and postpartum maturation (Clendeninn, Petraitis, and Simon, 1976; Coyle and Pert, 1976). These experiments indicate an approximately 15-fold increase in the number of opiate receptors from birth to 60 days of age. Although increased opiate receptor development might be expected to produce a concomitant increase in morphine effects, this is not the case for all morphine effects. The lethal dose ($LD_{50}$) of morphine, for example, is more directly related to BBB development than to opiate receptor development. Kupferberg and Way (1963) injected rats intraperitoneally with morphine at either 1, 2, 4, 8, 12, 16, 24, or 32 days of age and found that $LD_{50}$ was 45 mg/kg in 1-16 day-olds, 125 mg/kg in 24 day-olds and 220 mg/kg in 32 day-olds. This ontogenetic change in $LD_{50}$ was inversely correlated with brain permeability to morphine in blood. Since $LD_{50}$ remained essentially constant from 1 to 16 days of age despite an increasing number of opiate receptors during this period, it is unlikely that morphine lethality is directly related to opiate receptor development.

In contrast to morphine lethality, morphine-induced analgesic effects are thought to be more directly related to opiate receptor development, in part because morphine-induced analgesia is antagonized by naloxone, the
"pure" opiate antagonist which has greater affinity for the opiate receptor than morphine (Terenius, 1974), and also because morphine produces analgesia when microinjected into high density but not low density opiate receptor brain sites (Mayer and Murphin, 1976; Yaksh, Yeung and Rudy, 1976). Thus, morphine-induced analgesic effects might be expected to change as a function of the developmental increase in number of opiate receptors that occurs prior to BBB development. Consistent with this possibility, Auguy-Valette and co-workers (1978) tested rats at either 5, 10, 15, 20, 25, 30, 45, 60, or 120 days of age and observed an age-related biphasic change in morphine-induced analgesic effect. The first phase (5-15 days) was characterized by an incremental sensitivity to morphine-induced analgesia that was paralleled by an increase in number of opiate receptors. The second phase (15-30 days) was characterized by a decremental sensitivity to morphine-induced analgesia that was paralleled by a decrease in brain permeability to the drug. These observations indicate that both opiate receptor and BBB development influence morphine-induced analgesic effects, although their influence is evident at different stages of infancy.

Since acute morphine-induced analgesic effects are directly related to the number of opiate receptors prior to BBB development, chronic morphine-induced analgesic effects involved in tolerance may also be related to opiate receptor development during this same period of infancy. Several studies have demonstrated that morphine administration in infancy produces reliable and long-lasting tolerance (Sonderegger, Bromley and Zimmermann, 1977; Sonderegger and Zimmermann, 1976; Sonderegger and Zimmermann, 1978; Zimmermann, Branch, Newman Taylor, Young and Pang, 1974). In these in-
stances, however, treatment consisted of relatively long-term morphine exposure (either pellet implantation or daily injections for one week or longer) and thus the potential relationship between opiate receptor development and tolerance was not evaluated. Moreover, such long-term morphine exposure in infancy may produce increased mortality, decreased body weight, and delayed eye-opening onset (Zimmermann, Sonderegger, and Bromley, 1977). These profound physiological effects, which reflect retarded development, may be minimized by short-term rather than long-term morphine exposure at low dosage. Short-term, low-dose morphine exposure should also permit evaluation of the potential relationship between opiate receptor development and tolerance.

Some evidence suggests that early short-term exposure to a low dose of morphine may not lead to tolerance. Rats exposed to morphine on either the day before birth (Johannesson, Steele, and Becker, 1972) or the day after birth (Huidobro and Huidobro, 1973) display increased morphine-induced analgesic effects (hyperanalgesia) when tested at a later age. These observations contrast with reports of tolerance following single-dose morphine in adults (Cochin and Kornetsky, 1964; Kornetsky and Bain, 1968) and with reports of tolerance following long-term morphine exposure in infants (Sonderegger, Bromley, and Zimmermann, 1977; Sonderegger and Zimmermann, 1976; Sonderegger and Zimmermann, 1978; Zimmermann, Branch, Newman Taylor, Young, and Pang, 1974). These contrasting observations suggest the interesting possibility that short-term morphine exposure during early periods of opiate receptor development may induce increased sensitivity to subsequent morphine treatment, and that short-term morphine exposure during
later periods of opiate receptor development may induce decreased sensi-
tivity to subsequent morphine treatment. The research reported here ex-
amined that possibility.
EXPERIMENT 1

The purpose of this experiment was to determine if the observation of single-dose morphine hyperalgesia reported by Huidobro and Huidobro (1973) could be replicated. Huidobro and Huidobro (1973) used two main treatment groups to demonstrate this effect. One group was subcutaneously injected with 1 mg/kg morphine at one day of age and the second group was untreated. At 16 days of age, each animal from both groups was subcutaneously injected with 1 mg/kg morphine and subsequently assessed for pain responsivity with a standard hot plate procedure, i.e., latency to perform a paw-lick response to a noxious thermal stimulus was measured. Animals given morphine at one day of age and tested at 16 days of age displayed greater morphine analgesia (hyperalgesia) than untreated animals. However, since these two groups were not given similar handling-injection treatment at one day of age, it is not possible to conclude that the hyperalgesic effect reflects morphine exposure per se. Treatment of neonates which involves separation from the lactating mother may produce systematic behavioral and physiological changes in maternal care (Barnett and Burn, 1967; Bell, Nitschke, Bell, and Zachman, 1974; Smotherman, Wiener, Mendoza, and Levine, 1977). These stress-related effects can influence pain responsivity independently of morphine by affecting pituitary-adrenal activity which can influence pain responsivity (Bodnar, Glusman, Spiaggia, Brutus, and Kelly, 1978).

The present experiment examined the effects of early morphine and saline injection relative to no injection on subsequent morphine-induced changes in pain responsivity, activity, and body temperature. Tests of
these early treatments were given at 26 days of age rather than at the 16 day age used by Huidobro and Huidobro (1973) since preliminary data from this laboratory indicated that paw-lick responses on a hot plate surface were extremely variable in 16-day-old animals.

Method

Animals. Nine multiparous pregnant Long-Evans hooded rats (Blue Spruce Farms, New York) arrived in our laboratory before parturition. They were individually caged in standard stainless steel maternity cages (43 X 26 X 18 cm) and maintained in a temperature and humidity controlled room under a 12 hour light-dark cycle with water and Teklad pellets continuously available. Maternity cages were inspected twice daily during the light hours, and the day on which a litter was first observed was designated as the day of parturition (Day 0).

Apparatus. The hot plate apparatus consisted of a slide warming tray (Chicago Surgical & Electrical Co., 26020) with its temperature control dial set to remain constantly on at the highest temperature. Temperature was controlled and maintained at a relatively constant 52° C by a Variac (Standard Electrical Co., 300 BU). A 30 X 15 X 35 cm clear plastic chamber with a hinged top and open bottom was placed on the hot plate surface. The chamber was covered with brown adhesive paper except for the top and a 30 X 7 cm window along the bottom of the front wall through which animals could be observed. The hot plate apparatus was placed in a 100 X 30 X 90 cm wooden wall cabinet with the doors and shelves removed. A 15 W white light was mounted on the back wall of the cabinet interior and illuminated the
hot plate apparatus. Response latencies were recorded to the nearest 0.1 sec by a hand-operated electronic timer (Hunter, 120A). The activity apparatus consisted of a 30 X 15 X 35 cm brown adhesive-paper covered chamber with a hinged top and hardware cloth floor. Four photocell sensors and light sources were arranged to divide this chamber interior into nine approximately equal rectangular sections 3 cm above the floor. Solid state equipment (Massey Dickinson) transduced and recorded photobeam interruptions. A reinforced thermistor bead (Fenwal Electronics, FA41J1) and ohmmeter (Hewlett-Packard, 3476A) were used to assess rectal temperature. All apparatus was located in a room adjacent to where the animals were housed.

Procedure. On the day after parturition (Day 1), pups in each litter were sexed and the litters culled to 10 pups (5 male and 5 female) whenever possible. After sexing and culling, which took about five minutes, all pups from six randomly chosen litters were weighed to the nearest 0.1 g and injected sc at the nape of the neck with either morphine or saline; three litters received 1.0 mg/ml/kg morphine sulfate and three litters received an equivalent volume of 0.9% saline. These pups were separated from their mothers for about 15 minutes and were kept in huddles before and after injection. Pups from the remaining three litters were neither weighed nor injected but were returned to their mothers immediately after culling.

Following Day 1 treatment, all animals were left undisturbed until weaning. Animals were removed from their mothers and placed with littermates in a stainless steel group-cage on Day 21 and were individually caged in standard stainless steel cages on Day 25. In both group and individual housing conditions, Teklad pellets and water were continuously available.
On Day 26, each animal was injected with either morphine or saline and tested for pain responsivity, activity and body temperature. One half of the animals from each Day 1 treatment litter were injected sc with 5.0 mg/ml/kg morphine sulfate and the remaining animals were injected with 0.9% saline. Assignment of animals to either morphine or saline treatment was random with the stipulation that each group consist of an approximately equal number of males and females. Animals were tested for pain responsivity 30 minutes after injection. The animal was placed on the hot plate surface (52°C) and latency to perform a paw-lick response to a front or hind paw was recorded as paw-lick latency (PLL). If a paw-lick response was not observed within 120 sec, the test was terminated and PLL recorded as 120 sec. Activity was tested 45 minutes after injection. The animal was placed in the activity apparatus for 90 sec and each photobeam interruption was recorded as an activity count. Body temperature was recorded 60, 120, and 180 minutes after injection. The animal was hand-held and the thermistor bead inserted 1 cm into the rectum. After approximately 15 sec, resistance was recorded to the nearest 0.01 kΩ, and kΩ data were later transformed to °C data. In all cases, animals were returned to their home cages between postinjection tests.

Injections and postinjection tests on Day 26 (Test Day 1) were repeated on Days 27 and 28 (Test Days 2 and 3). On Day 29 (Test Day 4), all animals were injected with 5.0 mg/ml/kg morphine and tested for pain responsivity, activity and body temperature as described for Test Days 1–3. Summaries of the different treatment groups and postinjection tests are provided in Tables 1 and 2 respectively.
Table 1. Summary of treatment groups in Experiment 1.

<table>
<thead>
<tr>
<th>Group</th>
<th># Litters</th>
<th>Day 1</th>
<th>Sub-group</th>
<th>N</th>
<th>Days 26-28</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>None</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>11</td>
<td>Morphine</td>
<td>Morphine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>11</td>
<td>Saline</td>
<td>Morphine</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>Saline</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>12</td>
<td>Morphine</td>
<td>Morphine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>12</td>
<td>Saline</td>
<td>Morphine</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>Morphine</td>
<td>C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>9</td>
<td>Morphine</td>
<td>Morphine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C&lt;sub&gt;2&lt;/sub&gt;</td>
<td>9</td>
<td>Saline</td>
<td>Morphine</td>
</tr>
</tbody>
</table>

Table 2. Summary of postinjection tests on each test day.

<table>
<thead>
<tr>
<th>Postinjection Interval (min)</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>Pain Responsivity</td>
</tr>
<tr>
<td>45</td>
<td>Activity</td>
</tr>
<tr>
<td>60</td>
<td>Body Temperature</td>
</tr>
<tr>
<td>120</td>
<td>Body Temperature</td>
</tr>
<tr>
<td>180</td>
<td>Body Temperature</td>
</tr>
</tbody>
</table>
**Data analysis.** Potential sex and litter effects on pain responsivity, activity and body temperature were considered nuisance variables. Although potential sex differences were controlled experimentally by assigning approximately one half male and one half female to each treatment group, potential litter differences were not controlled. These potential litter effects were therefore assessed by separate hierarchial ANOVAs for each dependent measure in which animals were nested in litters and litters were nested in Day 1 treatment. In these preliminary analyses, data were collapsed across within-group factors.

Infant and test day treatment differences were analyzed as separate split-plot ANOVAs for each dependent measure. Comparisons between infant morphine treated and untreated animals were not of interest. Thus, data obtained from infant untreated and saline treated animals were analyzed separately from those obtained from infant saline treated and morphine treated animals. Significant interactions were subsequently analyzed for simple main effects.

**Results**

Table 3 summarizes the mortality data for each infant treatment group. Although there was no significant difference in number of mortalities between infant saline treated and untreated animals, morphine treatment produced significantly more mortalities than both saline and no treatments ($\chi^2 = 4.97; \text{df} = 1; p < .05$). All mortalities occurred prior to weaning at 21 days of age.

Hierarchal analyses of variance revealed no significant differences
Table 3. Number of mortalities from infant untreated, saline treated and morphine treated groups in Experiment 1.

<table>
<thead>
<tr>
<th>Group</th>
<th># Litters</th>
<th>Injection</th>
<th># Alive on Day 1</th>
<th># Alive on Day 26</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>None</td>
<td>29</td>
<td>22</td>
<td>24%</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>Saline</td>
<td>27</td>
<td>24</td>
<td>11%</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>Morphine</td>
<td>30</td>
<td>18</td>
<td>40%</td>
</tr>
</tbody>
</table>
among litters in either pain responsivity, activity, or body temperature on test days 1-3. In these analyses, animals tested following test injections of morphine displayed longer PLLs ($F = 22.49; df = 1, 6; p < .01$), less activity ($F = 71.59; df = 1, 6; p < .001$), and higher body temperature ($F = 71.59; df = 1, 6; p < .001$) than animals tested following saline. Since no litter effects were obtained on these measures, all subsequent statistical analyses were performed without regard to litter differences.

**Pain responsivity.** Figure 1 summarizes the pain responsivity data obtained from infant untreated (Panel A) and saline treated (Panel B) animals following either morphine or saline injections on test days 1-3. In these infant treatment groups, morphine on test day 1 produced significantly longer PLLs than saline ($F = 72.85; df = 1, 70; p < .001$). There was a significant decline in PLLs across test days 1-3 following either morphine ($F = 92.33; df = 2, 84; p < .001$) or saline ($F = 7.10; df = 2, 84; p < .01$). On test day 3, there was no significant difference in PLLs between morphine and saline injected animals. The magnitude of morphine-induced analgesic effect and rate of tolerance development were not influenced by saline injection in infancy, as there were no significant differences in PLLs between infant saline treated and untreated animals.

Figure 2 summarizes the data obtained from these same treatment groups when each animal was administered morphine on test day 4. For groups labelled Morph this was their fourth test injection of morphine and for groups labelled Sal this was their first test injection of morphine. Tolerance is evident in that Morph groups displayed significantly shorter PLLs
Figure 1. Mean paw-lick latencies obtained following either morphine or saline on test days 1-3 from animals untreated in infancy (Panel A) and animals treated with saline in infancy (Panel B) in Experiment 1.
The graph shows the paw-lick latency (in seconds) over test days for both morphine and saline treatments. **MORPHINE** showed a consistent decrease in latency across all test days, with the latency decreasing from approximately 70 seconds on day 1 to less than 10 seconds on day 3. **SALINE** also showed a decrease in latency, although it was not as steep as the morphine group. The latency for saline decreased from about 70 seconds on day 1 to around 20 seconds on day 3. The graph is split into two parts, **A** and **B**, with similar trends observed in both sections.
Figure 2. Mean paw-lick latencies obtained following morphine on test day 4 from animals untreated in infancy (Panel A) and animals treated with saline in infancy (Panel B) in Experiment 1. Morph groups previously received morphine injections on test days 1-3 and Sal groups previously received saline injections on test days 1-3.
All injections morphine

Drug treatment on test days 1-3

Paw-lick latency (sec)
than Sal groups ($F = 18.20; df = 1, 42; p < .001$). There was no significant difference in PLLs between infant untreated (Panel A) and saline treated (Panel B) animals. Moreover, regardless of infant treatment, animals given morphine for the first time on test day 4 (Fig 2, Sal groups) displayed significantly shorter PLLs than animals given morphine for the first time on test day 1 (Fig 1, Morphine groups on test day 1; $F = 10.44; df = 1, 44; p < .01$). This latter attenuation in PLL was produced by repeated hot plate tests independent of morphine and presumably reflects behavioral tolerance (e.g., Kayan, Ferguson, and Mitchell, 1973).

Although there were no significant differences in pain responsivity between infant saline treated and untreated animals, there were significant differences between infant morphine and saline treated animals. Figure 3 summarizes the PLL data obtained from infant saline treated (Panel A) and morphine treated (Panel B) animals following either morphine or saline injections on test days 1-3. In these infant treatment groups, morphine on test day 1 produced significantly longer PLLs than saline ($F = 30.14; df = 1, 63; p < .001$). There was a significant decline in PLLs across test days 1-3 following either morphine ($F = 44.49; df = 2, 76; p < .001$) or saline ($F = 4.69; df = 2, 76; p < .05$). On test day 3, there was no significant difference in PLLs between morphine and saline injected animals. Tolerance was potentiated by morphine treatment in infancy, as infant morphine treated animals displayed significantly shorter PLLs on test day 1 than infant saline treated animals ($F = 14.16; df = 1, 63; p < .05$). This decrease in PLLs produced by infant morphine treatment was obtained regardless of whether the injection on test day 1 was morphine
Figure 3. Mean paw-lick latencies obtained following either morphine or saline on test days 1-3 from animals treated with saline in infancy (Panel A) and animals treated with morphine in infancy (Panel B) in Experiment 1.
Figure 4. Mean paw-lick latencies obtained following morphine on test day 4 from animals treated with saline in infancy (Panel A) and animals treated with morphine in infancy (Panel B) in Experiment 1. Morph groups previously received morphine injections on test days 1-3 and Sal groups previously received saline injections on test days 1-3.
ALL INJECTIONS MORPHINE

DRUG TREATMENT ON TEST DAYS 1-3
or saline. Figure 4 summarizes the data obtained from these same treatment groups when each animal was administered morphine on test day 4. Tolerance resulting from morphine injections on test days 1-3 is evident in that Morph groups displayed shorter PLLs than Sal groups. This difference was significant for infant saline treated animals (Panel A; $F = 21.42; df = 1, 38; p < .05$) but not for infant morphine treated animals (Panel B). Moreover, tolerance resulting from infant morphine treatment was evident in that infant morphine treated animals displayed shorter PLLs than infant saline treated animals. This difference was significant for Sal groups ($F = 12.11; df = 1, 38; p < .05$) but not for Morph groups. These data indicate that infant morphine treatment potentiated the development of morphine analgesic tolerance.

**Activity.** Figure 5 summarizes the activity data obtained from infant untreated (Panel A) and saline treated (Panel B) animals following either morphine or saline injections on test days 1-3. In these infant treatment groups, morphine on test day 1 produced significantly less activity than saline ($F = 34.84; df = 1, 45; p < .001$). There was a significant increase in activity across test days 1-3 following morphine ($F = 7.61; df = 2, 68; p < .01$) and a significant decrease in activity across test days 1-3 following saline ($F = 5.63; df = 2, 68; p < .01$). On test day 3, there was no significant difference in activity between morphine and saline injected animals. Although the magnitude of morphine-induced hypoactive effect and rate of tolerance development were similar in both of these infant treatment groups, infant saline treated animals displayed significantly less activity than infant untreated animals ($F = 11.77; df = 1,$
Figure 5. Mean activity obtained following either morphine or saline on test days 1-3 from animals untreated in infancy (Panel A) and animals treated with saline in infancy (Panel B) in Experiment 1.
Figure 6. Mean activity obtained following morphine on test day 4 from animals untreated in infancy (Panel A) and animals treated with saline in infancy (Panel B) in Experiment 1. Morph groups previously received morphine injections on test days 1-3 and Sal groups previously received saline injections on test days 1-3.
ALL INJECTIONS MORPHINE

A

B

DRUG TREATMENT ON TEST DAYS 1-3
This depression in activity was evident on each test day 1-3 regardless of whether morphine or saline was administered. Figure 6 summarizes the data obtained from these same treatment groups when each animal was administered morphine on test day 4. For groups labelled Morph this was their fourth test injection of morphine and for groups labelled Sal this was their first test injection of morphine. Tolerance is evident in that Morph groups displayed significantly more activity than Sal groups ($F = 8.30; df = 1, 34; p < .01$). Moreover, infant saline treated animals (Panel B) displayed significantly less activity than infant untreated animals (Panel A; $F = 8.57; df = 1, 34; p < .01$). This depression in activity occurred in both Morph and Sal groups, indicating that the infant injection procedure per se depressed later activity independent of morphine.

Figure 7 summarizes the activity data obtained from infant saline treated (Panel A) and morphine treated (Panel B) animals following either morphine or saline injections on test days 1-3. In these infant treatment groups, morphine on test day 1 produced significantly less activity than saline ($F = 40.02; df = 1, 63; p < .001$). There was a significant increase in activity across test days 1-3 following morphine ($F = 11.26; df = 2, 76; p < .001$). On test day 3, there was no significant difference in activity between morphine and saline injected animals. Infant morphine treated animals displayed significantly more activity than infant saline treated animals on test day 2 ($F = 14.16; df = 1, 63; p < .05$) but not on test day 1. These data indicate that while tolerance was potentiated by infant morphine treatment, this potentiation was not evident
Figure 7. Mean activity obtained following either morphine or saline on test days 1-3 from animals treated with saline in infancy (Panel A) and animals treated with morphine in infancy (Panel B) in Experiment 1.
A

ACTIVITY

SALINE

MORPHINE

TEST DAY

B

0 20 40 60 80 100

1 2 3 1 2 3
Figure 8. Mean activity obtained following morphine on test day 4 from animals treated with saline in infancy (Panel A) and animals treated with morphine in infancy (Panel B) in Experiment 1. Morph groups previously received morphine injections on test days 1-3 and Sal groups previously received saline injections on test days 1-3.
ALL INJECTIONS MORPHINE

ACTIVITY

MORPH      SAL      MORPH      SAL

DRUG TREATMENT ON TEST DAYS 1-3
until the second test injection of morphine. Figure 8 summarizes the data from these same treatment groups when each animal was administered morphine on test day 4. Tolerance resulting from morphine injections on test days 1-3 is evident in that Morph groups displayed significantly more activity than Sal groups ($F = 24.25; df = 1, 38; p < .001$). However, tolerance resulting from infant morphine treatment is not evident, as there was no significant difference in activity between infant saline treated (Panel A) and morphine treated (Panel B) animals. These data, together with those summarized in Figure 7, suggest that more than one test injection of morphine may be necessary to observe hypoactive tolerance resulting from the infant morphine treatment.

**Body temperature.** Table 4 summarizes the body temperature data obtained from all treatment groups across all test days and all postinjection intervals. In both infant untreated and saline treated animals, morphine on test day 1 produced significantly higher body temperatures than saline 60 min after injection ($F = 123.00; df = 1, 70; p < .001$). On this test day, there was a significant decline in body temperature measured 60, 120 and 180 min after either morphine ($F = 252.58; df = 2, 84; p < .001$) or saline ($F = 21.23; df = 2, 84; p < .001$). There was no significant difference in body temperature between morphine and saline injected animals 180 min after injection. Tolerance to the morphine-induced hyperthermic effect was not obtained, as there was no significant decline in body temperature across test days 1-3. Independent of morphine, infant saline treated animals displayed higher body temperatures than infant untreated animals. This difference was significant 60 min after injection on test day 2 ($F = 4.13; \ldots$
Table 4. Mean body temperatures obtained from animals untreated, saline treated and morphine treated in infancy and tested following either morphine or saline injection in Experiment 1. The three values for each subgroup on each test day represent mean temperatures recorded 60, 120 and 180 min after injection respectively. On test day 4, all injections were morphine.

<table>
<thead>
<tr>
<th>Group</th>
<th>Infant Injection</th>
<th>Subgroup</th>
<th>Test Day Injection</th>
<th>Mean Body Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>A</td>
<td>None</td>
<td>A₁</td>
<td>Morphine</td>
<td>37.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>37.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A₂</td>
<td>Saline</td>
<td>36.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36.4</td>
</tr>
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<td>36.2</td>
</tr>
<tr>
<td>B</td>
<td>Saline</td>
<td>B₁</td>
<td>Morphine</td>
<td>37.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>37.1</td>
</tr>
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<td></td>
<td></td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B₂</td>
<td>Saline</td>
<td>36.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36.4</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>36.3</td>
</tr>
<tr>
<td>C</td>
<td>Morphine</td>
<td>C₁</td>
<td>Morphine</td>
<td>37.9</td>
</tr>
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<td></td>
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<td></td>
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<td>C₂</td>
<td>Saline</td>
<td>36.8</td>
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<td></td>
<td>36.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36.5</td>
</tr>
</tbody>
</table>
When each animal was administered morphine on test day 4, a similar pattern of results was obtained. Morphine hyperthermic tolerance was not evident, as there was no significant temperature difference between animals previously given either morphine or saline on test days 1-3. Moreover, infant saline treated animals displayed significantly higher body temperatures than infant untreated animals 60 min after injection ($F = 8.45; df = 1, 70; p < .01$). These data indicate that the infant injection procedure per se increased later body temperature independently of morphine.

Although the infant injection procedure influenced later body temperature, infant morphine exposure did not. In both infant saline and morphine treated animals, morphine on test day 1 produced significantly higher body temperatures than saline 60 min after injection ($F = 136.49; df = 1, 51; p < .001$). There was a significant decline in body temperature measured 60, 120 and 180 min after either morphine ($F = 277.53; df = 2, 76; p < .001$) or saline ($F = 27.05; df = 2, 76; p < .001$). Tolerance was not obtained, as there was no significant decline in body temperatures across test days 1-3. When each animal was administered morphine on test day 4, there was no significant temperature difference between animals previously given either morphine or saline on test days 1-3. Moreover, there was no significant difference between infant saline and morphine treated animals, indicating that infant morphine exposure did not influence later body temperature.
Discussion

The results of Experiment 1 demonstrate that both morphine and the injection procedure per se, administered one day after birth in rats, can produce long-lasting changes in pain responsivity, activity, and body temperature. Comparison of data obtained from infant saline treated and untreated animals indicated that the injection procedure at one day of age decreased activity and increased body temperature measured at 26-29 days of age. Comparisons between infant morphine treated and saline treated animals indicated that morphine exposure at one day of age decreased PLLs and increased activity measured at 26-29 days of age. These long-lasting changes related to the injection procedure and to morphine exposure were observed regardless of whether test injections were morphine or saline. These observations demonstrate the importance of using saline injected control animals when investigating the influence of acute morphine exposure during early infancy on later morphine effects.

One purpose of the present experiment was to replicate the observation of single-dose hyperanalgesia in one-day-old rats reported by Huidobro and Huidobro (1973). These investigators reported that animals given morphine one day after birth later displayed greater morphine analgesia (hyperanalgesia) than animals untreated one day after birth. In contrast, the results of the present experiment demonstrated that animals given morphine one day after birth later displayed less morphine analgesia (tolerance) than animals given saline one day after birth. Although the reasons for these conflicting results are unclear, it is unlikely that the difference in infant control groups (untreated vs. saline treated) is involved, as
there was no difference in pain responsivity between infant untreated and saline treated animals in the present experiment (see Figure 1). However, differences in test age, pre-injection training on the hot plate, and animal strain may be relevant considerations. Huidobro and Huidobro tested animals on the hot plate at 16 days of age. In the present experiment, animals were tested at 26-29 days of age. Preliminary data from this laboratory indicated that paw-lick responses following saline were extremely variable in 16-day-old rats and, with some animals, were not observed for up to 180 sec after contact with a 52° C hot plate. Further, Huidobro and Huidobro (1973) used albino rats and pretrained them on the hot plate prior to assessment of pain responsivity, while the present experiment involved hooded rats and no pretraining.

Regardless of the source of difference between the present results and those reported by Huidobro and Huidobro (1973), hyperalgesia is not a consistent result of early morphine exposure. The data indicate that a single dose of morphine at one day of age produced analgesic tolerance and are consistent with reports of single-dose tolerance in adults (Cochin and Kornetsky, 1964; Huidobro, Huidobro-Toro, and Way, 1976; Kornetsky and Bain, 1968). Previous reports have demonstrated long-lasting tolerance following morphine exposure in infants (Sonderegger, Bromley, and Zimmermann, 1977; Sonderegger and Zimmermann, 1976; Sonderegger and Zimmermann, 1978; Zimmermann, Branch, Newman Taylor, Young, and Pang, 1974). In these instances, however, morphine exposure was relatively long-term, consisting of either pellet implantation or daily injections for one week or longer. The present report clearly demonstrates long-lasting single-dose morphine anal-
gesic tolerance in infants. This tolerance was obtained with a morphine
dose of 1 mg/kg, which contrasts with the morphine dose of 5-20 mg/kg
required to produce single-dose tolerance in adults. This difference
between infants and adults in dosages required to produce single-dose
tolerance may reflect differences in brain permeability to morphine in
blood, as the blood-brain barrier to morphine develops between about 16-32
days of age (Johannesson and Becker, 1973; Johannesson, Steele, and Becker,
1972; Kupferberg and Way, 1963) and also may involve differences in opiate
receptor development since there is an approximately 15-fold increase in
the number of opiate receptors from birth to 60 days of age (Clendeninn,

Morphine analgesic tolerance obtained in the present experiment may
involve both pharmacological and behavioral (nonpharmacological) factors
(Adams, Yeh, Woods, and Mitchell, 1969; Gebhart and Mitchell, 1971; Kayan,
Ferguson, and Mitchell, 1973). Pharmacological tolerance has been de­
scribed as a diminished analgesic response brought about by morphine
exposure per se and may reflect alterations in neurotransmitter-receptor
or immunoreactive mechanisms (Clouet and Iwatsubo, 1975; Cochin, 1970;
Takemori, 1975). In contrast, behavioral tolerance has been described as
a diminished analgesic response brought about by experience with either a
functional or nonfunctional analgesiometric apparatus and may reflect clas­
sical conditioning of an hyperalgesic response (Siegel, 1975), instrumental
conditioning of a pain indicant response (Gebhart, Sherman, and Mitchell,
1971), or habituation to novel stimuli associated with the analgesiometric
apparatus (Bardo and Hughes, 1979). In the present experiment, tolerance
evident in infant morphine treated animals may best be described as phar-
macological, because tolerance was obtained on the first test with the not
plate apparatus. In contrast, tolerance evident in all infant treatment
groups across repeated morphine injections and tests may be described, at
least in part, as behavioral. This behavioral contribution to tolerance
was evident in the diminished analgesic response to morphine resulting
from prior hot plate tests without morphine (cf. Figure 2, Sal groups and
Figure 1, morphine groups on test day 1).

Infant morphine treatment produced tolerance to subsequent morphine-
induced analgesia and potentiated analgesic tolerance development to suc-
cessive morphine injections. The present results demonstrate similar ef-
facts of infant morphine treatment on subsequent morphine-induced hypo-
activity. Tolerance to morphine-induced hypoactivity developed more rapid-
ly across test days 1-3 in infant morphine treated animals than in infant
saline treated animals (see Figure 7). This tolerance, however, was not
evident on test day 1 but was evident on test day 2. These data contrast
with the observation of morphine analgesic tolerance obtained on test day
1 and demonstrate that the presence or absence of measurable tolerance on
a single test depends on which particular morphine effect is assessed.

Unlike analgesic and hypoactive tolerance, infant morphine treatment
failed to produce tolerance to morphine-induced hyperthermia. Regardless
of infant treatment, morphine produced a robust hyperthermic effect that
did not decline across repeated injections and tests. These data are not
consistent with at least one report of morphine hyperthermic tolerance
following repeated injections and tests in adult rats (Siegel, 1978). How-
ever, the conditions necessary for obtaining morphine hyperthermic tolerance are not clear, as several other reports indicate that morphine-induced hyperthermia may actually increase following repeated injections (Gunne, 1960; Martin, Pryzbylik, and Spector, 1977; Thornhill, Hirst, and Gowdey, 1978). Despite this discrepancy in the literature, the present failure to obtain hyperthermic tolerance is consistent with the observation that, relative to analgesic and hypoactive tolerance, the rate at which morphine tolerance develops to hyperthermia is extremely slow (Fernandes, Kluwe, and Coper, 1977).

Finally, acute morphine-induced analgesia has been reported to be positively correlated with the number of opiate receptors prior to BBB development (Auguy-Valette, Cros, Gouarderes, Gout, and Pontonnier, 1978). Morphine-induced analgesic tolerance may also be related to opiate receptor development during this same period of infancy. If morphine tolerance is directly related to number of opiate receptors prior to BBB development, then the degree of tolerance produced by a 1 mg/kg morphine injection one day after birth, as obtained in experiment 1, ought to decrease as a function of increased injection age. Experiment 2 examined this possibility.
EXPERIMENT 2

This experiment was designed to assess the potential influence of single-dose morphine treatment at either 1, 5, 9, or 13 days of age on later morphine-induced analgesia, hypoactivity and hyperthermia. This particular age range was chosen because it is characterized by rapid opiate receptor development with minimal BBB influence.

Method

Animals. Sixteen multiparous female Long-Evans hooded rats (Blue Spruce Farms, New York) were mated in this laboratory with male rats of the same strain. Mating consisted of randomly housing one female and one male together for 10 consecutive days. Following this, females were individually caged and maintained as described in Experiment 1.

Apparatus. Apparatus was that described in Experiment 1.

Procedure. Females were examined twice daily for parturition, once in the morning and once in the afternoon. The day on which a litter was first observed was designated as parturition for that litter (Day 0). On Day 1, each litter was sexed and culled to 10 pups (5 male and 5 female) whenever possible.

Treatment was generally similar to that described in Experiment 1 except that an untreated group was not used and an additional variable, age at which infants were initially administered morphine or saline, was investigated. On Day 1, animals from two randomly chosen litters were injected with 1 mg/ml/kg morphine sulfate and animals from two other randomly chosen
litters were injected with 0.9% saline. Twelve more litters were similarly treated except that treatment occurred on either Day 5, 9, or 13, with four litters (2 morphine and 2 saline) randomly treated at each age. Animals were weaned and housed as described in Experiment 1.

On Day 26, one half of the animals from each treatment litter were injected with 5 mg/ml/kg morphine and the other half were injected with saline. Assignment of animals to either the morphine or saline treatment group was random with the stipulation that each group consist of an approximately equal number of males and females. Each animal was subsequently tested for pain responsivity, activity, and body temperature after either morphine or saline injection as described in Experiment 1. These injections and tests were repeated on Days 27 and 28. On Day 29, all animals were injected with morphine and tested. The treatment groups are summarized in Table 5.

Data analysis. Statistical analyses of pain responsivity, activity and body temperature data generally paralleled those described in Experiment 1, except that day of infant morphine or saline treatment was included as an additional between-groups factor in the split-plot ANOVAs. Trend analyses were also used to assess differences between the ages at which morphine or saline treatments were administered.

Results

Table 6 summarizes the mortality data for each infant treatment group. There were no significant differences in number of mortalities between infant treatment groups. Although animals given saline at 9 days of age tend
Table 5. Summary of treatment groups in Experiment 2.

<table>
<thead>
<tr>
<th>Group</th>
<th># Litters</th>
<th>Age (days)</th>
<th>Injection</th>
<th>Sub-Group</th>
<th>N</th>
<th>Days 26-28</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>1</td>
<td>Morphine</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>10</td>
<td>Morphine</td>
<td>Morphine</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10</td>
<td>Saline</td>
<td>Morphine</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>1</td>
<td>Saline</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>10</td>
<td>Morphine</td>
<td>Morphine</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>9</td>
<td>Saline</td>
<td>Morphine</td>
</tr>
<tr>
<td>C</td>
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<td>Morphine</td>
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</tr>
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<td></td>
<td></td>
<td></td>
<td>C&lt;sub&gt;2&lt;/sub&gt;</td>
<td>9</td>
<td>Saline</td>
<td>Morphine</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>5</td>
<td>Saline</td>
<td>D&lt;sub&gt;1&lt;/sub&gt;</td>
<td>9</td>
<td>Morphine</td>
<td>Morphine</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D&lt;sub&gt;2&lt;/sub&gt;</td>
<td>8</td>
<td>Saline</td>
<td>Morphine</td>
</tr>
<tr>
<td>E</td>
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<td>9</td>
<td>Morphine</td>
<td>E&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>Saline</td>
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<td>6</td>
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<td>13</td>
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<td></td>
<td></td>
<td></td>
<td>G&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>Saline</td>
<td>Morphine</td>
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<tr>
<td>H</td>
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<td>10</td>
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<td>Morphine</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H&lt;sub&gt;2&lt;/sub&gt;</td>
<td>9</td>
<td>Saline</td>
<td>Morphine</td>
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</table>
Table 6. Mortality of infant treatment groups in Experiment 2.

<table>
<thead>
<tr>
<th>Group</th>
<th># Litters</th>
<th># Alive on Day 1</th>
<th>Age</th>
<th>Injection</th>
<th># Alive on Treatment Day</th>
<th># Alive on Day 26</th>
<th>Mortality Following Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>20</td>
<td>1</td>
<td>Morphine</td>
<td>20</td>
<td>20</td>
<td>0 %</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>20</td>
<td>1</td>
<td>Saline</td>
<td>20</td>
<td>19</td>
<td>5 %</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>20</td>
<td>5</td>
<td>Morphine</td>
<td>20</td>
<td>20</td>
<td>0 %</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>18</td>
<td>5</td>
<td>Saline</td>
<td>18</td>
<td>17</td>
<td>6 %</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>18</td>
<td>9</td>
<td>Morphine</td>
<td>17</td>
<td>17</td>
<td>0 %</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>20</td>
<td>9</td>
<td>Saline</td>
<td>16</td>
<td>13</td>
<td>19 %</td>
</tr>
<tr>
<td>G</td>
<td>2</td>
<td>19</td>
<td>13</td>
<td>Morphine</td>
<td>18</td>
<td>18</td>
<td>0 %</td>
</tr>
<tr>
<td>H</td>
<td>2</td>
<td>19</td>
<td>13</td>
<td>Saline</td>
<td>19</td>
<td>19</td>
<td>0 %</td>
</tr>
</tbody>
</table>
ed to displayed the highest mortality (19%), this tendency did not appear to reflect a treatment effect. In this infant treatment group, four animals died before and three animals died after saline injection at 9 days of age.

**Pain responsivity.** Figure 9 summarizes the pain responsivity data obtained from each infant treatment group following either morphine or saline on test days 1-4. Treatment groups are abbreviated as M-M, M-S, S-M, and S-S, where the first letter refers to treatment in infancy (M = morphine, S = saline) and the second letter (M or S) refers to injections on test days 1-3. Both infant treatment groups displayed morphine-induced analgesia on test Day 1. Regardless of infant treatment, morphine on this test day produced significantly longer PLLs than saline ($F = 87.45; \text{df} = 1, 212; p < .001$). There was a significant decline in PLLs across test days 1-3 following either morphine ($F = 153.43; \text{df} = 2, 254; p < .001$) or saline ($F = 14.35; \text{df} = 2, 254; p < .001$). On test day 3, there was no significant difference in PLLs between morphine and saline injected animals. On test day 4, when each animal received morphine, tolerance is evident in animals given morphine on test days 1-3, as they displayed significantly shorter PLLs than animals given saline on test days 1-3 ($F = 56.06; \text{df} = 1, 127; p < .001$).

Although test injections of morphine produced an initial analgesic effect and subsequent tolerance in both infant treatment groups, the magnitude of these effects was influenced by infant treatment. On test day 1, animals treated at one day of age with morphine displayed significantly shorter PLLs than animals treated at one day of age with saline ($F = 4.42; \text{df} = \ldots$).
Figure 9. Mean paw-lick latencies obtained following morphine or saline on test days 1-4 from animals treated with either morphine or saline at either 1, 5, 9, or 13 days of age in Experiment 2. M-M groups received morphine in infancy and morphine on test days 1-3; S-M groups received saline in infancy and morphine on test days 1-3; M-S groups received morphine in infancy and saline on test days 1-3; and S-S groups received saline in infancy and saline on test days 1-3. On test day 4, all injections were morphine.
This effect was evident in animals tested following morphine (cf M-M and S-M groups) but not following saline (cf M-S and S-S groups). These data demonstrate that morphine treatment at one day of age attenuated later morphine-induced analgesia. This effect on test day 1 was not obtained with morphine treatment at either 5, 9, or 13 days of age however, as there was no significant difference in PLLs between animals treated at these ages with either morphine (M-M and M-S groups) or saline (S-M and S-S groups). Trend analyses of test day 1 data revealed that infant saline treatment produced a significant linear decrease in PLLs as a function of increased treatment age ($F = 7.91; df = 1, 212; p < .05$). This effect demonstrates that the infant injection procedure influenced later pain responsivity. There was also a significant quadratic trend in PLLs as a function of infant morphine treatment age ($F = 12.55; df = 1, 212; p < .05$). On test day 4, there was a significant interaction effect ($F = 5.37; df = 3, 127; p < .01$) involving PLLs of animals treated with either morphine or saline at either 9 or 13 days of age and given saline of test days 1-3. The basis for interpreting this effect is not readily apparent.

**Activity.** Figure 10 summarizes the activity data obtained from each infant treatment group following either morphine or saline on test days 1-4. Animals given morphine on test day 1 displayed less activity than animals given saline. This effect, however, was statistically significant only for groups treated with saline at 1 or 5 days of age, and for groups treated with morphine at 5 or 13 days of age ($F > 6.10; df = 1, 212; p < .05$ in each case). Morphine on test day 2 produced less activity than
Figure 10. Mean activity obtained following morphine or saline on test days 1–4 from animals treated with either morphine or saline at either 1, 5, 9, or 13 days of age in Experiment 2. M–M groups received morphine in infancy and morphine on test days 1–3; S–M groups received saline in infancy and morphine on test days 1–3; M–S groups received morphine in infancy and saline on test days 1–3; and S–S groups received saline in infancy and saline on test days 1–3. On test day 4, all injections were morphine.
ALL INJECTIONS MORPHINE

INFANT TREATMENT AGE (DAYS)
saline in animals treated at one day of age with saline ($F = 13.17; df = 1, 212; p < .001$). On test day 3, morphine produced significantly greater activity than saline in animals treated with saline at 13 days of age and in animals treated with morphine at 9 or 13 days of age ($F \geq 4.48; df = 1, 212; p < .05$ in each case). Tolerance to morphine-induced hypoactivity was evident when all animals received morphine on test day 4, as animals given morphine on test days 1-3 displayed greater activity than animals given saline on test days 1-3 ($F = 73.30; df = 1, 127; p < .001$).

Although test injections of morphine produced an initial hypoactive effect to which tolerance developed, the magnitude of effect was influenced by infant treatment. Animals treated at one day of age with morphine displayed significantly more activity than animals treated at one day of age with saline when morphine was given on test day 1 ($F = 23.80; df = 1, 212; p < .001$), but not when saline was given on test day 1. These data demonstrate that morphine treatment at one day of age attenuated later morphine-induced hypoactivity. This effect on test day 1 was not obtained with morphine treatment at either 5, 9, or 13 days of age however, as there was no significant difference in activity between animals treated at these ages with either morphine (M-M and M-S groups) or saline (S-M and S-S groups). Trend analyses of test day 1 data as a function of increased treatment age revealed a significant linear trend in M-M animals ($F = 4.81; df = 1, 212; p < .05$) and S-M animals ($F = 10.93; df = 1, 212; p < .01$), and a significant quadratic trend in M-S animals ($F = 7.74; df = 1, 212; p < .01$). These data indicate that the age at which either morphine or saline was given in infancy...
influenced later activity. On test day 4, there were no significant differences in activity between animals treated at either 1, 5, 9, or 13 days of age with either morphine or saline.

**Body temperature.** Body temperatures of several animals treated at 9 days of age were not recorded due to an apparatus problem. To avoid extremely unequal cell sizes, ANOVA was performed only on body temperatures from animals treated at 1, 5, or 13 days of age. These data are summarized in Table 7. Morphine on test day 1 produced significantly higher body temperatures than saline 60 min after injection ($F = 171.40; df = 1, 168; p < .001$) and 120 min after injection ($F = 47.45; df = 1, 168; p < .001$), but not 180 min after injection. There was a significant decline in body temperatures measured 60, 120, and 180 min after either morphine ($F = 555.72; df = 2, 202; p < .001$) or saline ($F = 56.88; df = 2, 202; p < .001$). There was no significant change in morphine-induced hyperthermia across test days 1-3. On test day 4, when all injections were morphine, there was no significant difference in body temperature between animals given morphine on test days 1-3 and animals given saline on test days 1-3. These data indicate that tolerance to morphine-induced hyperthermia did not develop.

Infant morphine treatment also failed to produce tolerance to morphine-induced hyperthermia. Animals treated at either 1, 5, or 13 days of age with morphine displayed significantly higher body temperatures than animals treated at either 1, 5, or 13 days of age with saline ($F = 18.35; df = 1, 101; p < .001$). This effect was obtained on test days 1-3 following either morphine or saline, but not at each postinjection interval. On test day
Table 7. Mean body temperatures obtained from animals morphine or saline treated at 1, 5, or 13 days old and tested following either morphine or saline injection in Experiment 2. The three values for each subgroup on each test day represent mean temperatures recorded 60, 120 and 180 min after injection respectively. On test day 4, all injections were morphine.

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<th>Infant Treatment</th>
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4, infant morphine treated animals displayed significantly higher body temperatures than infant saline treated animals 60 min after injection ($F = 6.82; df = 1, 168; p < .05$) and 180 min after injection ($F = 7.75; df = 1, 168; p < .05$). These data indicate that morphine treatment at either 1, 5, or 13 days of age increased later body temperature.

Discussion

The results of Experiment 2 demonstrate that a single morphine dose administered at different periods of infancy can differentially influence later morphine-induced analgesic and hypoactive effects. Morphine exposure at one day of age decreased PLLs and increased activity measured after morphine injection at 26 days of age. In contrast, morphine exposure at either 5, 9, or 13 days of age did not influence PLLs or activity measured after morphine injection at 26 days of age. These results indicate that the efficacy of 1.0 mg/kg morphine in producing long-lasting single-dose tolerance in infants declines soon after one day of age.

Although early morphine treatment produced tolerance to later morphine induced analgesic and hypoactive effects, this treatment did not produce tolerance to later morphine-induced hyperthermia. Single-dose morphine administered at either 1, 5, or 13 days of age increased rather than decreased body temperatures measured at 26-29 days of age. Moreover, this effect was obtained regardless of whether body temperatures were measured after morphine or saline injections. These results indicate that morphine treatment in infancy can produce long-lasting alterations in body temperature regulation.
Infant morphine treatment produced long-lasting age-dependent changes in pain responsivity, activity, and body temperature. Long-lasting age-dependent changes in pain responsivity and activity were also produced by saline treatment. Animals given saline early in infancy displayed greater morphine-induced analgesic and hypoactive effects than animals given saline later in infancy (cf. S-M and S-S groups on test day 1 in Figures 9 and 10). Thus, the injection procedure per se administered at different infancy periods influenced later morphine effects. However, these age-related effects of infant saline treatment were not observed for morphine-induced hyperthermia.
GENERAL DISCUSSION

A single injection of 1 mg/kg morphine in one-day-old rats produced long-lasting tolerance to subsequent morphine-induced analgesic and hypoactive effects but not to hyperthermic effects. In Experiment 1, animals given morphine at one day of age displayed shorter PLLs and greater activity at 26-28 days of age than animals given saline at one day of age. These effects were obtained regardless of whether animals were tested following morphine or saline. The PLL results contrast with the observation by Huidobro and Huidobro (1973) that morphine treatment at one day of age produced relatively long PLLs indicative of morphine-induced hyperanalgesia. However, the present results are consistent with observations of single-dose tolerance in adults (Cochin and Kornetsky, 1964; Kornetsky and Bain, 1968) and with observations of tolerance following multiple doses or pellet implantation in infants (Sonderegger, Bromley, and Zimmermann, 1977; Sonderegger and Zimmermann, 1976; Sonderegger and Zimmermann, 1978; Zimmermann, Branch, Newman Taylor, Young, and Pang, 1974). Furthermore, these results are also consistent with the observation that, relative to analgesic and hypoactive effects, the development of tolerance to hyperthermia is extremely slow (Fernandes, Kluwe, and Coper, 1977).

Single-dose tolerance to 1 mg/kg morphine in infants was age-dependent. In Experiment 2, morphine treatment at 1 but not at 5, 9, or 13 days of age attenuated morphine-induced analgesic and hypoactive effects as assessed at 26-28 days of age. This age-dependent change in susceptibility to single-dose tolerance with 1 mg/kg morphine may reflect either a difference in the interval between infant morphine treatment and tolerance assessment,
a change in BBB development, or a change in opiate receptor development.

Age-dependent changes in susceptibility to single-dose tolerance may reflect a change in the interval between infant morphine treatment and tolerance assessment. Tolerance assessment was initiated at 26 days of age regardless of when morphine was administered in infancy. Thus, animals treated at 1 day of age had a longer interval between morphine treatment and tolerance assessment than animals treated at either 5, 9, or 13 days of age; i.e., animals treated at either 1, 5, 9, or 13 days of age had treatment-tolerance assessment intervals of either 25, 21, 17, or 13 days respectively. Tolerance obtained following morphine at 1 but not at 5, 9, or 13 days of age might therefore suggest that a 25-day "incubation" period is necessary for tolerance to be manifest. According to this interpretation, morphine administration at either 1, 5, 9, or 13 days of age would be expected to produce similar tolerance if assessed at either 26, 30, 34, or 38 days of age respectively. This possibility however, is not consistent with reports demonstrating that tolerance decreases when the interval between initial morphine treatment and tolerance assessment increases beyond about three days (Cochin and Korner, 1964; Huidobro, Huidobro-Toro, and Way, 1976). This evidence suggests that animals treated late in infancy should have displayed greater tolerance than animals treated early in infancy. Since this effect was clearly not obtained, it is unlikely that the age-dependent change in susceptibility to tolerance reported here reflects a difference in treatment-tolerance assessment intervals.
Age-dependent changes in susceptibility to morphine tolerance may reflect a change in BBB development. As previously described, the BBB to morphine is virtually nonexistent up to about 15 days of age, but rapidly develops from this time and becomes adult-like by about 32 days of age (Johannesson and Becker, 1973; Johannesson, Steele, and Becker, 1972; Kupferberg and Way, 1963). During this period of BBB development, there is a concomitant decrease in morphine-induced analgesic effect (Huidobro and Huidobro, 1973). These observations indicate that the magnitude of morphine-induced analgesic and perhaps hypoactive effects are potentially related to BBB maturation. If this is the case, then the attenuation in morphine-induced analgesic and hypoactive effects produced by morphine treatment at 1 but not at 5, 9, or 13 days of age in the present experiments may reflect a potentiation in BBB maturation induced by morphine treatment at 1 but not at 5, 9, or 13 days of age. This possibility could be investigated by assaying morphine levels in brain and blood after morphine injection at 26 days of age in animals previously treated with morphine at different periods of infancy.

Age-dependent changes in susceptibility to morphine tolerance may reflect a change in opiate receptor development. As previously described, opiate receptors are first evident two weeks into gestation and progressively increase in number up to adulthood (Clendeninn, Petraitis, and Simon, 1976; Coyle and Pert, 1976). These receptors appear to be involved in morphine-induced analgesia, as morphine-induced analgesia is antagonized by naloxone, an opiate antagonist which has greater affinity
for the opiate receptor than morphine (Terenius, 1974). Moreover, mor-
phine produces analgesia when microinjected into high density but not
low density opiate receptor sites (Mayer and Murphin, 1976; Yaksh, Yeung,
and Rudy, 1976), and finally the analgesic efficacy of morphine is direct-
ly correlated with the normal ontogenetic increase in number of opiate
receptors from birth to about 15 days of age in rats (Auguy-Valette, Cros,
Gouarderes, Gout, and Pontonnier, 1978). Opiate receptors may also be in-
volved in morphine-induced hypoactivity, as morphine-induced hypoactivity,
like analgesia, is antagonized by naloxone (Bhargava, 1978). These ob-
servations suggest that the magnitude of morphine-induced analgesic and
hypoactive effects may be related to the number of opiate receptors in
brain. If this is the case, then the attenuation in morphine-induced
analgesic and hypoactive effects produced by morphine treatment at 1 but
not at 5, 9, or 13 days of age in the present experiments may reflect an
attenuation in opiate receptor development induced by morphine treatment
at 1 but not at 5, 9, or 13 days of age. This possibility is consistent
with evidence indicating that the number of opiate receptors decreases
following morphine treatment. Although this effect has only been observed
in adult brain slices (Davis, Akera, and Brody, 1975), there is some ev-
idence that infant treatment can alter opiate receptor development. In
infants, the number of opiate receptors has been reported to increase fol-
lowing naloxone (Diaz, Paul, Frenk, and Bailey, 1978) or stress (Torda,
1977). One report, however, has failed to observe any alteration in the
number of opiate receptors in infants following morphine (Coyle and Pert,
1976). Clearly, further research is necessary to determine whether the
age-dependent change in susceptibility to tolerance reported here reflects
a change in opiate receptor development.

Changes in opiate receptor or BBB development may underly age-depen­
dent changes in morphine tolerance. The present experiments demonstrate
that these possibilities may be assessed following acute low-dose morphine
treatment. Previous reports of long-lasting morphine tolerance in infants
have administered morphine for one week or longer (Sonderegger, Bromley, and
Zimmermann, 1977; Sonderegger and Zimmermann, 1976; Sonderegger and
Zimmermann, 1978; Zimmermann, Branch, Newman Taylor, Young, and Pang, 1974).
Such long-term treatment does not allow for evaluation of age-dependent
changes in susceptibility to tolerance within a short period of infancy
such as from 1 to 13 days of age. Moreover, such long-term treatment in
infancy increases mortality (Zimmermann, Sonderegger and Bromley, 1977).
In the present experiments, mortality following morphine treatment was
minimized by acute injection and in fact was not observed following acute
morphine at either 1, 5, 9, or 13 days of age in Experiment 2.

Early morphine injection clearly influenced subsequent response mea­
sures. The present results also demonstrate that early saline injection
can produce robust long-lasting changes in pain responsivity, activity,
and body temperature. In Experiment 1, animals treated with saline at
one day of age displayed less activity and higher body temperatures than
animals untreated at one day of age. These effects were obtained at 26-28
days of age regardless of whether animals were tested following morphine
or saline. In Experiment 2, animals treated early in infancy with saline
displayed longer PLLs and less activity than animals treated late in in­
fancy with saline. These effects were obtained at 26-28 days of age when animals were tested following morphine. Some evidence suggests that these effects may be stress-related.

Stressful stimulation during infancy produces a variety of long-lasting physiological and behavioral changes in rats (Daly, 1973; Denenberg and Zarrow, 1971; Levine, 1969; Russell, 1971). These changes reflect direct effects on the infant, as well as indirect effects induced by alterations in maternal care (Barnett and Burn, 1967; Bell, Nitschke, Bell, and Zachman, 1974; Smotherman, Wiener, Mendoza, and Levine, 1977). Stress effects in infants have typically been produced by administering intense and chronic sensory stimulation over a long period of infancy; e.g., intermittent shock applied throughout preweaning development. In the present experiments however, changes in pain responsivity, activity, and body temperature were produced by administering relatively moderate and acute sensory stimulation over a brief period of infancy; i.e., saline injection applied at either 1, 5, 9, or 13 days of age. The injection procedure involved a 15-minute period of separation from the lactating mother, during which time infants were handled and injected with saline. Changes in pain responsivity, activity, and body temperature produced by the infant saline treatment may therefore reflect stress induced by separation from the mother, handling, and/or the injection procedure. Regardless of these factors played a role in the long-lasting changes produced by infant saline treatment reported here, the present observations illustrate the difference between saline-injected and untreated control groups for assessing long-lasting psychopharmacological consequences of infant drug treatment.
Finally, saline administration at 26-28 days of age also may have influenced pain responsivity, activity, and body temperature independent of morphine. This possibility was not directly assessed in the present experiments, as no control group was uninjected at 26-28 days of age. However, it has previously been shown that saline injection produces a transient increase in body temperature (Gunne, 1960), suggesting that it may be stressful. In the present experiments, body temperatures were higher 60 minutes after saline injection than 180 minutes after injection. Exposure to the hot plate may have contributed to this temperature increase, as hot plate tests preceded body temperature measurements and hot plate exposure is known to be stressful (Torda, 1977). Acute stress produces a transient elevation in endogenous morphine-like neuropeptides in brain (Guillemin, Vargo, Rossier, Minick, Ling, Rivier, Vale, and Bloom, 1977; Madden, Akil, Patrick, and Barchas, 1977) and produces various effects which are functionally similar to those produced by morphine, including analgesia (Akil, Madden, Patrick, and Barchas, 1976; Bodnar, Kelly, Spiaggia, and Glusman, 1978), hypoactivity (Gray, Solomon, Dunphy, Carr, and Hession, 1976; Pinel and Mucha, 1973) and hyperthermia (Delini-Stula, 1970). These observations indicate that stressful stimulation is an important factor to consider when assessing morphine effects across the life-span.
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