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Does the effect of morphine challenge change on maternal behaviour of dams chronically treated with morphine during gestation and further on during lactation?

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ABSTRACT

Opioids impair the maternal behaviour of rats. The effect of morphine on maternal behaviour in dams treated chronically with morphine during the whole pregnancy and lactation has not been analysed systematically. The aim of the present study was to investigate the possible differences in the disruptive effect of morphine on maternal behaviour following morphine challenges between dams treated chronically with saline or morphine during gestation and postpartum. The antinociceptive action of morphine was also studied in dams. The disruptive effect of morphine on maternal behaviour was not changed as the postpartum period passed. The duration of this effect of morphine lasted for about 2 h. The dose-dependent disruptive effect of acute doses of morphine on maternal behaviour was more marked in the morphine-treated dams, than in the saline-treated ones, indicating a tendency for sensitisation to this effect. A trend for tolerance was observed to the antinociceptive effect of morphine in animals treated daily with morphine during the entire gestational and lactation periods; however, this difference did not reach statistical significance. Our experimental protocol might be a predictive model of human opioid abuse. Sensitisation to the impairing effect of opiates on maternal behaviour may explain why a mother abusing heroin neglects her baby even if she does not experience euphoria.

1. Introduction

Endogenous opioids are important in maintaining pregnancy, inducing delivery and maternal behaviour (MB) (Brunton and Russell, 2008).

Previous studies demonstrated that opiates such as morphine (MO) disrupt and opiate antagonists restore MB (Blass et al., 1995; Bridges and Grimm, 1982; Brunton and Russell, 2008; Grimm and Bridges, 1983; Hammer and Bridges, 1987; Kinsley et al., 1993; Rosenblatt et al., 1988). Disruption of MB was demonstrated by various methods, like lack of retrieval (Grimm and Bridges, 1983), placentophagia (Mayer et al., 1985), impairment of pup care and nursing (Slamberova et al., 2001). Others demonstrated that activation of μ-opioid receptors by intracerebroventricular (i.c.v.) infusion of various opioid agonists dose-dependently impaired MB in primiparous lactating rats (Mann et al., 1991). The number of preceding pregnancies or parturitions seems to affect the action of MO; multiparous female rats were significantly less sensitive to the MB-disruptive effect of MO than primiparous ones (Kinsley and Bridges, 1988). The duration of acute disruptive effect of MO was found to be 2-4 hours (Grimm and Bridges, 1983).

It was shown that gradually increased dose of MO, administered i.c.v. by osmotic pump inhibited MB from the 2nd postpartum day (PD) until PD7-PD9. However, MO's impairing effect decreased by time; on PD6 and PD7 the difference in the MB of the vehicle- and the MO-treated dams was not significant (Rayner et al., 1988).

According to several studies, prenatal opioid exposure results in behavioural changes in the pups, e.g. enhanced locomotor activity (Hutchings et al., 1993), decreased adaptation to a novel environment (Yang et al., 2003) or increased sensitivity to the rewarding effect of opioids (Gagin et al., 1996; Gagin et al., 1997; Ramsey et al., 1993; Timár et al., 2010). Though these behavioural consequences can be explained mainly by alterations in the endogenous opioid functioning and disturbance in early neuronal development (Tenconi et al., 1996), the role of disrupted MB cannot be excluded.

Little data can be found in the literature about the consequences of chronic MO treatment during pregnancy on MB after parturition. It was reported that MO given twice daily in a dose of 10 mg/kg subcutaneously (s.c.) between the 11th and the 18th gestational

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days decreased the nursing and increased the latency in pup retrieval test (RT) compared to dams treated with saline (SAL) (Slamberova et al., 2001). According to other studies a 7 day-long MO treatment from the 17th day of pregnancy resulted in sensitisation to the MB-inhibitory effect of MO, while repeated MO treatments before mating did not influence the effect of a subsequent MO challenge on MB (Miranda-Paiva et al., 2001; Yim et al., 2006).

In a preliminary series of experiments, a modified form of treatment schedule for developing MO dependence was applied (Buckett, 1965). In this series, the starting dose was 5 mg/kg twice a day and the maximal dose (30 mg/kg twice daily) was reached on the 5th day, then this dose-schedule was continued. Although all the dams survived in the following weeks, there was no normal parturition among the MO-treated dams, but abortion, premature delivery and death of pups after birth could be observed. Furthermore, the continuous MO exposure in moderate doses is considered to be more relevant to the daily regular human opiate abuse (Carpenter et al., 1998; Crawford et al., 1983; Degenhardt et al., 2008). That is why in the present series of experiments a constant dose of MO (10 mg/kg) once a day was applied.

Hitherto, the challenging effect of MO on MB in dams treated chronically with MO during the entire pregnancy and further in the postpartum period has not been analysed systematically. Furthermore, no data was found on possible changes of any other effects of MO after MO treatment protocols similar or comparable to ours. In order to check whether our constant treatment with 10 mg/kg MO during the whole perinatal period (including the entire pregnancy and further in postpartum period) induces the usually observed tolerance to the antinociceptive effect of MO, a radiant tail-flick test was performed on dams after weaning.

The aim of the present study was to answer the following questions:

1. Is there any difference in MB-disruptive effect of MO as postpartum time passes?
2. How long is the duration of disruptive effect of MO on MB in dams treated with MO during gestation?
3. Does a dose-response relationship of MO exist on MB change in dams treated with MO during gestation?
4. How does the antinociceptive action of MO change in dams treated with MO during gestation?

2. Materials and methods

2.1. Animals

Male and female Wistar rats weighing 200-220 g (Charles River, Budapest, Hungary) were used in the experiments. Nulliparous female rats were mated with males at a ratio of 3:2 and the sperm positive females were than separated and housed individually. From the day of mating, the dams were divided into two groups. Group I was treated with saline (SAL). Group II was treated with MO s.c. once a day during the pregnancy and then further in the postpartum period. MO was given in a dose of 5 mg/kg/day on the first two days and 10 mg/kg/day afterwards. SAL or MO was administered at 8 a.m. every day. Constant rodent chow and water were available ad libitum. The animals were kept at a constant temperature (20-21 °C) and humidity (55 ± 5%) under a standard 12-12 h light/dark cycle (light on at 6.00 a.m.). Body weight was measured once a week during pregnancy and lactation. The day of delivery was considered to be PD1. Experiments were performed according to the Semmelweis University guidelines on the use of experimental animals under the license of the Ethical Committee. In all of the four series of experiments, separated groups of dams were used (see details in Section 2.4).

2.2. Materials

Morphine hydrochloride (ICN Hungary, Tiszavasvári, Hungary) was dissolved in physiological saline and given s.c. in a volume of 0.1 ml/100 g body weight.

2.3. Methods

2.3.1. Maternal behaviour

2.3.1.1. Observation. A modified method of Slamberova and Myers was used (Myers et al., 1989; Slamberova et al., 2001). The following behavioural parameters were checked: (i) Observed MB (MBo): active grooming (active nursing or cleaning of pups) passive nursing, littering or manipulating on nest shaves. (ii) Observed non–MB (non-MBo) (out-of-the-nest behaviour): eating, drinking, self-grooming and other behaviours, like rearing, ambulation, resting and sleeping. Besides these, non-MBo in the nest was also recorded. We observed animals for 2 hours; parameters were registered every 5 minutes. If any of the MBo or non-MBo parameters was observed, a score of "1" was given. The maximum score of the complex MBo or non-MBo was 24.

2.3.1.2. Retrieval test (RT). A modified method of Myers and Slamberova was used (Myers et al., 1989; Slamberova et al., 2001). All the pups were removed from the maternal cage. Five minutes after removal they were placed back in the corner of the maternal cages opposite to the nest. When a mother had less than 5 offsprings, the test-pups were completed to 5 from another litter. Latencies were measured until the 1st and the 5th pups were retrieved back to the nest. The maximum duration of the RT was 1200 seconds. When any of the tested offspring crawled back into the nest or the mother started to make a new nest the latency measured was considered invalid. Only valid data were analysed.

2.3.2. Tail-flick-test

The tail-flick test was performed as described earlier (Furst et al., 1993). At the beginning of the experiment the basal latency of the rats was measured. Animals with latencies over 4 sec were excluded from further testing. The latencies were tested 30 min after MO administration.

To avoid tissue damage, the cut-off was set to 8 sec. The antinociceptive effect was calculated as the Maximum Possible Effect: $MPE\% = \{(T' - T_0)/(\text{cut-off} - T_0)\} \times 100\%$.

2.4. Experimental protocols

2.4.1. Experiment 1. Maternal behaviour as the function of postpartum time

MB changes were investigated as the pups were aging. Both SAL- and MO-dams were tested for MB in the first period of lactation. According to previously reported data, time spent in active nursing and the frequency of maternal contact with the pups decreased between PD10 and PD13 (Slamberova et al., 2001). In our preliminary experiment, we found that RT could not be measured after PD10-12 because the pups climbed back alone into the nest (unpublished data). Therefore MB was tested in the first period of lactation i.e. on PD2, PD3, PD4, PD5, PD7 and PD9. Each dam was checked every day. Parameters of MBo and non-MBo activities were measured 30 minutes after the usual daily treatment (SAL in SAL-group, 10 mg/kg MO in MO-group) and after completing the MB observation (that is 150 min after treatment) RT was performed on the same dams. The number of dams was 6 and 7 in SAL- and MO-groups, respectively. Table 1 shows the protocol in this experiment.

2.4.2. Experiment 2. Duration of impairment of MB after MO challenge

MBo and non-MBo were observed in different time points: 30 min, 2 hours, 4 hours and 24 hours after the usual daily dose of MO (10 mg/

Table 1
Experimental protocols in experiment 1, 2 and 3.

Experimental protocol in experiment 1							
Treatment group	Challenge	Experiments on different postpartum days					
		PD2	PD3	PD4	PD5	PD7	PD9
SAL MO	10 mg/kg SAL 10 mg/kg MO	Each day 30 min after challenge MB observation and RT after MB observation was completed					

Experimental protocol in experiment 2					
Treatment group	Challenge	Experiments starting after challenge (PD2-3)			
		30 min	2 h	4 h	24 h
SAL	10 mg/kg SAL	MB observation and RT on a different population	-	-	MB observation and RT on a different population
MO	10 mg/kg MO	MB observation and RT on a different population	MB observation and RT on a different population	MB observation and RT on a different population	MB observation and RT on a different population

Experimental protocol in experiment 3						
Treatment group	Time and type of experiment	Challenge (PD2-3)				
SAL	Both MB observation and RT 30 min after challenge	SAL	1 mg/kg MO	3 mg/kg MO	10 mg/kg MO	20 mg/kg MO
MO		SAL	1 mg/kg MO	3 mg/kg MO	10 mg/kg MO	20 mg/kg MO

kg s.c.) in MO-group and 30 min and 24 hours after SAL in SAL-group. In the latter case, the 2 and 4 hours observations were omitted, since s.c. injection of SAL did not influence the MB. The experiments were performed on PD2-3. Different series of animals (two SAL-treated and four MO-treated groups) were used for each time-point. Since the aim of this experiment was to study the disruptive effect of MO on MB in the function of time after its administration, RT was performed in another six groups of animals. In this experiment RT was evaluated (without MB observation) also 30 min, 2 hours, 4 hours and 24 hours after the usual daily injection of MO (10 mg/kg s.c.) in MO-group and 30 min and 24 hours after SAL in SAL-group. The number of dams was 8-13 per group. Table 1 summarises the protocol in experiment 2.

2.4.3. Experiment 3. Dose-effect relationship of MO on MB

In this series of experiments both SAL-treated dams (SAL-dams) and MO-treated ones (MO-dams) were challenged with SAL or different doses of MO (1, 3, 10 and 20 mg/kg s.c.) on PD2-3. Different series of animals (five SAL-treated and five MO-treated groups) were used for each dose. Two-hour-observational tests and RT were made in another ten groups of dams. Both observations (MBo, non-MBo) and RT started 30 minutes after challenge. The number of animals was 9 and 8 in SAL- and MO-treated groups, respectively. Table 1 summarises the protocol in experiment 3.

2.4.4. Experiment 4. Antinociceptive effect of morphine

The antinociceptive effect of MO was determined two days after weaning by the radiant tail-flick test both in SAL- and MO-dams. These dams were treated with the same dose of MO (10 mg/kg) (or SAL) daily until the end of lactation. Animals used for this experiment were not tested in any of the previous experiments. Dose-response curves were established after injecting 1.5-3-6 mg/kg and 3-6-12 mg/kg MO to SAL-treated and to MO-treated dams, respectively. Five-five dams were used for testing each dose.

2.5. Statistical analysis

The data presented in the figures of the paper are mean values, with standard errors of the mean (S.E.M.).

In experiment 1, mixed ANOVA with a between groups factor of treatment (MO or SAL) and a repeated-measures factor of postpartum day (PD2, 3, 4, 5, 7, and 9) was performed on each outcome variable and multiple comparisons were performed by Dunnett's method; outcome variables on PD 3, 4, 5, 7, 9 were compared to that on PD2.

Kruskal-Wallis test and post-hoc testing by multiple comparisons after Dunn's method were used for statistical analysis in Experiment 2.

In experiment 3 statistical significance of difference between mean values was evaluated by two-way ANOVA. Dunnett post-hoc test was used, taking the SAL-treated SAL-challenged group as the control.

The difference between the birth weight of SAL- and MO-pups were analysed by Mann-Whitney U-test.

Results of the tail-flick test were analysed with nonlinear regression curve-fit analysis, ED_{50} values and their 95% confidence limits (95%CL) were calculated.

All of the analyses apart from mixed ANOVA were performed with GraphPad Prism version 3.00 for Windows, GraphPad Software, San

Table 2
Changes in body weight (g) of dams treated with SAL or MO from the 1st day of gestation (GD1) until the 14th postpartum day (PD14).

	GD1	GD7	GD14	GD21/PD1	PD7	PD14
SAL	211.9 ± 1.8	231.5 ± 3.4	281.9 ± 5.4	335.4 ± 4.7	373.8 ± 6.6	325.8 ± 3.8
MO	211.3 ± 3.48	231.3 ± 3.9	284.2 ± 4.3	336.3 ± 3.6	372.5 ± 2.4	322.1 ± 3.4

No difference between the two groups.
Data are shown in mean ± standard error of mean.

Diego California USA, www.graphpad.com Copyright (c) 1994-1999 by GraphPad Software. For mixed model ANOVA Splus 6.1 (Insightful, WA) was used.

Results were considered to be significant if *p* values were less than 0.05.

3. Results

3.1. Body weight and litter

During the whole gestation there was no difference in the body weight between SAL- and MO-dams (Table 2).

There was no statistically significant difference between SAL- and MO-groups in the number of litters, in the sex ratio of the litters, however the birth weight of prenatally MO exposed pups was significantly lower (5.67 ± 0.08 versus 5. 25 ± 0.07; $p<0.01$, Mann-Whitney-test).

3.1.1. Experiment 1. Maternal behaviour as the function of postpartum time during early postpartum period (PD2-9)

MO-treatment (factor: treatment) resulted in marked impairment in MBo ($F(1,11)=30.27$, $p=0.0002$), increase in non-MBo ($F(1,11)=27.17$, $p=0.0003$) and longer latencies in RT ($F(1,11)=55.42$, $p=0.00001$) for retrieving the 1st pup. MO treatment did not significantly lengthen the latency for retrieving the 5th pup back to the nest.

The effect of postpartum days (repeated measure factor PD) was significant in MBo ($F(1,63)=6.76$, $p=0.01$) and in non-MBo ($F(1,63)=4.06$, $p=0.05$) and was not in RT (either for the 1st or for the 5th pup). After performing multiple comparisons in SAL-group between days (PD), only the mean values of MBo scores on PD2 and 3 were significantly different (difference of means = 6.33, 95% CL = 0.4078 - 12.26, $p<0.05$). In the MO-group, the MBo scores were not different on the different days. After performing one-way repeated measures ANOVA in each treatment group the mean values of scores of non-MBo were not statistically different either in SAL-dams, or in MO-ones.

The interaction of treatment by days was not significant in either parameter (Fig. 1).

3.1.2. Experiment 2. Duration of impairment of MB after acute MO challenge

In the MO-group, 30 min and 2 h after 10 mg/kg s.c. MO challenge a marked, statistically significant impairment of MBo was found (difference in rank sum: 30.84, $p<0.001$ and 22.23, $p<0.01$, for 30 min and 2 h values, respectively). Similar results were obtained for non-MBo in the same group of animals (difference in rank sum: -30.04, $p<0.001$ and -21.81, $p<0.01$ for 30 min and 2 h values, respectively).

In RT, a similar time-effect relationship was observed. MO-treated dams retrieved their 1st pup and also the 5th pup into the nest significantly slower 30 min after the MO challenge (-19.61, $p<0.001$ and -23.00, $p<0.05$ for the 1st and 5th pup, respectively). Although the differences in rank sum of latencies between SAL- and MO-groups were big even 2 h after MO treatment (-16.26 and -16.75 for retrieval of the 1st and the 5th pup, respectively), these differences were not statistically significant.

There was no difference between SAL- and MO-groups either in the MBo, non-MBo or latencies in RT 24 h after the usual daily treatment (Fig. 2).

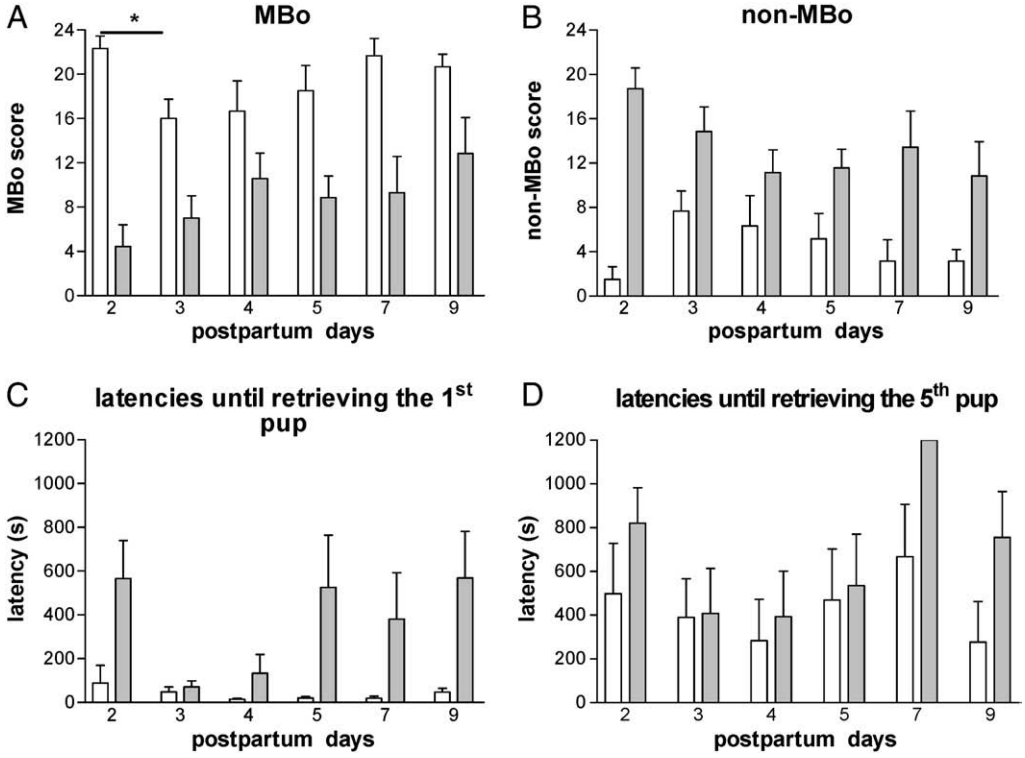

Fig. 1. MB as the function of postpartum time. Scores of MBo (A), non-MBo (B). Latencies in seconds retrieving the 1st pup (C) and the 5th pup (D) in group of dams treated with SAL or MO. Empty bars: SAL-treated group, grey bars: MO-treated group. MO-treatment resulted in marked impairment in MBo ($F(1,11)=30.27$, $p=0.0002$), increase in non-MBo ($F(1,11)=27.17$, $p=0.0003$) and longer latencies in RT ($F(1,11)=55.42$, $p=0.00001$ for retrieving the 1st pup. The effect of postpartum days (repeated measure factor PD) was significant in MBo ($F(1,63)=6.76$, $p=0.01$) and in non-MBo ($F(1,63)=4.06$, $p=0.05$ and was not in RT (either for the 1st or for the 5th pup). After performing multiple comparisons in the SAL-group between days (PD) only the mean values of MBo scores on PD2 and 3 were significantly different (difference of means = 6.33, 95% CI = 0.4078 - 12.26, $p<0.05$). Data are shown in mean ± standard error of mean, analysis: mixed ANOVA with a between groups factor of treatment (MO or SAL) and a repeated-measures factor of postpartum day (PD2, 3, 4, 5, 7, and 9), multiple comparisons were performed by Dunnett's method; outcome variables on PD 3, 4, 5, 7, 9 were compared to that on PD2.

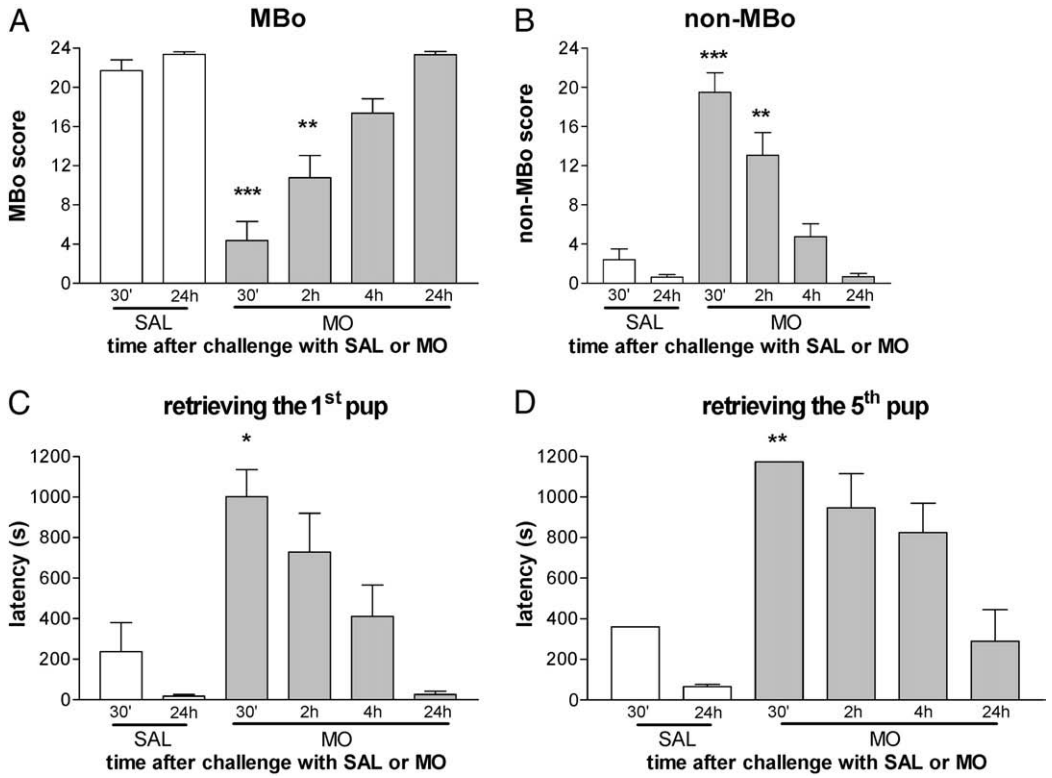

Fig. 2. Duration of impairment of MB after acute MO challenge. Scores of MBo (A) and non-MBo (B) in the group of dams treated with SAL or MO, 30 minutes, 2 hours, 4 hours and 24 hours after the daily dose. Latencies in seconds for retrieving the 1st pup (C) and the 5th pup (D) in the SAL- and MO-treated groups, 30 minutes, 2 hours, 4 hours and 24 hours after the daily dose. Empty bars: SAL-treated group, grey bars: MO-treated group. Data are shown mean and ± standard error of mean. *$p<0.05$; **$p<0.01$; ***$p<0.001$, sal 30 min group were used as the control. Analysis: Kruskal-Wallis-test, Dunn-post-hoc test.

3.2. Experiment 3. Dose-effect relationship of MO challenge on MB in dams treated with SAL or MO during pregnancy

As Fig. 3 shows, MO in an increasing dose range (0-1-3-10-20 mg/kg s.c.) dose-dependently disrupted the MB both in SAL- and MO-groups.

Statistically significant treatment effect (SAL or MO) was seen in MBo and non-MBo ($F(1,74) = 4.62$ and 4.73, respectively, $p<0.05$).

The effect of challenge (0-1-3-10-20 mg/kg MO) was statistically significant on all of the measured parameters ($F(4,74) = 29.16$, $F(4,74) = 30.61$, $F(4,76) = 31.66$ and $F(4,69) = 51.19$ for MBo, non-MBo, retrieving the 1st and the 5th pup, respectively, $p<0.0001$ in all tests).

After performing a post-hoc test, MB impairment proved to be statistically significant after administration of 10 and 20 mg/kg MO in all tests in both groups ($p<0.001$).

In SAL-groups, mean differences (95%CL) between MO-challenged and SAL-challenged animals were for the 10 and 20 mg/kg MO challenge, respectively: 10.67 (3.95-17.38) and 16.56 (9.63-23.48) in MBo; -10.44 (-17.09 to -3.80) and -16.39 (-23.24 to -9.54) in non-MBo; -645.4 s (-1154 s to -137.2 s) and -1192 s (-1661 s to -723.6 s), retrieving the 1st pup and -1054 s (-1458 s to -648.9 s) and -1054 s (- 1427 s to -680.4 s) retrieving the 5th pup.

In MO-groups, mean differences (95%CL) between MO-challenged and SAL-treated-SAL-challenged animals were also for the 10 and 20 mg/kg MO challenge, respectively: 12.18 (5.260-19.10) and 18.43 (11.51-25.35) in MBo; -12.14 (-18.99 to -5.291) and -18.76 (-25.61 to -11.92) in non-MBo; -700.4 s (-1126 s to -275.2 s) and -1061 s (-1529 s to -592.3 s) retrieving the 1st pup and -950.9 s (-1296 s to -605.7 s) and -1054 s (-1427 s to -680.4 s) retrieving the 5th pup.

Furthermore, in MO-dams (but not in SAL-dams), challenge with 3 mg/kg MO also resulted in statistically significant decrease in MBo scores: mean difference (95% CL): 7.06 (0.13 to 13.98); $p<0.05$ and significant increase in latency retrieving the 5th pup back to the nest (mean difference (95% CL): -531.4 s (-884.3 s to -178.5 s); $p<0.001$).

However, the treatment by challenge interaction was not significant.

3.3. Experiment 4. Antinociceptive effect of MO

MO exerted antinociceptive effect both in SAL and MO-treated dams (Fig. 4). Nonlinear regression curve-fit analysis did not reveal a significant difference. ED_{50} (95%CL) value was, however, lower in the SAL-group, than in the MO-group: 3.99 (2.23-7.13) and 5.92 (4.16-8.41) mg/kg, respectively.

4. Discussion

The major findings of the present study were: (i) there was no meaningful change in the MB-disruptive effect of MO as the postpartum period passed (PD2-9). (ii) The duration of the acute disruptive effect of MO on MB was 2 h. (iii) The disruptive effect of MO on MB was more pronounced in the MO-treated, than in the SAL-treated dams, indicating the development of sensitisation to this effect. (iv) A trend for tolerance was observed to the antinociceptive effect of MO in animals treated daily with MO during the whole gestation and lactation, however, the difference was not statistically significant.

Our data are in good agreement with that of others reporting that administration of MO during lactation disrupts the MB; prolongs the latency to retrieve the pups to the nest (Blass et al., 1995; Bridges and Grimm, 1982; Brunton and Russell, 2008; Grimm and Bridges, 1983; Hammer and Bridges, 1987; Kinsley et al., 1993; Rosenblatt et al., 1988), impairs pup care and nursing (Slamberova et al., 2001).

However, relatively few data can be found in the literature about the consequences of repeated MO administration on MB, especially

during pregnancy and in the period of lactation. It was demonstrated that a gradually increased dose of MO, administered i.c.v. from PD2 until PD7-9 inhibited MB (Rayner et al., 1988). Pre-partum MO treatment of dams resulted in sensitisation to the MB inhibitory effect of MO (Miranda-Paiva et al., 2001). The MB of dams treated with MO during late pregnancy decreased compared to the SAL-treated peers (Slamberova et al., 2001).

The main aim of this study was to analyse how chronic daily treatment with 10 mg/kg s.c. MO during pregnancy and further on after parturition influences the MB and also to study how the effects of subsequent MO challenges change.

First we planned to clarify whether the MB of dams changed parallel with the growing of pups. The only data found in the literature refers to measurement of drug-free MB on different postpartum days from PD1 until PD23 in dams treated with either MO or SAL from the 11th day of pregnancy to the 18th (Slamberova et al., 2001). A progressive decrease of active nursing, independent of the pre-treatment, was observed as the pups were aging. The dams spent less time in the litter by contacting or grooming their pups and displayed a higher frequency of total self-care as postpartum time progressed. No data was shown concerning RT as the function of postpartum time in this paper and the effect of postnatally given MO challenge was not checked.

In our experiment, both MBo and non-MBo, as well as pup retrieval was measured daily for up to 9 days. MO-treatment decreased MBo, increased non-MBo and resulted in longer latencies in RT, retrieving the 1st pup. The same effect of MO could not be demonstrated for retrieving the 5th pup which is not astonishing because RT for the 5th pup was measured more than 150 min after administration of MO. Unanimous, progressive differences in MB or in RT were not found between days during this early postpartum period. Though statistical analysis revealed significant repeated factor effect (PD) on scores of MBo and non-MBo, more detailed analysis showed consistent differences only between PD2 and 3 in MBo scores in SAL-dams. This difference between days (PD) did not appear either in non-MBo or in RT in SAL-group and also could not be seen in any parameters of the MO-group. It should be noted that on PD3 in the SAL-group, three of six MBo scores were unexpectedly low. Since this difference was observed only in one case and one test, the day-effect was ignored henceforth and all further experiments were performed during the early period of lactation.

The second question we intended to check was how long the disruptive effect of MO-challenge on MB lasts in dams treated with the constant 10 mg/kg dose of MO daily during the whole gestation. After s.c administration of 10 mg/kg MO its plasma concentrations were reported to be 1.1, 0.2 and about 0 μg/ml at 30 min, 2 h and 4 h, respectively. The same 30 min, 2 h and 4 h values in the rat brain extracellular fluid were at about 0.5, 0.5 and about 0 μg/ml, respectively (Stain et al., 1995).

The half-life of MO was found to be at about 30 minutes in rats (Aasmundstad et al., 1995; Stain et al., 1995).

This data indicates that MO's action generally declines about 2 h after its administration. In our experiments the inhibitory effect of MO on MB both in observational tests and RTs was still significant two hours after

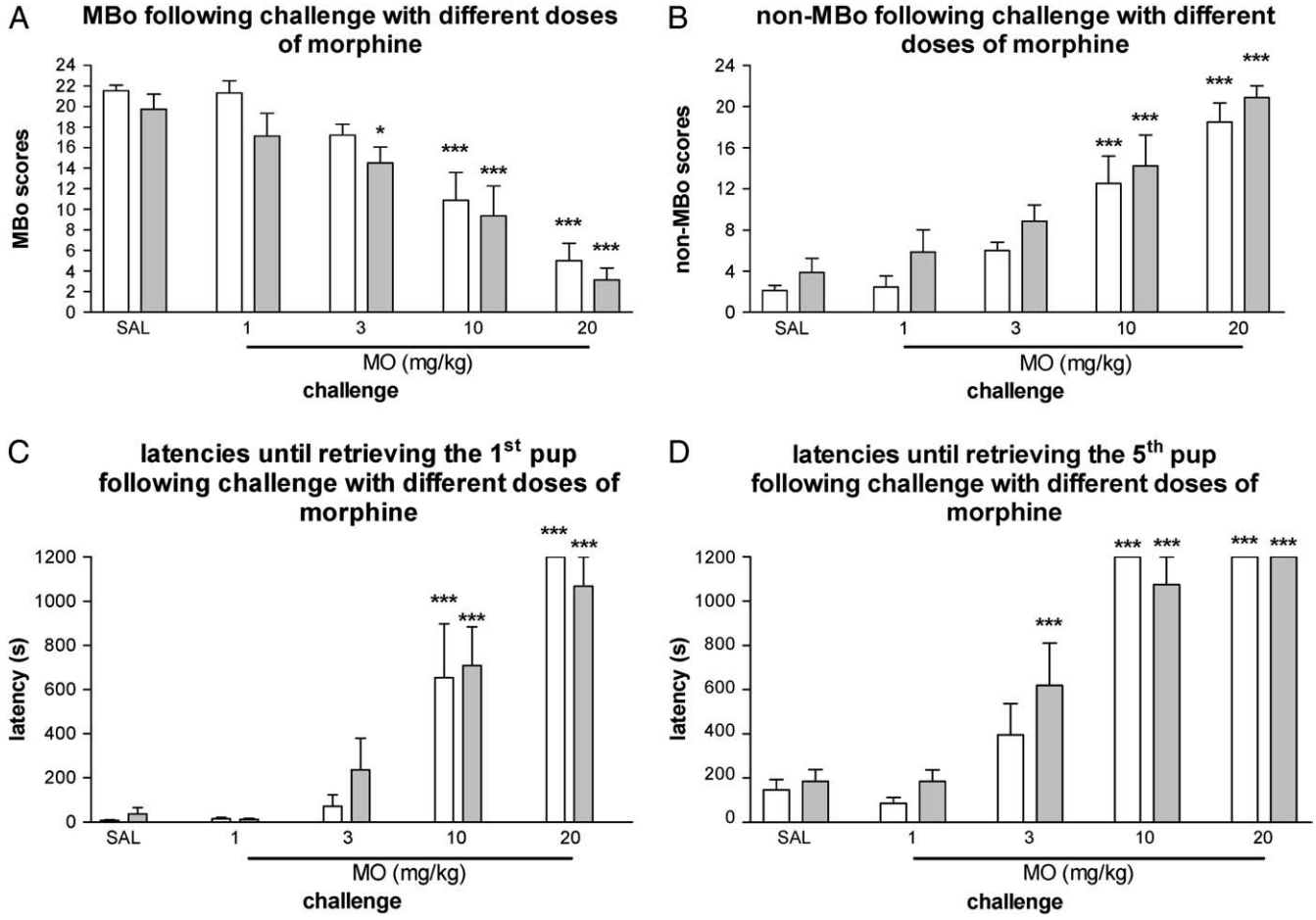

Fig. 3. Dose-effect relationship of MO challenge on MB in dams treated with SAL or MO during pregnancy. Scores of MBo (A) and non-MBo (B) in SAL- or MO-treated dams 30 minutes after challenged with SAL, 1, 3, 10 and 20 mg/kg MO s.c.. Latencies for retrieving the 1st (C) and the 5th (D) pup are in seconds. Empty bars: SAL-treated group, grey bars: MO-treated group. Data are shown as mean ± standard error of mean. *$p<0.05$; **$p<0.01$; ***$p<0.001$, versus control (SAL-treated, SAL-challenged) group. Analysis: two-way ANOVA, Dunnett-post-hoc test.

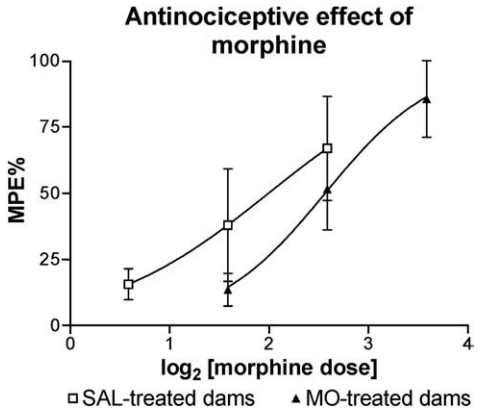

Fig. 4. Antinociceptive effect of MO. Antinociceptive effect of MO in dams treated with SAL or MO during the whole pregnancy and lactation. 1.5-3-6 mg/kg and 3-6-12 mg/kg MO was administered s.c. to SAL-treated and to MO-treated dams, respectively.

its administration and it was not detectable at 4 h after MO challenge. Four hours after treatment only a very mild, not significant decrease of MBo, increase of non-MBo and prolonged latency to retrieve the pups were measured.

The results show that time-dependency of MO's effects on MB is similar to that of other effects of MO e.g. antinociception and respiratory depression (Al-Khrasani et al., 2007; Shimoyama et al., 2001). Our results are in good agreement with the data of Grimm et al. who also reported that the disruptive effect of MO on MB lasted for about 2 h. (Grimm and Bridges, 1983).

On the basis of these results we may state that MB of dams treated with MO during lactation is impaired at least for 2-3 h daily. Both SAL- and MO-pre-treated dams displayed more MBo during the light and more non-MBo during the dark phase of the day, as reported earlier (Slamberova et al., 2001). So the daily MO exposure during the day even if the MB is inhibited only for a couple of hours may result in marked decrease in taking care of pups.

The inhibitory action completely disappears 24 h after MO challenge i.e. at the time-point of the next day treatment. This is in contrast with the results of Slamberova et al, who showed that administration of MO in the second half of pregnancy decreased MBo, enhanced non-MBo and prolonged latency to retrieve all the pups during lactation (Slamberova et al., 2001). However, they managed a rather different pre-partum MO exposure, MO administration started only on the 11th day of gestation and ended on the 18th, MO (10 mg/kg) was given twice a day. These treatment differences, by enhancing the possibility of development of dependence and withdrawal might explain the different results.

Chronic MO exposure results in the development of tolerance to several opioid effects e.g. analgesia, respiratory depression and nausea (Dumas and Pollack, 2008; Freye and Latasch, 2003; Furst, 2008; Kanjhan, 1995; Riba et al., 2002a,b), on the contrary MO administration during the late period of pregnancy results in sensitisation to the disruptive effect of MO on MB (Miranda-Paiva et al., 2001; Yim et al., 2006). In these experiments, however, MO was administered only from the 17th day of pregnancy in low doses (5 or 3.5 mg/kg/day) for a total of 5 doses and MB was measured 5 days later on PD5 (SAL-challenge) and PD6 (MO-challenge).

We intended to check how a constant dose (10 mg/kg/day) of MO during the whole pregnancy and then further on after parturition influenced the acute effect of MO on MB. As it was previously demonstrated in MO-naïve dams MO in doses of 5 and 10 mg/kg effectively disrupted MB whereas the lower doses were ineffective or only marginally disruptive (Kinsley and Bridges, 1988). The kinetics of MO in cerebrospinal fluid was reported to be linear: following s.c. administration of 1 and 3 mg/kg MO, cerebrospinal fluid concentrations of MO were at about 0.16 μM, 0.5 μM, respectively (Okura et al., 2007). In order to investigate the dose-effect relationship regarding MB, we administered 0-1-3-10-20 mg/kg doses in the early lactation period to dams treated with SAL or MO during gestation. In our experiment, MO dose-dependently disrupted MB in both groups. Statistical analysis revealed a significant treatment effect both in MBo and non-MBo. In addition, 3 mg/kg MO induced significant behavioural impairment only in MO-treated dams. These data indicate that our long-term constant dose MO pre-treatment may also result in development of sensitisation to the MB-disruptive effect of MO.

In order to check whether our constant treatment with 10 mg/kg MO during the complete perinatal period (during the entire pregnancy and lactation) induces the usually measured tolerance to the antinociceptive effect of MO or rather a sensitisation as to MB, a radiant tail-flick test was performed on dams after weaning. MO-treated dams showed a moderate statistically not significant decrease in the antinociceptive effect of MO. In the literature no data concerning changes in opioid antinociceptive tolerance during pregnancy were found. The lack of tolerance to the antinociceptive effect of MO is rather surprising. As previously described, after continuous treatment with constant doses of MO (10 mg/kg twice daily) complete antinociceptive tolerance developed in 7 days in male rats (Kiraly et al., 2006) One may speculate that hormonal and neural changes during pregnancy may similarly influence MB and antinociception.

Summarising our results, we may state that chronic MO exposure in a constant dose during the entire gestational period results in sensitisation to the MB-disruptive effect of MO in rats. As the duration of action of MO does not change in dams treated with MO during pregnancy, our results suggest that maternal care of these pups is decreased and this might be also responsible for the behavioural alterations demonstrated later in the pups. Our results might be of importance also in connection with human opioid abuse. Heroin abusers repeat and increase the dose of the drug to reach euphoria, since during the long term usage the positive reinforcement decreases and the main purpose of the abuse becomes the avoidance of withdrawal symptoms (Cheek et al., 1976; Handelsman et al., 1992; Hosztafi, 2003; Hutcheson et al., 2001; Sherman et al., 1989). If the continuous opioid use does not result in tolerance, but on the contrary, it results in sensitisation to the disruptive effect of opiates on MB, it might be expected that a mother who abuses heroin may neglect her baby even if she does not experience euphoria.

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