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μ-Opioid Receptors are not Involved in Acute Cocaine-Induced Locomotor Activity nor in Development of Cocaine-Induced Behavioral Sensitization in Mice

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Although μ-opioid receptors have been extensively investigated for their role in drug reinforcement, little is known about the contribution of these receptors to the acute and sensitized locomotor response to cocaine. In this study μ-opioid receptor involvement in acute cocaine-induced locomotor activity and in the development of cocaine-induced behavioral sensitization was evaluated using μ-opioid receptor knockout mice and chronic naltrexone (NTX) pretreatment as models. In addition, co-administration of the specific μ-opioid receptor antagonist CTOP with repeated saline or cocaine injections was used to establish the involvement of μ-opioid receptors in sensitization to the locomotor stimulant effects of cocaine. The acute locomotor response to cocaine (3, 10, 20, or 30 mg/kg i.p.) of μ-opioid receptor knockout or chronic NTX pretreated mice was not different from the cocaine response of their respective controls. With respect to cocaine-induced behavioral sensitization, induced by daily injections of 20 mg/kg cocaine for 11 subsequent days, μ-opioid receptor knockout mice developed behavioral sensitization to the locomotor stimulant effects of cocaine (challenge 10 mg/kg i.p.) comparable to wild-type littermates and the μ-opioid receptor antagonist CTOP did not affect cocaine-induced sensitization either. However, mice that were pretreated with NTX exhibited augmented cocaine-induced behavioral sensitization relative to placebo pretreated controls, which may be ascribed to increased δ-opioid receptor levels as has been described for chronic NTX pretreated mice. The present findings suggest that μ-opioid receptors are not required for the acute locomotor response to cocaine nor are they essential for the development of cocaine-induced behavioral sensitization.
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INTRODUCTION

Repeated intermittent exposure to cocaine enhances the locomotor stimulant effects of cocaine upon a subsequent exposure. This phenomenon, behavioral sensitization to the motor stimulant effects of drugs, is thought to reflect long-term adaptations to chronic drug exposure that may underlie certain aspects of drug addiction (Robinson and Berridge, 2000). A single exposure to cocaine can be sufficient to induce long-lasting sensitization to the locomotor stimulant effects of cocaine (Vanderschuren *et al*, 1999), possibly through long-term potentiation of dopamine neurons in the ventral tegmental area (VTA) (Ungless *et al*, 2001). Repeated intermittent pre-exposure to cocaine also facilitated the subsequent acquisition of cocaine self-administration (Horger *et al*, 1990; Piazza *et al*, 1990). Furthermore pre-exposure to, for example, amphetamine, cocaine, and morphine enhanced the conditioned motivational effects of the respective drug (Lett, 1989; Shippenberg and Heidbreder, 1995a). Thus, repeated exposure to drugs of abuse induces long-term adaptations, which contribute to sensitization to the locomotor stimulant and motivational effects of these drugs.

Involvement of endogenous opioid systems in drug reinforcement has been demonstrated repeatedly in laboratory animals (Herz, 1997; Van Ree *et al*, 1999). Opioid antagonists reduce cocaine and ethanol self-administration (De Vry *et al*, 1989; Froehlich *et al*, 1990; Kornet *et al*, 1991; Kuzmin *et al*, 1997; Stromberg *et al*, 1998), primarily through μ-opioid receptors in the VTA (Ramsey *et al*, 1999). Endogenous opioid systems have also been implicated in

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behavioral sensitization induced by psychostimulants. For example, the nonselective opioid antagonist naltrexone (NTX) prevented the development of cocaine-induced behavioral sensitization (Sala *et al*, 1995). The selective δ-opioid receptor antagonist naltrindole blocked the development, but not the expression, of sensitization to the locomotor stimulant effects of cocaine (Heidbreder *et al*, 1993a, 1996). Moreover, sensitization to the conditioned effects of cocaine was prevented when naltrindole was given together with repeated cocaine injections (Shippenberg and Heidbreder, 1995b). Effects of κ-selective opioid receptor agonists, both exogenous and endogenous, upon behavioral sensitization have been reviewed elsewhere (Shippenberg and Rea, 1997). In short, sensitization to the conditioned effects of cocaine or amphetamine, as apparent after pre-exposure to a psychostimulant, was abolished when κ-opioid receptor agonists were administered in combination with the psychostimulant (Shippenberg *et al*, 1996). In addition, κ-opioid receptor agonists reduced sensitization to the locomotor stimulant effects of cocaine (Heidbreder *et al*, 1993b, 1995), but (Vanderschuren *et al*, 2000). Taken together, these studies indicate that δ- and κ-opioid receptors are involved in the development of cocaine-induced behavioral sensitization. In contrast, little is known about the role of μ-opioid receptors in sensitization to the (locomotor) effects of cocaine. Interestingly, data from a recent study suggested that μ-opioid receptors might also contribute to cocaine-induced behavioral sensitization (Yoo *et al*, 2003).

The aim of this study was to establish the involvement of μ-opioid receptors in acute cocaine-induced locomotor activity and in cocaine-induced behavioral sensitization. The possible role of μ-opioid receptors in the acute locomotor response to cocaine and in cocaine-induced behavioral sensitization was investigated using μ-opioid receptor knockout mice, co-administration of the μ-opioid receptor selective antagonist CTOP (Hawkins *et al*, 1989), and by means of chronic NTX pretreated mice. Chronic NTX pretreatment was included in this study because chronic exposure of mice to NTX results in an increase in μ-opioid receptor binding sites, although δ-opioid receptor binding is also increased by this pretreatment albeit to a lower extent and upregulation of κ-opioid receptors after chronic NTX exposure is restricted to cortical regions (Yoburn *et al*, 1988; Lesscher *et al*, 2003).

MATERIALS AND METHODS

Animals

Male mice, either C57Bl/6Jico mice (Charles River, l'Arbresle, France) or μ-opioid receptor knockout and wild-type mice derived from heterozygous breeding (GDL, Utrecht), aged 2–3 months were group housed (2–4) in extended Macrolon© type I cages with water and food pellets available *ad libitum*. Environmental conditions were controlled (22°C and 50% humidity; lights on at 0700 and lights off at 1900, GDL Utrecht University). The experimental procedures were approved by the Ethical Committee for Animal Experiments of the University Medical Center Utrecht.

μ-Opioid Receptor Knockout Mice

The μ-opioid receptor knockout and wild-type mice used for this experiment have been described previously and were on a mixed 129Sv/C57Bl6 background (Schuller *et al*, 1999). No detectable binding of [^{3}H]DAMGO or μ-opioid receptor transcript was present in μ-opioid receptor knockout mice and there is no evidence for compensatory changes in other opioid receptor subtypes: binding to δ-opioid receptor subtypes was comparable between genotypes and δ- and κ- and ORL-1 receptor mRNA levels were also unchanged (Schuller *et al*, 1999). Wild-type (+/+) and homozygous knockout (−/−) mice were obtained from heterozygous breeding. The mice used in the present study were on a C57Bl6/Jico background after 6–7 backcrossings to C57Bl6/Jico mice (Charles River, l'Arbresle, France). The mice were genotyped by PCR on genomic DNA isolated from tail tips. The mutant product was 700 bp, the wild-type product 525 bp; the three primers used were outside the mutation site (5′ GAC TTT CCT GGC TGA TGC AAA CAA CCT 3′), within the mutation site (5′ CAT GGT TCT GAA TGC TTG CTG CGG ACT 3′) and within the neomycin box (5′ CTA CCT GCC CAT TCG ACC ACC AA 3′).

Chronic NTX Treated Mice

At least 1 week after transportation, C57Bl/6Jico mice from Charles River (L'Arbresle, France) received a pellet containing 15 mg NTX or a corresponding placebo pellet, which was implanted subcutaneously in the nape of the neck under isoflurane anesthesia (2%/53% N_2O/45% O_2) (day 1). NTX and placebo treatments were randomly assigned and mice of both treatment groups were housed together (two of both per cage). On day 8 the pellet was removed (2% isoflurane/53% N_2O/45% O_2). All experiments described here commenced 48 h after pellet removal, that is, on day 10.

Acute Cocaine-Induced Locomotor Activity

Experiment 1. μ-Opioid receptor knockout mice; experiment 2. Chronic NTX treated mice. Clear plexiglass cylinders of 20 cm in diameter and 30 cm in height were used as open fields. The mice were allowed to acclimatize to the experiment room for at least 1 h prior to placement in the open field. The mice were then placed in the open field and motor activity was monitored for 1 h. Thereafter, saline was injected i.p. and the mice were monitored for another hour. Finally, cocaine was injected i.p. (3, 10, 20, or 30 mg/kg) after which the mice were returned to the open field and their locomotor activity was determined for another 30 min. During the total 2.5 h of the experiment, the activity pattern of the mice was tracked and analyzed for the total distance moved in the open field per 5 min intervals using Ethovision Color-Pro 2.3 software (Noldus Information Technology, Wageningen, NL). $N = 6$ for 3, 20, and 30 mg/kg treatment groups. $N = 8$ for the 10 mg/kg cocaine treatment group of the μ-opioid receptor knockout experiment. $N = 10$ for the 10 mg/kg cocaine treated chronic NTX or placebo pretreated groups.

Cocaine-Induced Behavioral Sensitization

Experiment 3. Cocaine-induced behavioral sensitization in wild-type mice. In total, 16 C57Bl/6Jico mice were

randomly assigned to either cocaine or saline treatment groups. During 11 days the mice received a daily i.p. injection of either cocaine (20 mg/kg) or saline. At 72 h after the last cocaine or saline injection, the mice were transported and allowed to acclimatize to the experiment room for at least 1 h. Thereafter, the mice were placed for the first time in the open field as described. During the first hour in the field the locomotor activity was measured to monitor the adaptation of the mice to the novel environment. Subsequently, all mice received a saline injection (i.p.) after which they were returned to the open field and monitored for another hour. Thereafter, all mice received a cocaine challenge (10 mg/kg i.p.) and their locomotor activity in the open field was determined during 30 min. The distance moved in the open field was measured as described above.

Experiment 4. μ-Opioid receptor knockout and wild-type mice. At 2–3 months of age the behavioral sensitization commenced as described for experiment 3. Mice of both genotypes (wild-type +/+ and μ-opioid receptor knockout mice −/−) were randomly assigned to either saline or cocaine treatment groups. Group sizes were either seven mice for the +/+ saline treatment group or eight mice for +/+ cocaine, −/− saline, and −/− cocaine treatment groups.

Experiment 5. CTOP co-administration. For this experiment 32 male C57Bl/6JIco mice from Charles River (L'Arblesle, France) were used. After transportation, the mice were allowed to acclimatize for at least 1 week before the behavioral sensitization commenced. The mice were randomly assigned to one of four treatment groups: placebo/saline, placebo/cocaine, CTOP/saline, or CTOP/cocaine. CTOP (1 mg/kg) or placebo was administered i.p. 30 min prior to the daily saline or cocaine injections (co-administration). The 1 mg/kg dose for CTOP was chosen based on a previous reward-relevant study, which demonstrated effective reduction of alcohol consumption by both 0.1 and 1 mg/kg CTOP (Kim *et al*, 2000). Further procedures were similar to those described for experiment 3. $N=8$ per treatment group.

Experiment 6. Effects of chronic NTX pretreatment upon cocaine-induced behavioral sensitization. For this experiment mice were, prior to the behavioral sensitization protocol, pretreated with NTX or placebo by subcutaneous implanted pellets as described above for experiment 2. The sensitization protocol (see experiment 3) commenced 48 h after removal of the pellet, that is, on day 10. $N=8$ for placebo/saline, placebo/cocaine, NTX/saline, and NTX/cocaine treatment groups.

Drugs

Cocaine (cocaine-HCl, OPG, Utrecht, The Netherlands) and CTOP (Tocris, Bristol, UK) were dissolved in saline, control mice received saline injections. Cocaine, CTOP, and saline were injected i.p. in a volume of 5 ml/kg. NTX and corresponding placebo pellets (Research Triangle Institute, North Carolina, USA) were implanted subcutaneously in the nape of the neck as described above.

Statistical Analysis

For statistical analyses SPSS10.1 was used. Open field activity is expressed as distance moved in 5 min intervals. Analyses of variance (ANOVAs) with repeated measurements were used to analyze the data with distance moved as the dependent variable. For the acute experiment only the locomotor activity data for the 30 min after cocaine injection was analyzed with genotype (+/+ or −/−, experiment 1) or pretreatment (placebo or NTX, experiment 2) as factors. For the sensitization experiment, the data for the first hour, the hour after saline injection and the 30 min after cocaine challenge were analyzed separately. The independent factors were treatment (saline or cocaine) and either genotype (+/+ or −/−, experiment 4), co-administration (placebo or CTOP, experiment 5), or pretreatment (placebo or NTX, experiments 6). When appropriate, *post hoc* analyses were performed using Student's *t*-tests. The data are expressed as mean ± SEM distance travelled in 5 min intervals in centimetres. Statistical significance was accepted at $P<0.05$.

RESULTS

Acute Cocaine-Induced Locomotor Activity

Experiment 1. Acute locomotor response to cocaine in μ-opioid receptor knockout vs *wild-type mice.* After 2 h adaptation to the open field, the mice received 3, 10, 20, or 30 mg/kg cocaine i.p. There were no differences between wild-type and μ-opioid receptor knockout mice during habituation or during the hour after saline injection (data not shown). The dose–response curve for cocaine-induced locomotor activity of μ-opioid receptor knockout mice and wild-type mice is shown in Figure 1. Cocaine increased the locomotor activity of both μ-opioid receptor knockout and wild-type mice in the open field in a dose-dependent way (dose effect $F(3,48)=31$, $P<0.001$). The genotypes were comparable in their response to cocaine because there was no significant time × genotype × dose interaction, no significant effect of genotype, nor was there a genotype × dose effect.

Experiment 2. Effects of chronic NTX pretreatment upon acute cocaine-induced locomotor activity. After 2 h adaptation to the open field, the mice received 3, 10, 20, or 30 mg/kg cocaine i.p. There were no differences between placebo and chronic NTX pretreated mice during habituation or the hour after saline injection (data not shown). The dose–response curve for cocaine-induced locomotor activity of chronic NTX and placebo pretreated mice is depicted in Figure 2. Cocaine increased the locomotor activity of the mice in this experiment in a dose-dependent manner as is apparent from a significant dose effect ($F(3,47)=45$, $P<0.001$) and a significant time × dose interaction ($F(15,235)=4.2$, $P<0.001$). Further, a significant time × pretreatment × dose interaction was observed ($F(15,235)=2.9$, $P<0.001$), which was caused by a significant time × pretreatment interaction within the

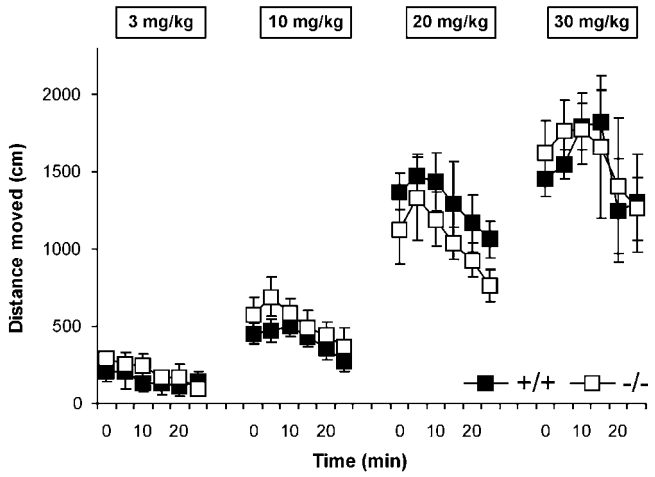

Figure 1 Acute cocaine-induced locomotor activity for μ-opioid receptor knockout (−/−) and wild-type (+/+) mice. The distance moved in cm during 30 min subsequent to 3, 10, 20, or 30 mg/kg cocaine administration i.p. is shown for both genotypes. *N* per genotype was 6 for 3, 20, or 30 mg/kg and *N* = 8 for 10 mg/kg cocaine.

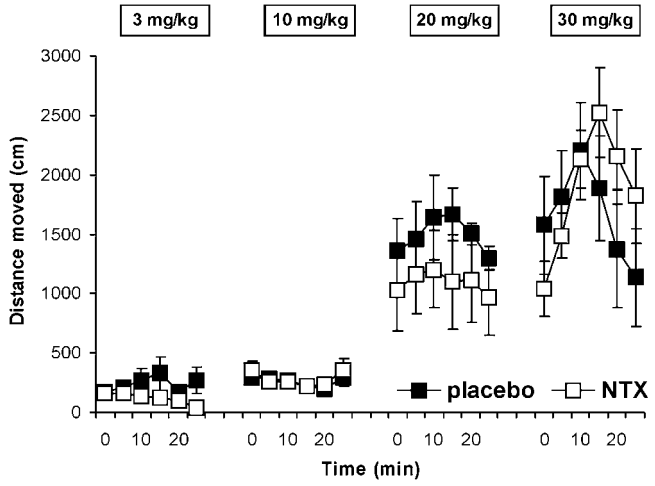

Figure 2 Effects of chronic NTX pretreatment upon the acute response to cocaine. The distance moved in cm during 30 min subsequent to 3, 10, 20, or 30 mg/kg cocaine administration i.p. is shown for both pretreatment groups. *N* per NTX or placebo pretreatment group was 6 for 3, 20, or 30 mg/kg and *N* = 10 for 10 mg/kg cocaine.

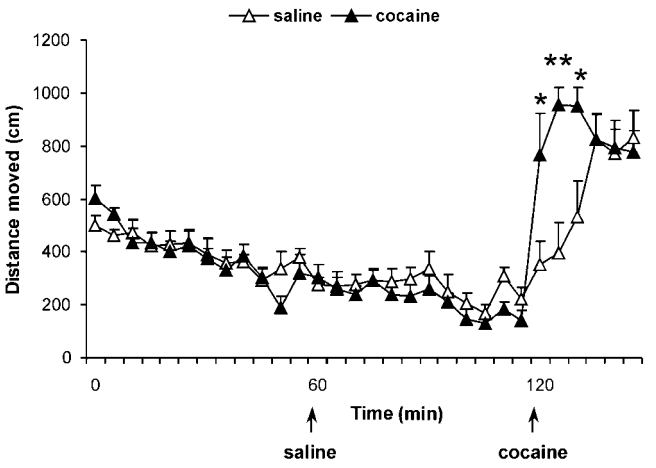

Figure 3 Development of behavioral sensitization in C57Bl6/Jico mice. Sensitization was induced by repeated intermittent treatment of C57Bl/6Jico mice with saline or cocaine (20 mg/kg) for 11 days. The time course of the activity in the open field, 72 h after cessation of the sensitization protocol, is shown with 1 h adaptation to the field, followed by 1 h in the field after an i.p. saline injection and 30 min after an i.p. injection of 10 mg/kg cocaine. The activity in the open field is expressed in the total distance moved in centimetres during 5 min intervals. *N* = 8 per treatment. **P* < 0.05, ***P* < 0.01, significant difference between saline and cocaine treated mice.

30 mg/kg cocaine dose group ($F(5,50) = 3.0$, $P < 0.05$). It appears that the response to cocaine is somewhat altered for the NTX pretreated mice as compared to the placebo controls (Figure 2).

Cocaine-Induced Behavioral Sensitization

Experiment 3. Cocaine-induced behavioral sensitization in wild-type mice. The locomotor activity in the open field and the response to a 10 mg/kg cocaine challenge of C57Bl/6Jico mice after repeated administration of 20 mg/kg cocaine or saline injections is shown in Figure 3.

During the first hour in the open field the activity of the mice declined (time $F(11,143) = 7.5$, $P < 0.001$) and there was no effect of cocaine treatment upon the activity in the unfamiliar open field. Subsequent to saline administration, a further decline in the activity of the mice was apparent (time $F(11,143) = 3.2$, $P < 0.01$) without cocaine treatment effects. After the 2 h of adaptation to the open field, 10 mg/kg cocaine was administered and induced an increase in the locomotor activity of the mice (Figure 3). The cocaine and saline treated mice responded differentially to the 10 mg/kg cocaine challenge, as is evident from a significant time-× treatment interaction ($F(5,65) = 4.8$, $P < 0.01$) and an overall effect of treatment ($F(1,13) = 4.9$, $P < 0.05$). *Post hoc* analyses revealed significant differences between saline and cocaine treated mice for the first 15 min after the 10 mg/kg cocaine challenge was administered; $P < 0.05$, < 0.01, and < 0.05 for the locomotor activity during 0–5, 5–10, and 10–15 min after cocaine injection, respectively. This sensitization protocol was subsequently used for the experiments 4, 5, and 6.

Experiment 4. Cocaine-induced behavioral sensitization in μ-opioid receptor knockout and wild-type mice. Locomotor activity and the response to a cocaine challenge of μ-opioid receptor knockout mice (−/−) and wild-type controls (+/+) are shown in Figure 4.

The locomotor activity of μ-opioid receptor knockout and wild-type mice reduced during the first hour in the open field (effect of time: $F(11,297) = 10$, $P < 0.001$). During the 60 min after saline injection, the locomotor activity of the mice did not further decline and a significant time-× genotype interaction was observed ($F(11,297) = 2.0$, $P < 0.05$): the μ-opioid receptor knockout mice were less active as compared to wild-type mice 15−20, 35−40, and 40−45 min after saline injection ($P < 0.05$). There was no effect of treatment upon the activity of the mice in the open

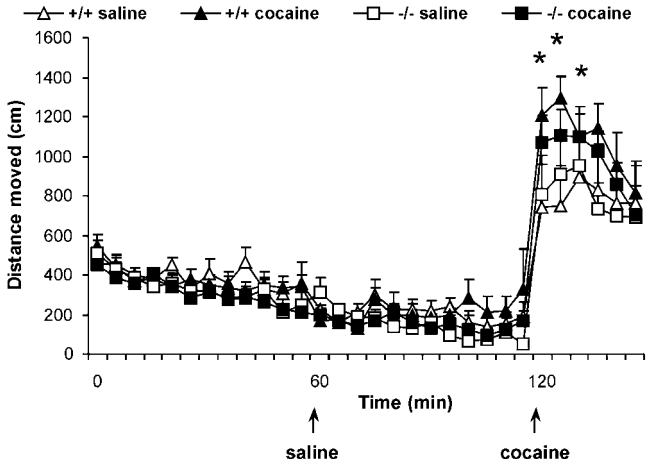

Figure 4 Behavioral sensitization in μ-opioid receptor knockout mice (−/−) and wild-type controls (+/+). Sensitization was induced by repeated intermittent treatment with saline or cocaine (20 mg/kg) for 11 days. The time course of the activity in the open field, 72 h after cessation of the sensitization protocol, is shown with 1 h adaptation to the field, followed by 1 h in the field after an i.p. saline injection and 30 min after an i.p. injection of a 10 mg/kg cocaine challenge. The activity in the open field is expressed in the total distance moved in centimetres during 5 min intervals. $N = 7$–8 per treatment per genotype. $^{*}P < 0.05$, significant difference between saline and cocaine treated subjects.

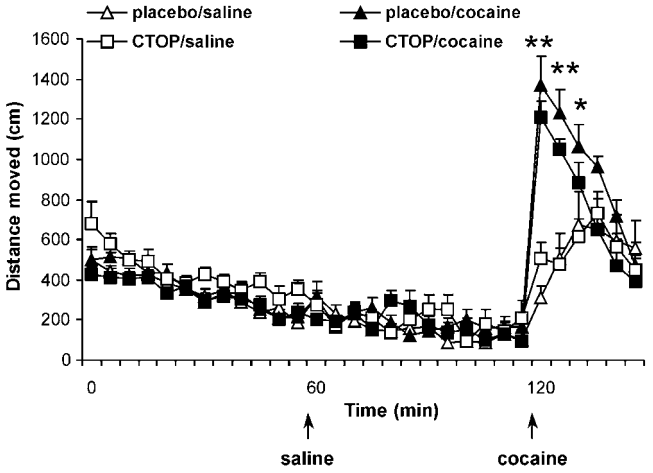

Figure 5 Effects of CTOP upon the development of cocaine-induced behavioral sensitization. CTOP or placebo were injected 30 min prior to repeated intermittent saline or cocaine (20 mg/kg) injections for 11 days (co-administration). The time course of the activity in the open field, 72 h after cessation of the sensitization protocol, is shown with 1 h adaptation to the field, followed by 1 h in the field after an i.p. saline injection and 30 min after injection of a 10 mg/kg cocaine challenge. The activity in the open field is expressed in the total distance moved in centimetres during 5 min intervals. $N = 8$ per treatment (saline or cocaine) per co-administration (placebo or CTOP). $^{*}P < 0.01$, $^{**}P < 0.001$, significant difference between saline and cocaine treatment groups.

field and all mice were comparable in their locomotor activity just prior to cocaine injection.

The locomotor response to the 10 mg/kg cocaine challenge was augmented in cocaine treated mice as compared to saline treated mice, illustrating the occurrence of behavioral sensitization to cocaine (time × treatment $F(5,135) = 2.3$, $P < 0.05$). Subsequent *Post hoc* tests revealed that cocaine treated mice responded with a higher increase in locomotor activity as compared to saline treated mice during 0–5, 5–10, and 15–20 min after the cocaine challenge was administered ($P < 0.05$). No genotype or genotype × treatment interaction was observed, suggesting that the development of cocaine-induced behavioral sensitization was comparable for μ-opioid receptor knockout and wild-type mice. There was no difference between saline treated wild-type and μ-opioid receptor knockout mice in their locomotor response to cocaine in the open field.

Experiment 5. Effects of CTOP co-administration upon cocaine-induced behavioral sensitization. In Figure 5 the locomotor activity and the locomotor response to a cocaine challenge is shown for mice, which received either CTOP or placebo co-administered with the repeated intermittent saline or cocaine injections.

During the first hour in the open field the mice reduced their activity (effect of time, $F(11,308) = 28$, $P < 0.001$), indicative of adaptation to the open field. There was a significant co-administration × treatment effect upon the activity during the first hour in the open field ($F(1,28) = 6.8$, $P < 0.05$), which was caused by higher locomotor activity of the CTOP/saline treated mice as compared to saline/saline and CTOP/cocaine treated mice. The locomotor activity declined further during the hour after saline injection (time effect $F(11,308) = 4.9$, $P < 0.001$) and a significant time × co-administration × treatment effect was observed ($F(11,308) = 2.1$, $P < 0.05$), which as *Post hoc* tests revealed was caused by minor differences between groups. That is, 0–5 min after saline administration the saline/cocaine treated mice were more active than CTOP/cocaine treated mice ($P < 0.05$). Further, 20–25 min after saline was administered, CTOP/cocaine treated mice were more active than mice from the CTOP/saline group ($P < 0.05$) and 35–40 min after saline injection, the mice that received repeated CTOP/saline treatment were more active in the open field as compared to saline/saline treated mice ($P < 0.05$). All groups were comparable in their locomotor activity during the last intervals prior to cocaine administration.

Overall analysis of the locomotor activity of all groups during the 30 min after administration of the 10 mg/kg cocaine challenge dose revealed a significant time × treatment (saline or cocaine) interaction ($F(5,140) = 28$, $P < 0.001$). Cocaine treated mice responded with a higher increase in locomotor activity to the 10 mg/kg cocaine challenge during 0–5, 5–10, and 10–15 min after cocaine was injected ($P < 0.001$, < 0.001, and < 0.01). There was no effect of CTOP co-administration nor was there a significant time × co-administration × treatment interaction, suggesting that the μ-opioid receptor antagonist CTOP did not affect the development of cocaine-induced behavioral sensitization. CTOP co-administration did not affect the acute locomotor response to cocaine as CTOP/saline treated mice were not significantly different in their response to cocaine from placebo/saline treated mice.

Experiment 6. Effects of chronic NTX pretreatment upon cocaine-induced behavioral sensitization. The locomotor activity in the open field and the response to a 10 mg/kg

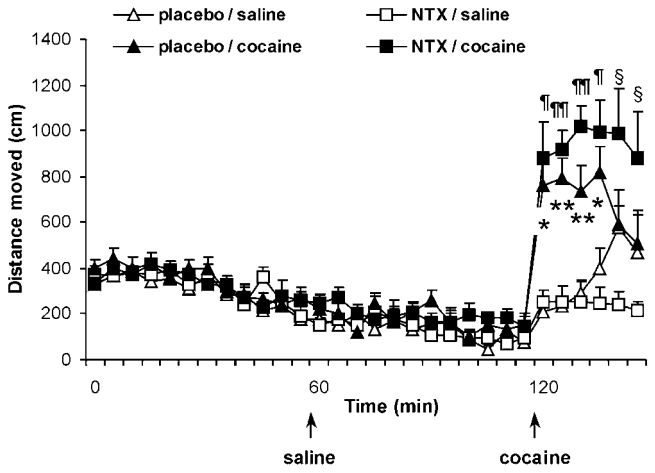

Figure 6 Effects of chronic placebo or NTX pretreatment upon the development of behavioral sensitization. Starting 48 h after placebo or NTX pellet removal, repeated intermittent daily injections of saline or cocaine (20 mg/kg) were given for 11 days. The time course of the activity in the open field, 72 h after cessation of the sensitization protocol, is shown with 1 h adaptation to the field, followed by 1 h in the field after an i.p. saline injection and 30 min after an i.p. injection of a 10 mg/kg cocaine challenge. The total distance moved in centimetres during 5 min intervals is shown. $N=8$ per treatment (saline or cocaine) per pretreatment (NTX or placebo). $^{*}P<0.05$, $^{**}P<0.01$, significant difference between saline and cocaine treated mice within the placebo pretreated group. $^{\S}P<0.05$, $^{\P}P<0.01$, $^{\P\P}P<0.001$, significant difference between NTX pretreated, saline controls, and NTX pretreated, cocaine treated mice.

cocaine challenge of chronic NTX and placebo pretreated mice is depicted in Figure 6.

During the first hour in the open field the mice adapted to the open field, as reflected by a decrease in locomotor activity (effect of time $F(11,308)=14$, $P<0.001$). There was no effect of chronic NTX pretreatment upon the locomotor activity during the first hour in the open field. The locomotor activity during the hour subsequent to saline injection declined further (effect of time: $F(11,308)=3.7$, $P<0.001$), without group differences.

The cocaine-induced locomotor response was augmented in cocaine treated mice (effect of treatment $F(1,28)=58$, $P<0.001$). Chronic NTX pretreatment enhanced the development of behavioral sensitization to cocaine, which was apparent from a significant pretreatment × treatment interaction ($F(1,28)=7.2$, $P<0.05$). Separate analyses of the groups confirmed the development of cocaine-induced behavioral sensitization in placebo pretreated mice (time × treatment $F(5,70)=4.8$, $P<0.01$ and treatment $F(1,14)=10$, $P<0.01$) and chronic NTX pretreated mice (treatment $F(1,14)=62$, $P<0.001$). *Post hoc* analyses revealed that placebo/cocaine treated mice were significantly more active than placebo/saline treated mice during the first 20 min after cocaine challenge injection ($P<0.01$). Across the entire 30 min after cocaine injection, the NTX pretreated mice that received repeated intermittent cocaine injections were significantly more active than NTX pretreated mice that received repeated saline injections ($P<0.01$). Placebo/saline pretreated mice were more active as compared to chronic NTX/saline pretreated mice 20–25 min after injection of 10 mg/kg cocaine ($P<0.01$).

DISCUSSION

Here we investigated the role of μ-opioid receptors in the acute locomotor response to cocaine and in the development of cocaine-induced behavioral sensitization using μ-opioid receptor knockout mice, co-administration of the μ-opioid receptor antagonist CTOP, and chronic NTX pretreatment. Chronic NTX pretreatment is known to induce increases in μ-opioid receptor binding, but also in δ- and κ-opioid receptor binding, although to a lesser extent (Lesscher *et al*, 2003). Our findings indicate that μ-opioid receptors are not required for acute cocaine-induced locomotor activity nor are they essential for cocaine-induced behavioral sensitization to develop.

Acute Cocaine-Induced Locomotor Activity

The locomotor response to cocaine was not different between μ-opioid receptor knockout and wild-type mice, suggesting that μ-opioid receptors are not required for cocaine-induced motor activity. Similarly, no differences were apparent between chronic NTX pretreated mice and placebo pretreated controls, except for the response to 30 mg/kg cocaine. A significant pretreatment time interaction for this dose suggests a different temporal pattern of the response to 30 mg/kg cocaine between placebo and chronic NTX pretreated mice. Cocaine-induced locomotor activity has not been described previously for chronic NTX pretreated animals. With respect to μ-opioid receptor knockout mice, the present findings are in agreement with those of Becker *et al* (2002) who also reported comparable cocaine-induced locomotor activity for μ-opioid receptor knockout mice, which lack exons 2 + 3 of the μ-opioid receptor gene, and wild-type controls. Taken together, μ-opioid receptors are not required for the acute locomotor response to cocaine in mice.

Cocaine-Induced Behavioral Sensitization

Opioid modulation of psychostimulant-induced behavioral sensitization has been described previously, at least for δ- and κ-opioid receptors. The δ-opioid receptor antagonist naltrindole and κ-opioid receptor selective agonists impaired the development of behavioral sensitization, which occurs after repeated intermittent treatment of rats with psychostimulant drugs (Heidbreder *et al*, 1993a, b, 1995, 1996; Shippenberg and Heidbreder, 1995b; Shippenberg *et al*, 1996; Shippenberg and Rea, 1997 but Vanderschuren *et al*, 2000). A role of μ-opioid receptors in psychostimulant sensitization was suggested first of all by impaired cocaine-induced sensitization after NTX co-administered with cocaine (Sala *et al*, 1995) and also by findings of two recent studies. Expression of mRNA encoding μ- and δ-opioid receptors was increased in the VTA of amphetamine-sensitized rats (Magendzo and Bustos, 2003). Another study dealt with cocaine-induced behavioral sensitization in mice with a targeted deletion of exons 2 and 3 of the μ-opioid receptor gene. These μ-opioid receptor knockout mice developed a sensitized locomotor response to cocaine, although the temporal pattern of responding was different from wild-type mice (Yoo *et al*, 2003).

The present data show that cocaine-induced behavioral sensitization is retained in exon 1 μ-opioid receptor knockout mice suggesting that μ-opioid receptors are not required for cocaine-induced behavioral sensitization. However, inherent to application of classic knockout strategies and hence lack of a specific gene from gestation, the possibility that for example δ-opioid receptors have compensated for the loss of μ-opioid receptors in this case cannot be ruled out. Retained cocaine-induced behavioral sensitization for μ-opioid receptor knockout mice is not in agreement with previous studies, which suggested a role of μ-opioid receptors in psychostimulant sensitization (Sala *et al*, 1995; Magendzo and Bustos, 2003; Yoo *et al*, 2003). Therefore, a subsequent experiment was performed to elucidate the role of μ-opioid receptors in cocaine-induced sensitization. For this experiment, the μ-opioid receptor selective antagonist CTOP, which is approximately 2000-fold more specific for μ- over δ-opioid receptors (Hawkins *et al*, 1989), was co-administered with cocaine according to a procedure similar to the previous study by Sala *et al* (1995) with the nonselective opioid antagonist NTX. If μ-opioid receptors were involved in cocaine-induced behavioral sensitization as the previous data of Sala *et al* (1995) suggest, then the μ-opioid receptor selective antagonist CTOP should impair cocaine-induced behavioral sensitization. We did not observe a difference in cocaine-induced behavioral sensitization between CTOP and saline treated groups suggesting that in fact that μ-opioid receptors are not required for behavioral sensitization to the locomotor stimulant effects of cocaine to develop, which is in agreement with the μ-opioid receptor knockout experiment. In further support of a lack of involvement of μ-opioid receptors in cocaine-induced behavioral sensitization, a recent study by Hummel *et al* (2004) reported cocaine-induced behavioral sensitization in exon 1 μ-opioid receptor knockout mice. As mentioned, Yoo *et al* (2003) reported differential cocaine-induced behavioral sensitization in μ-opioid receptor knockout mice. They observed cocaine-induced behavioral sensitization in μ-opioid receptor knockout mice, lacking exons 2 and 3, after 6 days of cocaine treatment whereas behavioral sensitization was only apparent after 12 days of cocaine treatment in wild-type controls. This suggests that cocaine-induced behavioral sensitization is potentiated in the absence of μ-opioid receptors. However, the lack of cocaine-induced behavioral sensitization in wild-type mice on day 6 together with the fact that both at the first day of testing and after 6 days of cocaine treatment, the locomotor response to cocaine was lower for μ-opioid receptor knockout mice as compared to wild-type controls complicate the interpretation of these data. Taken together, μ-opioid receptors appear not to be required for the development of cocaine-induced behavioral sensitization.

In the final experiment the effect of overexpression of predominantly μ-, but also δ-, opioid receptors by chronic NTX pretreatment upon cocaine-induced behavioral sensitization was assessed. Chronic NTX pretreatment was found to augment cocaine-induced behavioral sensitization. This is an interesting finding considering that the acute locomotor response to cocaine was not affected by chronic NTX pretreatment, although at the 30 mg/kg dose the cocaine response appeared higher for mice that were pretreated with NTX as opposed to placebo pretreated controls. However, μ-opioid receptors seem not to be required for cocaine-induced behavioral sensitization to develop, as can be concluded from the μ-opioid receptor knockout and CTOP experiments, respectively. Therefore, it appears unlikely that enhanced μ-opioid receptor levels in chronic NTX treated mice contribute to enhanced behavioral sensitization to the locomotor stimulant effects of cocaine in these mice. Rather, the augmented sensitized locomotor response to cocaine of chronic NTX pretreated mice may be attributable to increased number of δ-opioid receptors, the subtype of opioid receptors that has indeed been implicated in cocaine-induced behavioral sensitization (Heidbreder *et al*, 1993a, 1996; Shippenberg and Heidbreder, 1995b).

In contrast to retained cocaine-induced behavioral sensitization observed for μ-opioid receptor knockout mice, μ-opioid receptor knockout mice failed to self-administer cocaine (Mathon *et al*, in preparation), suggesting that μ-opioid receptors are critically involved in cocaine reinforcement but are not required for cocaine-induced behavioral sensitization. The sensitization protocol that was used for the present studies involved daily injections in an environment different from the test environment, similar to previous studies addressing the role of δ- and κ-opioid receptors in cocaine-induced behavioral sensitization (Heidbreder *et al*, 1995, 1996). With this approach mainly non-associative aspects of cocaine-induced behavioral sensitization are involved. It is therefore interesting to consider the possibility that μ-opioid receptors might be involved in associative aspects of cocaine-induced behavioral sensitization, which could be subject to future studies. For chronic NTX treatment, a striking parallel increase is apparent in the development of cocaine-induced sensitization and cocaine reinforcement, both of which are augmented after chronic NTX pretreatment. Chronic treatment with NTX facilitated the initiation of cocaine self-administration in rats (Ramsey and Van Ree, 1990) and initiation of alcohol consumption has been shown to be potentiated by chronic NTX treatment in mice (Phillips *et al*, 1997). It appears that similar mechanisms might be involved in both phenomena. However, since chronic NTX treatment causes increases both in μ- and δ-opioid receptor levels (Lesscher *et al*, 2003), the increased reinforcing effects of cocaine and the augmented cocaine-induced behavioral sensitization in chronic NTX pretreated mice are likely to entail distinct mechanisms, that is through increased μ- and δ-opioid receptors, respectively.

In conclusion, the present findings show that μ-opioid receptors are not required for the acute locomotor response to cocaine or for the development of cocaine-induced behavioral sensitization.

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