Morphine administration or sexual segregation in infancy affect the response to the same drug in adult mice

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Summary. - Several experiments indicate that CNS opioid regulatory systems show a remarkable plasticity during development. The same systems respond to a wide range of environmental stimuli, particularly those which can affect the threshold of pain sensitivity (e.g., Environmentally Induced Analgesia). This paper summarizes a series of studies using outbred CD-1 mice, aimed at assessing: a) morphine effects on pain sensitivity and locomotor activity at two ages during development, namely, before and after weaning, and b) the consequences of such exposure on adult sensitivity to the same drug. The development of hot-plate response consisted mainly of a progressive decrease of latencies and of a parallel reduction of sensitivity to morphine. While morphine depressed activity on day 14, it increased or had apparently no effect on day 21. With respect to carry-over consequences of early drug and test exposure, the animals with a history of testing at the preweaning stage were more sensitive to the depressant effect of morphine (10 mg/kg) than those pretested at a later stage. By contrast, morphine analgesia was attenuated by drug pre-exposure, independently of the age of previous testing. In sum, the age of early exposure and type of early treatment interacted to determine the level of adult pain sensitivity in the no-drug state. Finally, the long-term effects of sexual segregation in infancy on the response to painful stimulation and morphine were assessed. Adult male mice - reared from birth to weaning in litters containing either only male pups (MM), or both male and female pups (MF) - were challenged in a hot-plate test upon morphine or saline injection. In absence of significant differences between MM and MF mice in body weight gain and in achievement of a series of neurobehavioural landmarks, MF males showed longer latencies than MM animals, and such a difference increased upon morphine administration. The latter results indicate that the function of systems mediating response to painful stimulation and opiate analgesia can be affected by subtle variations in early social conditions.

Key words: infantile sexual segregation, long-term effects, analgesia, morphine, mouse.

Introduzione

Several experiments indicate that CNS opioid regulatory systems show a remarkable plasticity during development. In fact, systemic administration of morphine to altricial rodents results at different postnatal ages in quite different physiological and behavioral changes [1-4]. Specifically, the analgesic effect of the drug in rats increases from the early postnatal period until about postnatal day 15 [1] in parallel to a rapid rise of the number of ∝-receptors [5]. In the subsequent period, drug sensitivity declines [1, 2] in parallel to the reduction of general drug toxicity, and these changes are apparently related to the maturation of the blood/brain barrier [1], for literature data and discussion, see also [5].
Locomotor activity is also differentially affected by morphine as a function of postnatal age. One study showed a reduction of activity in rats of ten days, and either no effects or activity enhancements at later ages [3]. Another study, lnbred mice of the C57BL/6 strain showed mainly hyperactivity during ontogeny, except for a brief period (days 20-22), when the drug produced a marked catatonia [6].

More recently, attention has been given to the proactive effects of postnatal morphine exposure on subsequent responding to the drug. For example, extended morphine administration in rats from birth to weaning produced tolerance to the analgesic and hypoactivating effects of the drug at 22 days without, however, any appreciable effect on µ-receptors proliferation [7]. The same investigators also showed that a single dose of morphine administered to newborn rats sufficed to produce a similar change in sensitivity 26 days later. The same systems respond to a wide range of environmental stimuli, particularly those which can affect the threshold of pain sensitivity (e.g. Environmentally Induced Analgesia produced by different types of stressful experiences). In addition, there is now substantial evidence for a role of endogenous brain opioids in the regulation of social responses [8-11] and several experiments have shown an influence of social environment on opioid system functioning. Brief periods (1-4 days) of social isolation increased pain responsiveness in young rats, while concurrently decreasing the analgesic efficacy of morphine [12]. Conversely, when animals were isolated for prolonged periods (3-13 weeks), responsivity to morphine increased [13, 14]. The subtlety of these interactions is shown by another experiment using young rats isolated for 10 days, save for brief periods of social interaction (5 min of pairing on alternate days). In fact, at 33 days (3H)adrenorphine binding in brain was reduced in animals grouped for 30 min before sacrifice, relative to animals not grouped [15].

In this frame, we have performed over the past years a series of experiments aimed at assessing the effects of different doses of morphine on pain sensitivity and locomotor activity of mice at two ages during development, namely, before and after weaning, and the consequences of such exposure on adult sensitivity to the same drug. Animals were treated and tested for three consecutive days either during the last part of the previsual stage (preweanlings; days 14-16; eye opening is completed on day 17 in the strain used [19], or starting on the day after weaning (postweanlings; days 21-23). These treatment periods were chosen also on the basis of previous studies, which showed that rat and mouse pups of these two ages have quite different patterns of activity, habituation, and response to model drugs such as amphetamine and scopolamine [16, 17].

A second goal was to assess the long-term drug effects on morphine responding at the adult stage. All animals were thus retested at about 70 days of age for activity, pain reactivity, and morphine effects thereon.

Finally, the long-term effects of early manipulation of social environment (sexual segregation) on subsequent reactivity to painful stimulation and morphine challenge were assessed. At this aim, adult male mice - reared from birth to weaning in litters containing either only male pups (MM), or both male and female pups (MF) - were challenged in a hot-plate test upon morphine or saline injection.

A substantial part of the data has already been published [5, 18]. Therefore, the main purpose of the present paper is to address some interesting points which have emerged in the course of these studies.

Effects of morphine on activity and pain reactivity during development

Preliminary experiments showed a much more marked sensitivity to the depressant effects of the drug in preweanlings than in postweanlings, and a 20 mg/kg dose was sometimes lethal in the former. Therefore, the doses were 0, 0.5, 1, 5, or 10 mg/kg for preweanlings and 0, 1, 5, 10, or 20 mg/kg for postweanlings. On each of the following two days each pup was similarly weighed/injected, and tested for activity but not for pain reactivity.

On day 25, all litters were reduced to the four animals treated previously with either saline or 1, 5, or 10 mg/kg of morphine. On day 70 (+2 days) the mice were injected ip with saline, and 20 min later their activity was measured. Immediately after, the animals were exposed to the hot plate. Twenty-four hr later, activity and hot-plate reactivity were assessed again after morphine administration (10 mg/kg ip). All tests were carried out during the initial hours of the dark period. The experimental design was counterbalanced in order to equate the representation of various groups at different test times, while repeated testing of the same animal took place at about the same time of day.

The data on hot-plate responding at 14 and 21 days (Fig. 1, upper graph) clearly show maximal or near-maximal analgesic effects of morphine already at the lowest doses used. There were no significant differences between drug doses, which also applies to the apparently more marked analgesia produced at 21 days by 10 mg/kg of the drug, than by other doses.

The activity data did not show differences between successive daily sessions, therefore they are presented after pooling of the scores obtained on days 14-16 and 21-23, respectively (Fig. 1, lower graph). In preweanlings, morphine depressed activity already at the lowest dose used. In postweanlings, the drug effect was apparently nonmonotonic, with a slight stimulation at the lower dose and depression at the highest dose.
had been previously exposed to the same drug than in mice previously injected with saline. This was confirmed by the finding of a significant effect of prior treatment in the ANOVA.

The adult activity data (Fig. 3) failed to show significant differences between the various groups on the first day of testing upon saline injection. On the following day, after morphine injection (10 mg/kg), the animals previously exposed to either saline or drug at 21-23 days maintained a higher activity level than those pre-exposed at 14-16 days. This was confirmed by the ANOVA, which yielded a highly significant effect of age of prior testing without an effect of type of previous treatment.

Adult response to painful stimulation and morphine as a function of sexual segregation in infancy

Pregnant mice were randomly assigned to either the MM or the MF experimental group. At birth, litters of the MM group were culled to 6 male pups, while MF litters were culled to 3 male and 3 female pups. In four cases the gender composition of the litter made it impossible to respect the original assignment. These litters were excluded from the experiment, which used 13 litters in each of the two groups.

At weaning (day 21) all females and three randomly selected males from each of the MM litters were sacrificed. The remaining three males in each litter were left undisturbed (save for routine maintenance) in the maternity box until the day of hot-plate testing.

As shown in Table 1, body growth and scores of neurobehavioural development were also recorded at several ages during development, to take into account possible biases in offspring growth rate deriving from a different access to the dam. No significant differences as a function of social manipulation were evident.

These measures were selected as developmental markers of critical stages in mouse ontogeny according to a slightly modified Fox procedure. These tests assessed whether or not the animal had developed an adultlike performance. All measurements took place during the initial hours of the light-dark cycle. (Reprinted with permission from [19]).

The results on the hot-plate test are depicted in Fig. 4. Latencies to first paw lick were significantly affected by both the rearing and the drug variables. Between-group comparisons showed a significant difference between MF and MM males treated by morphine at either the 5 mg/kg (p < 0.05) or the 10 mg/kg doses (p < 0.01), but not in the saline treatment condition.

General discussion

The data obtained in the immature mice of the present experiments confirm and extend previous rat data showing marked changes of morphine effects during development (see “Introduction”). Specifically, the sensitivity to the
Fig. 2. - Hot-plate reactivity on first and second day of retesting at about 70 days (after saline and morphine, respectively) of mice previously exposed as preweanlings or postweanlings (see Fig. 1). Data are means (± S.E.M.) of 9 animals.

Fig. 3. - Activity on first and second day of retesting at about 70 days (after saline and morphine, respectively) of mice previously exposed as preweanlings or postweanlings (see Fig. 1). Data are means (± S.E.M.) of 9 animals.

depressant action of the drug on activity was markedly attenuated between postnatal days 14-16 and 21-23. It has also to be noticed that activity levels were substantially the same in successive daily tests, pointing to an absence of morphine tolerance with these combinations of testing and treatment schedules. By contrast, the differences in analgesic effects of morphine at the two ages, if any, were minimal. Therefore, the overall profile cannot be explained either by the increase of opioid receptors during the third week [20] or by an increased efficiency of the blood/brain barrier [1]. As concerns the latter, however, one cannot exclude differential maturation phenomena involving separate brain areas responsible for effects on activity and pain reactivity, respectively.
Table 1. - Postnatal somatic and neurobehavioural scores

<table>
<thead>
<tr>
<th></th>
<th>MM (n=78)</th>
<th>FF (n=78)</th>
<th>MF (n=78)</th>
<th>M(MF) (n=39)</th>
<th>F(MF) (n=39)</th>
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</thead>
<tbody>
<tr>
<td><strong>Day 3</strong></td>
<td></td>
<td></td>
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<tr>
<td>Body weight (g)</td>
<td>2.44 ± 0.28</td>
<td>2.46 ± 0.28</td>
<td>2.64 ± 0.30</td>
<td>2.64 ± 0.30</td>
<td>2.65 ± 0.30</td>
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<tr>
<td>Slow-righting (%)</td>
<td>62.82</td>
<td>65.38</td>
<td>62.82</td>
<td>66.67</td>
<td>56.41</td>
</tr>
<tr>
<td>Cliff-aversion (%)</td>
<td>42.85</td>
<td>42.85</td>
<td>38.09</td>
<td>42.86</td>
<td>33.33</td>
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<tr>
<td><strong>Day 7</strong></td>
<td></td>
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<tr>
<td>Body weight (g)</td>
<td>4.95 ± 0.56</td>
<td>4.92 ± 0.56</td>
<td>4.91 ± 0.56</td>
<td>4.93 ± 0.56</td>
<td>4.90 ± 0.50</td>
</tr>
<tr>
<td>Slow-righting (%)</td>
<td>92.30</td>
<td>78.20</td>
<td>88.46</td>
<td>87.18</td>
<td>89.74</td>
</tr>
<tr>
<td>Forelimb-placing (%)</td>
<td>91.12</td>
<td>94.87</td>
<td>98.71</td>
<td>97.44</td>
<td>100</td>
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<tr>
<td>Hindlimb-placing (%)</td>
<td>24.35</td>
<td>26.56</td>
<td>16.66</td>
<td>17.55</td>
<td>15.38</td>
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<td><strong>Day 14</strong></td>
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<tr>
<td>Body weight (g)</td>
<td>9.54 ± 1.08</td>
<td>9.64 ± 1.09</td>
<td>9.63 ± 1.09</td>
<td>9.62 ± 109</td>
<td>9.63 ± 109</td>
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<tr>
<td>Screen-climbing (%)</td>
<td>94.87</td>
<td>98.71</td>
<td>98.71</td>
<td>97.44</td>
<td>100</td>
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<tr>
<td>Ear-opening (%)</td>
<td>96.15</td>
<td>98.71</td>
<td>98.71</td>
<td>100</td>
<td>97.44</td>
</tr>
<tr>
<td>Eye-opening (%)</td>
<td>46.15</td>
<td>39.74</td>
<td>42.30</td>
<td>46.15</td>
<td>38.46</td>
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<tr>
<td><strong>Day 18</strong></td>
<td></td>
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<tr>
<td>Body weight (g)</td>
<td>11.68 ± 1.32</td>
<td>11.59 ± 1.31</td>
<td>11.39 ± 1.29</td>
<td>11.41 ± 1.30</td>
<td>11.37 ± 1.29</td>
</tr>
<tr>
<td>Ear-opening (%)</td>
<td>100</td>
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<td>100</td>
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<tr>
<td>Eye-opening (%)</td>
<td>100</td>
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On the other hand, it must be emphasized that major changes in activity patterns and response to model drugs such as amphetamine and scopolamine occur during the third week of postnatal development (for rat and mouse data, see [16, 17]). This suggests that differences in morphine responding between preweanlings and postweanlings may not be due to differences in the drug effect per se, but rather to modified profile of interactions between opioid modulatory mechanisms and other regulatory systems (for literature and discussion, see [21, 22]).

In fact, morphine effects on activity during development appear to be markedly affected by organismic and test variables, which are known to influence activity patterns and responses to neurotransmitter agonists and antagonists. Specifically, Filibeck et al. [6], using an inbred mouse strain (C57BL/6) and a different (unfamiliar) test environment, found an increase of baseline activity during the third postnatal week much larger than that observed in the present experiments. Moreover, these investigators found morphine effects which were almost constantly in the direction of a marked hyperactivity, in spite of different developmental ages and activity baselines.

With respect to long-term consequences of early morphine and text exposure, the results of the present experiment further support the view that the drug affects activity and pain reactivity by different mechanisms [23]. Prior morphine exposure did not influence activity and response to morphine at the young-adult stage. But the animals with a history of testing at the preweaning stage were more sensitive than those pretested at a later stage to the depressant effect of a 10 mg/kg drug dose. By contrast, morphine analgesia was attenuated by prior drug exposure, independently of age of previous testing.

Fig. 4. - Mean latency (± S.E.M.) to first paw lick in a hot-plate test at 40 days of male mice reared until weaning in litters composed of all male (MM) or male and female (MF) pups. One animal from each litter was injected IP with either saline, 5, or 10 mg/kg of morphine 15 min before testing (N = 13 per group).

This picture is further complicated by the finding that age of early exposure and type of early treatment interacted to determine the level of adult pain sensitivity in the no-drug state. The lack of controls left undisturbed at the time of early testing, however, does not allow to discriminate between two main possibilities. The first is that all combinations of early treatment and time of early exposure produced hyperalgesia, except exposure in the no-drug state at the preweanling stage. The second is that of a hypalgesia which was produced only by exposure at the preweanling stage and was prevented if such exposure took place in the morphine state.

It appears difficult to suggest any mechanism that might account for the former type of effect. (For example, a conditioned hypalgesia [24] could explain the profiles of the groups previously exposed to morphine, but a host of additional assumptions would be necessary to account for a hyperalgesia in the group treated with saline at the postweanling stage). On the other hand, some indications are available, which indirectly support the alternative explanation outlined above. Specifically, the reduced sensitivity to pain of animals exposed at the preweanling stage resembles the well-known phenomenon of environmentally induced analgesia (EIA; [25]), which can follow a wide variety of stressful experiences (for literature and discussion, see also [21]). The fact that a similar long-term effect did not occur in the case of early exposure in the morphine state could be ascribed to an attenuation of the pain experience and/or to a state-dependence phenomenon. At this point, one can speculate about the difference between the groups exposed in the no-drug state at the preweanling and the postweanling stage, respectively. Some critical differences between the two developmental ages are well-known; for example, cholinergic (muscarinic) mechanisms which contribute to the control of the organism’s activity become functional during the third postnatal week [16]. On the other hand, opioid and non-opioid mechanisms, including muscarinic ones, are known to interact in a complex fashion to produce various components of EIA [24-26]. Therefore, one should test the hypothesis that changes in the interactions between different systems, particularly opioid, GABAergic and cholinergic ones, may make the organism progressively more selective with respect to long-term repercussions of early environmental stimulation (for literature and discussion, see [21]).

With respect to the effects of sexual segregation in infancy on adult pain response and morphine analgesia, namely, male mice raised in all-male litters showed a less marked analgesic response to morphine than male mice reared in litters with a balanced gender composition; this difference is apparently specific since it was not related to variation of somatic and neurobehavioural development as a function of sex ratio in the litter. Moreover, it was obtained by a manipulation quite different from those used in previous studies, which compared the different effects of group and individual housing.

It is also worth noting that the results so far reported in the literature were obtained by testing animals shortly after the end of the period of enforced social condition [11, 14]. In the present study, the interval between the end of exposure to different social conditions and testing was 20 days. Therefore, the results suggest that manipulation of social milieu during infancy has a long-term influence on systems which serve general reactivity and pain sensitivity by modulating response to painful stimulation.

At the present stage of the work it is not possible to determine which factor was responsible for the differences related to early social environment condition. As outlined above, the opioid receptors mature early in ontogeny, and may have an important physiological role in mediating the affiliative bonds early in development (see “Introduction”). Therefore, one working hypothesis with respect to such a “sibling effect” [28], may be that the nature of the interactions between pups of the same litter (e.g., different levels of social grooming or playful interactions in infancy [29]) can affect the development and subsequent function of this system. On the other hand, differential postnatal maternal effects due to variation of the dam’s behaviour depending on litter gender composition must also be considered [19].

The different individual response to the analgesic effect of morphine apparently reflects the individual variation in sensitivity of central mechanisms mediating the action of opiate agents. An intriguing possibility that morphine administration can also affect in vivo the release of the endogenous ligands for opiate binding sites cannot be excluded. Therefore, a tentative hypothesis is that, in morphine-responders (i.e., the MF-mice), the concentration of the endogenous ligands (in CNS) is higher than in MM-mice (morphine-noresponders). If it is the case or not, the use of MM and MF animals, or in general this type of early social manipulation, offers an additional tool for analyzing the functional significance of the relation between different ligand sites at the opioid receptor complex (see e.g. [30]).

With respect to the mechanisms involved in the production of the observed differences, subsequent research will need to consider both opioid and non-opioid systems which interact in the modulation of pain sensitivity and of the various components of the response to pain (see e.g. [30]). The fact that artificial manipulation of social environment in infancy can qualitatively affect some aspects of the animal’s behavioral repertoire at adulthood raises the question of whether comparable effects might occur naturally in view of the large difference in sex ratio of natural mouse litters. Perhaps, quantitative and/or qualitative variation in social interaction with the opposite sex in infancy is a contributor to the interindividual variation in the capacity of coping with environmental challenges, including drug administration or toxicant exposure (for literature and discussion see [30]).
Acknowledgements

This study was performed as part of the sub-project Behavioural Pathophysiology (project Non-infectious Pathology, Istituto Superiore di Sanità). We thank Dr. Flavia Chiarotti for expert statistical advice.


REFERENCES


