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RESEARCH PAPER

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

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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 α7 preferring nAChR antagonist methyllycaconitine (MLA; 5 mg·kg^{-1}, i.p.) or the β2* nAChR antagonist dihydro-β-erythroidine (DHβE; 2 mg·kg^{-1}, i.p.). Quantitative autoradiography was used to examine the effect of cocaine exposure on α7 and α4β2* nAChRs, and on the high-affinity choline transporter.

KEY RESULTS

MLA+cocaine administration induced an intense self-grooming behaviour, indicating a likely role for α7 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 DHβE) administration. DHβE treatment prevented the induction of stereotypy sensitisation to cocaine but prolonged locomotor sensitisation, implicating heteromeric β2* nAChRs in the neuroadaptations mediating cocaine-induced behavioural sensitisation. 'Binge' cocaine treatment region-specifically increased α4β2* 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; DHβE, 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 α7 receptors, and into α-bungarotoxin insensitive, heteropentameric, non-α7 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 α4β2 combination (α4β2*, 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 β2 subunits reduces the conditioned rewarding effects of cocaine (Zachariou *et al.*, 2001), and treatment with the non-α7, β2* nAChR antagonist dihydro-β-erythroidine (DHβE) prevents cocaine-induced locomotor sensitisation (Schoffelmeer *et al.*, 2002; Champtiaux *et al.*, 2006), indicating that heteromeric nAChR activation contributes to cocaine reinforcement. The α7 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 (Champtiaux *et al.*, 2006). At the neurochemical level, while administration of MLA in the NAc has been shown to further enhance, and DHβE 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 α7 and β2* 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, purposeless 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 α7 and non-α7, β2* 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 α7 and β2* nAChR blockade on cocaine-induced behaviour was studied over 14 days using MLA and DHβE respectively. The administration of antagonists with different selectivity profiles for nAChRs induced distinct behavioural alterations in cocaine-treated mice, which suggests that α7 and non-α7, β2* 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+DHβE (2 mg·kg^{-1}; weight as hydrobromide salt), DHβE+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 DHβE were used to block α7 and non-α7, β2* 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 (±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 (±nAChR antagonists). Behavioural activities were monitored after each of the three daily injections. Six groups of mice were treated with saline or cocaine (±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 (±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 α7 and non-α7 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 [^{3}H]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 microscales 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 α7 and non-α7 nAChRs, and of the high-affinity CHTs, in brain regions of saline and cocaine (±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 DHβE 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}·), DHβE+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

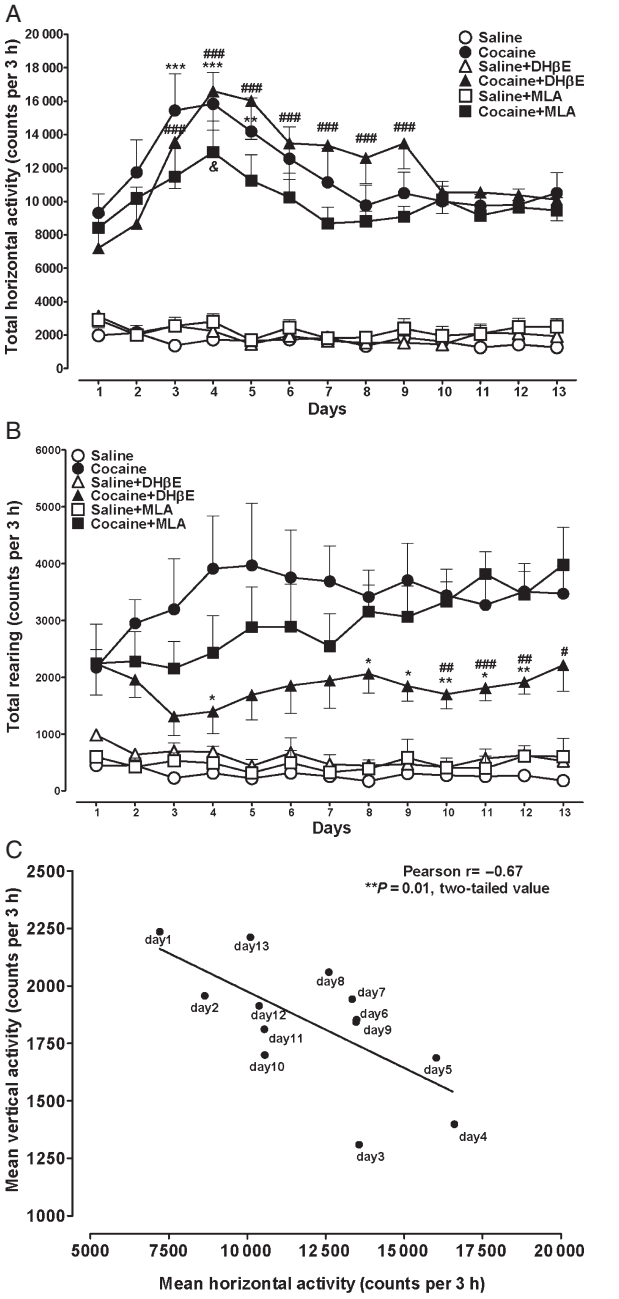

Figure 1

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 ± 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, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus MLA+cocaine treatment. (C) Correlation analysis between the mean daily vertical and horizontal activity of DHβE+cocaine-treated mice.

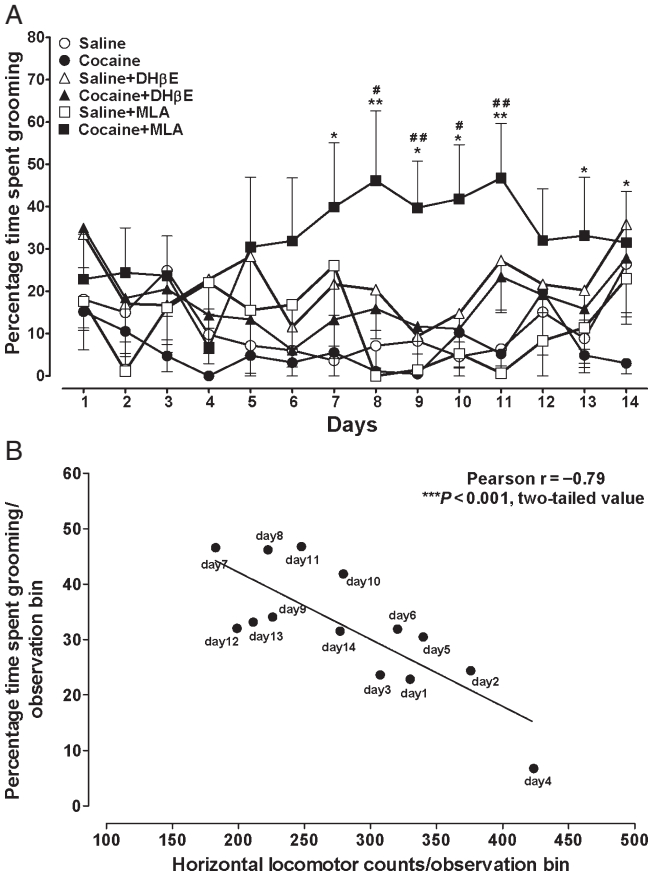

Figure 2

'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 ± SEM percentage time self-grooming of six to seven animals per group. Repeated measures ANOVA showed significant cocaine×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×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 (±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 [^{125}I]epibatidine, [^{125}I]α-bungarotoxin and [^{3}H]hemicholinium binding sites.

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

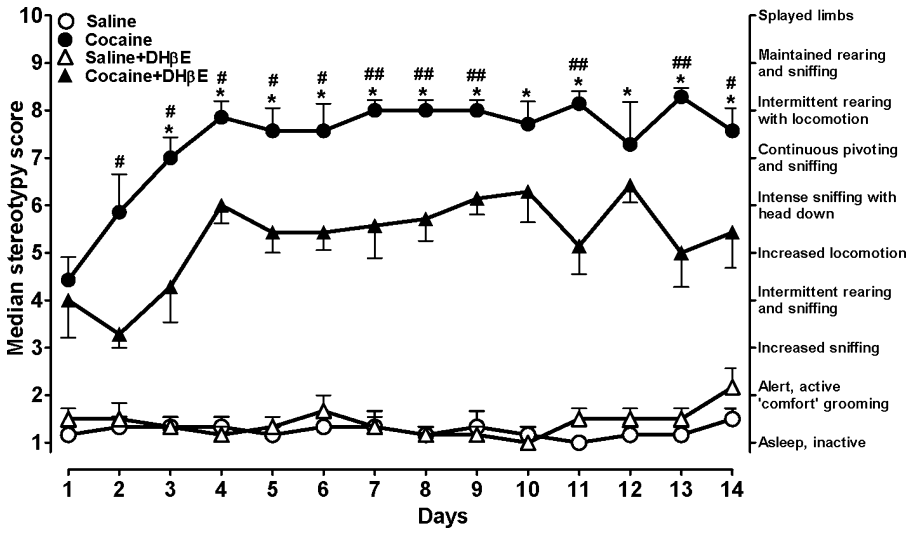

Figure 3

'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 (±DHβE). Data are presented as the median ± 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.

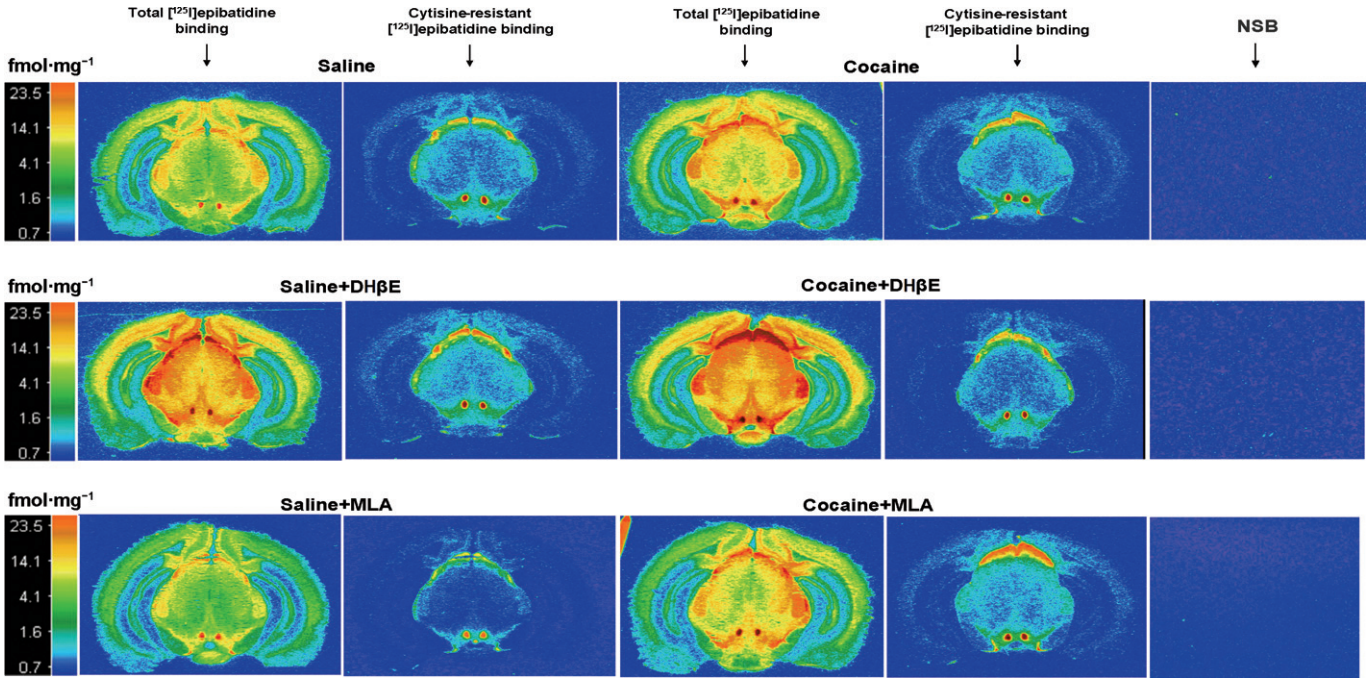

Figure 4

α4β2* nAChR binding is region-specifically increased in the VTA and the SNc following 'binge' cocaine treatment. Computer enhanced colour autoradiograms of total and cytisine-resistant [^{125}I]epibatidine binding in coronal brain sections of C57BL/6J mice treated with a chronic 'binge' saline or cocaine (±DHβE or MLA) administration protocol. The adjacent sections shown are from the level of the ventral tegmental area (bregma −3.16 mm). To label α4β2* receptors, sections were incubated for 2 h with 100 pmol of [^{125}I]epibatidine, alone or in the presence of 20 nmol cytisine. Specific α4β2* nAChR binding was calculated following the subtraction of cytosine resistant from total [^{125}I]epibatidine images. Adjacent sections were incubated with 300 μmol (–) 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 (±DHβE or MLA)-treated mice were processed in parallel.

treatment groups. Non-specific binding was indistinguishable from film background. For α4β2* nAChRs, two-way ANOVA revealed significant main effects of treatment and region, with no significant treatment×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 α4β2* 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 DHβE with either saline or cocaine increased α4β2* 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 DHβE-treated mice, administration of the antagonist did not further increase α4β2* 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, DHβE+cocaine versus MLA+cocaine, saline+DHβE versus DHβE+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×region interaction effects [$F_{(60,\ 429)} = 0.5$, $P > 0.05$] were observed on heteromeric, non-α4β2*-binding sites, following 14 days of 'binge' drug administration (Table 2; two-way ANOVA). For homomeric α7 nAChRs, there were no significant treatment [$F_{(5,\ 689)} = 1.1$, $P > 0.05$] or treatment×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 DHβE-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 DHβE+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+DHβE ($P < 0.05$) and DHβE+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 α7 and β2* nAChRs play distinct roles in modulating cocaine-induced behaviour. We show for the first time that the α7 preferring antagonist MLA induces a marked self-grooming behaviour in cocaine-treated mice, whereas the non-α7, β2* nAChR antagonist DHβE 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 α7 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 α7 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 α6 subunits (α6*) (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 α7, but also heteromeric α6* nAChRs, at least partially. Nevertheless, the lack of self-grooming in animals treated either with cocaine or the broad spectrum heteromeric nAChR antagonist DHβE (Jensen *et al.*, 2005) strongly indicates that α7 receptor inhibition is required for the manifestation of the grooming response. In other words, as both MLA and DHβE can inhibit α6* nAChRs, the differential effects of these antagonists must preferentially reveal α7- and β2*-mediated behaviours respectively. Thus, while the relative contribution of MLA-sensitive nAChRs to cocaine-induced behaviour can be further examined using specific α6* nAChR ligands or nicotinic subunit knockout mice, the current data argue for a predominant role of α7 nAChRs in modulating MLA+cocaine-induced grooming, by demonstrating that the behaviour does not develop in cocaine-treated mice, unless α7 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 α4β2* nAChRs, following chronic 'binge' saline and cocaine (± DHβE or MLA) administration

Region	Cytisine-sensitive [^{125}I]epibatidine binding (fmol·mg^{-1} tissue equivalent)					
	Saline	Cocaine	Saline+DHβE	Cocaine+DHβE	Saline+MLA	Cocaine+MLA
Olfactory tubercle	6.9 ± 0.5	6.4 ± 0.8	6.8 ± 0.5	9.4 ± 0.7	7.8 ± 0.8	6.3 ± 0.9
Prelimbic cortex	8.1 ± 0.9	6.6 ± 0.8	7.4 ± 0.6	8.0 ± 0.8	6.6 ± 0.5	8.2 ± 0.6
Cingulate cortex	7.7 ± 0.9	7.5 ± 0.7	9.1 ± 0.7	9.7 ± 0.9	7.8 ± 0.6	7.9 ± 0.4
Nucleus accumbens						
Core	6.2 ± 0.6	5.9 ± 0.5	7.4 ± 0.9	7.8 ± 0.4	6.8 ± 0.7	5.9 ± 0.5
Shell	6.1 ± 0.7	5.7 ± 0.5	6.5 ± 0.5	7.4 ± 0.3	6.8 ± 0.6	5.7 ± 0.6
Caudate putamen	8.1 ± 0.9	8.3 ± 0.7	9.5 ± 1.0	10.1 ± 0.9	9.0 ± 0.9	8.3 ± 0.5
Primary motor cortex	8.2 ± 0.6	7.6 ± 0.7	9.8 ± 0.7	10.2 ± 0.8	8.0 ± 0.6	8.2 ± 0.5
Secondary motor cortex	8.2 ± 0.7	7.4 ± 0.6	9.6 ± 0.7	10.3 ± 0.9	8.3 ± 0.9	8.2 ± 0.5
Hippocampus	3.0 ± 0.4	3.3 ± 0.4	3.4 ± 0.5	3.2 ± 0.4	2.7 ± 0.4	3.2 ± 0.3
Subiculum	13.4 ± 1.7	14.7 ± 1.8	14.6 ± 2.2	13.3 ± 1.2	12.1 ± 1.0	12.5 ± 1.4
Posterior thalamic nuclear group	13.4 ± 1.2	13.7 ± 1.1	16.2 ± 1.5	16.4 ± 1.6	16.2 ± 1.9	15.4 ± 2.6
Lateral posterior thalamic nucleus	13.7 ± 1.2	13.3 ± 1.0	16.6 ± 1.4	15.7 ± 1.5	15.9 ± 1.5	14.8 ± 2.5
Medial geniculate nucleus	12.8 ± 1.7	16.6 ± 1.6	18.5 ± 3.1**	18.9 ± 2.2**	14.0 ± 1.5	15.4 ± 1.5
Ventral lateral geniculate nucleus	12.1 ± 1.5	11.0 ± 1.4	13.6 ± 2.1	15.4 ± 1.8$^{\#}$	12.9 ± 2.1	13.0 ± 2.5
Dorsal lateral geniculate nucleus	14.0 ± 1.4	12.5 ± 1.4	16.3 ± 2.0	16.5 ± 4.5$^{\#}$	15.6 ± 2.0	14.8 ± 2.8
Visual cortex	8.1 ± 0.7	7.7 ± 0.3	9.2 ± 0.7	9.6 ± 0.5	8.5 ± 0.6	8.4 ± 0.9
Superficial grey layer of the superior colliculus	8.0 ± 2.0	12.1 ± 1.8	18.7 ± 3.7***	16.8 ± 2.8***,$^{\#}$	10.4 ± 3.5	12.8 ± 1.4*
Ventral tegmental area	9.7 ± 1.0	14.9 ± 1.4** (54%)	15.8 ± 2.3**	16.9 ± 2.0** (7%)	12.5 ± 1.6	14.1 ± 1.5* (13%)
Substantia nigra, pars compacta	12.0 ± 1.0	17.4 ± 1.6** (45%)	18.6 ± 3.0**	18.0 ± 1.8** (–3%)	14.7 ± 1.3	17.6 ± 1.5** (20%)

Animals were injected with cocaine (15.0 mg·kg^{-1}), cocaine+DHβE (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 cytisine-resistant [^{125}I]epibatidine binding was subtracted from total [^{125}I]epibatidine binding values in order to quantify the density of α4β2* nAChRs. The values of cytisine-sensitive [^{125}I]epibatidine binding represent the mean ± 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×region interaction effects ($P > 0.05$) on cytisine-sensitive [^{125}I]epibatidine binding. Within-region differences were examined using Fisher LSD *post hoc* tests. Percentage change in α4β2* nAChR binding between cocaine-treated mice and their respective vehicle controls is shown in parenthesis 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 cytisine-resistant nAChR binding, following chronic 'binge' saline and cocaine (±DHβE or MLA) administration

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

Values of cytisine-resistant [^{125}I]epibatidine binding represent mean ± 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×region interaction effects ($P > 0.05$) on cytisine-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 α7 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 α7 agonism may be a viable therapeutic approach for the anxiogenic effects of 'binge' cocaine. Impairment of α7 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 α7 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 (±MLA)-treated mice. The chronic administration of MLA, with or without cocaine, reduced [^{3}H]hemicholinium binding compared with saline and DHβE 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 hypocholinergic 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 α7 nAChRs, following chronic 'binge' saline and cocaine (±DHβE or MLA) administration

Region	[^{125}I]α-bungarotoxin binding (fmol·mg^{-1} tissue equivalent)					
	Saline	Cocaine	Saline+DHβE	Cocaine+DHβE	Saline+MLA	Cocaine+MLA
Frontal association cortex	20.4 ± 6.9	19.6 ± 6.3	19.7 ± 4.2	23.5 ± 4.5	12.6 ± 2.8	17.4 ± 1.9
Cingulate cortex	25.3 ± 5.6	21.4 ± 7.9	21.2 ± 2.9	25.5 ± 4.6	15.5 ± 3.1	20.4 ± 4.4
Caudate putamen	33.1 ± 8.5	31.2 ± 7.3	29.1 ± 4.2	37.6 ± 5.3	23.7 ± 5.1	29.2 ± 5.3
Endopiriform nucleus, dorsal	41.5 ± 6.9	46.0 ± 6.5	38.6 ± 8.7	49.6 ± 9.6	36.0 ± 9.0	36.4 ± 7.3
Primary motor cortex	30.6 ± 5.4	35.1 ± 7.0	26.4 ± 2.7	38.8 ± 3.6	19.6 ± 1.8	24.1 ± 4.5
Claustrum	40.6 ± 10.1	40.5 ± 5.9	28.1 ± 7.5	38.3 ± 5.5	30.9 ± 6.7	21.2 ± 3.6
Posterior thalamic nuclear group	28.6 ± 11.6	28.7 ± 7.0	23.3 ± 3.3	24.9 ± 4.8	20.9 ± 5.0	25.7 ± 5.1
Hippocampus, CA1	30.3 ± 4.9	32.3 ± 5.4	27.6 ± 8.3	33.7 ± 4.3	32.0 ± 8.5	25.8 ± 3.7
Hippocampus, CA3	34.6 ± 6.2	37.1 ± 5.1	33.2 ± 6.8	41.0 ± 6.3	34.8 ± 8.2	33.8 ± 5.8
Dentate gyrus	22.5 ± 6.1	32.5 ± 7.2	29.8 ± 2.9	28.0 ± 4.6	26.9 ± 6.0	24.6 ± 3.2
Basolateral amygdaloid nucleus	36.0 ± 8.7	34.6 ± 3.6	28.1 ± 6.6	35.7 ± 6.3	32.1 ± 6.5	34.9 ± 5.5
Posteromedial cortical amygdaloid nucleus	100.1 ± 23.1	67.6 ± 12.0	70.9 ± 12.0	90.9 ± 18.8	84.0 ± 14.6	69.7 ± 6.4
Medial amygdaloid nucleus	49.2 ± 9.7	41.3 ± 2.2	45.5 ± 9.5	46.2 ± 5.5	49.7 ± 13.0	52.9 ± 6.6
Dorsomedial hypothalamic nucleus	33.9 ± 9.7	29.3 ± 2.5	28.5 ± 3.5	38.3 ± 6.5	35.5 ± 11.8	39.5 ± 4.9
Ventromedial hypothalamic nucleus	30.5 ± 6.6	43.1 ± 6.2	43.7 ± 3.8	39.9 ± 6.6	46.1 ± 13.5	42.6 ± 4.3
Lateral hypothalamus	28.0 ± 4.4	19.4 ± 4.1	22.3 ± 2.8	20.5 ± 2.9	27.5 ± 4.6	25.3 ± 4.0
Zona incerta	41.9 ± 8.6	35.2 ± 9.3	35.9 ± 10.1	30.3 ± 4.8	29.9 ± 7.8	41.2 ± 7.3
Ventral lateral geniculate nucleus	54.0 ± 8.9	32.3 ± 4.9	52.9 ± 6.8	42.7 ± 6.5	53.9 ± 12.4	53.6 ± 7.9
Superficial grey layer of the superior colliculus	86.7 ± 12.8	89.9 ± 12.1	94.3 ± 10.8	94.3 ± 13.2	91.7 ± 16.2	91.2 ± 13.7
Ventral tegmental area	40.8 ± 4.7	41.8 ± 5.8	49.8 ± 3.2	50.7 ± 9.9	49.7 ± 6.5	46.4 ± 7.0
Substantia nigra	21.2 ± 6.1	37.5 ± 6.7	24.1 ± 4.1	31.7 ± 5.5	19.8 ± 4.0	25.8 ± 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 ± SEM specific binding of 3 nmol of [^{125}I]α-bungarotoxin, in brain regions of 6–7 animals per group. No significant treatment ($P > 0.05$) or treatment×region interaction effects ($P > 0.05$) were observed on α7 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-α7, β2* nAChR antagonist DHβE did not induce grooming, but led to distinct behavioural alterations in 'binge'-treated mice. The ability of DHβE 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 DHβE+cocaine-treated mice. The blockade of stereotypy sensitisation with DHβE 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 β2* 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 α4β2* nAChRs in the VTA and the SNc. Our binding data show a clear association between stereotypy and α4β2* nAChR regulation in the midbrain, as in groups of mice that showed large up-regulation of α4β2* nAChRs, compared with their respective vehicle controls (cocaine vs. saline), larger stereotypy scores were observed, whereas in animals with small alterations in α4β2* nAChR binding (DHβE+cocaine vs. DHβE+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 α4β2* 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 α4β2* nAChRs to the development of cocaine-

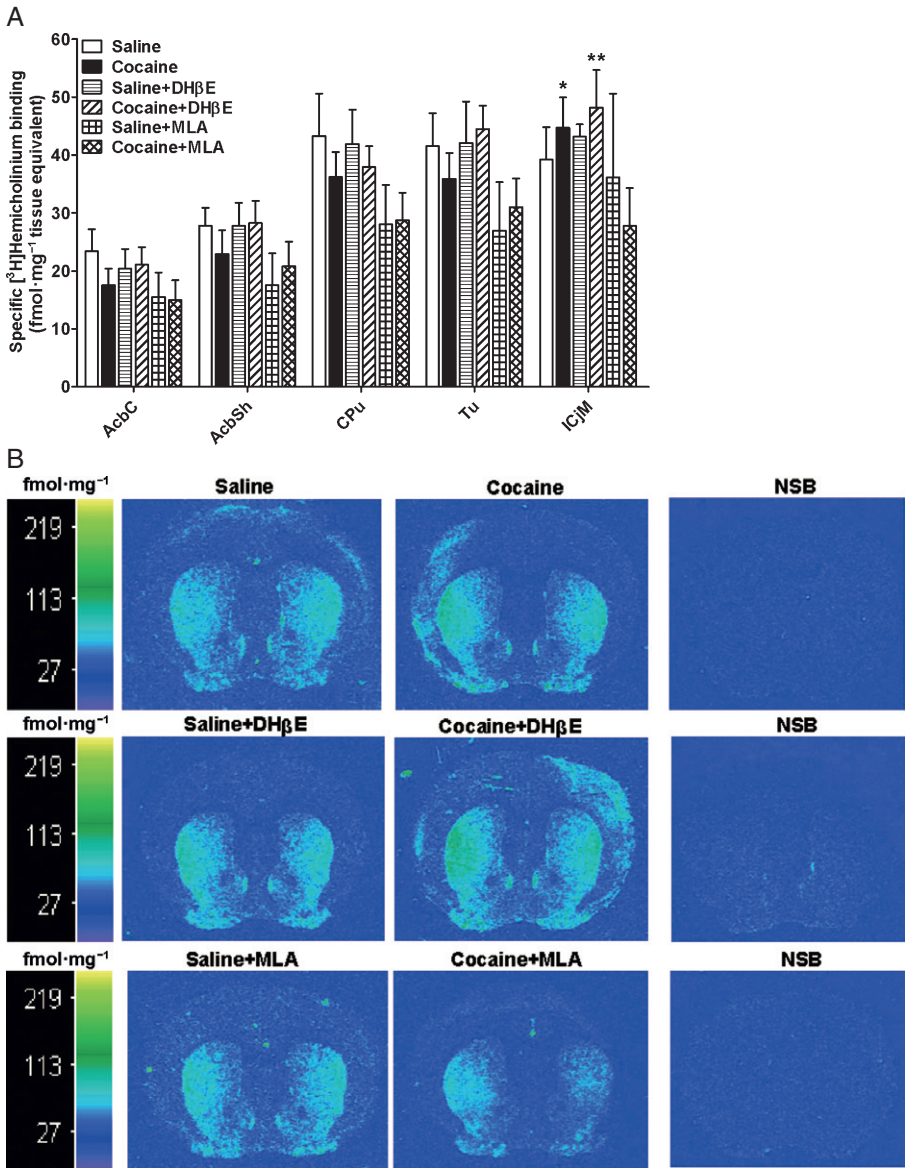

Figure 5

Binding to the high-affinity choline transporters is reduced following chronic MLA administration. (A) Data represent the mean ± SEM specific [^{3}H]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 [^{3}H]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 α4β2* nAChRs for the alleviation of motor stereotypy.

The behaviour of DHβE+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 DHβE administration, indicating that heteromeric β2* 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 Schoffelmeer *et al.* (2002), who showed that systemic DHβE 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 Schoffelmeer *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 β2* 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 β2* and α7 nAChRs in order to exert these effects.

The observation of increased α4β2* 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). α4β2* 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 DHβE 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 α4β2* up-regulation in mediating the behavioural effects of chronic cocaine. As in the case of nicotine-induced up-regulation of α4β2* 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 D_2 or D_3 receptors, which are activated following chronic exposure to cocaine, induces up-regulation of α4β2* 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 α7 nAChRs induces an excessive self-grooming behaviour during the repeated administration of cocaine. Second, the inhibition of non-α7, β2* 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.

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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.

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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 tartratewas delivered at a dose of 24 $mg \cdot kg^{-1} \cdot day^{-1}$, at a rate of 0.5 µl/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 ± 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+DHβE.

Movie S3 Cocaine+MLA.

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