RESEARCH PAPER

The stereotypy-inducing and OCD-like effects of chronic 'binge' cocaine are modulated by distinct subtypes of nicotinic acetylcholine receptors

A Metaxas, HL Keyworth, JH Yoo, Y Chen, I Kitchen* and A Bailey*

Department of Biochemistry and Physiology, Faculty of Health and Medical Sciences, Institute of Health and Medical Sciences, University of Surrey, Guildford, UK

Correspondence

Athanasios Metaxas, VU Medical Center, Nuclear Medicine and PET Research, Location Radionuclide Center, De Boelelaan 1085 c, 1081 HV Amsterdam, the Netherlands.
E-mail: a.metaxas@vumc.nl

*Joint senior author

Keywords

cocaine; nicotinic receptors; grooming; sensitisation; repetitive/stereotyped disorders; autoradiography

Received

28 March 2012

Revised

27 April 2012

Accepted

30 April 2012

BACKGROUND AND PURPOSE

High rates of cigarette smoking occur in cocaine-dependent individuals, reflecting an involvement of nicotinic acetylcholine receptors (nAChRs) in cocaine-elicited behaviour. This study was designed to assess the contribution of different nAChR subtypes to the behavioural and neurochemical effects of chronic cocaine treatment.

EXPERIMENTAL APPROACH

Cocaine (15 mg·kg⁻¹, i.p.) was administered to male C57BL/6J mice in a chronic 'binge' paradigm, with and without the coadministration of the $\alpha 7$ preferring nAChR antagonist methyllycaconitine (MLA; 5 mg·kg⁻¹, i.p.) or the $\beta 2^*$ nAChR antagonist dihydro- β -erythroidine (DH β E; 2 mg·kg⁻¹, i.p.). Quantitative autoradiography was used to examine the effect of cocaine exposure on $\alpha 7$ and $\alpha 4\beta 2^*$ nAChRs, and on the high-affinity choline transporter.

KEY RESULTS

MLA+cocaine administration induced an intense self-grooming behaviour, indicating a likely role for $\alpha 7$ nAChRs in modulating this anxiogenic, compulsive-like effect of cocaine. In the major island of Calleja, a key area of action for neuroleptics, MLA+cocaine reduced choline transporter binding compared with cocaine (with or without DH β E) administration. DH β E treatment prevented the induction of stereotypy sensitisation to cocaine but prolonged locomotor sensitisation, implicating heteromeric $\beta 2^*$ nAChRs in the neuroadaptations mediating cocaine-induced behavioural sensitisation. 'Binge' cocaine treatment region-specifically increased $\alpha 4\beta 2^*$ nAChR binding in the midbrain dopaminergic regions: ventral tegmental area and substantia nigra pars compacta.

CONCLUSIONS AND IMPLICATIONS

We have shown a differential, subtype-selective, contribution of nAChRs to the behavioural and neurochemical sequelae of chronic cocaine administration. These data support the clinical utility of targeting specific nAChR subtypes for the alleviation of cocaine-abuse symptomatology.

Abbreviations

CHT, choline transporter; DH β E, dihydro- β -erythroidine; MLA, methyllycaconitine; NAc, nucleus accumbens; nAChR, nicotinic acetylcholine receptor; OCD, obsessive-compulsive disorder; SNc, substantia nigra pars compacta; VTA, ventral tegmental area

Introduction

Epidemiological and clinical reports demonstrate that cocaine-dependent individuals exhibit high rates of cigarette smoking and nicotine addiction compared with the general population (Weinberger and Sofuoglu, 2009). Presumably, this high degree of co-morbidity exists because nicotine potentiates the desired rewarding properties of cocaine and/or ameliorates the adverse psychotomimetic effects of the psychostimulant (Bolla *et al.*, 1998). Indeed, cocaine users in the clinical setting report both stimulating and sedating or calming effects of cigarette smoking, the latter including a reduction of cocaine-induced psychosis, paranoia and anxiety (Wiseman and McMillan, 1998; Adler *et al.*, 2001). Pharmacologically, the modulatory effects of nicotine intake on the behavioural states induced by cocaine begin with high-affinity binding of nicotine to brain cholinergic receptors. Therefore, the clinical observations described above imply an important role for nicotinic acetylcholine receptors (nAChRs) in mediating both the reinforcing and psychotomimetic properties of cocaine.

Neuronal nAChRs constitute a heterogeneous family of pentameric, ligand-gated ion channels, which can be classified according to subunit composition and pharmacology into α -bungarotoxin sensitive, homopentameric $\alpha 7$ receptors, and into α -bungarotoxin insensitive, heteropentameric, non- $\alpha 7$ nAChRs (Albuquerque *et al.*, 2009; receptor nomenclature follows Alexander *et al.*, 2011). The latter can be assembled by combinations of different α and β nicotinic subunits, among which the most prevalent is the $\alpha 4\beta 2$ combination ($\alpha 4\beta 2^*$, with the asterisk indicating the possible participation of additional subunits). Preclinical evidence suggests that heteromeric and homomeric nAChRs play distinct roles in modulating the addictive-related properties of cocaine. The genetic ablation of $\beta 2$ subunits reduces the conditioned rewarding effects of cocaine (Zachariou *et al.*, 2001), and treatment with the non- $\alpha 7$, $\beta 2^*$ nAChR antagonist dihydro- β -erythroidine (DH β E) prevents cocaine-induced locomotor sensitisation (Schoffelmeer *et al.*, 2002; Champiaux *et al.*, 2006), indicating that heteromeric nAChR activation contributes to cocaine reinforcement. The $\alpha 7$ preferring antagonist methyllycaconitine (MLA) attenuates the intracranial self-stimulation rewarding effect of cocaine, suggesting that homomeric nAChRs are also involved in cocaine reinforcement (Panagis *et al.*, 2000). Surprisingly, however, neither microinjections of MLA in the ventral tegmental area (VTA) nor in the nucleus accumbens (NAc) alter locomotor sensitisation to cocaine (Champiaux *et al.*, 2006). At the neurochemical level, while administration of MLA in the NAc has been shown to further enhance, and DH β E not alter, cocaine-induced increases in accumbal dopamine levels, intra-VTA administration of either antagonist increases cocaine's effect on mesolimbic neurotransmission, indicating that multiple nicotinic cholinergic pathways influence the dopamine enhancing effects of cocaine, in complex patterns (Zanetti *et al.*, 2007).

Although substantial evidence implicates $\alpha 7$ and $\beta 2^*$ nAChRs in cocaine reinforcement, the role of specific nicotinic subtypes in modulating the neuropsychiatric consequences of chronic cocaine use is not well characterised. Humans addicted to cocaine can exhibit repetitive, purpose-

less and complex stereotyped behaviours, referred to as punding (Fasano and Petrovic, 2010). These cocaine-induced stereotypies are phenotypically similar to those seen in several neurological and neuropsychiatric disorders (Silver *et al.*, 2001; Evans *et al.*, 2009) and involve changes in striatal cholinergic neurotransmission (Aliane *et al.*, 2009; 2011). Cocaine dependence has also been associated with co-morbid psychotic and anxiety spectrum disorders (Tang *et al.*, 2007), including obsessive-compulsive disorder (OCD) (Crum and Anthony, 1993; Goodwin *et al.*, 2002). Interestingly, higher rates of cigarette smoking are observed both in individuals suffering from neuropsychiatric illness (Dome *et al.*, 2010) and in cocaine-dependent individuals (Kalman *et al.*, 2005), implying the existence of common substrates in these conditions.

The current study was designed to assess the contribution of $\alpha 7$ and non- $\alpha 7$, $\beta 2^*$ nAChRs to the behavioural sequelae of chronic cocaine administration in mice. Based on the documented involvement of nAChRs in cocaine reinforcement, as well as in the pathophysiology of various neuropsychiatric disorders (Wallace *et al.*, 2011), we reasoned that distinct effects of chronic cocaine treatment may be mediated and/or modulated by different nAChR subtypes. To assess this hypothesis, the effect of $\alpha 7$ and $\beta 2^*$ nAChR blockade on cocaine-induced behaviour was studied over 14 days using MLA and DH β E respectively. The administration of antagonists with different selectivity profiles for nAChRs induced distinct behavioural alterations in cocaine-treated mice, which suggests that $\alpha 7$ and non- $\alpha 7$, $\beta 2^*$ nAChRs play unique roles in modulating the effects of chronic cocaine administration.

Methods

Animals and drug treatment

All animal care and experimental studies complied with the UK Home Office, Animals (Scientific Procedures) Act, 1986. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (McGrath *et al.*, 2010). Six- to 8-week-old male C57BL/6J mice (Charles River, Margate, Kent, UK) were used in these tests ($n = 6-7$ per treatment group; total = 39). Animals were single-housed in a temperature-controlled room, under a 12 h light/dark schedule. Food and water in the home cage were available *ad libitum*. Mice were habituated to handling for a period of 5 days prior to treatment. Following acclimatisation, i.p. injections (all dose shown are per injection) of saline (5 mL·kg⁻¹), cocaine (15 mg·kg⁻¹; weight as free base), saline+DH β E (2 mg·kg⁻¹; weight as hydrobromide salt), DH β E+cocaine, saline+MLA (5.0 mg·kg⁻¹; weight as citrate salt) and MLA+cocaine were administered to six groups of animals, using a steady dose, 'binge' treatment protocol. Three daily injections were administered at 1 h intervals for a period of 14 days, with the first injection delivered 1 h after the beginning of the light phase of the light/dark cycle. MLA and DH β E were used to block $\alpha 7$ and non- $\alpha 7$, $\beta 2^*$ nAChRs respectively. Doses of nicotinic antagonists were based on previous reports (Schoffelmeer *et al.*, 2002; Damaj *et al.*, 2003) and their potency to antagonise

nicotine-induced effects was confirmed in preliminary experiments (Supporting Information Figures S1 and S2). All drugs were dissolved in sterile 0.9% saline and purchased from Sigma-Aldrich, Gillingham, Dorset, UK.

Measurement of cocaine-induced horizontal and vertical activity

Motor activity was measured in 12 motility chambers (40 × 20 × 20 cm; Linton Instrumentation, Norfolk, UK). Each cage had two sets of 16 photocells located at right angles to each other, projecting horizontal infrared beams 2.5 cm apart, and 1 and 6 cm above the cage floor. Cocaine-induced locomotor activity testing was performed as detailed previously (Bailey *et al.*, 2007a; 2008). Animals were habituated for 2 days for 60 min per day in the motility chambers prior to the start of treatment. Each daily session began with assessing basal activity for a period of 60 min. Subsequently, mice received an i.p. injection of saline or cocaine ($\pm$ nAChR antagonists) and were immediately returned to the chambers where horizontal and vertical activity was measured for 60 min. Animals received a second and third injection 60 and 120 min after the first one. Total horizontal and vertical motor activity was defined by the measurement of sequential infrared beam breaks, recorded every 5 min, beginning immediately after placing the animals in the cage following an injection of saline or cocaine ($\pm$ nAChR antagonists). Behavioural activities were monitored after each of the three daily injections. Six groups of mice were treated with saline or cocaine ($\pm$ nAChR antagonists) in four cohorts, and response data were pooled after completion of all studies.

Cocaine-induced stereotypy and grooming

Animals were videotaped 20 min after each daily injection for 40 s, and the tapes were later rated for stereotypy and grooming by a trained observer, unaware of the animal's treatment group. Stereotypy was scored using the rating system of Daunais and McGinty (1995), which consists of a graded scale of drug-induced behaviours: (i) asleep, inactive; (ii) alert, 'comfort' grooming; (iii) increased sniffing (occasional light sniffing, often while exploring the cage); (iv) intermittent rearing and sniffing (two or three rears in a 20 s period, with sniffing frequently at the apex of the rear); (v) increased locomotion; (vi) intense sniffing (rapid, often with head down); (vii) continuous pivoting and sniffing (no rearing, no locomotion); (viii) intermittent rearing behaviour with locomotion; (ix) maintained rearing and sniffing; and (x) splayed limbs. Each mouse received a single score following each injection, which corresponded to the stereotypic behaviour predominantly observed during the observation period. For each animal, the median score of the three daily injections was used for analysis (Schlussman *et al.*, 2005; Bailey *et al.*, 2007b).

Self-grooming was measured following each of the three daily injections. A daily percentage of time spent grooming was calculated for each animal for the duration of the study and used for statistical analysis (Peca *et al.*, 2011).

Quantitative autoradiography

Mice were killed by cervical dislocation 30 min following the last injection of saline or cocaine ($\pm$ nAChR antagonists) on day 14 of the study, and brains were immediately removed,

frozen in isopentane on dry ice and stored at -80°C for subsequent processing. Tissue sectioning was carried out at -21°C , using a Microm HM505E cryostat (Carl Zeiss, MICROM Laborgerate, GmbH, Waldorf, Germany). 20 μm frozen coronal sections were cut at 300 μm intervals, from rostral to caudal levels, and stored at -20°C for radioligand binding. Quantitative autoradiography was performed to measure binding to $\alpha 7$ and non- $\alpha 7$ nAChRs, and to the high-affinity choline transporter (CHT), using 3 nmol [^{125}I] α -bungarotoxin (specific activity 152 Ci·mmol $^{-1}$), 100 pmol [^{125}I]epibatidine (specific activity 2200 Ci·mmol $^{-1}$), and 8 nmol [^3H]Hemicholinium-3 (specific activity 144.5 Ci·mmol $^{-1}$) respectively. All radioligands were purchased from PerkinElmer, Boston, MA, USA. On the day of each binding experiment, sections were thawed and processed according to established protocols (Orr-Urtreger *et al.*, 1997; Besson *et al.*, 2007; Metaxas *et al.*, 2010) with minor modifications. Detailed autoradiographic procedures are shown in Supporting Information. Radioligand-bound sections were apposed to Kodak BioMax MR film (Sigma-Aldrich), along with autoradiographic microscopies of known radioactive concentration (GE Healthcare, Buckinghamshire, UK) to allow quantification. All brain structures were identified by reference to the mouse atlas of Franklin and Paxinos (2001), and analysed using an MCID image analyser (InterFocus Imaging, Linton, UK), as detailed previously (Kitchen *et al.*, 1997).

Statistical analysis

Motor activity and self-grooming were analysed using a mixed model ANOVA, with cocaine and nAChR antagonist as the between subject variables, and time as the within-subject, repeated variable. When significant overall interactions were found, a further analysis of partial interactions was performed. Tukey's *post hoc* analysis was applied when the initial *P* value was <0.05 .

Stereotypy ratings were initially analysed using Friedman's non-parametric ANOVA. When significant effects were observed, between-group comparisons on individual test days were performed using Mann-Whitney *U*-tests. The development of stereotypy sensitisation in individual treatment groups was performed using two-tailed Wilcoxon signed-rank comparisons.

Two-way ANOVA for the factors treatment and brain region was used for the comparison of quantitative measures of $\alpha 7$ and non- $\alpha 7$ nAChRs, and of the high-affinity CHTs, in brain regions of saline and cocaine ($\pm$ nAChR antagonists)-treated mice. Where ANOVA yielded significant treatment effects, Fisher LSD posttests were used to investigate differences in binding between groups in individual regions.

Correlation analysis employed the parametric Pearson's product moment test. All data were analysed using the Statistica software (Statsoft Inc., Maisons-Alfort, France).

Results

Effect of MLA and DH β E on motor activity in mice treated with a chronic 'binge' cocaine administration paradigm

C57BL/6J mice were injected (doses shown are per injection) with cocaine (15 mg·kg $^{-1}$), DH β E+cocaine (2 mg·kg $^{-1}$),

MLA+cocaine (5 mg·kg⁻¹) or their respective vehicle controls, three times daily at hourly intervals. Cocaine-treated animals showed greater horizontal and vertical activity compared with their respective vehicle controls on all days of treatment (Figure 1A and B; repeated measures ANOVA). There were distinct changes in both horizontal and vertical activity of mice that were co-treated with either DHβE or MLA, compared with animals receiving cocaine alone. Figure 1A shows the effect of nAChR antagonism on daily horizontal activity. On day 1, there was no effect of DHβE or MLA on the acute locomotor response to cocaine (Figure 1A; $P > 0.05$, Tukey posttests). On days 3–5, an augmentation of horizontal activity counts was observed in mice treated with cocaine alone compared with day 1, indicating the initiation of locomotor sensitisation (Figure 1A; Tukey posttests). In DHβE-treated mice, the sensitisation effect of cocaine on horizontal activity was prolonged until day 9 (days 3–9 vs. day 1, $P < 0.001$, Tukey posttests). In MLA+cocaine-treated animals, increased horizontal activity was noted only on day 4 versus day 1 ($P < 0.05$, Tukey posttests). Repeated measures ANOVA confirmed significant main effects of cocaine [$F_{(1, 33)} = 146.3$, $P < 0.001$] and time [$F_{(12, 396)} = 9.7$, $P < 0.001$], with significant cocaine×time [$F_{(12, 396)} = 10.2$, $P < 0.001$] and cocaine×time×nAChR antagonist interaction effects on horizontal motor activity [$F_{(24, 396)} = 1.7$, $P < 0.05$]. No main effect of nAChR antagonism on horizontal activity was observed [$F_{(2, 33)} = 0.5$, $P > 0.05$].

Figure 1B shows the effect of nAChR blockade on total vertical activity, representing rearing. There was no effect of DHβE or MLA on the acute rearing effect of cocaine on day 1 (Figure 1B; $P > 0.05$, Tukey posttests). Between-group comparisons showed a marked reduction in rearing activity of DHβE + cocaine-treated mice compared with cocaine ($P < 0.01$) and MLA+cocaine treatment ($P < 0.05$, repeated measures ANOVA, followed by Tukey posttests). Statistical analysis confirmed significant main effects of cocaine [$F_{(1, 33)} = 93.8$, $P < 0.001$] and significant cocaine×nAChR antagonist interaction effects [$F_{(2, 33)} = 5.5$, $P < 0.01$] on total rearing activity. No significant main effects of time [$F_{(12, 396)} = 0.9$, $P > 0.05$] or nAChR antagonist [$F_{(2, 33)} = 2.7$, $P > 0.05$] were observed, whereas the cocaine × time interaction effect was nearly significant [$F_{(12, 396)} = 1.8$, $P = 0.054$]. Correlation analysis indicated an inverse association between the mean daily vertical and horizontal activity of DHβE + cocaine-treated mice, indicating that the antagonist prolonged horizontal locomotor activity at the expense of rearing (Pearson $r = -0.67$, $P = 0.01$; Figure 1C).

Effect of MLA and DHβE on self-grooming behaviour in mice treated with a chronic 'binge' cocaine administration paradigm

Grooming behaviour was measured daily, 20 min after each of the three hourly injections. It should be noted that assessing the effects of nAChR blockade on self-grooming was not an original aim of the present study, but this response was analysed because of an intense and previously uncharacterised behavioural alteration that developed following the coadministration of cocaine with MLA.

Animals treated with MLA+cocaine showed a dramatic increase in grooming bouts, spending as much as 50% of the

Figure 1

The effect of DHβE or MLA coadministration with chronic 'binge' cocaine on motor activity. Horizontal (A) and vertical (B) motor activity (representing rearing) was monitored over the 14-day duration of the study. For each mouse, the mean motor activity of the three daily 1-h periods was calculated and later used for analysis. Each data point represents the daily mean $\pm$ SEM activity of six to seven animals per group. (A) In DHβE-treated animals, the sensitisation effect of cocaine on horizontal activity was prolonged until day 9. $**P < 0.01$, $***P < 0.001$ versus day 1 cocaine, $###P < 0.001$ versus day 1 DHβE+cocaine, $*P < 0.05$ versus day 1 MLA+cocaine. (B) Total rearing activity was markedly reduced in DHβE+cocaine-treated mice, compared with cocaine and MLA+cocaine treatment. $*P < 0.05$, $**P < 0.01$ versus cocaine, $*P < 0.05$, $###P < 0.001$ versus MLA+cocaine treatment. (C) Correlation analysis between the mean daily vertical and horizontal activity of DHβE+cocaine-treated mice.

Figure 2

'Binge' MLA+cocaine treatment induces excessive self-grooming behaviour. (A) A daily percentage of time spent grooming was calculated for each animal throughout the study. Data are presented as the mean $\pm$ SEM percentage time self-grooming of six to seven animals per group. Repeated measures ANOVA showed significant cocaine $\times$ nAChR antagonist interaction effects on grooming behaviour. * $P < 0.05$, ** $P < 0.01$ versus cocaine, # $P < 0.05$, ## $P < 0.01$ versus saline+MLA (B) Correlation analysis between horizontal locomotor and self-grooming activities in MLA+cocaine-treated mice. Each data point represents the mean daily horizontal locomotor activity of MLA+cocaine-treated animals, plotted against their corresponding time spent grooming on individual test days.

observation period engaged in self-grooming on days 7–11 (Figure 2A). The mice were constantly repeating components of the grooming sequence, at the expense of other forms of activity (Figure 2B). Their behaviour was intense and interrupted, progressed randomly rather than sequentially and led to hair loss, a phenotype that is reminiscent of animal models of OCD (Supporting Information Movie S1). A negative correlation between the increased time spent grooming and the horizontal locomotor activity of MLA+cocaine-treated animals was observed, indicating the competing nature of the measured behaviours and the shift from horizontal locomotor stimulation to excessive self-grooming (Figure 2B; Pearson $r = -0.79$, $P < 0.001$). None of the other groups presented this aberrant and disorganised self-grooming behaviour. Repeated measures ANOVA confirmed significant cocaine $\times$ nAChR

antagonist interaction effects on grooming behaviour [$F_{(2, 33)} = 3.5$, $P < 0.05$], and significant main effects of nAChR antagonist [$F_{(2, 33)} = 3.3$, $P < 0.05$]. Between-group comparisons showed that coadministration of MLA+cocaine significantly increased time spent grooming, compared with the administration of cocaine ($P < 0.01$) and saline+MLA ($P < 0.01$, Tukey posttests). There were no significant main effects of cocaine [$F_{(1, 33)} = 0.6$, $P > 0.05$] or time [$F_{(13, 429)} = 1.5$, $P > 0.05$] on self-grooming activity.

Effect of DHβE on cocaine-induced behavioural stereotypy in mice treated with a chronic 'binge' cocaine administration paradigm

The stereotypy score assigned to each animal was its median stereotypy score, which was calculated from the three daily injections of saline or cocaine. The intense grooming behaviour that developed in MLA+cocaine-treated animals constituted a significant deviation from the stereotypic repertoire that is typically anticipated following the administration of psychostimulants in rodents (Creese and Iversen, 1974; Daunais and McGinty, 1995), and therefore the evaluation of stereotypy in this group of mice was not included in the analysis.

Treatment with cocaine (with or without DHβE) resulted in the expression of behavioural stereotypy, compared with vehicle administration ($P < 0.001$, Friedman's test). The stereotypy response to 15 mg·kg⁻¹ of cocaine was increased on day 3 compared with day 1, an enhancement that persisted for the duration of the study, indicating the development of cocaine-induced stereotypy sensitisation (days 3–14 vs. day 1, $P < 0.05$, Wilcoxon test; Figure 3). DHβE+cocaine-treated mice exhibited lower levels of stereotypy compared with cocaine-treated animals on days 2–9, 11, 13 and 14 (Mann–Whitney U -tests). Moreover, cocaine-induced stereotypy sensitisation was attenuated by the administration of DHβE, with animals in this group maintaining constant levels of stereotypy during the course of study (days 2–14 vs. day 1, $P > 0.05$, Wilcoxon test). The median stereotypy score for DHβE+cocaine-treated mice throughout all 14 days was 5, corresponding to behaviour characterised by increased locomotor activity, whereas for cocaine-treated animals the predominant median stereotypy score was 8, corresponding to behaviour characterised by intermittent rearing and sniffing with locomotion.

The different behaviours exhibited by animals receiving cocaine ($\pm$ nAChR antagonists) are shown in representative videos from the seventh day of treatment in Supporting Information Movies S1–S3.

Quantitative autoradiography of nAChRs and of high-affinity CHTs

Mice were killed 30 min following the last injection of saline or cocaine (with or without nAChR antagonists), and their brains were processed for quantitative autoradiography of [¹²⁵I]epibatidine, [¹²⁵I] α -bungarotoxin and [³H]hemicholinium binding sites.

Figure 4 shows representative autoradiograms of total and cytosine-resistant [¹²⁵I]epibatidine binding at the level of the VTA, in coronal brain sections from animals of each of the six

Figure 3

'Binge' DHβE+cocaine treatment lowers stereotypy scores and prevents cocaine-induced stereotypy sensitisation. The daily score assigned to each animal was its median stereotypy score, which was calculated from the three daily injections of saline or cocaine ($\pm$ DHβE). Data are presented as the median $\pm$ SEM stereotypy scores of six to seven animals per group and were analysed using non-parametric statistics. * $P < 0.05$ versus day 1 cocaine, # $P < 0.05$, ## $P < 0.01$ versus DHβE+cocaine.

Figure 4

$\alpha 4 \beta 2^*$ nAChR binding is region-specifically increased in the VTA and the SNc following 'binge' cocaine treatment. Computer enhanced colour autoradiograms of total and cytosine-resistant [125 I]epibatidine binding in coronal brain sections of C57BL/6 mice treated with a chronic 'binge' saline or cocaine ($\pm$ DHβE or MLA) administration protocol. The adjacent sections shown are from the level of the ventral tegmental area (bregma -3.16 mm). To label $\alpha 4 \beta 2^*$ receptors, sections were incubated for 2 h with 100 pmol of [125 I]epibatidine, alone or in the presence of 20 nmol cytosine. Specific $\alpha 4 \beta 2^*$ nAChR binding was calculated following the subtraction of cytosine resistant from total [125 I]epibatidine images. Adjacent sections were incubated with 300 μ mol (–) nicotine hydrogen tartrate to calculate non-specific binding (NSB), which was indistinguishable from film background. The colour bar represents a pseudo-colour interpretation of black and white film images in fmol·mg $^{-1}$ tissue equivalent. Sections from chronic saline, cocaine ($\pm$ DHβE or MLA)-treated mice were processed in parallel.

treatment groups. Non-specific binding was indistinguishable from film background. For $\alpha 4\beta 2^*$ nAChRs, two-way ANOVA revealed significant main effects of treatment and region, with no significant treatment $\times$ region interaction effects [treatment: $F_{(5, 625)} = 11.4$, $P < 0.001$; region: $F_{(18, 625)} = 46.5$, $P < 0.001$; interaction: $F_{(90, 625)} = 0.7$, $P > 0.05$]. Region-specific increases in $\alpha 4\beta 2^*$ nAChR binding were detected in the substantia nigra pars compacta (SNc) and the VTA of mice treated with cocaine alone, compared with saline controls ($P < 0.01$, Fisher LSD posttests; Table 1). Chronic administration of the heteromeric antagonist DH β E with either saline or cocaine increased $\alpha 4\beta 2^*$ nAChR binding in most regions examined, compared to saline treatment (Table 1). Although increased binding levels were observed in the VTA and the SNc of DH β E-treated mice, administration of the antagonist did not further increase $\alpha 4\beta 2^*$ nAChR binding in these brain regions, compared with cocaine treatment ($P > 0.05$, Fisher LSD posttests). In addition, no differences were detected in cocaine versus MLA+cocaine, DH β E+cocaine versus MLA+cocaine, saline+DH β E versus DH β E+cocaine, saline+MLA versus MLA+cocaine, and saline versus saline+MLA-treated animals, in any of the brain areas examined ($P > 0.05$, Fisher LSD posttests).

No significant main effect of treatment [$F_{(5, 429)} = 1.2$, $P > 0.05$] and no significant treatment $\times$ region interaction effects [$F_{(60, 429)} = 0.5$, $P > 0.05$] were observed on heteromeric, non- $\alpha 4\beta 2^*$ -binding sites, following 14 days of 'binge' drug administration (Table 2; two-way ANOVA). For homomeric $\alpha 7$ nAChRs, there were no significant treatment [$F_{(5, 689)} = 1.1$, $P > 0.05$] or treatment $\times$ region interaction effects [$F_{(100, 689)} = 0.05$, $P > 0.05$] on [125 I] α -bungarotoxin binding (Table 3; two-way ANOVA).

For the high-affinity CHT, ANOVA revealed significant main effects of treatment and region, with no significant treatment by region interaction effects [treatment: $F_{(5, 163)} = 4.6$, $P < 0.001$; region: $F_{(4, 163)} = 16.9$, $P < 0.001$; interaction: $F_{(20, 163)} = 0.3$, $P > 0.05$]. Between-group comparisons showed a decrease in the density of CHT across all regions examined in MLA+cocaine-treated mice, compared with saline ($P < 0.01$), cocaine ($P < 0.05$) and DH β E-treated groups ($P < 0.001$; Figure 5 A and B). [3 H]hemicholinium binding was markedly reduced in the major island of Calleja of MLA+cocaine-treated mice, compared with cocaine ($P < 0.05$) and DH β E+cocaine treatment ($P < 0.01$, Fisher LSD posttests). Moreover, chronic saline+MLA treatment reduced CHT binding across all areas examined, compared with saline ($P < 0.05$), saline+DH β E ($P < 0.05$) and DH β E+cocaine treatment ($P < 0.01$; Fisher LSD posttests).

Discussion and conclusions

The present results demonstrate that subtype-selective antagonism of nAChRs can progressively shift the effects of chronic cocaine towards two markedly different directions, suggesting that $\alpha 7$ and $\beta 2^*$ nAChRs play distinct roles in modulating cocaine-induced behaviour. We show for the first time that the $\alpha 7$ preferring antagonist MLA induces a marked self-grooming behaviour in cocaine-treated mice, whereas the non- $\alpha 7$, $\beta 2^*$ nAChR antagonist DH β E prevents the induction

of stereotypy sensitisation to cocaine and lowers stereotypy scores.

The uncharacteristic, excessive and repetitive grooming response to chronic MLA+cocaine treatment represents a novel behavioural modification, not previously associated either with the effects of cocaine in rodents or with a dysfunction of $\alpha 7$ nAChRs. Moreover, it establishes the role of a particular nAChR subgroup in modulating a distinct behavioural effect of chronic cocaine administration. Videotape analysis revealed that time spent grooming was significantly increased following MLA+cocaine treatment, compared with cocaine and saline+MLA administration. Animals self-groomed to the extent of hair removal, indicating the intense nature of their behaviour. The fact that increased grooming was displayed in a novel context (i.e. activity chambers) also reflects its severity, because novel environments have been shown to suppress grooming duration (Berridge *et al.*, 2005). Importantly, studies examining the effects of cocaine on self-grooming show that the psychostimulant is a potent and dose-dependent grooming suppressant (Cooper and van der Hoek, 1993; Desai and Terry, 2003). Therefore, the excessive behaviour observed exclusively in MLA+cocaine-treated mice demonstrates an interaction between cocaine and the nAChR antagonist in inducing this aberrant response and introduces a prominent role for nAChRs in regulating the grooming suppressant properties of cocaine.

MLA has been commonly used *in vivo* as a selective $\alpha 7$ nAChR antagonist, suggesting that homomeric nAChRs modulate self-grooming behaviour in cocaine-treated mice. Considerable *in vitro* evidence, however, shows that MLA also has activity at heteromeric nAChRs, containing $\alpha 6$ subunits ($\alpha 6^*$) (Mogg *et al.*, 2002; Zoli *et al.*, 2002; Salminen *et al.*, 2007). Hence, despite the scarcity of studies measuring the brain concentration of MLA following its systemic administration (Turek *et al.*, 1995; Welch *et al.*, 2009), it is likely that at doses similar to those used here, MLA levels will be high enough to inhibit not only $\alpha 7$, but also heteromeric $\alpha 6^*$ nAChRs, at least partially. Nevertheless, the lack of self-grooming in animals treated either with cocaine or the broad spectrum heteromeric nAChR antagonist DH β E (Jensen *et al.*, 2005) strongly indicates that $\alpha 7$ receptor inhibition is required for the manifestation of the grooming response. In other words, as both MLA and DH β E can inhibit $\alpha 6^*$ nAChRs, the differential effects of these antagonists must preferentially reveal $\alpha 7$ - and $\beta 2^*$ -mediated behaviours respectively. Thus, while the relative contribution of MLA-sensitive nAChRs to cocaine-induced behaviour can be further examined using specific $\alpha 6^*$ nAChR ligands or nicotinic subunit knockout mice, the current data argue for a predominant role of $\alpha 7$ nAChRs in modulating MLA+cocaine-induced grooming, by demonstrating that the behaviour does not develop in cocaine-treated mice, unless $\alpha 7$ nAChRs are blocked.

Grooming is an innate behaviour that is represented across most animal species, including humans (Sachs, 1988). It is a spontaneous stereotyped activity that occurs as transition behaviour between rest and action (Fentress, 1988), and it is known to be increased in periods of low stress ('relaxation') and high stress ('anxiety') (Kalueff and Tuohimaa, 2004). Unlike low stress, high stress grooming in rodents is characterised by frequent bursts of rapid, truncated self-grooming activity (van Erp *et al.*, 1994; Kalueff and

Table 1

Quantitative autoradiography of $\alpha 4\beta 2^*$ nAChRs, following chronic 'binge' saline and cocaine ($\pm$ DH β E or MLA) administration

Region	Cytisine-sensitive [125 I]epibatidine binding (fmol·mg $^{-1}$ tissue equivalent)			
	Saline	Cocaine	Saline+DH β E	Cocaine+DH β E
Olfactory tubercle	6.9 $\pm$ 0.5	6.4 $\pm$ 0.8	6.8 $\pm$ 0.5	9.4 $\pm$ 0.7
Prelimbic cortex	8.1 $\pm$ 0.9	6.6 $\pm$ 0.8	7.4 $\pm$ 0.6	8.0 $\pm$ 0.8
Cingulate cortex	7.7 $\pm$ 0.9	7.5 $\pm$ 0.7	9.1 $\pm$ 0.7	9.7 $\pm$ 0.9
Nucleus accumbens				
Core	6.2 $\pm$ 0.6	5.9 $\pm$ 0.5	7.4 $\pm$ 0.9	7.8 $\pm$ 0.4
Shell	6.1 $\pm$ 0.7	5.7 $\pm$ 0.5	6.5 $\pm$ 0.5	7.4 $\pm$ 0.3
Caudate putamen	8.1 $\pm$ 0.9	8.3 $\pm$ 0.7	9.5 $\pm$ 1.0	10.1 $\pm$ 0.9
Primary motor cortex	8.2 $\pm$ 0.6	7.6 $\pm$ 0.7	9.8 $\pm$ 0.7	10.2 $\pm$ 0.8
Secondary motor cortex	8.2 $\pm$ 0.7	7.4 $\pm$ 0.6	9.6 $\pm$ 0.7	10.3 $\pm$ 0.9
Hippocampus	3.0 $\pm$ 0.4	3.3 $\pm$ 0.4	3.4 $\pm$ 0.5	3.2 $\pm$ 0.4
Subiculum	13.4 $\pm$ 1.7	14.7 $\pm$ 1.8	14.6 $\pm$ 2.2	13.3 $\pm$ 1.2
Posterior thalamic nuclear group	13.4 $\pm$ 1.2	13.7 $\pm$ 1.1	16.2 $\pm$ 1.5	16.4 $\pm$ 1.6
Lateral posterior thalamic nucleus	13.7 $\pm$ 1.2	13.3 $\pm$ 1.0	16.6 $\pm$ 1.4	15.7 $\pm$ 1.5
Medial geniculate nucleus	12.8 $\pm$ 1.7	16.6 $\pm$ 1.6	18.5 $\pm$ 3.1**	18.9 $\pm$ 2.2**
Ventral lateral geniculate nucleus	12.1 $\pm$ 1.5	11.0 $\pm$ 1.4	13.6 $\pm$ 2.1	15.4 $\pm$ 1.8 [#]
Dorsal lateral geniculate nucleus	14.0 $\pm$ 1.4	12.5 $\pm$ 1.4	16.3 $\pm$ 2.0	16.5 $\pm$ 4.5 [#]
Visual cortex	8.1 $\pm$ 0.7	7.7 $\pm$ 0.3	9.2 $\pm$ 0.7	9.6 $\pm$ 0.5
Superficial grey layer of the superior colliculus	8.0 $\pm$ 2.0	12.1 $\pm$ 1.8	18.7 $\pm$ 3.7***	16.8 $\pm$ 2.8*** [#]
Ventral tegmental area	9.7 $\pm$ 1.0	14.9 $\pm$ 1.4** (54%)	15.8 $\pm$ 2.3**	16.9 $\pm$ 2.0** (7%)
Substantia nigra, pars compacta	12.0 $\pm$ 1.0	17.4 $\pm$ 1.6** (45%)	18.6 $\pm$ 3.0**	18.0 $\pm$ 1.8** (−3%)
				14.7 $\pm$ 1.3
				17.6 $\pm$ 1.5** (20%)

Animals were injected with cocaine (15.0 mg·kg $^{-1}$), cocaine+DH β E (2.0 mg·kg $^{-1}$) or cocaine+MLA (5.0 mg·kg $^{-1}$) three times daily, for a period of 14 days. Thirty minutes after the last injection, the mice were killed by cervical dislocation, and their brains were removed and processed for quantitative receptor autoradiography of heteromeric nAChRs. Specific cytosine-resistant [125 I]epibatidine binding was subtracted from total [125 I]epibatidine binding values in order to quantify the density of $\alpha 4\beta 2^*$ nAChRs. The values of cytosine-sensitive [125 I]epibatidine binding represent the mean $\pm$ SEM fmol·mg $^{-1}$ of tissue equivalent, in brain regions of six to seven animals per group. Two-way ANOVA showed significant main effects of treatment ($P < 0.001$) and region ($P < 0.001$), with no significant treatment $\times$ region interaction effects ($P > 0.05$) on cytosine-sensitive [125 I]epibatidine binding. Within-region differences were examined using Fisher LSD *post hoc* tests. Percentage change in $\alpha 4\beta 2^*$ nAChR binding between cocaine-treated mice and their respective vehicle controls is shown in parentheses for the VTA and SNC.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus saline, [#] $P < 0.05$ versus cocaine.

Table 2

Quantitative autoradiography of cytosine-resistant nAChR binding, following chronic 'binge' saline and cocaine ($\pm$ DH β E or MLA) administration

Region	Cytosine-resistant [125 I]epibatidine binding (fmol-mg $^{-1}$ tissue equivalent)					
	Saline	Cocaine	Saline+DH β E	Cocaine+DH β E	Saline+MLA	Cocaine+MLA
Olfactory tubercle	1.1 $\pm$ 0.3	1.2 $\pm$ 0.3	1.4 $\pm$ 0.2	1.2 $\pm$ 0.3	1.0 $\pm$ 0.4	1.0 $\pm$ 0.2
Nucleus accumbens						
Core	1.7 $\pm$ 0.4	1.9 $\pm$ 0.3	1.7 $\pm$ 0.3	1.9 $\pm$ 0.3	1.6 $\pm$ 0.3	1.5 $\pm$ 0.3
Shell	1.6 $\pm$ 0.4	1.7 $\pm$ 0.3	1.7 $\pm$ 0.3	1.7 $\pm$ 0.3	1.5 $\pm$ 0.3	1.3 $\pm$ 0.3
Caudate putamen	1.7 $\pm$ 0.4	1.7 $\pm$ 0.3	1.6 $\pm$ 0.3	1.8 $\pm$ 0.3	1.6 $\pm$ 0.3	1.3 $\pm$ 0.3
Subiculum	2.9 $\pm$ 0.5	3.5 $\pm$ 0.2	3.1 $\pm$ 0.5	3.4 $\pm$ 0.5	3.0 $\pm$ 0.3	3.1 $\pm$ 0.3
Posterior thalamic nuclear group	1.9 $\pm$ 0.4	2.2 $\pm$ 0.2	2.3 $\pm$ 0.2	2.3 $\pm$ 0.4	1.9 $\pm$ 0.3	2.1 $\pm$ 0.2
Lateral posterior thalamic nucleus	2.5 $\pm$ 0.4	3.3 $\pm$ 0.4	3.1 $\pm$ 0.4	3.5 $\pm$ 0.6	3.1 $\pm$ 0.5	3.3 $\pm$ 0.5
Medial geniculate nucleus	2.1 $\pm$ 0.3	1.8 $\pm$ 0.4	3.5 $\pm$ 1.5	2.5 $\pm$ 0.2	2.0 $\pm$ 0.4	2.3 $\pm$ 0.4
Ventral lateral geniculate nucleus	9.7 $\pm$ 1.1	11.3 $\pm$ 0.9	10.8 $\pm$ 0.7	11.2 $\pm$ 1.6	11.1 $\pm$ 1.2	10.6 $\pm$ 0.9
Dorsal lateral geniculate nucleus	8.8 $\pm$ 1.1	10.5 $\pm$ 0.8	10.6 $\pm$ 0.7	10.7 $\pm$ 1.4	10.5 $\pm$ 1.0	10.2 $\pm$ 0.8
Superficial grey layer of the superior colliculus	12.0 $\pm$ 1.2	10.6 $\pm$ 2.6	7.6 $\pm$ 1.7	12.0 $\pm$ 2.4	10.3 $\pm$ 2.0	9.4 $\pm$ 1.7
Ventral tegmental area	3.3 $\pm$ 0.6	3.5 $\pm$ 0.6	4.6 $\pm$ 0.5	4.4 $\pm$ 0.2	3.5 $\pm$ 0.4	3.7 $\pm$ 0.4
Substantia nigra, pars compacta	2.7 $\pm$ 0.5	2.8 $\pm$ 0.4	3.3 $\pm$ 0.4	3.2 $\pm$ 0.3	2.7 $\pm$ 0.6	2.3 $\pm$ 0.5

Values of cytosine-resistant [125 I]epibatidine binding represent mean $\pm$ SEM fmol-mg $^{-1}$ of tissue equivalent in brain regions of six to seven animals per group. Two-way ANOVA showed no significant treatment effects ($P > 0.05$), and no significant treatment $\times$ region interaction effects ($P > 0.05$) on cytosine-resistant [125 I]epibatidine binding sites.

Tuohimaa, 2005). In this study, MLA+cocaine-treated mice constantly disrupted their activity in order to repeat short components of the grooming sequence, indicating the stress-derived nature of their behaviour. Indeed, excessive grooming is typically encountered in animal models of anxiety spectrum disorders, particularly of OCD (Feusner *et al.*, 2009). For instance, mice lacking the *Sapap3* or *Hoxb8* genes are considered to be animal models of OCD-like behaviours and exhibit intense self-grooming leading to hair removal and skin lesions (Greer and Capecchi, 2002; Welch *et al.*, 2007). Although it is not possible to determine the exact nature of the self-grooming activity we observed (e.g. obsession, intrusive thoughts, dysfunction of impulse control, punding), our findings indicate a key role for $\alpha 7$ nAChRs in modulating aspects of cocaine-induced behaviour that are characteristic of anxiety spectrum disorders. The abuse of cocaine is known to have anxiogenic effects (Gawin, 1991), triggering psychotic-like symptoms in as many as 50% of abusing individuals (Williamson *et al.*, 1997). Moreover, there is high co-morbidity between severe mood and anxiety spectrum disorders and chronic cocaine use (Crum and Anthony, 1993; Goodwin *et al.*, 2002). Despite this, the management of the symptomatology of cocaine abuse has been given very little attention in a clinical or research setting (Mendelson and Mello, 1996). The present data thus suggest that $\alpha 7$ agonism may be a viable therapeutic approach for the anxiogenic effects of 'binge' cocaine. Impairment of $\alpha 7$ nAChRs has been implicated in neuropsychiatric illness, including schizophrenia, autism, attention deficit hyperactivity disorders, mood and anxiety disorders (Lightfoot *et al.*, 2008). The current findings extend this literature, in providing evidence that

the $\alpha 7$ preferring antagonist MLA induces an aberrant, compulsive-like behaviour during chronic cocaine treatment.

To investigate the neurochemical mechanism underlying MLA+cocaine-induced grooming, binding to nAChRs and to the high-affinity CHT was analysed in brain regions of cocaine and saline ($\pm$ MLA)-treated mice. The chronic administration of MLA, with or without cocaine, reduced [3 H]hemicholinium binding compared with saline and DH β E treatment. Because the high-affinity uptake of choline into presynaptic cholinergic terminals is the rate limiting step in acetylcholine synthesis, the selective labelling of CHT by [3 H]hemicholinium is considered to provide an index of *in vivo* cholinergic neuron activity (Ribeiro *et al.*, 2006). Therefore, the observed overall decrease in choline uptake sites indicates a reduced cholinergic tone following chronic MLA administration. Based on the mutually opposing relationship between cholinergic and dopaminergic neurotransmission in the striatum (Hikida *et al.*, 2001; 2003), it is likely that a reduced cholinergic tone can exacerbate the effects of cocaine on dopamine neurotransmission. Indeed, intra-VTA or intra-NAc administration of MLA has been shown to further enhance cocaine-induced dopamine release in the NAc, compared with the administration of cocaine alone (Zanetti *et al.*, 2007). Given that hyperactivation of the dopaminergic system has been implicated in the manifestation of anxiety spectrum disorders and stress-induced grooming (Homberg *et al.*, 2002; Berridge *et al.*, 2005), it is plausible that a hypo-cholinergic tone contributes significantly towards the behaviour observed in MLA+cocaine-treated animals. Interestingly, reduced choline uptake sites in MLA+cocaine-treated mice were observed in the major island of Calleja, a brain region

Table 3

Quantitative autoradiography of $\alpha 7$ nAChRs, following chronic 'binge' saline and cocaine ($\pm$ DH β E or MLA) administration

Region	Saline	[¹²⁵ I] α -bungarotoxin binding (fmol·mg ⁻¹ tissue equivalent)				
		Cocaine	Saline+DH β E	Cocaine+DH β E	Saline+MLA	Cocaine+MLA
Frontal association cortex	20.4 $\pm$ 6.9	19.6 $\pm$ 6.3	19.7 $\pm$ 4.2	23.5 $\pm$ 4.5	12.6 $\pm$ 2.8	17.4 $\pm$ 1.9
Cingulate cortex	25.3 $\pm$ 5.6	21.4 $\pm$ 7.9	21.2 $\pm$ 2.9	25.5 $\pm$ 4.6	15.5 $\pm$ 3.1	20.4 $\pm$ 4.4
Caudate putamen	33.1 $\pm$ 8.5	31.2 $\pm$ 7.3	29.1 $\pm$ 4.2	37.6 $\pm$ 5.3	23.7 $\pm$ 5.1	29.2 $\pm$ 5.3
Endopiriform nucleus, dorsal	41.5 $\pm$ 6.9	46.0 $\pm$ 6.5	38.6 $\pm$ 8.7	49.6 $\pm$ 9.6	36.0 $\pm$ 9.0	36.4 $\pm$ 7.3
Primary motor cortex	30.6 $\pm$ 5.4	35.1 $\pm$ 7.0	26.4 $\pm$ 2.7	38.8 $\pm$ 3.6	19.6 $\pm$ 1.8	24.1 $\pm$ 4.5
Clastrum	40.6 $\pm$ 10.1	40.5 $\pm$ 5.9	28.1 $\pm$ 7.5	38.3 $\pm$ 5.5	30.9 $\pm$ 6.7	21.2 $\pm$ 3.6
Posterior thalamic nuclear group	28.6 $\pm$ 11.6	28.7 $\pm$ 7.0	23.3 $\pm$ 3.3	24.9 $\pm$ 4.8	20.9 $\pm$ 5.0	25.7 $\pm$ 5.1
Hippocampus, CA1	30.3 $\pm$ 4.9	32.3 $\pm$ 5.4	27.6 $\pm$ 8.3	33.7 $\pm$ 4.3	32.0 $\pm$ 8.5	25.8 $\pm$ 3.7
Hippocampus, CA3	34.6 $\pm$ 6.2	37.1 $\pm$ 5.1	33.2 $\pm$ 6.8	41.0 $\pm$ 6.3	34.8 $\pm$ 8.2	33.8 $\pm$ 5.8
Dentate gyrus	22.5 $\pm$ 6.1	32.5 $\pm$ 7.2	29.8 $\pm$ 2.9	28.0 $\pm$ 4.6	26.9 $\pm$ 6.0	24.6 $\pm$ 3.2
Basolateral amygdaloid nucleus	36.0 $\pm$ 8.7	34.6 $\pm$ 3.6	28.1 $\pm$ 6.6	35.7 $\pm$ 6.3	32.1 $\pm$ 6.5	34.9 $\pm$ 5.5
Posteromedial cortical amygdaloid nucleus	100.1 $\pm$ 23.1	67.6 $\pm$ 12.0	70.9 $\pm$ 12.0	90.9 $\pm$ 18.8	84.0 $\pm$ 14.6	69.7 $\pm$ 6.4
Medial amygdaloid nucleus	49.2 $\pm$ 9.7	41.3 $\pm$ 2.2	45.5 $\pm$ 9.5	46.2 $\pm$ 5.5	49.7 $\pm$ 13.0	52.9 $\pm$ 6.6
Dorsomedial hypothalamic nucleus	33.9 $\pm$ 9.7	29.3 $\pm$ 2.5	28.5 $\pm$ 3.5	38.3 $\pm$ 6.5	35.5 $\pm$ 11.8	39.5 $\pm$ 4.9
Ventromedial hypothalamic nucleus	30.5 $\pm$ 6.6	43.1 $\pm$ 6.2	43.7 $\pm$ 3.8	39.9 $\pm$ 6.6	46.1 $\pm$ 13.5	42.6 $\pm$ 4.3
Lateral hypothalamus	28.0 $\pm$ 4.4	19.4 $\pm$ 4.1	22.3 $\pm$ 2.8	20.5 $\pm$ 2.9	27.5 $\pm$ 4.6	25.3 $\pm$ 4.0
Zona incerta	41.9 $\pm$ 8.6	35.2 $\pm$ 9.3	35.9 $\pm$ 10.1	30.3 $\pm$ 4.8	29.9 $\pm$ 7.8	41.2 $\pm$ 7.3
Ventral lateral geniculate nucleus	54.0 $\pm$ 8.9	32.3 $\pm$ 4.9	52.9 $\pm$ 6.8	42.7 $\pm$ 6.5	53.9 $\pm$ 12.4	53.6 $\pm$ 7.9
Superficial grey layer of the superior colliculus	86.7 $\pm$ 12.8	89.9 $\pm$ 12.1	94.3 $\pm$ 10.8	94.3 $\pm$ 13.2	91.7 $\pm$ 16.2	91.2 $\pm$ 13.7
Ventral tegmental area	40.8 $\pm$ 4.7	41.8 $\pm$ 5.8	49.8 $\pm$ 3.2	50.7 $\pm$ 9.9	49.7 $\pm$ 6.5	46.4 $\pm$ 7.0
Substantia nigra	21.2 $\pm$ 6.1	37.5 $\pm$ 6.7	24.1 $\pm$ 4.1	31.7 $\pm$ 5.5	19.8 $\pm$ 4.0	25.8 $\pm$ 5.3

Animals were treated using a steady dose, 'binge' administration protocol, receiving three daily injections of either saline or cocaine (with or without nAChR antagonists), 1 h apart, for a period of 14 days. Autoradiographic data are presented as the mean $\pm$ SEM specific binding of 3 nmol of [¹²⁵I] α -bungarotoxin, in brain regions of 6–7 animals per group. No significant treatment ($P > 0.05$) or treatment $\times$ region interaction effects ($P > 0.05$) were observed on $\alpha 7$ nAChRs binding levels.

with substantial cholinergic (Schafer *et al.*, 1998) and dopaminergic (Halliwell and Horne, 1998) input, and an important site for the action of neuroleptics (Guo *et al.*, 1998; McCormick *et al.*, 2010).

Chronic administration of the non- $\alpha 7$, $\beta 2^*$ nAChR antagonist DH β E did not induce grooming, but led to distinct behavioural alterations in 'binge'-treated mice. The ability of DH β E to reduce stereotypy and prevent the induction of stereotypic sensitisation to cocaine, while prolonging horizontal locomotor sensitisation, indicates the prevalence of a locomotor-stimulant over a 'stereotypy-prone' behavioural phenotype in DH β E+cocaine-treated mice. The blockade of stereotypy sensitisation with DH β E is consistent with the antagonist's ability to produce the same effect when delivered within the VTA of cocaine-treated rats (Champtiaux *et al.*, 2006), suggesting that $\beta 2^*$ nAChRs in this brain region mediate the neuroadaptive changes underlying stereotypy sensitisation. In support of this suggestion, we show that chronic cocaine induces a marked region-specific up-regulation of $\alpha 4\beta 2^*$ nAChRs in the VTA and the SNc. Our binding data show a clear association between stereotypy and

$\alpha 4\beta 2^*$ nAChR regulation in the midbrain, as in groups of mice that showed large up-regulation of $\alpha 4\beta 2^*$ nAChRs, compared with their respective vehicle controls (cocaine vs. saline), larger stereotypy scores were observed, whereas in animals with small alterations in $\alpha 4\beta 2^*$ nAChR binding (DH β E+cocaine vs. DH β E+saline), stereotypy scores were significantly lower. Interestingly, activation of the striatonigral pathway is known to be associated with psychostimulant-induced stereotypy behaviour (Amalric and Koob, 1993; Aliane *et al.*, 2009). As a result, the up-regulation of SN-located $\alpha 4\beta 2^*$ nAChRs may also be involved in the behavioural sensitisation effect of cocaine.

Motor stereotypies are cardinal symptoms of psychostimulant abuse disorders and of various neurological and neuropsychiatric disorders, including Tourette's syndrome (Silver *et al.*, 2001) and Parkinson's disease (Evans *et al.*, 2009). Despite the effectiveness of acetylcholine inhibitors in treating stereotyped behaviour, severe and disabling side effects occur as a result of treatment. Therefore, the present data not only provide neurochemical evidence for the contribution of $\alpha 4\beta 2^*$ nAChRs to the development of cocaine-

Figure 5

Binding to the high-affinity choline transporters is reduced following chronic MLA administration. (A) Data represent the mean $\pm$ SEM specific [^3H]hemicholinium binding in striatal brain regions of six to seven animals per group. * $P < 0.05$, ** $P < 0.01$ versus MLA+cocaine. (B) The panels show coronal brain section cut at the level of the nucleus accumbens (bregma 0.86 mm). To label choline transporters, adjacent sections were incubated at 4°C for 60 min with 8 nmol of [^3H]hemicholinium alone or in the presence of 100 μmol unlabelled hemicholinium, to calculate nonspecific binding (NSB). The colour bar indicates a pseudo-colour interpretation of black and white image density, calibrated in fmol·mg $^{-1}$ of tissue equivalent.

induced stereotypy, but are also in favour of a therapeutic strategy that selectively targets $\alpha 4\beta 2^*$ nAChRs for the alleviation of motor stereotypy.

The behaviour of DH β E+cocaine-treated mice implies different roles for heteromeric nAChRs in modulating the intensity and time course of cocaine-induced stereotypy and locomotion. Contrary to our stereotypy results, cocaine-induced horizontal locomotor sensitisation was prolonged following DH β E administration, indicating that heteromeric $\beta 2^*$ nAChRs mediate the initiation of the psychomotor response to cocaine. Although the observed prolongation was

short lasting, our data do not corroborate the finding of Schoffelmeier *et al.* (2002), who showed that systemic DH β E administration completely prevents the development/expression of locomotor sensitisation in rats. This discrepancy could either reflect a species difference or the use of different behavioural protocols, as the initiation of locomotor sensitisation was investigated here by applying a steady dose, 'binge' treatment protocol, whereas the development/expression of long-term sensitisation was examined in the Schoffelmeier *et al.* (2002) study following a withdrawal period from repeated cocaine treatment. Nevertheless, our

data are in line with Zanetti *et al.* (2006), who demonstrated that blocking $\beta 2^*$ nAChRs in mice does not prevent the development of sensitisation to cocaine-elicited increases in mesolimbic dopamine levels and, consequently, of cocaine-induced locomotor sensitisation, highlighting the requirement of simultaneously blocking $\beta 2^*$ and $\alpha 7$ nAChRs in order to exert these effects.

The observation of increased $\alpha 4\beta 2^*$ nAChR binding in cocaine-treated mice further supports the concept that the primary events leading to the induction of psychostimulant behavioural sensitisation involve neuroadaptive changes within the VTA microcircuitry (Vanderschuren and Kalivas, 2000). $\alpha 4\beta 2^*$ nAChR binding was exclusively increased in the midbrain, and no changes were observed in the projection areas of dopamine cells, including the NAc. This finding ties in with the lack of effect of intra-accumbal DH β E injections on cocaine-induced behavioural sensitisation (Champtiaux *et al.*, 2006) and on dopamine release in the NAc (Zanetti *et al.*, 2007), and underlines the potentially crucial role of midbrain $\alpha 4\beta 2^*$ up-regulation in mediating the behavioural effects of chronic cocaine. As in the case of nicotine-induced up-regulation of $\alpha 4\beta 2^*$ nAChRs, cocaine-induced increases in nAChR binding could either reflect the enhancement of functional, 'activatable' nAChRs or simply that of desensitised receptors, or both (Picciotto *et al.*, 2008). Although the mechanism mediating these alterations is unknown, we postulate that a dopamine-stimulated cholinergic mechanism may be involved, as self-administered or passively received cocaine increases cholinergic input in the VTA (Maskos, 2008; You *et al.*, 2008), and the chronic stimulation of dopamine D₂ or D₃ receptors, which are activated following chronic exposure to cocaine, induces up-regulation of $\alpha 4\beta 2^*$ nAChRs in the midbrain (Tizabi *et al.*, 1999).

In conclusion, we demonstrate a profound and distinct involvement of nAChRs in the manifestation of the behavioural effects of chronic cocaine treatment. First, the inhibition of $\alpha 7$ nAChRs induces an excessive self-grooming behaviour during the repeated administration of cocaine. Second, the inhibition of non- $\alpha 7$, $\beta 2^*$ nAChRs protects against cocaine's stereotypy-inducing effects. To the best of our knowledge, this report is the first to demonstrate a subtype-selective contribution of nAChRs to the repetitive/stereotyped disorders associated with the chronic use of cocaine. Our results may have validity for the neuropsychiatric sequelae of cocaine addiction in humans, and invite further consideration of the nicotinic cholinergic system as an important target for the diagnosis and treatment of cocaine-abuse symptomatology. Although the extrapolation from rodent studies to human psychopharmacology is tenuous, the current data provide a pharmacological basis to explain the variable effects that cocaine users report following cigarette smoking, and imply that the perceived effects of nicotine intake in cocaine addicts depend on the availability and function of distinct nAChR subtypes.

Acknowledgements

This work was supported by the European Commission (Contract Number: LSHM-CT2004-005166).

Conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest with respect to the funding or completion of this work.

References

- Adler LE, Olincy A, Cawthra E, Hoffer M, Nagamoto HT, Amass L *et al.* (2001). Reversal of diminished inhibitory sensory gating in cocaine addicts by a nicotinic cholinergic mechanism. *Neuropsychopharmacology* 24: 671–679.
- Albuquerque EX, Pereira EF, Alkondon M, Rogers SW (2009). Mammalian nicotinic acetylcholine receptors: from structure to function. *Physiol Rev* 89: 73–120.
- Alexander SP, Mathie A, Peters JA (2011). Guide to receptors and channels (GRAC), 5th edition. *Br J Pharmacol* 164: S1–S324.
- Aliane V, Pèrez S, Nieoullon A, Deniau JM, Kemel ML (2009). Cocaine-induced stereotypy is linked to an imbalance between the medial prefrontal and sensorimotor circuits of the basal ganglia. *Eur J Neurosci* 30: 1269–1279.
- Aliane V, Perez S, Bohren Y, Deniau JM, Kemel ML (2011). Key role of striatal cholinergic interneurons in processes leading to arrest of motor stereotypies. *Brain* 134: 110–118.
- Amalric M, Koob GF (1993). Functionally selective neurochemical afferents and efferents of the mesocorticolimbic and nigrostriatal dopamine system. *Prog Brain Res* 99: 209–226.
- Bailey A, Gianotti R, Ho A, Kreek MJ (2007a). Downregulation of kappa-opioid receptors in basolateral amygdala and septum of rats withdrawn for 14 days from an escalating dose 'binge' cocaine administration paradigm. *Synapse* 61: 820–826.
- Bailey A, Yoo JH, Raciz I, Zimmer A, Kitchen I (2007b). Preprodynorphin mediates locomotion and D2 dopamine and mu-opioid receptor changes induced by chronic 'binge' cocaine administration. *J Neurochem* 102: 1817–1830.
- Bailey A, Metaxas A, Yoo JH, McGee T, Kitchen I (2008). Decrease of D2 receptor binding but increase in D2-stimulated G-protein activation, dopamine transporter binding and behavioural sensitization in brains of mice treated with a chronic escalating dose 'binge' cocaine administration paradigm. *Eur J Neurosci* 28: 759–770.
- Berridge KC, Aldridge JW, Houchard KR, Zhuang X (2005). Sequential super-stereotypy of an instinctive fixed action pattern in hyper-dopaminergic mutant mice: a model of obsessive compulsive disorder and Tourette's. *BMC Biol* 3: 4.
- Besson M, Granon S, Mameli-Engvall M, Cloez-Tayarani I, Maubourguet N, Cormier A *et al.* (2007). Long-term effects of chronic nicotine exposure on brain nicotinic receptors. *Proc Natl Acad Sci U S A* 104: 8155–8160.
- Bolla KI, Cadet JL, London ED (1998). The neuropsychiatry of chronic cocaine abuse. *J Neuropsychiatry Clin Neurosci* 10: 280–289.
- Champtiaux N, Kalivas PW, Bardo MT (2006). Contribution of dihydro-beta-erythroidine sensitive nicotinic acetylcholine receptors in the ventral tegmental area to cocaine-induced behavioral sensitization in rats. *Behav Brain Res* 168: 120–126.

- Cooper SJ, van der Hoek GA (1993). Cocaine: a microstructural analysis of its effects on feeding and associated behaviour in the rat. *Brain Res* 608: 45–51.
- Creese I, Iversen SD (1974). The role of forebrain dopamine systems in amphetamine induced stereotyped behavior in the rat. *Psychopharmacologia* 39: 345–357.
- Crum RM, Anthony JC (1993). Cocaine use and other suspected risk factors for obsessive-compulsive disorder: a prospective study with data from the Epidemiologic Catchment Area surveys. *Drug Alcohol Depend* 31: 281–295.
- Damaj MI, Kao W, Martin BR (2003). Characterization of spontaneous and precipitated nicotine withdrawal in the mouse. *J Pharmacol Exp Ther* 307: 526–534.
- Daunais JB, McGinty JF (1995). Cocaine binges differentially alter striatal preprodynorphin and zif/268 mRNAs. *Brain Res Mol Brain Res* 29: 201–210.
- Desai RI, Terry P (2003). Evidence of cross-tolerance between behavioural effects of nicotine and cocaine in mice. *Psychopharmacology* 166: 111–119.
- Dome P, Lazary J, Kalapos MP, Rihmer Z (2010). Smoking, nicotine and neuropsychiatric disorders. *Neurosci Biobehav Rev* 34: 295–342.
- van Erp AM, Kruk MR, Meelis W, Willekens-Bramer DC (1994). Effect of environmental stressors on time course, variability and form of self-grooming in the rat: handling, social contact, defeat, novelty, restraint and fur moistening. *Behav Brain Res* 65: 47–55.
- Evans AH, Strafella AP, Weintraub D, Stacy M (2009). Impulsive and compulsive behaviors in Parkinson's disease. *Mov Disord* 24: 1561–1570.
- Fasano A, Petrovic I (2010). Insights into pathophysiology of punning reveal possible treatment strategies. *Mol Psychiatry* 15: 560–573.
- Fentress JC (1988). Expressive contexts, fine structure, and central mediation of rodent grooming. *Ann N Y Acad Sci* 525: 18–26.
- Feusner JD, Hembacher E, Phillips KA (2009). The mouse who couldn't stop washing: pathologic grooming in animals and humans. *CNS Spectr* 14: 503–513.
- Franklin KBJ, Paxinos G (2001). *The Mouse Brain in Stereotaxic Coordinates*. Academic Press: San Diego, CA.
- Gawin FH (1991). Cocaine addiction: psychology and neurophysiology. *Science* 251: 1580–1586.
- Goodwin RD, Stayner DA, Chinman MJ, Wu P, Tebes JK, Davidson L (2002). The relationship between anxiety and substance use disorders among individuals with severe affective disorders. *Compr Psychiatry* 43: 245–252.
- Greer JM, Capecchi MR (2002). Hoxb8 is required for normal grooming behavior in mice. *Neuron* 33: 23–34.
- Guo N, Vincent SR, Fibiger HC (1998). Phenotypic characterization of neuroleptic-sensitive neurons in the forebrain: contrasting targets of haloperidol and clozapine. *Neuropsychopharmacology* 19: 133–145.
- Halliwel JV, Horne AL (1998). Evidence for enhancement of gap junctional coupling between rat island of Calleja granule cells in vitro by the activation of dopamine D3 receptors. *J Physiol* 506: 175–194.
- Hikida T, Kaneko S, Isobe T, Kitabatake Y, Watanabe D, Pastan I *et al.* (2001). Increased sensitivity to cocaine by cholinergic cell ablation in nucleus accumbens. *Proc Natl Acad Sci U S A* 98: 13351–13354.
- Hikida T, Kitabatake Y, Pastan I, Nakanishi S (2003). Acetylcholine enhancement in the nucleus accumbens prevents addictive behaviors of cocaine and morphine. *Proc Natl Acad Sci U S A* 100: 6169–6173.
- Homberg JR, van den Akker M, Raaso HS, Wardeh G, Binnekade R, Schoffeleer AN *et al.* (2002). Enhanced motivation to self-administer cocaine is predicted by self-grooming behaviour and relates to dopamine release in the rat medial prefrontal cortex and amygdala. *Eur J Neurosci* 15: 1542–1550.
- Jensen AA, Frolund B, Liljefors T, Krogsgaard-Larsen P (2005). Neuronal nicotinic acetylcholine receptors: structural revelations, target identifications, and therapeutic inspirations. *J Med Chem* 48: 4705–4745.
- Kalman D, Morissette SB, George TP (2005). Co-morbidity of smoking in patients with psychiatric and substance use disorders. *Am J Addict* 14: 106–123.
- Kalueff AV, Tuohimaa P (2004). Grooming analysis algorithm for neurobehavioural stress research. *Brain Res Brain Res Protoc* 13: 151–158.
- Kalueff AV, Tuohimaa P (2005). Mouse grooming microstructure is a reliable anxiety marker bidirectionally sensitive to GABAergic drugs. *Eur J Pharmacol* 508: 147–153.
- Kitchen I, Slowe SJ, Matthes HW, Kieffer B (1997). Quantitative autoradiographic mapping of mu-, delta- and kappa-opioid receptors in knockout mice lacking the mu-opioid receptor gene. *Brain Res* 778: 73–88.
- Lightfoot AP, Kew JN, Skidmore J (2008). Alpha7 nicotinic receptor agonists and positive allosteric modulators. *Prog Med Chem* 46: 131–171.
- McCormick PN, Kapur S, Graff-Guerrero A, Raymond R, Nobrega JN, Wilson AA (2010). The antipsychotics olanzapine, risperidone, clozapine, and haloperidol are D2-selective ex vivo but not in vitro. *Neuropsychopharmacology* 35: 1826–1835.
- McGrath J, Drummond G, Kilkenny C, Wainwright C (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. *Br J Pharmacol* 160: 1573–1576.
- Maskos U (2008). The cholinergic mesopontine tegmentum is a relatively neglected nicotinic master modulator of the dopaminergic system: relevance to drugs of abuse and pathology. *Br J Pharmacol* 153 (Suppl 1): S438–S445.
- Mendelson JH, Mello NK (1996). Management of cocaine abuse and dependence. *N Engl J Med* 334: 965–972.
- Metaxas A, Bailey A, Barbano MF, Galeote L, Maldonado R, Kitchen I (2010). Differential region-specific regulation of alpha4beta2* nAChRs by self-administered and non-contingent nicotine in C57BL/6J mice. *Addict Biol* 15: 464–479.
- Mogg AJ, Whiteaker P, McIntosh JM, Marks M, Collins AC, Wonnacott S (2002). Methyllycaconitine is a potent antagonist of alpha-conotoxin-MII-sensitive presynaptic nicotinic acetylcholine receptors in rat striatum. *J Pharmacol Exp Ther* 302: 197–204.
- Orr-Urtreger A, Goldner FM, Saeki M, Lorenzo I, Goldberg L, De Biasi M *et al.* (1997). Mice deficient in the alpha7 neuronal nicotinic acetylcholine receptor lack alpha-bungarotoxin binding sites and hippocampal fast nicotinic currents. *J Neurosci* 17: 9165–9171.
- Panagis G, Kastellakis A, Spyraiki C, Nomikos G (2000). Effects of methyllycaconitine (MLA), an alpha 7 nicotinic receptor antagonist, on nicotine- and cocaine-induced potentiation of brain stimulation reward. *Psychopharmacology* 149: 388–396.

- Peca J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN *et al.* (2011). Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* 472: 437–442.
- Picciotto MR, Addy NA, Mineur YS, Brunzell DH (2008). It is not 'either/or': activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. *Prog Neurobiol* 84: 329–342.
- Ribeiro FM, Black SA, Prado VF, Rylett RJ, Ferguson SS, Prado MA (2006). The 'ins' and 'outs' of the high-affinity choline transporter CHT1. *J Neurochem* 97: 1–12.
- Sachs BD (1988). The development of grooming and its expression in adult animals. *Ann N Y Acad Sci* 525: 1–17.
- Salminen O, Drapeau JA, McIntosh JM, Collins AC, Marks MJ, Grady SR (2007). Pharmacology of alpha-conotoxin MII-sensitive subtypes of nicotinic acetylcholine receptors isolated by breeding of null mutant mice. *Mol Pharmacol* 71: 1563–1571.
- Schafer MK, Eiden LE, Weihe E (1998). Cholinergic neurons and terminal fields revealed by immunohistochemistry for the vesicular acetylcholine transporter. I. Central nervous system. *Neuroscience* 84: 331–359.
- Schlussman SD, Zhou Y, Bailey A, Ho A, Kreek MJ (2005). Steady-dose and escalating-dose 'binge' administration of cocaine alter expression of behavioral stereotypy and striatal preprodynorphin mRNA levels in rats. *Brain Res Bull* 67: 169–175.
- Schoffelmeer AN, De Vries TJ, Wardeh G, van de Ven HW, Vanderschuren LJ (2002). Psychostimulant-induced behavioral sensitization depends on nicotinic receptor activation. *J Neurosci* 22: 3269–3276.
- Silver AA, Shytle RD, Philipp MK, Wilkinson BJ, McConville B, Sanberg PR (2001). Transdermal nicotine and haloperidol in Tourette's disorder: a double-blind placebo-controlled study. *J Clin Psychiatry* 62: 707–714.
- Tang YL, Kranzler HR, Gelernter J, Farrer LA, Cubells JF (2007). Comorbid psychiatric diagnoses and their association with cocaine-induced psychosis in cocaine-dependent subjects. *Am J Addict* 16: 343–351.
- Tizabi Y, Copeland RL Jr, Brus R, Kostrzewa RM (1999). Nicotine blocks quinpirole-induced behavior in rats: psychiatric implications. *Psychopharmacology* 145: 433–441.
- Turek JW, Kang CH, Campbell JE, Arneric SP, Sullivan JP (1995). A sensitive technique for the detection of the alpha 7 neuronal nicotinic acetylcholine receptor antagonist, methyllycaconitine, in rat plasma and brain. *J Neurosci Methods* 61: 113–118.
- Vanderschuren LJ, Kalivas PW (2000). Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology* 151: 99–120.
- Wallace TL, Ballard TM, Pouzet B, Riedel WJ, Wettstein JG (2011). Drug targets for cognitive enhancement in neuropsychiatric disorders. *Pharmacol Biochem Behav* 99: 130–145.
- Weinberger AH, Sofuoglu M (2009). The impact of cigarette smoking on stimulant addiction. *Am J Drug Alcohol Abuse* 35: 12–17.
- Welch JM, Lu J, Rodriguiz RM, Trotta NC, Peca J, Ding JD *et al.* (2007). Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. *Nature* 448: 894–900.
- Welch KD, Green BT, Panter KE, Gardner DR, Pfister JA, Cook D *et al.* (2009). Investigation of the susceptibility of various strains of mice to methyllycaconitine toxicosis. *J Anim Sci* 87: 1558–1564.
- Williamson S, Gossop M, Powis B, Griffiths P, Fountain J, Strang J (1997). Adverse effects of stimulant drugs in a community sample of drug users. *Drug Alcohol Depend* 44: 87–94.
- Wiseman EJ, McMillan DE (1998). Rationale for cigarette smoking and for mentholation preference in cocaine- and nicotine-dependent outpatients. *Compr Psychiatry* 39: 358–363.
- You Z-B, Wang B, Zitzman D, Wise RA (2008). Acetylcholine release in the mesocorticolimbic dopamine system during cocaine seeking: conditioned and unconditioned contributions to reward and motivation. *J Neurosci* 28: 9021–9029.
- Zachariou V, Caldarone BJ, Weathers-Lowin A, George TP, Elsworth JD, Roth RH *et al.* (2001). Nicotine receptor inactivation decreases sensitivity to cocaine. *Neuropsychopharmacology* 24: 576–589.
- Zanetti L, de Kerchove D'Exaerde A, Zanardi A, Changeux JP, Picciotto MR, Zoli M (2006). Inhibition of both alpha7* and beta2* nicotinic acetylcholine receptors is necessary to prevent development of sensitization to cocaine-elicited increases in extracellular dopamine levels in the ventral striatum. *Psychopharmacology* 187: 181–188.
- Zanetti L, Picciotto MR, Zoli M (2007). Differential effects of nicotinic antagonists perfused into the nucleus accumbens or the ventral tegmental area on cocaine-induced dopamine release in the nucleus accumbens of mice. *Psychopharmacology* 190: 189–199.
- Zoli M, Moretti M, Zanardi A, McIntosh JM, Clementi F, Gotti C (2002). Identification of the nicotinic receptor subtypes expressed on dopaminergic terminals in the rat striatum. *J Neurosci* 22: 8785–8789.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Antagonism of nicotine-induced hypolocomotion by DHβE. Male C57BL/6J mice were habituated to the locomotor chambers for 4 days prior to treatment. On the day of the experiment, animals were pretreated with saline (5 ml·kg⁻¹) or DHβE (2 mg·kg⁻¹, salt weight), 5 min prior to the injection of saline or nicotine (1 mg·kg⁻¹, free-base weight). Five minutes after acute nicotine, total locomotor activity was measured for 10 min. All injections were given i.p.. DHβE antagonised nicotine-induced hypolocomotion without affecting baseline locomotor activity, and therefore this dose was used to block heteromeric nAChRs in subsequent experiments. Data are presented as the mean ± SEM of six animals per group and were analysed using one-way ANOVA. **P* < 0.05 versus all other groups, Bonferroni's post test.

Figure S2 MLA-precipitated nicotine withdrawal. Total (A) and individual (B) somatic signs precipitated by acute injection of MLA (5 mg·kg⁻¹, i.p., salt weight) following chronic infusion of nicotine or saline. C57BL/6J mice were anaesthetised with isoflurane, and a single incision (1 cm long) was made along the midline of the back, to reveal the subcutaneous layer. Blunt-ended scissors were used to open up a subcutaneous compartment, where the osmotic minipump

(ALZET® 2002 model, Charles River) was placed, with the flow moderator pointing away from the incision, parallel to the spine, containing either (–) nicotine hydrogen tartrate salt in 0.9% sterile saline, or vehicle (0.9% sterile saline). The incision was closed with 1–2 Michelle clips. Nicotine hydrogen tartrate was delivered at a dose of $24 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, at a rate of $0.5 \mu\text{l/h}$, for 14 days. On day 14, animals received an acute i.p. injection of MLA, and withdrawal signs were measured for 20 min in an observation chamber. MLA-precipitated somatic signs of withdrawal, and therefore, this dose was used to block homomeric nAChRs in subsequent experiments. Data are presented as the mean $\pm$ SEM of six

animals per group. $**P < 0.01$ versus control, Mann–Whitney *U*-test's non-parametric test.

Supporting Information Quantitative autoradiography protocols.

Movie S1 Cocaine.

Movie S2 Cocaine+DH β E.

Movie S3 Cocaine+MLA.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.