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Different time schedules affect conditioned place preference after morphine and morphine-6-glucuronide administration ☆

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Abstract

A number of studies have investigated the reward potential of morphine, using the Conditioned Place Preference (CPP) procedure. The morphine-metabolite morphine-6-glucuronide (M6G) is known to have analgesic activity comparable to morphine, but its reward properties are unclear.

An unbiased two compartment counterbalanced procedure was used to investigate the induction of CPP by morphine or M6G in C57BL/6J-Bom mice using different conditioning schedules. The conditioning sessions took place either immediately after the injections and lasted either 20 or 40 min, or were delayed until 15 min after the injections and lasted for 20 min. Locomotor activity was recorded during the conditioning sessions.

Morphine induced CPP when the 20-minute conditioning sessions were conducted directly after the injections, but not when they were delayed. M6G induced CPP when the 20-minute conditioning sessions were delayed, but not when the animals were conditioned directly after the injections. Neither morphine nor M6G induced CPP after 40-minute direct conditioning sessions. M6G had a biphasic effect on locomotor activity, with an initial decrease followed by excitation.

This study indicates that M6G has rewarding effects, and might contribute to the development of addiction after heroin or morphine administration. However, in any attempts to explore the reward properties of M6G, the choice of time schedule should be carefully considered.

Keywords: Morphine-6-glucuronide; M6G; Morphine; CPP; Reward; Conditioning; Pharmacokinetic; Biphasic locomotor activity

1. Introduction

Heroin is metabolised to 6-monoacetylmorphine within a few minutes of intake, and then to morphine (Rook et al., 2006). Morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) are the major metabolites of morphine in humans (Christrup, 1997). After administration of heroin or morphine, these morphine metabolites are present in higher concentrations for most of the time than the parent drug (Lotsch, 2005). The effects on analgesia, nausea, respiration etc, are different for morphine and the morphine glucuronides. For analgesia, it has been shown that morphine and M6G have agonist activity (Kilpatrick and Smith, 2005), while M3G is neutral or an antagonist (Gong et al., 1992; Suzuki et al., 1993; Lipkowski et al., 1994; Smith 2000). It has also been shown that M6G induces locomotor activity and conditioned place preference (CPP), (Morland et al., 1994; Handal et al., 2002; Vindenes et al., 2006), while M3G does not (Handal et al., 2002; Vindenes et al., 2006). CPP is a model used to assess the rewarding properties of drugs, and it is thought that CPP can measure a substance's ability to induce addiction (Bardo et al., 1995). Because M6G may contribute to the addiction potential of the parent drug, we wanted to investigate the effects of M6G in the CPP model. Despite the large number of studies using the CPP paradigm to investigate the reward effects of morphine (Tzschentke, 1998), only three studies have investigated the rewarding properties of M6G. Abbot and Franklin have shown that

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M6G can induce CPP in rats (Abbott and Franklin, 1991), but Baumeister et al. were not able to cause CPP after intranigral injection of M6G in rats (Baumeister et al., 1993). In a recent study from our laboratory, using a biased CPP model, subcutaneous administration of M6G induced CPP in mice, although to a lesser extent than morphine, when very long conditioning sessions (120 min) were used (Vindenes et al., 2006). M6G also induced an increase in locomotor activity which was delayed compared to morphine (Handal et al., 2002; Vindenes et al., 2006), indicating different pharmacokinetic or pharmacodynamic properties. A previous study from our laboratory showed that the half-life in C57BL mice blood is similar for both morphine and M6G, and estimated to be 28 and 25 min respectively (Handal et al., 2002). However, in the same study, the administration of M6G caused a delayed and longer lasting increase in locomotor activity compared to morphine. We have previously studied the morphine-metabolite M3G using the CPP model, but found only a tendency towards aversive properties (Vindenes et al., 2006). The contribution of M3G to the development of dependence and addiction is still unclear.

Since the reward effects of M6G may contribute to the addiction potential of the parent drugs morphine and heroin, we considered it important to see whether the delayed effect after administration of M6G might influence the induction of CPP. The aim of this study was to investigate whether different time schedules were required to induce CPP after treatment with M6G rather than morphine. The sessions took place either immediately or 15 min after the injections. The hypothesis tested was, 'Different time schedules are required to induce CPP after treatment with M6G as opposed to morphine.'

2. Materials and methods

2.1. Animals

A total of 156 male C57BL/6J-Bom mice (from Bomholt, Denmark), weighing 18–27 g at testing, were used in the experiments. Six animals were housed in each standard plastic cage, containing small red shelters to enrich the environment, for at least 5 days prior to the experiments. Food and water were freely available, except during the behavioural tests.

The temperature in the colony room was 24±1 °C and the room was illuminated with a 12-hour light–dark schedule, with the light period from 07:00 to 19:00. The mice were not handled prior to the experiment. The experiments were carried out during the light cycle in a testing room with dimmed light.

The Norwegian Review Committee for the use of Animal Subjects approved the experimental protocol of this study.

2.2. Recording of CPP and locomotor activity

Place preference and locomotor activity were measured with a Versamax animal activity monitoring system (AccuScan Instruments Inc., Colombus, USA). The cage size was 40×40 cm with infrared beams at 2.5 cm spacings. Each cage was divided into two distinct compartments, connected by an opening in the centre of the box that could be closed during the conditioning sessions. One compartment had white walls with a meshed metal plate as its floor. The other compartment had vertical black and white stripes (2 cm wide) on the walls and a metal plate with holes (4 mm Ø) as its floor. Both compartments had a transparent ceiling. In pre-tests with drug-naïve mice, the mean time in the striped compartment was 565±36 s and in the white compartment 635±36 s. Paired sample *t*-test showed no significant preference for either of the two compartments ($p=0.37$). We therefore consider this to be an unbiased CPP apparatus.

2.3. Drugs

Morphine hydrochloride was purchased from Norsk Medisinaldepot (Oslo, Norway) and morphine-6-β-D-glucuronide hydrate from Lipomed (Arlesheim, Switzerland). The drugs were dissolved in 0.9% saline. The injection volumes were 0.01 ml/gram mouse.

2.4. Treatment and procedures

The animals were randomly assigned to different groups (6–8 animals per group) and injected subcutaneously on the back, about 1 cm from the tail, with either 10, 20, 30, 50 or 80 μmol/kg morphine, corresponding to 3.8, 7.5, 11.3, 18.8 or 30 mg/kg, or 5, 10, 20, 30, 50 or 80 μmol/kg M6G, respectively 2.3, 4.6, 9.2, 13.8, 23.1 or 36.8 mg/kg. The doses chosen in this experiment, are based on the results from pilot studies and our previous study (Vindenes et al., 2006). The 30 μmol/kg doses used in the 40 min group have been administered in previous tests and induce CPP both for morphine and M6G. The intention of the dose–response study was to investigate low doses that did not induce CPP for morphine and M6G, and try to find doses that gave maximum CPP. For the M6G delayed group, 30 μmol/kg is not investigated, since the results from the 20 and 50 μmol/kg groups were quite similar.

The experiment included two phases, namely, conditioning (six sessions), where the mice were injected with either morphine or M6G and testing (one session), where the mice received no drug. Conditioning was carried out for 3 consecutive days. During conditioning, the mice received two injections each day, one with drug and one with saline, with a 6-hour interval. Half of each experimental group received saline as the first daily injection and the other half an opiate, every second day. Half of the mice in each group were conditioned with the drug to be tested in the white compartment and the other half in the striped compartment. A control group was injected with only saline before both of the conditioning sessions each day, and conditioned for 20 min immediately after the injections, following the same schedule as the drug injections.

Different conditioning schedules were used for the different groups:

1. 20 min conditioning immediately after drug injections (direct conditioning)
   Animals were placed in the conditioning cages immediately after drug administration. Five groups of mice were injected with 10, 20, 30, 50 or 80 μmol/kg morphine. Four groups were

treated with 10, 20, 30 or 80 μmol/kg M6G. The saline injections followed the same procedures as the drug injections.

2. 20 min conditioning with a 15 min delay after drug injections (delayed conditioning)
   Three groups were injected with morphine 10, 30 or 80 μmol/kg and five groups were injected with M6G 5, 10, 20, 50 or 80 μmol/kg. After the injections, the mice were carefully placed into their home cages for 15 min, before the 20-minute conditioning sessions took place. The saline injections followed the same procedures as the drug injections.
3. 40 min conditioning immediately after drug injections (direct conditioning)
   Mice were placed in the conditioning cages for 40 min directly after injections with 30 μmol/kg morphine or M6G. The saline injections followed the same procedures as the drug injections.

Locomotor activity was recorded for all the conditioning sessions. Only the results from the 3rd conditioning day are shown.

On day 4, all the mice were injected with saline only before a 20 min test session to see if their behaviour was affected by the conditioning. During testing, the mice had free access to both the white and the striped compartments. All the animals, whether conditioned immediately or with a delay after the injections, followed the same procedures during the testing session.

2.5. Statistical analysis

Time spent in the drug-paired compartment minus time spent in the unpaired compartment was used as a measure of preference for the drug-paired (conditioned) compartment. A positive result was interpreted as CPP. Statistical differences were

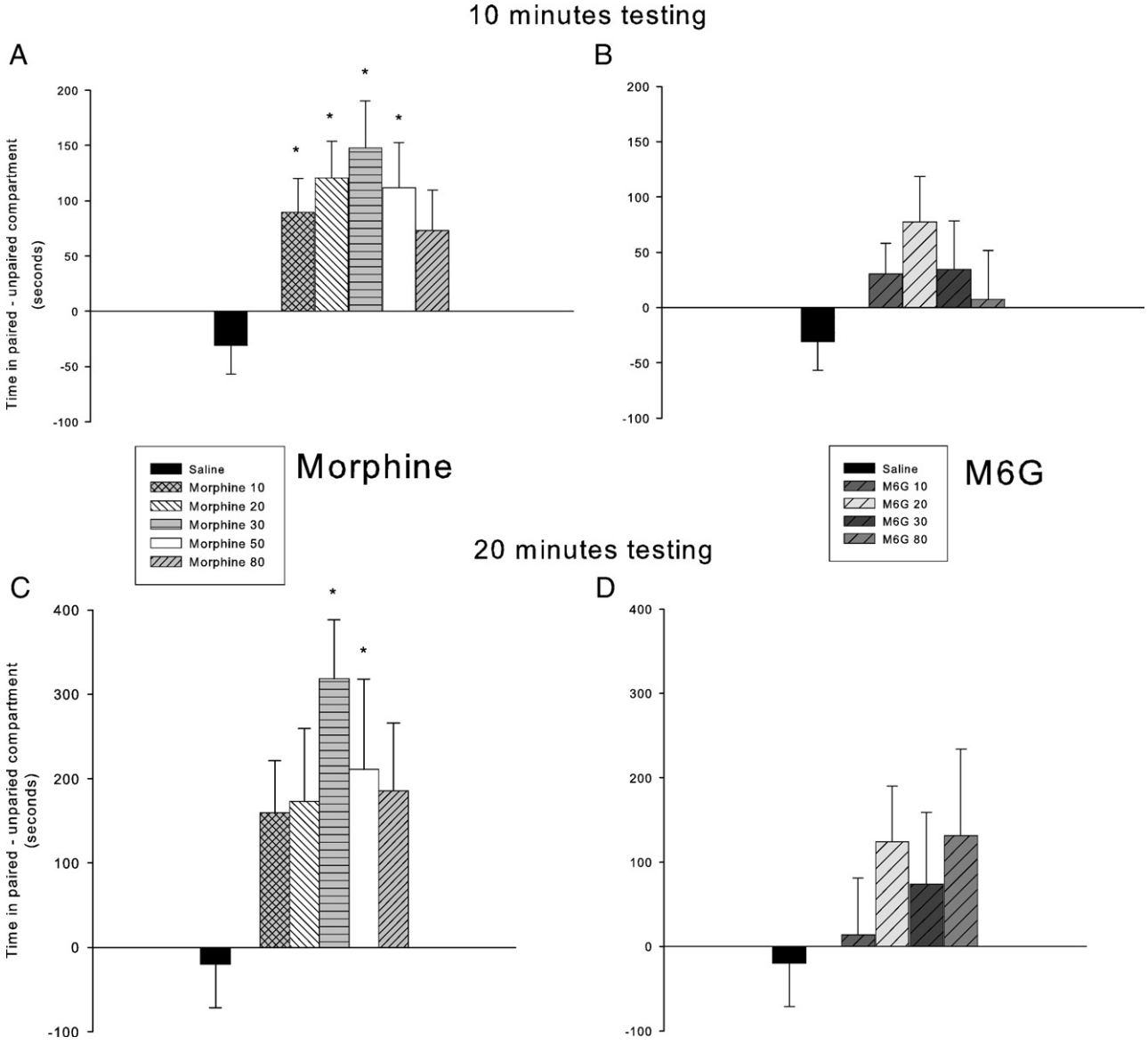

Fig. 1. CPP after treatment with different doses (μmol/kg) of morphine (A and C) and M6G (B and D) when conditioning was performed immediately after drug administration. Comparison to saline treated mice. The results after 10 (A and B) and 20 min (C and D) testing are presented. Bars represent mean time in paired compartment minus time in unpaired compartment±SEM. *$p<0.05$ ($n=6$–8 mice).

revealed by ANOVA, and Dunnetts post-hoc test was used for comparison with the saline group, for the animals conditioned for 20 min. For the animals conditioned for 40 min, independent sample Student's *t*-test was used for comparison with the saline treated animals, since only two groups were compared. Time spent in the white versus the striped compartment for the saline group was compared using paired *t*-test.

The CPP results during the first 10 min and from the whole 20 min testing session are presented. Both the distance travelled during each 5-minute period and the total distance travelled (cm) during the 20-minute session on conditioning day 3 after morphine or M6G treatment were compared to the movement of mice in the saline group, using ANOVA and Dunnetts post-hoc test. p values less than 0.05 were taken as statistically significant. Data are presented as mean±S.E.M. The statistical analyses were conducted using the statistical package SPSS 14.0.

3. Results

3.1. 20 min conditioning immediately after drug injections (direct conditioning)

Mice treated with morphine showed a significant CPP compared to the saline group both after 10 [$F(5, 52)=4.61$,

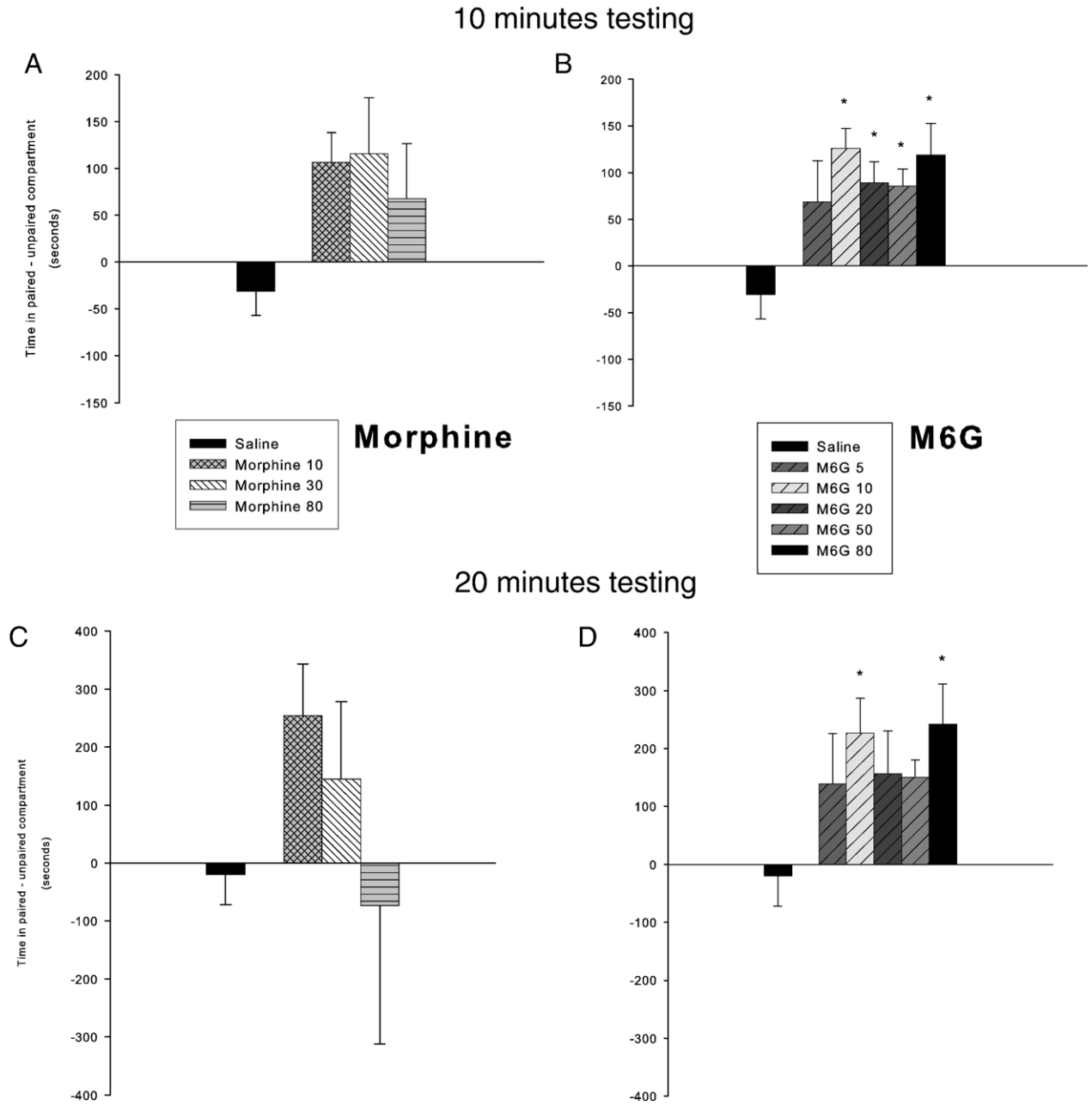

Fig. 2. CPP after treatment with different doses (μmol/kg) of morphine (A and C) and M6G (B and D) when conditioning was delayed until 15 min after drug administration, compared to saline treated mice. The results after 10 (A and B) and 20 min (C and D) testing are presented. Bars represent mean time in paired compartment minus time in unpaired compartment±SEM. *$p<0.05$ ($n=6$–8 mice).

$p<0.001$] and 20 min testing [$F(5, 52)=3.00$, $p=0.019$] (Fig. 1A and C). Dunnetts post-hoc comparisons revealed a significant CPP for the doses of 10, 20, 30 and 50 μmol/kg morphine after 10 min testing and for the doses 30 and 50 μmol/kg after 20 min testing. Mice injected with M6G did not show significant CPP either after 10 [$F(4, 43)=1.45$, $p=0.24$] or 20 min [$F(4, 43)=0.98$, $p=0.43$] testing (Fig. 1B and D), neither post-hoc analysis were significant. The results from each 5-minute bin of the test period showed the same tendency as the results in Fig. 1 for both morphine and M6G (data not shown).

3.2. 20 min conditioning with a 15 min delay after drug injections (delayed conditioning)

CPP was not seen for the animals treated with morphine either after 10 [$F(3, 31)=2.32$, $p=0.10$] or 20 min testing [$F(3, 31)=1.57$, $p=0.22$] (Fig. 2A and C), neither post-hoc analysis were significant. M6G induced CPP after 10 [$F(5, 50)=5.50$, $p<0.001$] and 20 min testing [$F(5, 50)=3.12$, $p=0.016$] (Fig. 2B and D). Dunnetts post-hoc comparisons revealed that 10, 20, 50 and 80 μmol/kg M6G induced a statistically significant CPP after 10 min testing and 10 and 80 μmol/kg M6G after 20 min testing. The results from each 5-minute bin of the test period showed the same tendency as the results in Fig. 2 for both morphine and M6G (data not shown).

3.3. 40 min conditioning immediately after drug injections (direct conditioning)

Statistically significant CPP was not seen in the animals conditioned for 40 min after treatment with 30 μmol/kg morphine either after 10 ($p=0.59$) or 20 min ($p=0.69$) testing.

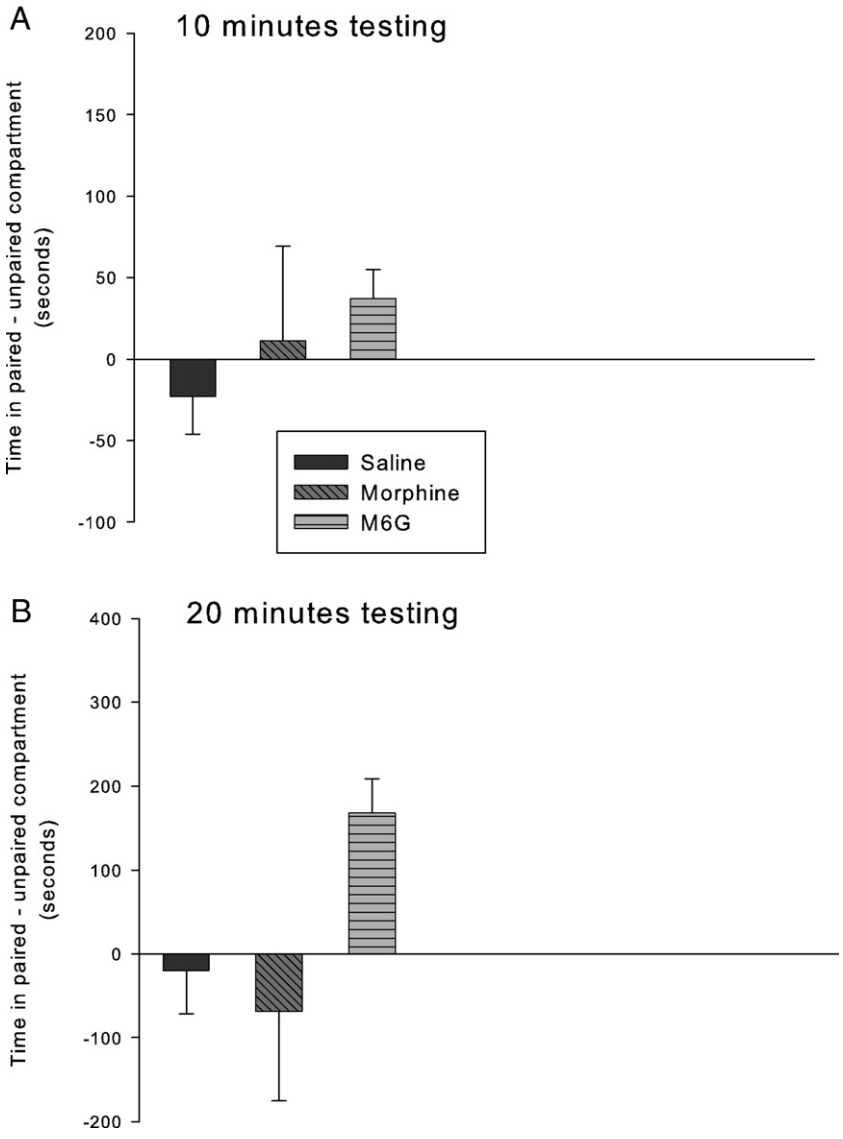

Fig. 3. CPP after 40 min conditioning immediately after the treatment with 30 μmol/kg morphine or M6G. The results after 10 (A) and 20 min (B) testing are presented. Bars represent mean time in paired compartment minus time in unpaired compartment±SEM ($n=6$–8 mice).

After treatment with 30 μmol/kg M6G, a tendency to CPP was seen after both 10 ($p=0.052$) and 20 min ($p=0.056$) testing (Fig. 3A and B). The results from each 5-minute bin of the test period showed the same tendency as the results in Fig. 3 for both morphine and M6G (data not shown).

3.4. Control group

The saline treated mice showed no statistically significant preference for the white or the striped compartment after 10 ($p=0.90$), 15 ($p=0.35$) or 20 ($p=0.21$) min of testing (data not shown).

3.5. Locomotor activity data

Both morphine [$F(8, 67)=62.04$, $p<0.001$] and M6G [$F(9, 76)=48.84$, $p<0.001$] increased the total distance travelled by the mice receiving these injections, compared to the saline treated mice (Fig. 4). Morphine increased activity following a dose–response pattern for both the direct and delayed

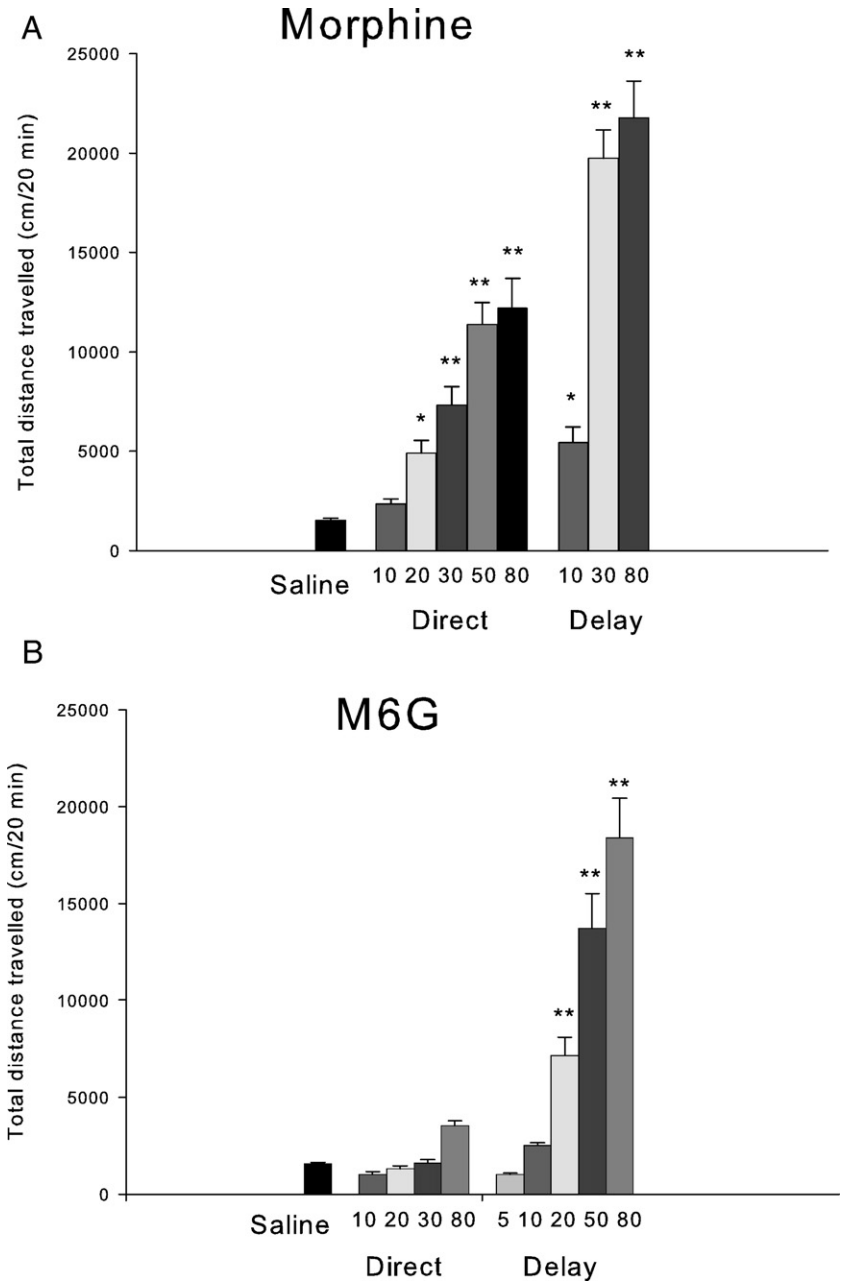

Fig. 4. Mean total distance travelled (cm/20 min) after different doses (μmol/kg) of morphine (A) or M6G (B) when the conditioning sessions were performed either immediately ("direct") or 15 min after the injections ("delay") on conditioning day three. The distance is illustrated as mean ± SEM. *$p<0.05$. **$p<0.001$ ($n=6$–8 mice).

conditioned mice. M6G led to increased activity only in the mice whose conditioning had been delayed. A dose–response curve was seen for delayed conditioning with M6G. After delayed conditioning, 10 μmol/kg morphine increased locomotor activity but the lowest M6G dose that significantly increased the activity, was 20 μmol/kg of M6G.

By studying the distance travelled each 5 min, it is possible to observe a biphasic effect on the locomotor activity of the mice after M6G treatment. The inset in Fig. 5C shows an initial reduction in the distance recorded for the 5–10 min period [$F(4, 43)=2.92$, $p<0.05$]. Dunnetts post-hoc comparisons revealed that only the dose of 20 μmol/kg M6G significantly reduced locomotion to below the values of the saline treated mice ($p<0.05$), although there was a clear tendency for the doses 10 μmol/kg ($p=0.071$), 30 μmol/kg ($p=0.055$) and 80 μmol/kg ($p=0.057$) M6G to cause reduced locomotor activity. Fig. 5D shows the dose-dependent increase in locomotor activity for the mice that were conditioned 15 min after the drug injections (Fig. 5D).

We cannot find any systematic differences in the locomotor activity on the test day, between the different drugs and doses administered, that could explain the CPP results (results not shown).

4. Discussion

In this study, we observed that different conditioning schedules affect CPP after treatment with morphine or M6G. To induce statistically significant CPP after treatment with M6G, the conditioning sessions had to be delayed for 15 min after the drug injections, whereas morphine induced statistically significant CPP only when the conditioning sessions were performed immediately after the injections. The 40-minute conditioning sessions, carried out immediately after drug injections, did not induce CPP for either morphine or M6G, although a clear tendency to CPP was seen for M6G. Morphine induced a dose-dependent increase in locomotor activity, both after direct and delayed conditioning. M6G caused a biphasic effect on

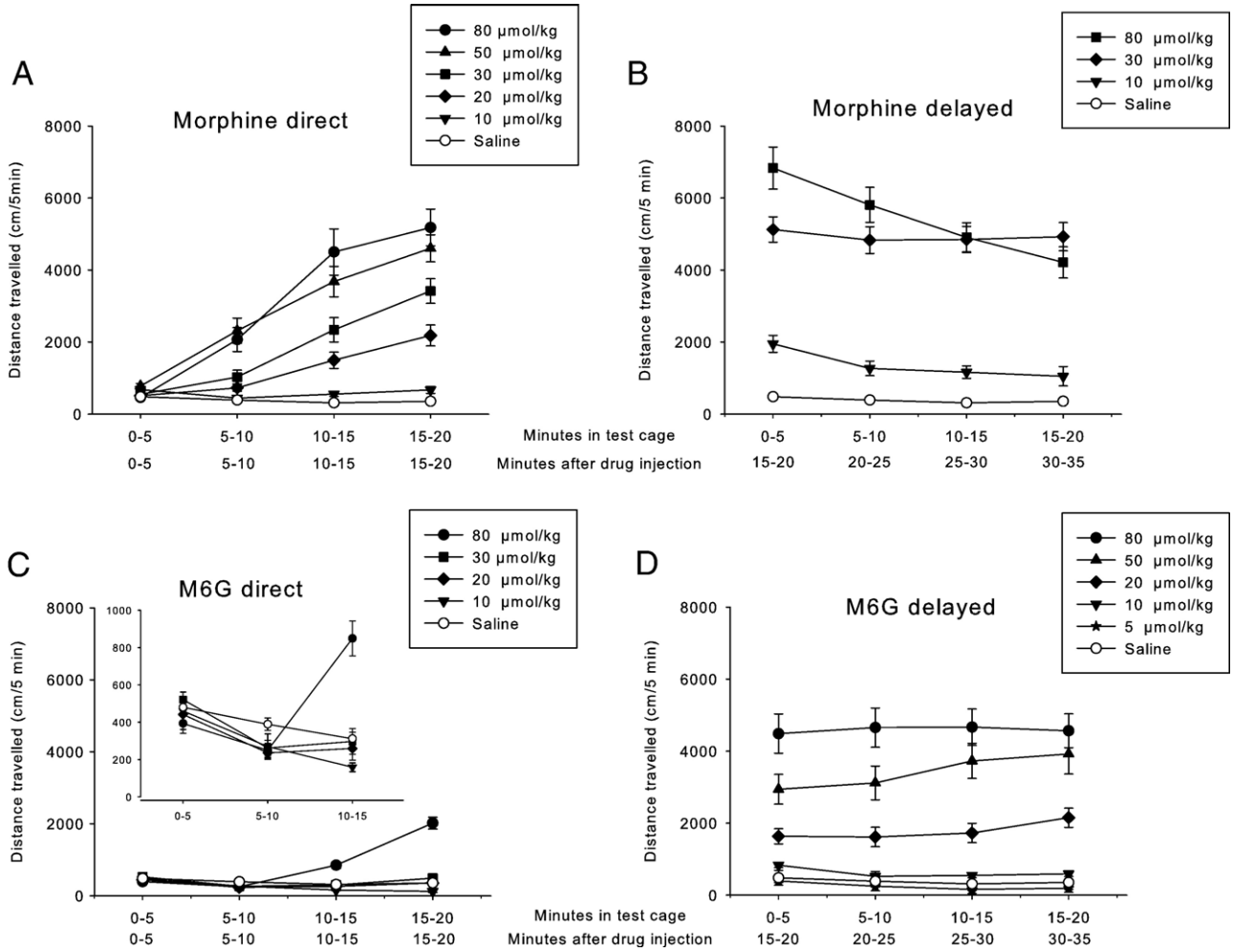

Fig. 5. Distance travelled (mean (cm/5 min)±SEM) on day three when conditioning was performed directly (A) or delayed (B) after morphine injections or directly (C) or delayed (D) after injections with M6G ($n=6$–8 mice). Both the minutes in test cage and the minutes after drug injection can be seen on the abscissa. In figure C, the first 15 min is enlarged to illustrate the initial sedation after the M6G injections during the 5–10 min period. The subsequent increase in locomotor activity can be seen in figure D.

locomotor activity: An initial hypoactivity could be seen for mice conditioned immediately after the M6G injections, while a dose-dependent increase in locomotor activity was seen when conditioning was delayed.

Despite the fact that M6G is known to contribute to several effects seen after morphine treatment (Kilpatrick and Smith, 2005), little is known about its abuse potential and few studies have investigated the reward effects of M6G using the CPP paradigm (Abbott and Franklin, 1991; Baumeister et al., 1993; Vindenes et al., 2006). Abbot and Franklin administered two doses (0.25 and 2.0 mg/kg) of M6G subcutaneously and only the highest dose induced CPP in rats which had a 40 min conditioning session (Abbott and Franklin, 1991). To our knowledge, this is the only study that shows CPP after systematic administration of M6G to rats. In another study, no CPP was observed after intranigral administration of M6G in rats (Baumeister et al., 1993). In a previous study at our laboratory, we found that subcutaneously administration of M6G induced CPP in mice, although to a lesser extent than morphine, if very long conditioning sessions (120 min) were used (Vindenes et al., 2006). A meta-analysis has reported that, after treatment with morphine, short-length (less than 20 min) and long-length (45 min or more) conditioning trials yield larger effect sizes than intermediate-length (25–30 min) trials (Bardo et al., 1995). In addition to the length of the conditioning sessions, our study has shown that the time from drug injection to conditioning seems to be important. M6G induced CPP only when the 20-minute conditioning sessions were delayed until 15 min after the injections. When the conditioning sessions lasted for 40 min directly after the injection, no CPP was obtained. The interval between the injections and the conditioning sessions was chosen in accordance with data from a previous study where locomotor activity time-curves during the conditioning trials showed that an increase in locomotor activity for M6G was delayed compared to morphine (Vindenes et al., 2006).

Other CPP-studies using 40 min conditioning sessions immediately after drug injections, have shown statistically significant CPP (Sakoori and Murphy, 2005; Orsini et al., 2005; Tahsili-Fahadan et al., 2006). This discrepancy could be due to the use of different mice strain (Tahsili-Fahadan et al., 2006), or different number of conditioning sessions, i.e. four instead of three (Sakoori and Murphy, 2005; Orsini et al., 2005). These factors, together with apparatus with different environment, could affect the development and appearance of CPP (Tzschentke, 1998). Mice that were conditioned for 20 min, 15 min after treatment with morphine, did not show statistically significant CPP, although a clear tendency was seen. When the 20 min conditioning sessions were performed immediately after morphine injections, statistically significant CPP was however induced, as expected due to previous studies.

Both the CPP results for the whole test session (20 min) and for the first half of the test session (10 min) are presented. We have broken down the test session into 5-minute bins, and these results show the same tendency as the 10 and the 20-minute period. During testing, there was no tendency towards an initial Conditioned Place Aversion (CPA) or a delayed induction of CPP after M6G treatment, compared to morphine. The biphasic locomotor activity seen for M6G treated mice during conditioning is probably a result of the M6G effect. A similar biphasic effect on CPP during testing has not been recorded, and this is probably because the testing session is in a drug-free state. The locomotor activity during testing does not reveal any systematic difference between the groups that can help us explain the different CPP results.

A potential problem when two conditioning sessions are carried out each day is that drug effects from the first session might be carried over to the second session and interfere with the results (Tzschentke, 1998). Morphine has been one of the drugs concerned, but since M6G has a similar half-life to the parent drug (Handal et al., 2002), this issue is just as important for M6G. In our study, this concern seems to be of less importance, suggesting that it is only a limited period of time after drug injection that is of significance for the reward effect and acquisition of CPP. The time after drug injection where locomotor activity increases might be important, but doses that induce CPP do not necessarily increase locomotor activity and vice versa. Several studies have stated that, despite the fact that both locomotor activity and CPP are associated with an increase in dopamine in the nucleus accumbens, there is little or no correlation between these two phenomena (Tzschentke, 1998; Parkinson et al., 1999; Cadoni and Di Chiara, 2000). Our study revealed an increased locomotor activity and induction of CPP only when the conditioning sessions with M6G were delayed, but a direct association between these effects cannot necessarily be assumed.

The locomotor activity data after drug treatment from the three conditioning sessions were in concordance, and only the results from day three are presented. Administration of morphine induced a dose-dependent increase in locomotor activity (Fig. 5A and B), but after injection with M6G, a biphasic locomotor effect was seen. This was characterized by an initial locomotor depression during the 5–10 min period (Fig. 5C) for the animals conditioned directly after administration, followed by hyperlocomotor activity (Fig. 5D) seen after the delayed conditioning. The same phenomenon was not seen for morphine. A biphasic locomotor effect after injections with morphine is well known for rats (Babbini and Davis, 1972; Vanderschuren et al., 1997; Timar et al., 2005) and hamsters (Schnur et al., 1983), but, to our knowledge, this has not been seen in mice, and we did not see it in our study. We have not found any literature describing a biphasic effect on locomotor activity after M6G injections, either in rats or mice. However, when we looked back at the locomotor activity curves from our previous study, the same biphasic effects were observed (Vindenes et al., 2006). The mechanisms underlying the biphasic effect of morphine are not fully understood, but explanations like different drug-receptor interactions, changes in acetylcholine levels in the brain and binding to opioid receptors located at two different sites, near the surface of certain medullated axons or in the cell body, have been suggested (Babbini and Davis, 1972; Schnur et al., 1983; Vanderschuren et al., 1997; Timar et al., 2005).

The reason why induction of CPP is difficult when conditioning is performed directly after administration of M6G might be its delayed effects, as we have observed for the increase in locomotor activity. We can assume that this delay is due to the

different pharmacokinetic properties of M6G as opposed to morphine. Human studies report a long delay between the time course of M6G plasma concentration and its effects and one explanation is that M6G equilibrates very slowly between plasma and the effect site in the central nervous system (Lotsch 2005). Earlier it was believed that the polar morphine-metabolites M6G and M3G could not penetrate the blood–brain-barrier (BBB), but a number of studies have revealed their access into the brain (Yoshimura et al., 1973; Barjavel et al., 1994; Aasmundstad et al., 1995; Okura et al., 2003). The BBB permeability of M6G is, however, reported to be 32–57 times lower than for morphine (Bickel et al., 1996; Wu et al., 1997). Yoshimura recorded a delay in brain concentration for the glucuronides compared to morphine (Yoshimura et al., 1973). Later studies did not confirm this delay (Yoshimura et al., 1973; Barjavel et al., 1994; Aasmundstad et al., 1995; Okura et al., 2003). A possible explanation might be that the glucuronides can act as molecular chameleons, hiding their hydrophilic groups (Carrupt et al., 1991). Aasmundstad et al. measured the concentrations of M6G and morphine in striatal extracellular fluid (ECF) in rats after s.c. administration of morphine or M6G, and no difference in t_{max} was seen (Aasmundstad et al., 1995). Barjavel et al. have also shown similar t_{max} for M3G, M6G and morphine in the brain cortex for rats (Barjavel et al., 1994). Handal et al. have investigated the concentrations in brain and serum after s.c. injections of M6G or morphine in mice and revealed no difference in time to maximum concentrations for either of the drugs, in serum or in the brain (Handal et al., 2007). However, the locomotor activity versus serum concentrations showed a more pronounced counter clockwise hysteresis plot for M6G, indicating a delay in the central nervous effect of M6G (Handal et al., 2002). In the rat brain, the concentration of M6G molecules in the extracellular fluid (ECF) is reported to be 125 times higher than in the intracellular space (ICS), whereas morphine levels were four times greater in ICS than in ECF (Stain-Texier et al., 1999). While morphine has to be loaded into the brain ICS, M6G is only distributed to the small hydrophilic space representing the ECF (Aasmundstad et al., 1995). This smaller volume of distribution (V_d) for M6G might compensate for the lower BBB permeability compared to morphine, when t_{max} is measured. The affinity for the μ receptors is reported to be 3–5 times lower for M6G compared to morphine, both in vitro and in vivo (Stain-Texier et al., 1999). A slow onset and prolonged duration of the drug effect, as observed for M6G, has been also described for the partial agonist buprenorphine, both in vitro and in vivo (Husbands and Lewis, 2003), but the molecular mechanisms underlying this observation are not yet known.

So, the pharmacokinetic and pharmacodynamic differences between morphine and M6G cannot explain our dissimilar observations regarding locomotor activity and CPP for these two drugs. The initial reduction in locomotor activity followed by a delayed hyperlocomotor effect after treatment with M6G might be due to central molecular mechanisms, like activation of different receptors, dimerization, or differences in intracellular signalling, but this needs to be explored further. The reason why a 15-minute delay in conditioning (20 min) induces CPP after M6G administration, but not 40 min direct conditioning, cannot be explained from the previous reports. This shows a need for studies to investigate whether M6G causes delayed receptor activation or a delayed dopamine response in the nucleus accumbens, compared to morphine.

5. Conclusion

These results indicate that M6G has rewarding effects, but requires different conditioning schedules from morphine to induce CPP. The mechanisms underlying these differences in the effects of morphine and M6G are unknown and more studies are warranted. It seems likely that M6G can contribute to the development of addiction after administration of heroin or morphine, but the exact role is still unclear.

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