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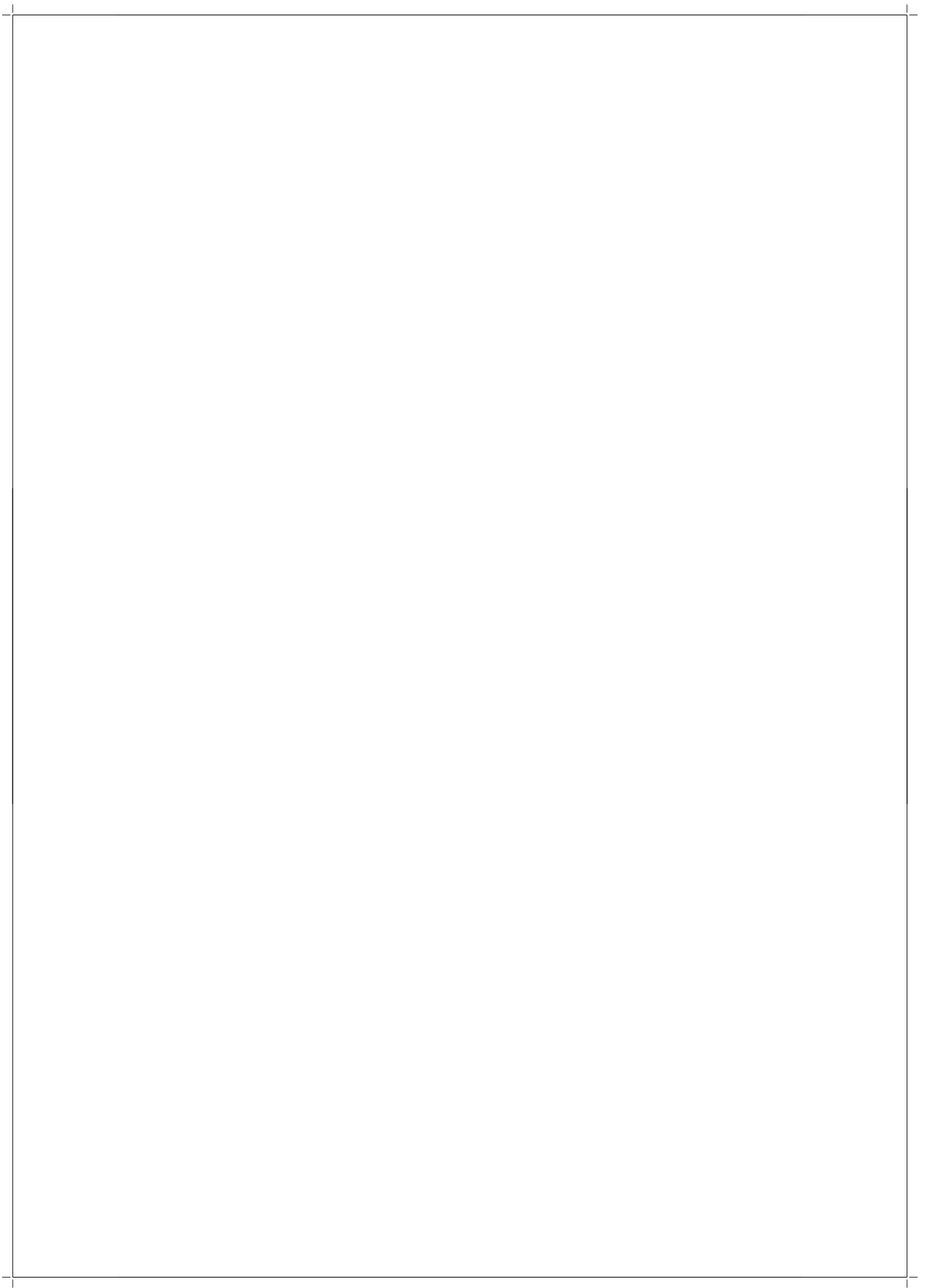
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Chapter 2

μ -Opioid receptors
in the nucleus
accumbens
shell region mediate
the effects of
amphetamine
on inhibitory
control and not
impulsive choice

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Chapter 2 Opioid receptors and amphetamine-induced impulsivity

Abstract

Acute challenges with psychostimulants such as amphetamine affect impulsive behavior in both animals and humans. With regard to amphetamine, it is important to unravel how this drug affects impulsivity since it is not only a widely-abused recreational drug but also regularly prescribed to ameliorate maladaptive impulsivity. Therefore, we studied the effects of amphetamine in two rat models of impulsivity, the 5-choice serial reaction time task and the delayed reward task, providing measures of inhibitory control and impulsive choice, respectively. We focused on the role of opioid receptor activation in amphetamine-induced impulsivity as there is ample evidence indicating an important role for endogenous opioids in several behavioral and neurochemical effects of amphetamine. Results showed that amphetamine-induced inhibitory control deficits were dose-dependently attenuated by the preferential μ -opioid receptor antagonist naloxone, but not by the selective δ -opioid receptor antagonist naltrindole or κ -opioid receptor antagonist nor-BNI. In contrast, naloxone did not affect amphetamine-induced improvements in impulsive decision making. Naloxone also completely prevented inhibitory control deficits induced by GBR 12909, a selective dopamine transporter inhibitor. Intracranial infusions of naloxone, the selective μ -opioid receptor antagonist CTAP, morphine, and the selective μ -opioid receptor agonist DAMGO revealed that μ -opioid receptor activation in the shell rather than the core subregion of the nucleus accumbens (NAc) modulates inhibitory control and subserves the effect of amphetamine thereon. Together, these results indicate an important role for NAc shell μ -opioid receptors in the regulation of inhibitory control, probably via an interaction between these receptors and the mesolimbic dopamine system.

Introduction

Impulsivity is a multifaceted construct covering various, largely independent, behavioral measures ranging from impulsive actions, e.g. disturbed inhibitory control or response inhibition, to impulsive decisions, e.g. delay aversion (Evenden, 1999; Moeller et al., 2001; Winstanley et al., 2006a; Pattij and Vanderschuren, 2008). Maladaptive impulsivity has been implicated in a wide range of psychiatric disorders, including Attention-Deficit/Hyperactivity Disorder (ADHD), bipolar disorders, and substance use disorders (American Psychiatric Association, 2000). Psychostimulant drugs such as methylphenidate and amphetamine targeting monoaminergic neurotransmission are nowadays leading prescription drugs to treat ADHD (Elia et al., 1999; Kutcher et al., 2004). Particularly for amphetamine, it is important to understand the neuronal mechanisms by which this psychostimulant affects

different aspects of impulsivity as it is also a widely abused addictive compound. In both humans and rodents, acute challenges with amphetamine impair inhibitory control, at least when operationalized as the inability to restrain inappropriate behavior (Cole and Robbins, 1987, 1989; Fillmore et al., 2003; Van Gaalen 2006a, 2009; Pattij et al., 2007b), and reduce impulsive choice, often measured as intolerance to delayed gratification or delay aversion (Wade et al., 2000; De Wit et al., 2002; Winstanley et al., 2003, 2005; Van Gaalen 2006b).

Although the effects of amphetamine on impulsivity depend on enhanced dopamine transmission (Cole and Robbins, 1987, 1989; Winstanley et al., 2003, 2005; Van Gaalen 2006a,b, 2009; Pattij et al., 2007b), other neurotransmitter systems may also play crucial roles in regulating impulsive behavior, including endogenous opioid systems (Madden et al., 1997; Kieres et al., 2004; Mitchell et al., 2007; Boettiger et al., 2009; Love et al., 2009; Olmstead et al., 2009; Pattij et al., 2009; Zacny and De Wit, 2009). Indeed, there is ample evidence showing that amphetamine activates endogenous opioid systems (Wang and McGinty, 1995, 1996; Olive et al., 2001; Gonzalez-Nicolini et al., 2003), and the effects of amphetamine on dopamine release and behavioral measures including locomotion, reward, and amphetamine-induced reinstatement of amphetamine seeking involve opioid transmission (Trujillo et al., 1991; Hooks et al., 1992; Schad et al., 1995; Jayaram-Lindstrom et al., 2004, 2008b; Haggkvist et al., 2009). This raises the question as to whether endogenous opioids also mediate amphetamine-induced impulsivity.

The nucleus accumbens (NAc) contains a dense expression of opioid receptors, particularly μ - and κ -opioid receptors (Mansour et al., 1987; Dilts and Kalivas, 1989; Svingos et al., 1997). This region modulates (amphetamine-induced changes in) inhibitory control and impulsive choice (Cole and Robbins, 1987, 1989; Cardinal et al., 2001; Christakou et al., 2004), with putative differential involvement of the NAc shell and core subregions (Murphy et al., 2008; Pattij et al., 2007b). The present aim was to investigate the role of opioid receptors in the NAc shell and core in amphetamine-induced changes in two aspects of impulsivity, inhibitory control and impulsive choice. To that end, we employed systemic and intracranial drug injections to manipulate behavioral performance in the 5-choice serial reaction time task (5-CSRTT) and delayed reward task (DRT), measuring inhibitory control and impulsive choice, respectively.

Materials and Methods

Subjects

Male Wistar rats were obtained from Harlan CPB (Horst, The Netherlands). At the start of the experiments animals weighed approximately 250 grams, and were housed two per cage in macrolon cages (42.5 × 26.6 × 18.5 cm; length × width × height) under a reversed 12 hr light/dark cycle (lights on at 7.00 p.m.) at controlled room temperature (21 ± 2 °C) and relative humidity of 60 ± 15%. Animals were maintained at approximately 90% of their free-

feeding weight, starting one week prior to the beginning of the experiments by restricting the amount of standard rodent food pellets (Harlan Teklad Global Diet, Blackthorn, UK). Water was available *ad libitum* throughout the entire experiment. All experiments were conducted with the approval of the animal ethical committee of the VU University, Amsterdam, The Netherlands.

Drugs

(+)-Amphetamine sulfate and morphine hydrochloride (both OPG, Utrecht, The Netherlands), as well as naloxone hydrochloride, H-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂ (CTAP), and [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-Enkephalin acetate salt (DAMGO) (all Sigma-Aldrich, St. Louis, MO, USA), and *nor*-Binaltorphimine dihydrochloride (*nor*-BNI; Tocris Bioscience, Bristol, UK) were dissolved in sterile saline. GBR 12909 dihydrochloride and naltrindole hydrochloride (both Sigma, St. Louis, MO, USA) were dissolved in sterile water. Drug doses and injection times were based on previous studies. *Nor*-BNI was injected 22–24 hours prior to testing, naloxone and naltrindole 30 min prior to testing, amphetamine and GBR 12909 20 min prior to testing. In the intracranial infusion experiments, systemic amphetamine/saline was administered immediately following intracranial infusion, i.e. 15 min prior to testing. Drugs were freshly prepared each day before testing and injected intraperitoneally (i.p.) in a volume of 1 ml/kg bodyweight or infused intracranially (i.c.) in a volume of 0.5 µl/hemisphere according to a Latin square within-subjects design for all experiments except for the tests with *nor*-BNI. As *nor*-BNI is very long-acting, we first conducted two test days to assess the effects of vehicle and amphetamine (in random order) in a group of rats, and then split the group of rats into two subgroups with equal behavioral results during the first two test days (amphetamine data not shown for *nor*-BNI alone group) to test the effects of *nor*-BNI alone or in combination with amphetamine on a final third test day. Drug tests were conducted on Tuesdays and Fridays with baseline training sessions on the other weekdays. Prior to the first test day, all animals had been habituated twice to i.p. saline injections. Importantly, behavioral sensitization might occur to the behavioral effects of amphetamine, even after a single exposure to 5 mg/kg, a tenfold higher dose than employed here (Vanderschuren et al., 1999). However, as shown previously in our laboratory, in an experimental design similar to those used in the present study the behavioral response to 0.5 mg/kg amphetamine was not altered by repeated exposure to this dose of amphetamine (van Gaalen et al., 2006b). Moreover, to limit the number of amphetamine challenges each subject received, in the present study animals were only tested in one particular drug Latin square except for animals used in the intracranial infusion experiments.

Apparatus

Experiments were conducted in identical rat five hole nose poke operant chambers with stainless steel grid floors (MED-NPW-5L, Med Associates Inc., St. Albans, VT, USA) housed in sound-insulating and ventilated cubicles. Set in the curved wall of each box was an array of five holes. Each nose poke unit was equipped with an infrared detector and a yellow light emitting diode (LED) stimulus light. Rodent food pellets (45 mg, Formula P, Bio-Serv,

Frenchtown, USA) could be delivered at the opposite wall via a dispenser. In addition, a white house light could illuminate the chamber. A computer equipped with MED-PC version 1.17 (Med Associates Inc.) controlled experimental sessions and recorded data. Animals were tested once daily from Monday until Friday, during the dark phase of the light/dark cycle.

Behavioral procedures

Separate groups of animals were trained for each experiment ($n = 16$ for systemic pharmacology; $n = 10$ and $n = 12$ for intracranial infusions), unless stated otherwise. For all paradigms similar habituation and magazine training protocols were followed. This protocol consisted of a habituation exposure to the operant chambers for 20 minutes (min) with the house light on and the food cup containing three food pellets during the first session. Subsequently, in the next two sessions, in total 75 pellets were delivered with an average delay of 15 seconds (s) to allow the animals to associate the sound of pellet delivery with reward.

5-Choice Serial Reaction Time Task

A detailed description of the 5-CSRTT behavioral procedure in our laboratory has been provided previously (Van Gaalen 2006a). In short, rats were trained to detect and respond to a brief visual stimulus in one of 5 nose poke units in order to obtain a food reward. Each session terminated after 100 trials or 30 min, whichever occurred first. Initially the duration of this stimulus was 32 and was gradually decreased to 1 s over sessions until animals reached stable baseline performance (accuracy >80% correct choice and <20% errors of omission). Responding during stimulus presentation or within the limited hold (LH) period of 2 s was counted as a correct response. Incorrect, premature responses during the fixed 5 s intertrial interval, and errors of omission (no responses or a response after the LH) did not lead to the delivery of a food reward and resulted in a 5 s time-out period during which the houselight was extinguished. Perseverative responses after correct choice, i.e. repeated responding during stimulus presentation into any stimulus unit following correct stimulus detection and before pellet collection, were measured but did not have any programmed consequences. The primary measure for inhibitory control was the number of premature responses. In some cases however, the percentage of premature responses before stimulus onset, calculated as $[\text{premature responses} / (\text{premature} + \text{correct} + \text{incorrect} + \text{perseverative responses})] * 100$ was used as an additional measure. This measure was only calculated to control for significant drug-induced increments in omission errors and amphetamine-induced differences in the number of premature responses between NAc core and shell drug infusion groups. Furthermore, the following other behavioral parameters were measured that reflect task performance: 1) accurate choice, i.e. percentage correct responses calculated as $[\text{number correct trials} / (\text{correct} + \text{incorrect trials})] * 100$; 2) omission errors, i.e. the total number of omitted trials during a session; 3) the total number of perseverative responses after correct choice, measuring aspects of compulsive behavior (Robbins, 2002); 4) latency to make a correct choice, i.e. the mean time between stimulus onset and nose poke in the illuminated unit; and 5) feeder latency, i.e. the latency to collect a pellet

following correct choice.

Delayed Reward Paradigm

The delayed reward paradigm used in our laboratory has been described previously (Van Gaalen 2006b). Briefly, in the final stages of training and during drug testing, a session was divided into 5 blocks of 12 trials, each block started with 2 forced choice trials. Each rat received a left forced and a right forced trial. The order of these was counterbalanced between subjects. In the next 10 trials, the animals had a free choice and both the left and right unit were illuminated. Poking into one position resulted in the immediate delivery of a small reinforcer (1 food pellet), whereas a nose poke into the other position resulted in the delivery of a large, but delayed, reinforcer (4 food pellets). If an animal omitted a response during this choice phase within 10 s, an intertrial interval was initiated and the trial was counted as an omission. The position associated with the small and large reinforcer was always the same for each individual, and counterbalanced for the group of rats. Delays for the large reinforcer progressively increased within a session per block of 12 trials as follows: 0, 5, 10, 20 and 40 s. Responding into non-illuminated units during the test was recorded, but had no further programmed consequences. The behavioral measure to assess task performance, i.e. the percentage preference for the large reinforcer as a function of delay, was calculated as $[\text{number of choices for the large reinforcer} / (\text{number choices large} + \text{small reinforcers})] * 100$. Furthermore, we calculated the total number of omitted choice trials per block of 10 trials within a session.

Surgery

Upon stable baseline performance in the 5-CSRTT, separate groups of animals were prepared for cannulation surgery by terminating the food restriction and providing free access to food for three days prior to surgery. Animals were anaesthetized using a combination of xylazine (Rompun; Bayer AG, Leverkusen, Germany; 7 mg/kg, i.p.) and ketamine (Alfasan, Woerden, The Netherlands; 100 mg/kg, i.m.), then placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA) for bilateral placement of indwelling guide cannulae (Plastics One, Roanoke, VA, USA). Guide cannulae were positioned 1 mm above the NAc core (8° angle relative to the midline sagittal plane of the skull), or the NAc shell (12° angle), and anchored to the skull with four stainless steel screws and dental acrylic cement. Cannulae were inserted under a sagittal angle to prevent possible intrusion of the lateral ventricles, thereby minimizing the possibility of drug diffusion into the lateral ventricles. The coordinates (in mm, relative to bregma) used for placement of intracranial cannulae were A/P +2.3, M/L \pm 2.7, D/V -6.4 ventral to skull for the NAc core groups, A/P +2.4, M/L \pm 2.6, and D/V -7.4 ventral to skull for the NAc shell groups, calculated from Paxinos and Watson (1998). The tooth bar was set to -2.5 mm. Rats received 0.5 ml/kg of the analgesic Ketofen (1%; Merial, Amstelveen, The Netherlands) and 0.3 ml/kg of the antibiotic Baytril (2.5%; Bayer, Mijdrecht, The Netherlands) at the end of surgery. Following surgery, the animals were housed individually and had *ad libitum* access to food for a week before retraining in the 5-CSRTT.

Infusion procedure

Intracranial infusions were carried out when stable baseline performance was re-established. Initially, during a sham infusion session, animals were habituated to insertion of the injectors (31 gauge and extending $1 (\pm 0.04)$ mm beyond the guide cannulae; Plastics One, model C316) into the guide cannulae. During the infusion studies, drugs were infused on Tuesdays and Fridays, with baseline training sessions in between during which no infusions were conducted. Rats were bilaterally infused with either saline, naloxone, CTAP, morphine or DAMGO over a period of 60 s at a rate of 0.5 μ l/min using 10 μ l Hamilton syringes driven by a syringe infusion pump (Harvard Apparatus, South Natick, MA, USA). Following infusion, the injectors remained in place for an additional 60 s to allow diffusion of the drug, and rats were tested 15 min later. For the combination studies of i.c. naloxone/CTAP pretreatment and i.p. amphetamine, directly after removal of the injectors, rats were injected with vehicle or amphetamine (0.5 mg/kg) and tested 15 min later.

Assessment of cannula placement

Following completion of the behavioral procedures, animals were deeply anaesthetized using sodium pentobarbital (Ceva Sante Animale BV, Maassluis, The Netherlands; 60 mg/ml, i.p.). Subsequently, animals were perfused transcardially with 100 ml 0.9% NaCl, followed by 500 ml 4% paraformaldehyde in 0.1M phosphate buffered saline (PBS, pH 7.2). Brains were removed rapidly and post-fixed for 1 h in the same fixative at room temperature, then stored in 5% sucrose in 0.1M PBS at 4 °C. Coronal sections of 35 μ m were cut on a cryostat and subsequently stained with thionine for the determination of the infusion sites. Only animals with correct cannulae placements were included in the analyses.

Statistical analyses

All data were analyzed using NCSS2007 version 07.1.18 (NCSS, LLC., Kaysville, UT, USA). Data were subjected to repeated measures analysis of variance (ANOVA) with drug treatment (5-CSRTT, DRT) and delay to large reinforcer (DRT) as within subjects variables. The homogeneity of variance across groups was determined using Mauchly's tests for equal variances and in case of violation of homogeneity, Huynh-Feldt epsilon (ϵ) adjusted degrees of freedom and resulting more conservative probability values were depicted and used for subsequent analyses. In case of statistically significant main effects, further post-hoc comparisons were conducted using Newman-Keuls multiple comparison tests. The level of probability for statistically significant effects was set at 0.05. All graphs were produced using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Effects of naloxone on amphetamine-induced impulsivity

To test the putative involvement of endogenous opioids in amphetamine-induced impulsivity, the effects of amphetamine alone and in combination with the opioid receptor

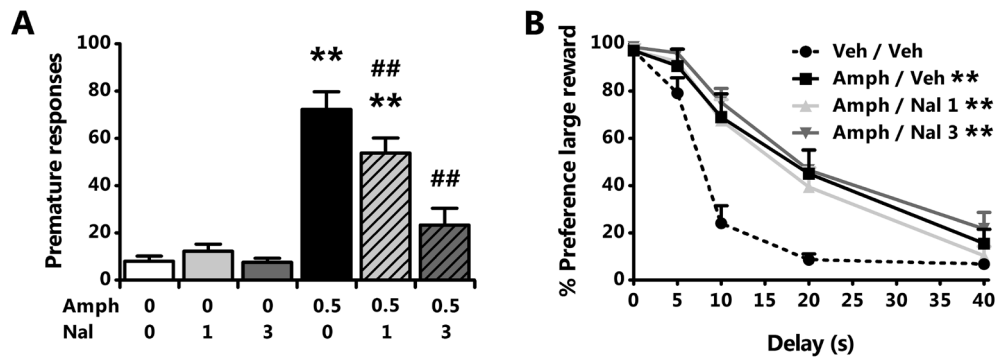


Figure 2.1. Pretreatment with naloxone attenuates the effects of amphetamine on inhibitory control, but not impulsive choice. Effects of 0.5 mg/kg amphetamine (AMPH), naloxone (NAL), and their combination on the mean (\pm SEM) number of premature responses made in the 5-CSRTT (A) and percentage preference for the larger, delayed reinforcer in the DRT (B). In total $n = 12$ and $n = 14$ animals were included in the analyses for respectively the 5-CSRTT and DRT data. Drug doses are expressed as mg/kg. * $p < 0.05$ and ** $p < 0.005$ versus Vehicle-Vehicle; # $p < 0.05$ and ## $p < 0.005$ compared to Amphetamine-Vehicle.

Table 2.1. Effects of 0.5 mg/kg amphetamine (AMPH), naloxone (NAL) and their combination on measures of attentional function, compulsivity, and motivation in the 5-CSRTT.

Treatment (mg/kg)	Accuracy (%)	Perseverative Responses	Correct response latency (ms)	Omissions	Feeder latency (ms)
Vehicle – Vehicle	95.0 \pm 0.9	5.9 \pm 1.1	303 \pm 15	8.4 \pm 2.7	1083 \pm 68
Vehicle – NAL 1	94.4 \pm 1.1	5.3 \pm 1.2	293 \pm 13	6.8 \pm 1.5	1120 \pm 89
Vehicle – NAL 3	93.3 \pm 1.2	7.7 \pm 2.2	296 \pm 16	8.9 \pm 2.0	1377 \pm 194
AMPH – Vehicle	85.9 \pm 1.5**	7.5 \pm 2.1	265 \pm 10*	8.8 \pm 2.6	1009 \pm 89
AMPH – NAL 1	88.6 \pm 1.5**	8.8 \pm 2.2	267 \pm 10*	5.9 \pm 0.9	1133 \pm 135
AMPH – NAL 3	92.0 \pm 1.6##	13.9 \pm 3.5*#	289 \pm 10	9.6 \pm 3.1	1797 \pm 387

In total $n = 12$ animals were included in the analyses and data depict mean \pm SEM. * $p < 0.05$ and ** $p < 0.005$ versus Vehicle-Vehicle; # $p < 0.05$ and ## $p < 0.005$ compared to AMPH-Vehicle.

antagonist naloxone were studied, first in the 5-CSRTT. Four animals were excluded from the analyses due to consistent high omission rates during baseline training and drug testing (>35 omissions/session). In line with previous reports (e.g. Cole and Robbins, 1987, 1989; Van Gaalen 2006a; Pattij et al., 2007b), a systemic injection of amphetamine (0.5 mg/kg) significantly increased premature responding in the 5-CSRTT (Figure 2.1a) and

prior administration of naloxone dose-dependently attenuated this effect ($F_{5,55} = 33.22$, $p < 0.001$, $\epsilon = 0.88$). Further comparisons revealed that both naloxone doses attenuated the effects of amphetamine, with a larger effect size of 3.0 mg/kg. This dose reversed the effects of amphetamine on premature responding to the extent that it was statistically not different from vehicle-vehicle treatment ($p = 0.070$), although the number of premature responses was still 2.9 fold higher. Importantly, in keeping with previous data naloxone by itself did not affect premature responding (Pattij et al., 2009), although it is conceivable that the somewhat lower baseline response levels prevented large reductions of response numbers. As summarized in Table 2.1, significant treatment effects were also observed on accurate choice ($F_{5,55} = 11.06$, $p < 0.001$), with amphetamine reducing the percentage of correct choices and naloxone dose-dependently antagonizing this effect. Amphetamine further decreased correct response latencies ($F_{5,55} = 4.48$, $p = 0.002$), and this effect was partially reversed by prior treatment with 3 mg/kg naloxone as this drug combination did neither differ from vehicle-vehicle nor vehicle-amphetamine treatment. Finally, there was a small but significant treatment effect on perseverative responding ($F_{5,55} = 2.90$, $p = 0.044$, $\epsilon = 0.66$) due to a significant increase thereof following amphetamine in combination with 3 mg/kg naloxone. No significant effects of any treatment were observed for the number of errors of omission ($F_{5,55} = 0.61$, NS, $\epsilon = 0.43$) or feeder latency ($F_{5,55} = 3.38$, NS, $\epsilon = 0.34$).

To test whether opioids also mediate the effects of amphetamine on another form of impulsivity, the effects of amphetamine alone and in combination with naloxone were also studied in the DRT. Two animals were removed from the analysis, one due to failure to show delay-dependent discounting of the large reinforcer, and one due to a consistent high rate of omissions (>70% of trials). As previously observed (e.g. Wade et al., 2000; Winstanley et al., 2003, 2005; Van Gaalen 2006b), amphetamine in this task reduced impulsive choice as reflected by an increased preference for the larger reinforcer over increasing delays as compared to vehicle treatment (dose: $F_{3,39} = 12.36$, $p < 0.001$; dose x delay: $F_{12,156} = 4.48$, $p < 0.001$). In contrast to what was observed in the 5-CSRTT, however, prior administration of naloxone did not alter the effects of amphetamine in the DRT (Figure 2.1b). Together, these results indicate that endogenous opioids are involved in mediating amphetamine-induced impulsivity in the 5-CSRTT, but not the DRT. The detrimental effects of amphetamine on accurate choice in the 5-CSRTT were only significant in the naloxone experiments and the observation that naloxone reversed these effects might suggest opioid involvement. Nonetheless, this notion is not paralleled by opposing effects on this parameter by intracranial infusion of opioid receptor agonists in this study, or systemic morphine as reported previously (Pattij et al., 2009).

Effects of naltrindole and nor-BNI on amphetamine-induced impulsivity

There are three main classes of opioid receptors in the brain, μ -, δ -, and κ -opioid receptors, each with their own function and distribution (e.g. Mansour et al., 1987, 1995; Waldhoer et al., 2004). Naloxone preferentially blocks μ -opioid receptors, but also has affinity for the other opioid receptor subtypes (Schmidhammer et al., 1989; Eguchi, 2004). To study the involvement of δ - and/or κ -opioid receptors in amphetamine-induced inhibitory control

deficits, the effects of amphetamine alone and in combination with naltrindole and nor-BNI, representing selective δ - or κ -opioid receptor antagonists, respectively, were tested in the 5-CSRTT. Naltrindole did not affect amphetamine-induced increments in premature responding in the 5-CSRTT and did not alter inhibitory control when administered by itself (Figure 2.2a; $F_{5,75} = 7.65$, $p < 0.001$, $\epsilon = 0.78$). No significant treatment effects were found on other behavioral parameters in the 5-CSRTT (Table 2.2; accurate choice: $F_{5,75} = 1.30$, NS; perseverative responses: $F_{5,75} = 0.76$, NS, $\epsilon = 0.49$; response latency: $F_{5,75} = 2.19$, NS, $\epsilon = 0.64$; omissions: $F_{5,75} = 0.51$, NS, $\epsilon = 0.82$; feeder latency: $F_{5,75} = 0.91$, NS, $\epsilon = 0.48$). The results obtained with nor-BNI (Figure 2.2b) were more difficult to interpret. Nor-BNI on itself did not affect any behavioral parameter in the 5-CSRTT (premature responding: $F_{1,7} = 1.71$, NS; accuracy: $F_{1,7} = 0.013$, NS; perseverative responses: $F_{1,7} = 0.057$, NS, $\epsilon = 0.49$; response latency: $F_{1,7} = 0.94$, NS; omissions: $F_{1,7} = 0.21$, NS; feeder latency: $F_{1,7} = 0.02$, NS). Results from the combination experiment with amphetamine and nor-BNI showed that there was an overall effect of treatment on inhibitory control ($F_{2,14} = 4.41$, $p = 0.033$). Post-hoc analysis showed that amphetamine-induced inhibitory control deficits were not significantly reduced by pretreatment with nor-BNI. However, there was also no significant increase in premature responding with the amphetamine and nor-BNI combination. The latter results may, however, have been confounded as there also was a significant effect of nor-BNI on the number of omissions (Table 2.2; $F_{2,14} = 4.11$, $p = 0.039$). Post-hoc analysis revealed that nor-BNI in combination with amphetamine significantly raised the number of omissions, suggesting a general decrease in responding under this condition. To control for these confounding effects, an additional analysis in which the number of premature responses was expressed as the percentage of total number of responses was performed (see materials and methods section for formula). This analysis revealed that there was an overall effect of treatment on premature responding ($F_{2,14} = 7.79$, $p = 0.005$), with amphetamine both alone and in combination with nor-BNI significantly increasing premature responding and no significant difference between the amphetamine and amphetamine/nor-BNI condition (vehicle-vehicle: $11.22 \pm 2.43\%$ premature responses; amphetamine-vehicle: $25.40 \pm 4.25\%$ and amphetamine/nor-BNI: $22.72 \pm 4.82\%$). There were no further treatment effects (accuracy: $F_{2,14} = 2.25$, NS; perseverative responses: $F_{2,14} = 1.18$, NS; response latency: $F_{2,14} = 2.85$, NS; feeder latency: $F_{2,14} = 1.51$, NS, $\epsilon = 0.65$). To further examine a role for endogenous opioid systems in regulating amphetamine-induced impulsivity in the DRT, the effects of amphetamine alone and in combination with naltrindole and nor-BNI were tested in this paradigm (Figure 2.3). Naltrindole alone did not significantly alter impulsive choice on itself and also did not affect the beneficial effects of amphetamine on impulsive choice (Figure 2.3a; dose: $F_{3,45} = 18.99$, $p < 0.001$; dose \times delay: $F_{12,180} = 6.05$, $p < 0.001$, $\epsilon = 0.59$). In the nor-BNI experiment, one animal unexpectedly died during task acquisition, hence, only 15 animals were tested. Results showed that similar to naltrindole, nor-BNI alone (Figure 2.3b) did not change impulsive decision making (dose: $F_{1,6} = 2.71$, NS; dose \times delay: $F_{4,24} = 0.98$, NS, $\epsilon = 0.62$). However, this κ -opioid receptor antagonist partially reversed the effects of amphetamine in the DRT (Figure 2.3c; dose: $F_{2,14} = 14.76$, $p < 0.001$; dose \times delay: $F_{8,56} = 3.32$, $p = 0.004$). Together, these data suggest that endogenous opioids and presumably μ -opioid receptors mediate amphetamine-induced deficits in inhibitory control, whereas κ -opioid

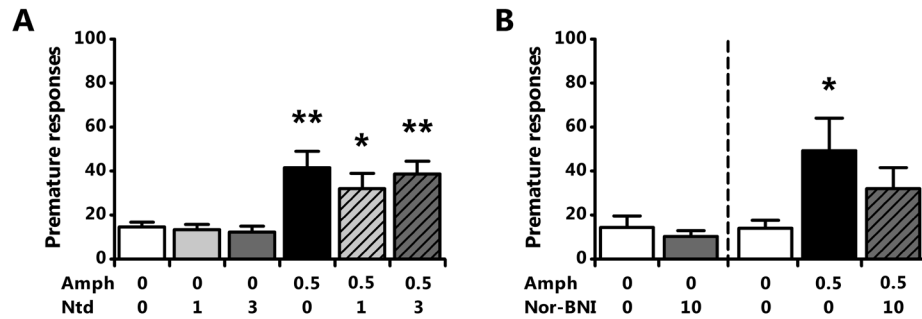


Figure 2.2. Effects of naloxone on amphetamine-induced inhibitory control deficits are not due to blockade of δ - or κ -opioid receptors. Effects of amphetamine (AMPH), naltrindole (NTD), nor-BNI and their combinations on mean (\pm SEM) number of premature responses made in the 5-CSRTT. In total $n = 16$ and $n = 8$ animals were included in the analyses for respectively the NTD (A) and nor-BNI data (B,C). Drug doses are expressed as mg/kg. * $p < 0.05$ and ** $p < 0.005$ versus Vehicle-Vehicle.

Table 2.2. Effects of 0.5 mg/kg amphetamine (AMPH), naltrindole (NTD), nor-BNI and their combinations on measures of attentional function, compulsivity, and motivation in the 5-CSRTT.

Treatment (mg/kg)	Accuracy (%)	Perseverative Responses	Correct response latency (ms)	Omissions	Feeder latency (ms)
Amphetamine + Naltrindole					
Vehicle – Vehicle	84.5 \pm 2.5	9.6 \pm 1.4	309 \pm 9	11.3 \pm 2.4	1404 \pm 223
Vehicle – NTD 1	85.2 \pm 2.3	11.6 \pm 2.4	301 \pm 10	10.2 \pm 2.4	1168 \pm 155
Vehicle – NTD 3	86.2 \pm 2.8	11.8 \pm 3.5	308 \pm 9	8.8 \pm 1.6	1189 \pm 199
AMPH – Vehicle	83.3 \pm 1.8	12.1 \pm 3.6	298 \pm 10	12.8 \pm 3.2	1135 \pm 224
AMPH – NTD 1	83.7 \pm 2.1	6.6 \pm 1.9	292 \pm 7	10.5 \pm 2.1	1073 \pm 154
AMPH – NTD 3	81.9 \pm 2.2	12.5 \pm 2.9	284 \pm 9	11.0 \pm 2.9	1665 \pm 431
Nor-Binaltorphimine alone					
Vehicle – Vehicle	89.0 \pm 2.0	10.5 \pm 3.8	290 \pm 8	8.1 \pm 2.0	1369 \pm 427
Vehicle – Nor-BNI 10	89.2 \pm 1.6	11.3 \pm 2.5	283 \pm 10	9.0 \pm 1.9	1297 \pm 232
Amphetamine + Nor-Binaltorphimine					
Vehicle – Vehicle	89.0 \pm 1.8	5.6 \pm 2.3	267 \pm 12	5.8 \pm 1.0	860 \pm 61
AMPH – Vehicle	85.6 \pm 2.6	4.0 \pm 1.2	253 \pm 10	6.3 \pm 1.9	730 \pm 80
AMPH – Nor-BNI 10	85.2 \pm 2.5	3.0 \pm 0.7	280 \pm 18	14.4 \pm 4.4 [#]	728 \pm 99

Respectively $n = 16$ animals and $n = 8$ animals were included in the analyses with NTD and nor-BNI, and data depict mean \pm SEM. [#] $p < 0.05$ compared to AMPH-Vehicle.

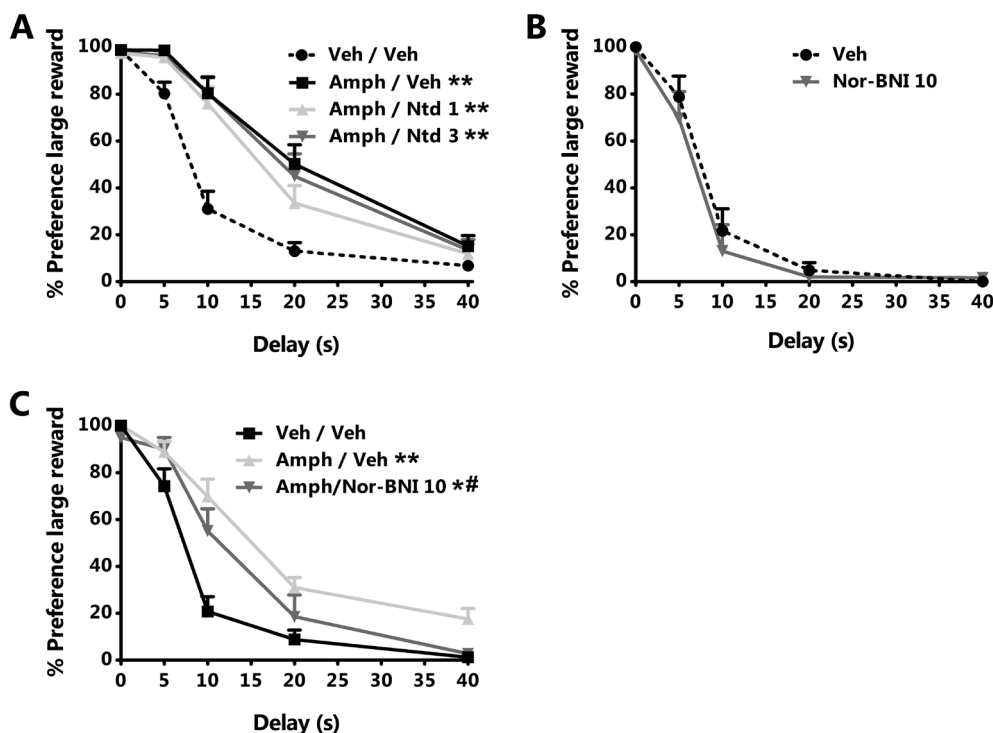


Figure 2.3. Effects of blockade of δ - or κ -opioid receptors on amphetamine-induced reductions in impulsive choice. Effects of amphetamine (AMPH), naltrindole (NTD), nor-BNI and their combinations on mean (\pm SEM) percentage preference for the larger, delayed reinforcer in the DRT. In total $n = 7/8$ and $n = 16$ animals were included in the analyses for respectively the NTD (A) and nor-BNI (B,C) data. Drug doses are expressed as mg/kg. * $p < 0.05$ and ** $p < 0.005$ versus Vehicle-Vehicle; # $p < 0.05$ compared to Amphetamine-Vehicle.

receptors might be involved in the effects of amphetamine on impulsive decision making.

Effects of naloxone on GBR 12909-induced impulsivity in the 5-CSRTT

Amphetamine's effects in the brain depend on the function of dopamine, norepinephrine, and serotonin transporters and as such this psychostimulant acts as an indirect agonist for these monoamine neurotransmitter systems (Seiden et al., 1993; Rothman et al., 2001; Robertson et al., 2009). As it has previously been shown that amphetamine-induced inhibitory control deficits critically depend on increased dopamine transmission (Cole and Robbins, 1987, 1989; Van Gaalen 2006a, 2009; Pattij et al., 2007b), it was tested whether prior administration of naloxone would also attenuate the effects of the selective dopamine transporter inhibitor GBR 12909 in the 5-CSRTT (Van Gaalen 2006a). In this experiment, one animal was excluded from analysis because of a high omission rate under vehicle condition. As shown in Figure 2.4, naloxone abolished the detrimental effects of GBR 12909 on inhibitory control ($F_{3,42} = 7.60$, $p < 0.001$). There were no significant overall treatment effects on other behavioral parameters (Table 2.3; accurate choice: $F_{3,42} = 1.57$,

NS; perseverative responding: $F_{3,42} = 0.73$, NS; response latency: $F_{3,42} = 1.52$, NS; errors of omission: $F_{3,42} = 1.42$, NS, $\epsilon = 0.69$; feeder latency: $F_{3,42} = 0.93$, NS). Thus, it seems that dopamine-induced inhibitory control deficits depend on endogenous opioid signaling, presumably via μ -opioid receptor activation.

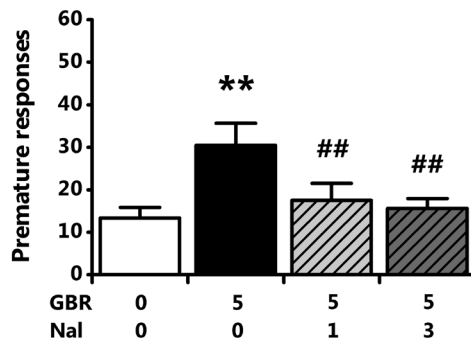


Figure 2.4. Dopamine-induced inhibitory control deficits can be blocked with naloxone. Effects of GBR 12909 (GBR), naloxone (NAL) and their combination on mean (\pm SEM) number of premature responses made in the 5-CSRTT. In total $n = 15$ animals were included in the analysis. ** $p < 0.005$ versus Vehicle-Vehicle; ## $p < 0.005$ compared to GBR 12909-Vehicle.

Table 2.3. Effects of 5 mg/kg GBR 12909 (GBR), naloxone (NAL) and their combination on measures of attentional function, compulsivity, and motivation in the 5-CSRTT.

Treatment (mg/kg)	Accuracy (%)	Perseverative Responses	Correct response latency (ms)	Omissions	Feeder latency (ms)
Vehicle – Vehicle	85.0 \pm 1.5	14.4 \pm 2.1	310 \pm 8	9.1 \pm 2.1	1716 \pm 178
GBR – Vehicle	82.2 \pm 1.9	17.1 \pm 3.9	292 \pm 8	7.7 \pm 1.4	1990 \pm 343
GBR – NAL 1	85.4 \pm 1.7	18.9 \pm 3.8	302 \pm 7	9.2 \pm 2.7	2216 \pm 339
GBR – NAL 3	84.8 \pm 2.1	16.7 \pm 3.3	304 \pm 9	11.5 \pm 2.2	2017 \pm 350

In total $n = 15$ animals were included in the analyses and data depict mean \pm SEM.

Effects of intra-nucleus accumbens infusion of naloxone on amphetamine-induced impulsivity in the 5-CSRTT

To find an anatomical locus for endogenous opioid-mediated effects of amphetamine in the 5-CSRTT, effects of naloxone infusion into either the NAc shell or NAc core alone or in combination with a systemic injection of amphetamine were determined. One rat from the NAc core group was excluded from analysis due to cannulae misplacement. Results showed that a systemic injection of amphetamine (0.5 mg/kg) robustly increased premature responding in both the NAc shell and NAc core group (Figure 2.5a,b). However, only in the NAc shell group this detrimental effect was dose-dependently attenuated by prior infusion of naloxone (NAc shell: $F_{4,36} = 14.01$, $p < 0.001$, $\epsilon = 0.53$; NAc core: $F_{4,32} = 6.11$, $p < 0.001$). Post-hoc analyses revealed that both doses of naloxone infused into the NAc

shell significantly attenuated amphetamine-induced deficits in inhibitory control, with a larger effect size of 5 μ g naloxone. This high dose reversed the effects of amphetamine on premature responding to the extent that this statistically did not differ from vehicle-vehicle treatment ($p = 0.067$), although the number of premature responses still was 2.3 fold higher. Data on other parameters in the 5-CSRTT are summarized in Table 2.4. For both groups significant treatment effects on accurate choice were found (NAc shell: $F_{4,36} = 5.07$, $p = 0.002$; NAc core: $F_{4,32} = 2.90$, $p = 0.037$). However, post-hoc analysis only showed a significant effect in the former group, in which infusion of 5 μ g naloxone alone increased accurate choice. With respect to correct response latency, a significant treatment effect was found for the NAc shell group only (NAc shell: $F_{4,36} = 7.64$, $p < 0.001$; NAc core: $F_{4,32} = 3.25$, $p = 0.07$, $\varepsilon = 0.49$), with amphetamine decreasing response latencies, an effect that was not altered by naloxone. Furthermore, there was a significant treatment effect on feeder latency in the NAc core group (NAc core: $F_{4,32} = 3.11$, $p = 0.046$, $\varepsilon = 0.75$; NAc shell: $F_{4,36} = 0.18$, NS, $\varepsilon = 0.52$), which seemed to be related to the highest dose of naloxone as post-hoc

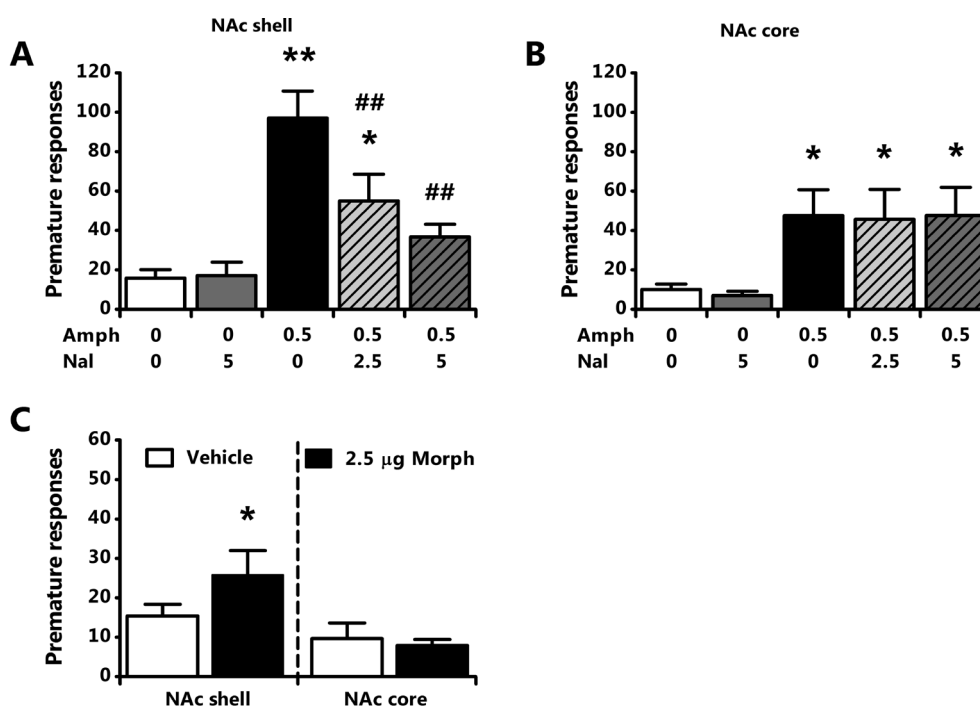


Figure 2.5. Involvement of μ -opioid receptors in the nucleus accumbens shell, not core, in amphetamine-induced inhibitory control deficits. Effects of systemic amphetamine (AMPH), intracranial naloxone (NAL) and their combination (A,B), or intracranial morphine (Morph; C) on mean (\pm SEM) number of premature responses made in the 5-CSRTT. In total $n = 10$ and $n = 9$ animals were included in the data analyses for the experimental groups with intracranial NAc shell and NAc core infusions. AMPH doses are expressed as mg/kg, NAL and morphine doses as μ g/hemisphere. * $p < 0.05$ and ** $p < 0.005$ versus Vehicle or Vehicle-Vehicle; # $p < 0.05$ compared to Amphetamine-Vehicle.

Table 2.4. Effects of 0.5 mg/kg amphetamine (AMPH), intracranial naloxone (NAL; μ g/hemisphere) into the nucleus accumbens shell (NAc shell) and core (NAc core) subregions and their combination, or intracranial morphine (MORPH; μ g/hemisphere) on measures of attentional function, compulsivity, and motivation in the 5-CSRTT.

Treatment (systemic – local)	Accuracy (%)	Perseverative Responses	Correct response latency (ms)	Omissions	Feeder latency (ms)
NAc shell					
Vehicle – Vehicle	79.0 \pm 3.6	12.6 \pm 2.6	368 \pm 15	12.4 \pm 1.5	1948 \pm 270
Vehicle – NAL 5	87.4 \pm 3.1*	7.3 \pm 1.7	362 \pm 19	20.1 \pm 5.1	2197 \pm 612
AMPH – Vehicle	75.4 \pm 3.2	8.9 \pm 2.6	321 \pm 15*	12.7 \pm 2.4	2029 \pm 718
AMPH – NAL 2.5	80.0 \pm 2.8	10.1 \pm 4.8	306 \pm 11**	20.7 \pm 5.4	1677 \pm 364
AMPH – NAL 5	80.9 \pm 3.3	12.9 \pm 6.9	302 \pm 14**	17.9 \pm 6.5	1911 \pm 522
Vehicle	85.2 \pm 2.8	10.5 \pm 2.6	347 \pm 16	12.9 \pm 2.8	1821 \pm 313
MORPH 2.5	85.4 \pm 2.1	10.5 \pm 1.6	321 \pm 16	10.0 \pm 2.2	1336 \pm 217
NAc core					
Vehicle – Vehicle	85.5 \pm 2.5	8.0 \pm 3.3	319 \pm 8	16.1 \pm 3.8	1441 \pm 278
Vehicle – NAL 5	86.9 \pm 2.5	16.4 \pm 6.6	312 \pm 9	12.2 \pm 2.0	2419 \pm 742
AMPH – Vehicle	81.6 \pm 1.9	8.7 \pm 3.9	297 \pm 13	10.2 \pm 2.2	1369 \pm 240
AMPH – NAL 2.5	83.1 \pm 3.3	8.0 \pm 3.7	293 \pm 8	9.7 \pm 2.7	1876 \pm 564
AMPH – NAL 5.0	79.6 \pm 2.7	29.3 \pm 13.9	290 \pm 8	11.2 \pm 2.7	3822 \pm 1086*#
Vehicle	86.4 \pm 2.2	8.1 \pm 2.0	337 \pm 12	13.6 \pm 3.3	1400 \pm 179
MORPH 2.5	88.5 \pm 2.4	6.2 \pm 1.5	310 \pm 8*	9.9 \pm 2.0	1163 \pm 178

In total $n = 10$ (shell region) and $n = 9$ (core region) animals were included in the analyses and data depict mean \pm SEM. * $p < 0.05$ and ** $p < 0.005$ versus Vehicle-Vehicle; # $p < 0.05$ compared to AMPH-Vehicle.

analysis revealed a trend towards a significant increase in feeder latencies after infusion of 5 μ g naloxone alone, and a significant increase after 5 μ g naloxone in combination with an amphetamine injection. No significant treatment effects were found for perseverative responding (NAc shell: $F_{4,36} = 0.39$, NS, $\epsilon = 0.67$; NAc core: $F_{4,32} = 2.28$, NS, $\epsilon = 0.38$) or errors of omission (NAc shell: $F_{4,36} = 1.61$, NS; NAc core: $F_{4,32} = 1.96$, NS). Finally, to rule out that any differences in the effects of naloxone observed between the NAc core and shell group were based on differential reactivity to amphetamine between groups, additional analyses were performed with treatment (vehicle-vehicle vs. vehicle-AMPH) as within-subjects factor and infusion site (NAc shell vs. NAc core) as between-subjects factor. These analyses

revealed no significant site or treatment by site interaction effects except for the parameters correct response latency (site: $F_{1,17} = 6.63$, $p = 0.020$; treatment \times site: $F_{1,17} = 1.04$, NS) and number of premature responses (site: $F_{1,17} = 5.73$, $p = 0.029$; treatment \times site: $F_{1,17} = 7.37$, $p = 0.015$). The latter observation might relate to minor damage to the core region in the NAc shell group due to guide cannulae insertion. Previously, it was found that NAc core and not shell lesions potentiate the effects of amphetamine on premature responding in the 5-CSRTT (Murphy et al., 2008). Subsequent analyses controlling for these infusion group differences by expressing the number of premature responses as a percentage of the total number of responses revealed that there were no significant site or treatment by site interaction effects (site: $F_{1,17} = 2.52$, NS; treatment \times site: $F_{1,17} = 2.15$, NS), indicating that when controlled for response rate differences both groups reacted similarly to the effects of amphetamine on inhibitory control. Thus, the differential effects of naloxone infusions into the NAc shell and core region on amphetamine-induced premature responding reflect distinct effects of naloxone in both regions rather than differential responsiveness to amphetamine in the two experimental groups. Together, these data reveal that endogenous opioids in the NAc shell, but not NAc core, may mediate amphetamine-induced inhibitory control deficits.

Effects of intra-nucleus accumbens infusion of morphine in the 5-CSRTT

After completion of the amphetamine and intra-NAc naloxone experiment, the effects of intracranial infusion of the μ -opioid receptor agonist morphine (2.5 μ g bilaterally) into the NAc shell and core on impulsivity in the 5-CSRTT were examined in the same groups of animals. Results showed that morphine significantly increased premature responding in the NAc shell, but not NAc core group (Figure 2.5c; NAc shell: $F_{1,9} = 6.07$, $p = 0.036$; NAc core: $F_{1,8} = 0.19$, NS). In addition, as shown in Table 2.4, morphine decreased the number

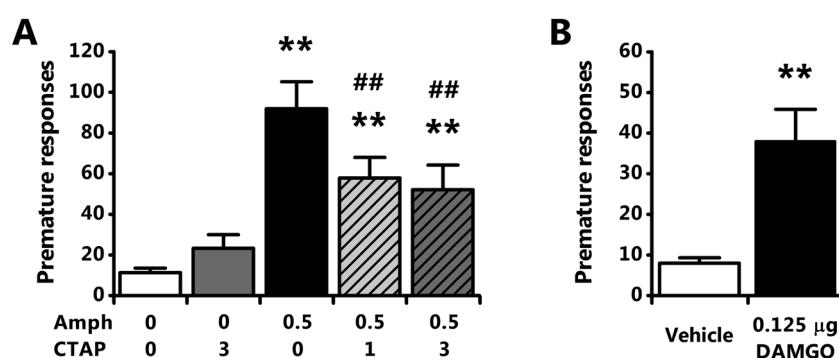


Figure 2.6. μ -Opioid receptors in the nucleus accumbens shell critically regulate (amphetamine-induced) inhibitory control deficits. Effects of systemic amphetamine (AMPH), intracranial CTAP into the NAc shell and their combination (A), or intra-NAc shell-infused DAMGO (B) on mean (\pm SEM) number of premature responses made in the 5-CSRTT. In total $n = 10$ animals were included in the data analyses. AMPH doses are expressed as mg/kg, CTAP and DAMGO doses as μ g/hemisphere. ** $p < 0.005$ versus Vehicle or Vehicle-Vehicle; ## $p < 0.005$ compared to Amphetamine-Vehicle.

Table 2.5. Effects of 0.5 mg/kg amphetamine (AMPH), intracranial CTAP ($\mu\text{g}/\text{hemisphere}$) into the nucleus accumbens shell (NAc shell) subregion and their combination, or intracranial DAMGO ($\mu\text{g}/\text{hemisphere}$) into the NAc shell on measures of attentional function, compulsivity, and motivation in the 5-CSRTT.

Treatment (systemic – local)	Accuracy (%)	Perseverative Responses	Correct response latency (ms)	Omissions	Feeder latency (ms)
Vehicle – Vehicle	86.7 \pm 2.9	12.4 \pm 2.0	334 \pm 11	15.3 \pm 2.1	2016 \pm 351
Vehicle – CTAP 3	84.1 \pm 2.5	13.5 \pm 3.7	330 \pm 8	19.0 \pm 3.4	2235 \pm 367
AMPH – Vehicle	73.7 \pm 3.6**	11.7 \pm 4.0	321 \pm 14	16.5 \pm 1.6	2274 \pm 483
AMPH – CTAP 1	80.2 \pm 3.1 [#]	8.3 \pm 2.3	317 \pm 10	19.2 \pm 2.5	2227 \pm 499
AMPH – CTAP 3	80.9 \pm 2.7 [#]	7.9 \pm 2.9	351 \pm 13	25.1 \pm 4.2	2037 \pm 525
Vehicle	86.7 \pm 2.0	10.2 \pm 3.4	342 \pm 15	14.9 \pm 1.2	2147 \pm 477
DAMGO 0.125	84.1 \pm 0.9	10.6 \pm 3.8	339 \pm 19	17.2 \pm 3.4	1759 \pm 461

In total $n = 10$ animals were included in the analyses and data depict mean \pm SEM. ** $p < 0.005$ versus Vehicle-Vehicle; [#] $p < 0.05$ compared to AMPH-Vehicle.

of omissions made in the NAc shell group (NAc shell: $F_{1,9} = 5.78$, $p = 0.04$; NAc core: $F_{1,8} = 2.88$, NS) and response latencies in the NAc core group (NAc shell: $F_{1,9} = 1.50$, NS; NAc core: $F_{1,8} = 12.76$, $p = 0.008$). Morphine did not cause significant changes in accurate choice (NAc shell: $F_{1,9} = 0.002$, NS; NAc core: $F_{1,8} = 1.99$, NS), perseverative responding (NAc shell: $F_{1,9} = 0.00$, NS; NAc core: $F_{1,8} = 0.43$, NS), or feeder latency (NAc shell: $F_{1,9} = 3.97$, NS; NAc core: $F_{1,8} = 0.79$, NS). These data indicate that in the NAc shell, unlike the NAc core, activation of most likely μ -opioid receptors is sufficient to impair inhibitory control in rats.

Effects of intra-nucleus accumbens shell infusion of the μ -opioid receptor antagonist CTAP on amphetamine-induced impulsivity in the 5-CSRTT

To confirm that μ -opioid receptor activation in the NAc shell is critical for amphetamine-induced inhibitory control deficits and promotes these behavioral impairments on itself, two additional experiments were performed. First, the effects of intracranial infusion of the selective μ -opioid receptor antagonist CTAP into the NAc shell alone or combined with systemic amphetamine were determined. In total, two rats were excluded from the analyses due to cannulae misplacement. Amphetamine (0.5 mg/kg) robustly increased premature responding in this group of rats (Figure 2.6a), and this detrimental effect was attenuated by prior infusion of CTAP ($F_{4,36} = 15.94$, $p < 0.001$). Post-hoc analyses revealed that both 1 and 3 μg CTAP infused into the NAc shell significantly and partially reversed amphetamine-induced deficits in inhibitory control. Data on other parameters in the 5-CSRTT are summarized in Table 2.5. Only significant treatment effects were found with respect to accurate choice ($F_{4,36} = 7.49$, $p < 0.001$). Here, amphetamine reduced accurate

choice, an effect that was completely restored by prior infusion of both CTAP doses into the NAc shell. No significant treatment effects were found for any other behavioral parameter (perseverative responding: $F_{4,36} = 1.30$, NS; correct response latency: $F_{4,36} = 2.60$, NS; errors of omission: $F_{4,36} = 1.66$, NS; and feeder latency: $F_{4,36} = 0.12$, NS).

Following completion of the amphetamine and intra-NAc shell CTAP experiment, the potent selective μ -opioid receptor agonist DAMGO (0.125 μ g bilaterally) was infused into the NAc shell. Results showed that DAMGO significantly increased premature responding (Figure 2.6b; $F_{1,9} = 17.53$, $p = 0.002$). As shown in Table 2.5, DAMGO did not significantly affect any other behavioral parameter (accuracy: $F_{1,9} = 3.11$, NS; perseverative responding: $F_{1,9} = 0.05$, NS; correct response latency: $F_{1,9} = 0.04$, NS; errors of omissions: $F_{1,9} = 0.41$, NS and feeder latency: $F_{1,9} = 3.62$, NS). Collectively, these data demonstrate that activation of μ -opioid receptors in the NAc shell sufficiently impairs inhibitory control and might thus represent a critical step in the neuronal mechanism underlying amphetamine's effects on inhibitory control in the 5-CSRTT.

Histology

Figure 7 depicts cannulae placements for animals included in the intracranial infusion experiments. For the amphetamine and naloxone/morphine experiments (Figure 7a), initially, 20 animals were trained in the 5-CSRTT with cannulae directed at either the NAc shell or core subregion ($n = 10$ each). One individual had to be excluded from the NAc core group, as for this animal histological examination showed cannula placement outside the borders of the NAc core. For the amphetamine and CTAP/DAMGO experiments (Figure 7b), 12 animals were trained in the 5-CSRTT with cannulae directed at the NAc shell subregion. Two individuals were excluded from these experiments due to cannulae placement outside the borders of the NAc shell.

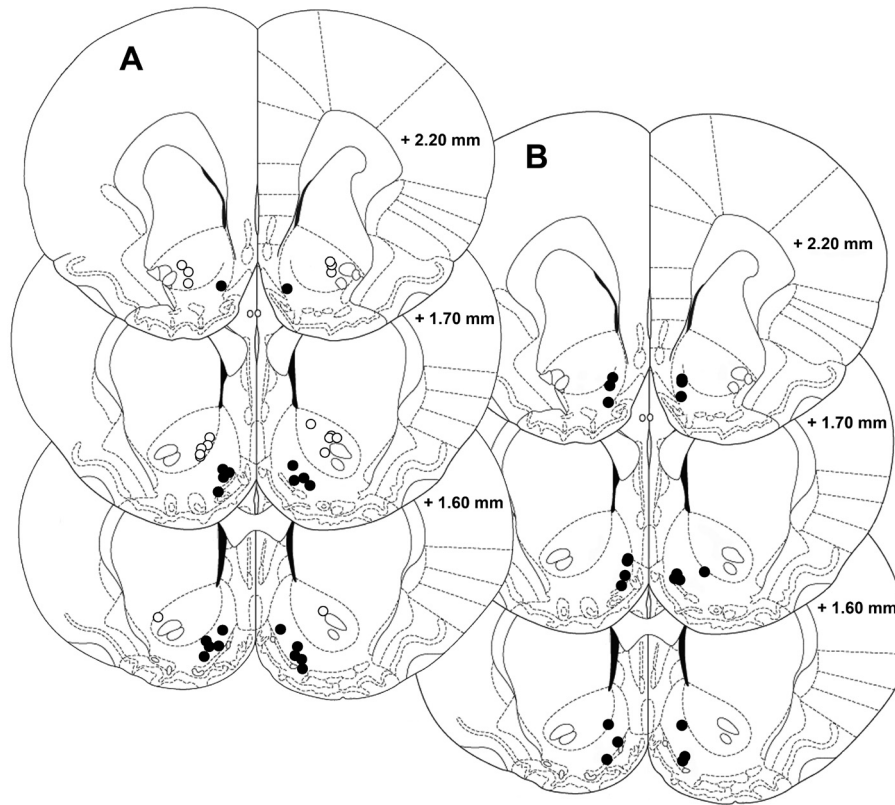


Figure 2.7. Assessment of cannula placement. Schematic drawing of coronal sections of the rat brain depicting cannula placements into either the nucleus accumbens (NAc) shell (closed circles) or core (open circles) subregion of rats used in the systemic amphetamine/intracranial naloxone and morphine experiments (A) and the systemic amphetamine/intracranial CTAP and DAMGO experiments (B). Numbers indicate anterior distance from bregma. Pictures are adapted from Paxinos & Watson (1998).

Discussion

The present results indicate that μ -opioid receptors in the NAc shell and not core region play an important role in amphetamine-induced inhibitory control deficits, probably via an interaction between μ -opioid receptors and mesolimbic dopamine transmission. In contrast, amphetamine's beneficial effects on impulsive choice appear independent from opioid signaling.

Opioids, amphetamine, and inhibitory control

Blocking opioid receptors by naloxone, either peripherally or intracranially in the NAc shell, attenuated amphetamine-induced premature responding in the 5-CSRTT. By contrast, the selective δ - or κ -opioid receptor antagonists naltrindole and nor-BNI did not affect

these behavioral effects of amphetamine, indicating involvement of μ -opioid receptors in amphetamine-induced inhibitory control deficits. Indeed, in further support, intra-NAc shell injections with the highly selective μ -opioid receptor antagonist CTAP also attenuated amphetamine-induced premature responding. The μ -opioid receptor agonists morphine and DAMGO induced premature responding when infused into the NAc shell but not core region, thereby revealing a neuroanatomical locus for the previously reported detrimental effects of morphine on inhibitory control (Pattij et al., 2009). The lack of nor-BNI altering inhibitory control extends previous reports showing no effect of κ -opioid receptor agonists on premature responding (Paine et al., 2007; Shannon et al., 2007). Interestingly, it was recently shown that δ - and μ -opioid receptor knockout mice are hyper- and hypo-impulsive, respectively (Olmstead et al., 2009), whereas in the present study neither naltrindole nor naloxone affected inhibitory control under baseline conditions. This discrepancy might be due to species differences, compensatory neuroadaptations in these mutants or differences in the behavioral paradigms used.

Effects of amphetamine on inhibitory control in the 5-CSRTT largely depend on increased (accumbal) dopamine transmission (Cole and Robbins, 1987, 1989; Van Gaalen 2006a, 2009; Pattij et al., 2007b). Moreover, naloxone attenuated inhibitory control deficits induced by amphetamine and the selective dopamine transporter inhibitor GBR 12909, indicating that an interaction between dopamine signaling and μ -opioid receptors mediates inhibitory control. It remains as yet unclear whether impulsivity-related μ -opioid receptor activation in the NAc shell is downstream of increased dopamine release, affecting activity in NAc projection areas (Svingos et al., 1997), or alternatively is merely a required step potentiating amphetamine-induced dopamine release (Yoshida et al., 1999; Hirose et al., 2005). Nonetheless, the current data extend previous data showing that acute challenges with psychostimulants such as amphetamine and cocaine activate endogenous opioid systems via dopamine-dependent mechanisms (Hurd and Herkenham, 1992; Wang and McGinty, 1995, 1996; Olive et al., 2001; Roth-Deri et al., 2003). Moreover, opioid receptors mediate amphetamine's effects on dopamine release and behavioral measures including locomotion, reward, and amphetamine-induced reinstatement of amphetamine seeking (Trujillo et al., 1991; Hooks et al., 1992; Schad et al., 1995; Jayaram-Lindstrom et al., 2004, 2008b; Haggkvist et al., 2009). Particularly the latter finding is of interest in view of the close interrelationship between impulsivity and relapse vulnerability (Perry and Carroll, 2008; Verdejo-Garcia et al., 2008). Thus, opioid receptor antagonists might be effective anti-relapse agents because of their beneficial effects on impulsivity.

Given the importance of (NAc) opioid systems in food-motivated behavior (Kelley et al., 2002; Cota et al., 2006) and amphetamine-induced hyperlocomotion (Hooks et al., 1992; Schad et al., 1995; Gonzalez-Nicolini et al., 2003), it could be argued that the observed effects of opioid (ant)agonists on (amphetamine-induced) impulsivity might be related to direct effects on food motivated-behavior and/or somatomotor activity. However, this seems very unlikely since none of the tested compounds had clear effects on behavioral measures that could be interpreted as indices of motivation for food, namely errors of

omission and feeder latencies. Moreover, systemic administration of naloxone attenuated both amphetamine-induced impulsivity and speeding of response latencies, a parameter reflecting aspects of somatomotor activity in the 5-CSRTT. Nonetheless, blocking μ -opioid receptors in the NAc shell was found to attenuate amphetamine-induced inhibitory control deficits while simultaneously potentiating rather than reducing amphetamine-induced speeding of response latencies. Collectively, these observations suggest that distinct pathways are mediating amphetamine's effects on food- and somatomotor-related behaviors.

Opioids, amphetamine, and impulsive choice

In contrast to its effects on inhibitory control, amphetamine generally reduces impulsive choice as measured in delayed reward paradigms, primarily via a dopamine-dependent mechanism (Wade et al., 2000; De Wit et al., 2002; Winstanley et al., 2003, 2005; Van Gaalen 2006b). The present results confirm and extend this conclusion by indicating that blocking opioid transmission did not modify amphetamine's beneficial effects on impulsive decisions. This observation may be somewhat surprising since μ -opioid receptors are positioned to modulate mesolimbic and mesocortical dopamine transmission. Indeed, morphine activates both aforementioned dopamine projections (Di Chiara and Imperato, 1988; Devoto et al., 2002), and blocking μ -opioid receptors reduces amphetamine-induced increments in striatal dopamine release (Hooks et al., 1992; Schad et al., 1995). Furthermore, the current data with nor-BNI and naltrindole suggest that δ - and κ -opioid receptors are not involved in (amphetamine-induced) impulsive choice. Pretreatment with nor-BNI somewhat attenuated the beneficial effects of amphetamine on impulsive choice. Nonetheless, the biological relevance of this finding is questionable given that κ -opioid receptors are well-known to negatively modulate mesocorticolimbic dopamine release (Spanagel et al., 1990; Devine et al., 1993; Margolis et al., 2006) and, hence, blocking κ -opioid receptors would be expected to potentiate rather than reduce amphetamine-induced dopamine release and subsequent impulsive decisions. Interestingly, there is ample evidence linking the opioid system, particularly μ -opioid receptors, to regulation of impulsive decision making (Kieres et al., 2004; Mitchell et al., 2007; Boettiger et al., 2009; Love et al., 2009; Pattij et al., 2009). It can therefore be concluded that 1) psychostimulants alter impulsive choice via opioid-independent mechanisms, and 2) opioids may modify this behavior via distinct neuronal mechanisms. Taken together, our findings lend further support to the idea that distinct dopamine pathways modulate inhibitory control and impulsive choice (Winstanley et al., 2006a; Pattij and Vanderschuren, 2008). In this regard, it is important to acknowledge that conceptually inhibitory control processes can be further dissected into action restraint (current study) and action cancellation mechanisms, that might rely on partially overlapping circuits and differentially react to drugs such as amphetamine (Feola et al., 2000; De Wit et al., 2002; for reviews, see Schachar et al., 2007; Eagle et al., 2008). Consequently, the critical involvement of μ -opioid transmission might not extrapolate to inhibitory control processes involving cancellation of ongoing behavioral responses and this warrants further investigation.

Nucleus accumbens, opioids, and impulsivity

In line with previous studies (Pattij et al., 2007b; Murphy et al., 2008), a differential role for NAc shell and core regions in amphetamine-induced inhibitory control deficits was found here, with a dopamine-dependent increase in μ -opioid receptor tone being important for the effects of amphetamine in the NAc shell and not core region. The discrepancy between both NAc regions observed here may relate to higher μ -opioid receptor densities in the shell compared with the core region (Dilts and Kalivas, 1989; Pickel et al., 2004). Alternatively, the divergent connectivity of both regions may be critical. Whereas the core region is mainly connected to extrapyramidal motor nuclei, the shell is preferentially embedded within limbic circuitries (Heimer et al., 1991; Berendse et al., 1992). Interestingly, a previous study (Pattij et al., 2007b) showed that dopamine in the NAc core rather than shell is critical for amphetamine-induced premature responding, suggesting that dopamine receptors mediating amphetamine-induced activation of endogenous opioid systems are located outside the NAc shell. In this respect, it is noteworthy that acute challenges with psychostimulants including amphetamine induce release of the endogenous opioid β -endorphin, which has high affinity for μ -opioid receptors (Schoffelmeer et al., 1991; Zadina et al., 1997), into the NAc shell (Olive et al., 2001; Roth-Deri et al., 2003; Doron et al., 2006). Interestingly, this phenomenon depends on activation of dopamine D2 receptors in the hypothalamic arcuate nucleus (Doron et al., 2006), a brain region in which the majority of β -endorphin cell bodies terminating in the NAc are located (Finley et al., 1981). Finally, bearing in mind the widespread distribution of μ -opioid receptors in the brain, it is conceivable that the NAc shell is not the only brain site in which μ -opioid receptors regulate inhibitory control. For instance, efferent brain nuclei such as the ventral pallidum (Napier and Mitrovic, 1999) or afferent sites including the ventral tegmental area (Schad et al., 2002) that densely express μ -opioid receptors may play a role in this respect.

Concluding remarks

In conclusion, the present study reveals that μ -opioid receptor signaling in the NAc shell importantly modulates inhibitory control and the effects of amphetamine thereon. The current study adds to literature suggesting that μ -opioid receptors may be a pharmacotherapeutical target to treat maladaptive impulsivity (Mitchell et al., 2007; Boettiger et al., 2009; Love et al., 2009). Indeed, the preferential μ -opioid receptor antagonist naltrexone has been shown to relieve a variety of impulse control disorders in humans including psychostimulant and alcohol addiction, pathological gambling, binge eating, compulsive sexual behavior, self-injurious behavior, and kleptomania as discussed previously (Boettiger et al., 2009). In view of our data, it may also be worthwhile to investigate the effectiveness of naloxone to treat patient groups suffering from reduced inhibitory control such as ADHD patient



