Effects of blockade of α4β2 and α7 nicotinic acetylcholine receptors on cue-induced reinstatement of nicotine-seeking behaviour in rats

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Abstract
Exposure to environmental stimuli conditioned to nicotine consumption critically contributes to the high relapse rates of tobacco smoking. Our previous work demonstrated that non-selective blockade of nicotinic acetylcholine receptors (nAChRs) reversed the cue-induced reinstatement of nicotine seeking, indicating a role for cholinergic neurotransmission in the mediation of the conditioned incentive properties of nicotine cues. The present study further examined the relative roles of the two major nAChR subtypes, α4β2 and α7, in the cue-induced reinstatement of nicotine seeking. Male Sprague–Dawley rats were trained to intravenously self-administer nicotine (0.03 mg/kg/infusion, free base) on a fixed-ratio 5 schedule of reinforcement. A nicotine-conditioned cue was established by associating a sensory stimulus with each nicotine infusion. After nicotine-maintained responding was extinguished by withholding the nicotine infusion and its paired cue, reinstatement test sessions were conducted with re-presentation of the cue but without the availability of nicotine. Thirty minutes before the tests, the rats were administered the α4β2-selective antagonist dihydro-β-erythroidine (DHβE) and α7-selective antagonist methyllycaconitine (MLA). Pretreatment with MLA, but not DHβE, significantly reduced the magnitude of the cue-induced reinstatement of responses on the active, previously nicotine-reinforced lever. In different sets of rats, MLA altered neither nicotine self-administration nor cue-induced reinstatement of food seeking. These results demonstrate that activation of α7 nAChRs participates in the mediation of the conditioned incentive properties of nicotine cues and suggest that α7 nAChRs may be a promising target for the development of medications for the prevention of cue-induced smoking relapse.

Introduction
Tobacco smoking is a leading preventable cause of death in the United States. Currently, approximately 46 million American adults are smokers, representing approximately 20% of the population (Jemal et al., 2011). The majority of smokers (approximately 80%) who attempt to quit on their own return to smoking within 1 month. Each year, only 3% of smokers quit successfully (Benowitz, 2010; Shiffman et al., 1998). The high relapse rates of tobacco smoking present a formidable challenge for the success of smoking cessation efforts.

Drug-associated environmental cues critically contribute to the maintenance of, and relapse to, drug-seeking behaviour (Caggiula et al., 2001; Conklin et al., 2010; O’Brien et al., 1998). Tobacco smoking is particularly effective in establishing the incentive properties of associated environmental cues because smoking rituals contain more drug-cue pairings than other drugs of abuse. Smoking-related cues (e.g. the visual and olfactory stimuli associated with each puff) elicit subjective states that can trigger smoking and nicotine-seeking behaviour (Caggiula et al., 2001; Carter and Tiffany, 1999; Conklin et al., 2008;
Alkondon and Albuquerque, 1993; Colquhoun and Patrick, 1997; Flores et al., 1992; Glennon and Dukat, 2012; Miranda et al., 2008; Niaura et al., 1988; O'Cui et al., 2013; Garcia-Rodriguez et al., 2012; Gass et al., 2012; Miranda et al., 2008; Niaura et al., 1988; O'Brien et al., 1998; Parker and Gilbert, 2000; Rose, 2006; Tong et al., 2007; Winkler et al., 2011; Zhou et al., 2009). Accumulating data from animal studies have demonstrated a significant contribution of nicotine-associated cues to the resumption of nicotine-seeking behaviour (Abdolahi et al., 2010; Chiamulera et al., 2010; Cohen et al., 2005; Feltenstein et al., 2012; Fowler and Kenny, 2011; LeSage et al., 2004; Liu, 2010; Liu et al., 2006, 2008; Paterson et al., 2005; Shaham et al., 1997). Despite our increasing knowledge of the significance of cue exposure in triggering smoking relapse, little is known about the neurobiological mechanisms that underlie the motivational effects of nicotine cues.

Nicotine exerts its reinforcing actions by activating nicotinic acetylcholine receptors (nAChRs). To date, 12 nAChR subunits have been identified: nine α-subunits (α2–α10) and three β-subunits (β2–β4). These subunits assemble nAChRs into either heteromeric (α- and β-subunits) or homomeric (α-subunit only) combinations (Dani and Bertrand, 2007; Gotti and Clementi, 2004; McGehee and Role, 1995; Sargent, 1993). Although more nAChR subtypes continue to be identified, heteromeric α4β2- and homomeric α7-containing receptors are the most abundant and widespread, comprising more than 90% of the nAChRs in the brain (Albuquerque et al., 2009; Flores et al., 1992; Gotti and Clementi, 2004; Millar and Gotti, 2009; Sargent, 1993; Zoli et al., 1998). These two major nAChR subtypes show considerable differences in many aspects, including localization, density, functional characteristics (e.g. kinetics of activation, desensitization and recovery from desensitization), and Ca²⁺ permeability (Albuquerque et al., 1997; Alkondon and Albuquerque, 1993; Colquhoun and Patrick, 1997; Flores et al., 1992; Glennon and Dukat, 2000; Lippiello, 1989; McGehee and Role, 1995; Papke and Thomschmidt, 1998; Tribollet et al., 2001; Wonnacott et al., 2006). Accumulating evidence has established a pivotal role for α4β2 nAChRs in the mediation of the reinforcing actions of nicotine (Exley and Cragg, 2008; Levin et al., 2010; Mineur and Picciotto, 2008; O’Connor et al., 2010; Rezvani et al., 2010; Tapper et al., 2004; Tobey et al., 2012; Vieyra-Reyes et al., 2008; Watkins et al., 1999; Wonnacott et al., 2005). In contrast, research on α7 nAChRs has been inconclusive; most studies have not established the necessity of these receptors for nicotine reward (e.g. Grottick et al., 2000; Pons et al., 2008; Stolerman et al., 2004; van Haaren et al., 1999; Walters et al., 2006), whereas several other studies have reported the involvement of α7 nAChRs in nicotine reinforcement (Besson et al., 2012; Brunzell and McIntosh, 2012; Markou and Paterson, 2001).

Recently, our laboratory demonstrated that mecamylamine, a nonselective nAChR antagonist, effectively reversed the cue-induced reinstatement of nicotine-seeking behaviour (Liu et al., 2007a). These results extended the role of nicotinic neurotransmission in the mediation of the reinforcing actions of nicotine to the conditioned motivational properties of nicotine-associated cues. Building on this line of research, the present study further targeted the α4β2 and α7 subtypes of nAChRs to determine their involvement in the mediation of the conditioned motivation exerted by nicotine cues. Specifically, the present study used an extinction–reinstatement model of relapse to examine the effects of the α4β2-selective antagonist dihydro-β-erythroidine (DHβE) and α7 nAChR-selective antagonist methyllycaconitine (MLA) on the cue-induced reinstatement of nicotine-seeking behaviour.

Methods

Subjects

Male Sprague–Dawley rats (Charles River, USA), 201–225 g upon arrival, were used. The animals were individually housed in a humidity- and temperature-controlled (21–22 °C) colony room on a reverse light/dark cycle (lights on 20:00 hours, lights off 08:00 hours). After 1 wk of habituation, the rats were placed on a food-restriction regimen (20 g chow/day) throughout the experiments, which allowed the rats to have consistent but low weight gain at approximately 85% of their free-feeding condition. The rats had unlimited access to water. The training and experimental sessions were conducted during the dark phase at the same time each day (09:00 hours–15:00 hours). All of the experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

Self-administration apparatus

Experimental sessions were conducted in standard operant conditioning chambers located inside sound-attenuating, ventilated cubicles (Med Associates, USA). The chambers were equipped with two retractable response levers on one side panel, a 28 V white
light above each lever, and a red house light on top of the chambers. Between the two levers was a food pellet trough. Intravenous nicotine injections were delivered by a drug delivery system with a syringe pump (model PHM100-10 r/min, Med Associates, USA). Experimental events and data collection were automatically controlled by an interfaced computer and software (Med-PC version IV, Med Associates, USA).

**Lever-press training**

To facilitate the learning of operant responding for nicotine self-administration (see below), the rats underwent lever-press training. One day after the beginning of the food-restriction regimen, the rats were placed in the experimental chambers, and the training sessions began with the introduction of the levers. Responding on the active lever was rewarded with the delivery of a food pellet (45 mg). The sessions lasted 1 h, with a maximum delivery of 45 food pellets on a fixed-ratio 1 (FR1) schedule. After the rats learned to respond, the reinforcement schedule was increased to FR5. The training ended after the rats earned 45 food pellets on the FR5 schedule. Successful lever-press training with food pellets as reinforcers was achieved within 2–5 sessions.

**Surgery**

Intravenous catheterization was performed after food training. The rats were anesthetized with isoflurane (1–3% in 95% O₂ and 5% CO₂). An indwelling catheter was inserted into the right external jugular vein. The catheters were constructed using a 15 cm piece of Silastic tubing (0.31 mm inner diameter, 0.63 mm outer diameter; Dow Corning Corporation, USA) attached to a 22-gauge stainless-steel guide cannula. The latter was bent and molded onto a durable polyester mesh (Plastics One, USA) with dental cement and became the catheter base. Through an incision on the rat’s back, the base was anchored underneath the skin at the level of the scapulae, and the catheter passed subcutaneously to the ventral lower neck region and inserted into the right jugular vein (3.5 cm). The animals were allowed at least 7 d to recover from surgery. During the recovery period, the catheters were flushed daily with 0.1 ml of sterile saline that contained heparin (30 U/ml) and Timentin (66.7 mg/ml) to maintain catheter patency and prevent infection. Thereafter, the catheters were flushed with heparinized saline before and after the experimental sessions.

**Nicotine self-administration and conditioning training**

After recovery from intravenous catheterization surgery, the rats were subjected to nicotine self-administration and conditioning training sessions. In the daily 1 h training sessions, the rats were placed in the operant conditioning chambers and connected to the intravenous drug infusion system. The sessions began with extension of the two levers and illumination of the red house light. Once the rats reached the FR requirement at the active lever, an infusion of nicotine (0.03 mg/kg, free base) was delivered in a volume of 0.1 ml in approximately 1 s, depending on the rats’ body weights. To establish a nicotine-conditioned cue, each nicotine infusion was paired with the presentation of an auditory/visual stimulus that consisted of a 5 s tone and 20 s illumination of the light above the active lever. A 20 s timeout period followed each nicotine infusion, during which time responses were recorded but not reinforced. An FR1 schedule was used for days 1–5, an FR2 schedule was used for days 6–8, and an FR5 schedule was used for the remaining days of the experiments. Throughout the experiments, responses at the inactive lever were recorded, but had no programmed consequences. Stable nicotine self-administration was considered to be established once the rats self-administered ≥10 infusions per session with ≤20% variation for at least three consecutive sessions. Four rats failed to meet the criterion and were eliminated from the subsequent experimental procedures.

**Extinction**

After the completion of the self-administration and conditioning training phase, the rats were subjected to extinction sessions. In the daily sessions, nicotine-maintained lever responding was extinguished by withholding nicotine and its associated cue. Responses on the active lever resulted in the delivery of saline rather than nicotine, and the cue was not presented. The FR5 schedule and 20 s timeout period were still in effect for saline infusions. The criterion for extinction was three consecutive sessions in which the number of responses per session was ≤20% of the responses averaged across the last three sessions of the self-administration and conditioning training phase.

**Reinstatement**

One day after the final extinction session, reinstatement tests were performed, in which introduction of the two levers and illumination of the red house light signaled
the beginning of the sessions. Immediately after the sessions began, a single response-noncontingent cue was presented to inform the rats of the availability of the nicotine cue. Throughout the test sessions, responses on the active lever on the FR5 schedule resulted in re-presentation of the cue and delivery of saline rather than nicotine. Responses on the inactive lever were recorded, but had no programmed consequences. The test sessions lasted 1 h.

Test 1, effects of DHβE and MLA on cue-induced reinstatement of nicotine seeking

After completion of extinction phase, rats were divided into two groups in a pseudo-random manner (n=10 each group) based on similar lever responses emitted during the self-administration and conditioning phases. Thirty minutes before the reinstatement test sessions, one group of rats was subcutaneously administered DHβE (0, 3 and 9 mg/kg), and the other group was intraperitoneally administered MLA (0, 2.5 and 10 mg/kg). The DHβE and MLA pretreatments were scheduled in a within-subjects Latin-square design in the respective groups. For both groups, the reinstatement test sessions were separated by two daily extinction sessions to determine the extinction baseline before each reinstatement test.

Test 2, effects of MLA on nicotine self-administration

Eight rats were used for testing effects of MLA on nicotine self-administration. After stable nicotine self-administration was established, the test sessions began. Thirty minutes before tests, MLA (0, 2.5 and 10 mg/kg) was intraperitoneally administered in a within-subjects Latin-square design. The test sessions were separated by two no-drug-treatment sessions.

Test 3, effects of MLA on cue-induced food seeking

Eight rats were trained to self-administer food pellets and a food-conditioned cue was established under conditions identical to that for nicotine rats, except that food pellets rather than nicotine infusions were delivered upon lever responses. After the food-maintained responses were extinguished, effects of MLA on cue-induced reinstatement of food seeking were examined. In the reinstatement test sessions, responses on the active lever resulted in presentations of the food cue on the FR5 schedule while there was no availability of food pellets. Thirty minutes before the test sessions, MLA (0, 2.5 and 10 mg/kg) was intraperitoneally administered in a within-subjects Latin-square design. The test sessions were separated by two extinction sessions.

Statistical analyses

The data are expressed as the mean±S.E.M. number of lever responses and nicotine infusions earned. The self-administration data averaged across the final three sessions were analysed using one-way analysis of variance (ANOVA). The data obtained in the extinction phase were analysed using two-way repeated ANOVA with session as the within-subjects factor and antagonist as the between-subjects factor. The data collected from the reinstatement tests with the two antagonists were separately analysed using one-way repeated-measures ANOVA with drug dose as the within-subjects factor. Differences among individual means were verified by subsequent Newman–Keuls post-hoc tests.

Results

Nicotine self-administration and extinction

The rats successfully acquired stable levels of nicotine self-administration in the 25 daily 1 h self-administration and conditioning training sessions. Averaged across the final three sessions, the rats (n=20) emitted a mean±S.E.M. number of responses of 81.8±14.7 on the active lever and 9.3±2.2 on the inactive lever. The animals correspondingly self-administered 14.3±2.2 infusions of nicotine at a unit dose of 0.03 mg/kg/infusion. Because grouping the rats for the subsequent nicotine-seeking tests was performed in a pseudo-random manner, the two groups for the following reinstatement/antagonist test had similar levels of responses on the active lever (F_{1,18}=0.10, p=0.76) and number of nicotine infusions (F_{1,18}=0.09, p=0.81). Details are shown in Table 1.

In the extinction phase, although these two groups did not differ (F_{1,18}=0.01, p=0.93), there was a significant effect of sessions (F_{9,162}=24.10, p<0.0001). Responses on the active lever in the two groups of rats similarly decreased across the daily sessions, indicating extinction of nicotine-seeking responses. All of the rats reached the extinction criterion in 10 d. Table shows the numbers of lever responses in the last extinction session.
Effects of DHβE on cue-induced reinstatement of nicotine-seeking behaviour

As shown in Fig. 1, DHβE pretreatment did not change lever-press responses in the cue-induced reinstatement tests conducted after extinction. The one-way repeated-measure ANOVA of the number of active lever responses did not reveal a significant effect of DHβE dose ($F_{2,18} = 0.51, p = 0.61$). Responses on the inactive lever remained unchanged.

Effects of MLA on cue-induced reinstatement of nicotine-seeking behaviour

Figure 2 shows a suppressant effect of MLA pretreatment on the cue-induced reinstatement of responses on the active, previously nicotine-reinforced lever. The one-way repeated-measure ANOVA of the number of active lever responses revealed a significant effect of MLA dose ($F_{2,18} = 13.23, p < 0.001$). Further Newman–Keuls post-hoc tests confirmed significant differences in the number of active lever responses between the 2.5 mg/k and 10 mg/kg ($p < 0.05$) and 10 mg/kg ($p < 0.01$) doses of MLA and the saline control condition and between the 10 mg/kg ($p < 0.05$) and 2.5 mg/kg doses of MLA, indicating a dose-dependent suppressant effect of MLA pretreatment on the cue-induced reinstatement of nicotine-seeking responses. However, responses on the inactive lever remained low and indistinguishable among the different dose conditions.

Effects of MLA on nicotine self-administration

MLA pretreatment did not change lever responses for nicotine self-administration (Table 2). There was

### Table 1. Similar lever-press response profiles in the two antagonist test groups either averaged across the final three sessions of the self-administration/conditioning phase or obtained from the last session of the extinction phase

<table>
<thead>
<tr>
<th>Group</th>
<th>DHβE (n=10)</th>
<th>MLA (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-administration/conditioning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active lever responses</td>
<td>82±16</td>
<td>79±13</td>
</tr>
<tr>
<td>Inactive lever responses</td>
<td>10±4</td>
<td>8±3</td>
</tr>
<tr>
<td>Extinction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active lever responses</td>
<td>15±5</td>
<td>16±5</td>
</tr>
<tr>
<td>Inactive lever responses</td>
<td>7±3</td>
<td>6±3</td>
</tr>
</tbody>
</table>

### Table 2. Methyllycaconitine pretreatment changed neither nicotine self-administration nor cue-induced reinstatement of food seeking. The numbers of responses on the active lever were presented

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>2.5 mg/kg</th>
<th>10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine self-administration (n=8)</td>
<td>91±14</td>
<td>95±21</td>
<td>87±12</td>
</tr>
<tr>
<td>Cue-induced food seeking (n=8)</td>
<td>45±9</td>
<td>36±10</td>
<td>42±8</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of dihydro-β-erythroidine pretreatment on lever-press responses in the reinstatement test sessions in rats (n=10). The data are expressed as the mean±S.E.M. number of lever responses made during the 1 h test sessions.

Fig. 2. Effect of methyllycaconitine pretreatment on lever-press responses in the reinstatement test sessions in rats (n=10). The data are expressed as the mean±S.E.M. number of lever responses made during the 1 h test sessions. *p<0.05, **p<0.01, significant difference from saline control condition (0); +p<0.05, significant difference from 2.5 mg/kg.
no significant effect of MLA dose on the number of responses on the active lever ($F_{2,14}=0.66$, $p=0.53$).

**Effects of MLA on cue-induced reinstatement of food seeking**

As shown in Table 2, lever responses made by the food-trained rats in the cue-induced reinstatement tests were not altered by pretreatment with MLA. A one-way repeated-measures ANOVA of the number of active lever responses did not reveal a significant effect of MLA dose ($F_{2,14}=0.20$, $p=0.82$).

**Discussion**

Based on our previous work, in which nonselective blockade of nAChRs attenuated the cue-induced reinstatement of nicotine-seeking behaviour (Liu et al., 2007a), the present study further examined the relative roles of $\alpha_4\beta_2$ and $\alpha_7$ nAChRs in the behaviour-related motivational effects of nicotine-conditioned cues. The results demonstrated that the $\alpha_7$-selective antagonist MLA but not $\alpha_4\beta_2$-selective antagonist DHβE effectively suppressed the cue-induced reinstatement of nicotine-seeking responses. These results indicate that cholinergic neurotransmission via activation of the $\alpha_7$ subtype of nAChRs plays a role in the mediation of the conditioned incentive properties of nicotine cues measured in the extinction-reinstatement procedure. Therefore, manipulation of $\alpha_7$ nAChR activity may prove to be a promising target for the development of pharmacotherapies for the prevention of smoking relapse triggered by exposure to environmental cues.

In the reinstatement test sessions, responses on the previously nicotine-reinforced, active lever were significantly attenuated after MLA pretreatment, indicating a suppressant effect of MLA on cue-induced reinstatement of nicotine-seeking behaviour. The involvement of $\alpha_7$ nAChRs in the conditioned motivational effect of nicotine cues is specific because of the following reasons. Pretreatment with MLA changed neither responses on the inactive lever during these reinstatement tests nor nicotine self-administering responses tested in a different group of rats. In the sessions to test cue-induced reinstatement of food seeking, MLA pretreatment did not alter lever responses, which is consistent with our previous work demonstrating that nonselective antagonism of nAChRs by mecamylamine changed neither food self-administration nor food-seeking responses reinstated by re-presentation of a food-associated cue (Liu et al., 2007a), indicating that nAChRs may not critically participate in food reinforcement and its related associative learning process. Moreover, MLA at 10 mg/kg, the highest dose used in the present study, did not alter nicotine-enhanced lever pressing in response to presentation of an intrinsically reinforcing sensory stimulus (Liu et al., 2007b). Altogether, these data exclude the possibility that MLA nonspecifically impaired general locomotor activity, arousal state, the motivation to earn rewards, operant goal-directed behaviour and the conditioned effect of food-conditioned cues. Therefore, the present results demonstrate a specific suppressant effect of MLA on the cue-induced reinstatement of nicotine seeking and suggest that activation of $\alpha_7$ nAChRs may be required for the expression of conditioned incentive motivation induced by nicotine-related cues. A role for $\alpha_7$ nAChRs in the effects of nicotine cues is consistent with evidence obtained from studies that used other learning-assessment paradigms. For example, Quarta et al. (2009) found that MLA attenuated the discriminative stimulus effect of nicotine, and dopamine release in the striatal region appeared to be involved in this effect. The deletion of $\alpha_7$ nAChRs was reported to impair responses in an appetitive learning task established by a natural reward, sucrose (Keller et al., 2005). A recent study showed that activation of $\alpha_7$ nAChRs participated in trace eyeblink conditioning, a hippocampus-dependent conditioning process (Brown et al., 2010).

There have been several studies examining the issue of whether $\alpha_7$ nAChRs are required for the primary reinforcing effects of nicotine. Two earlier studies tested the effects of $\alpha_7$ nAChR blockade on operant intravenous nicotine self-administration. One study showed that MLA did not interfere with nicotine self-administration (Grottick et al., 2000), but the other study demonstrated a suppressant effect of MLA on nicotine self-administration (Markou and Paterson, 2001). Conditioned place preference studies negated a possible role for $\alpha_7$ nAChRs in the mediation of nicotine reward. For example, mice that were either treated with MLA or deficient in $\alpha_7$ nAChRs developed nicotine-induced conditioned place preference at a level similar to their control counterparts (Grabus et al., 2006; Walters et al., 2006). A recent study, in which mice self-administered nicotine directly into the ventral tegmental area, showed that MLA pretreatment decreased self-administration responses in wildtype animals, whereas $\alpha_7$ nAChR knockout mice self-administered less nicotine only when nicotine unit doses were low (Besson et al., 2012). In contrast, Brunzell and McIntosh (2012) found that the $\alpha_7$ nAChR-selective antagonist...
α-conotoxin ArlB [VIIL,Vl6D], when microinjected into rat nucleus accumbens shell and anterior cingulate cortex, significantly increased nicotine self-administration behaviour under a progressive-ratio schedule of reinforcement. The results of the present study showing a lack of effect of MLA pretreatment on lever responses for nicotine self-administration suggest that activation of α7 nAChRs may not play an indispensable role in the mediation of nicotine primary reinforcement.

Although α4/2 nAChRs play a pivotal role in the mediation of the reinforcing effects of nicotine, neurotransmission via these receptors is not required for the expression of the behavioural motivational effects of nicotine cues. In the present study, DHβE pretreatment did not interfere with the cue-induced reinstatement of nicotine-seeking responses. The doses used should be sufficient to antagonize the receptors because such a dose range has often been used in the literature, including self-administration studies (e.g. Grottick et al., 2000; Paterson et al., 2010; Watkins et al., 1999) and our own work, that showed that 1–9 mg/kg DHβE effectively decreased nicotine-enhanced lever-pressing in response to the presentation of a reinforcing stimulus (Liu et al., 2007b). Therefore, cholinergic neurotransmission via activation of α4/2 nAChRs does not appear to be required for the mediation of conditioned incentive motivation elicited by nicotine cues. These results are consistent with three other studies published recently. Varenicline, a partial agonist at α4/2 nAChRs, had no effect on the cue-induced reinstatement of nicotine seeking assessed using similar extinction–reinstatement procedures in rodents (O’Connor et al., 2010; Wouda et al., 2011) and did not change cue-specific craving in smokers (Gass et al., 2012). However, varenicline after a longer pretreatment time did reduce the ability of nicotine cue to reinstate nicotine seeking in rats (Le Foll et al., 2012) and in a 3 wk treatment regimen in smokers without abstinence diminish smoking cue-elicited craving (Franklin et al., 2011). Interestingly, Wouda et al. (2011) found that varenicline effectively attenuated the cue-induced reinstatement of alcohol-seeking behaviour. Together with another report (Guillem and Peoples, 2010), in which varenicline at lower doses reduced the cue-induced reinstatement of cocaine-seeking, these results suggest a role for α4/2 nAChRs in the motivational effects of cues conditioned to alcohol and cocaine but not nicotine. Elucidating such a significant difference between nicotine and other drugs of abuse and the involvement of associative learning and memory processes warrants future studies.

Notably, α4/2 and α7 nAChRs play differential roles in nicotine-induced reinforcement and the conditioned reinforcement induced by nicotine cues. α4/2 nAChRs appear to participate in nicotine reinforcement but not conditioned reinforcement induced by nicotine cues, whereas α7 nAChRs do the opposite. The differential involvement of these two nAChR subtypes indicates a dissociation of the neurobiological mechanisms that underlie the primary reinforcing actions of nicotine and secondary reinforcement induced by nicotine cues. This hypothesis is supported by a recent study. O’Connor et al. (2010) reported that the α4/2 nAChR partial agonist varenicline suppressed nicotine self-administration and the reinstatement of nicotine seeking induced by nicotine priming and the combination of nicotine and its cue but did not affect reinstatement induced by the nicotine cue alone. Such a dissociation was also revealed at the opioidergic neurotransmission level. Our previous study showed that nonselective blockade of opioid receptors by naltrexone attenuated the cue-induced reinstatement of nicotine seeking but had no effect on nicotine self-administration (Liu et al., 2009). A similar dissociation was found with other drugs of abuse and signaling pathways. For example, we reported that the inhibition of nitric oxide synthesis attenuated the conditioned reinstatement of ethanol seeking but not the primary reinforcing actions of ethanol (Liu and Weiss, 2004). Similarly, Martin-Fardon et al. (2007) found that antagonism of orphan sigma-1 receptors reversed cue-induced cocaine-seeking but did not change cocaine self-administration. Even in cases in which one drug produced effects on both conditioned and primary reinforcement, the sensitivity of the effect was different. For example, responding motivated by stimuli conditioned to cocaine was more sensitive to glutamate antagonists than behaviour maintained by cocaine itself (Baptista et al., 2004; Newman and Beardsley, 2006). Therefore, the conditioned incentive properties of nicotine cues and primary reinforcing actions of nicotine may be mediated by different neurobiological substrates.

Finally, the brain exhibits wide expression of α7 nAChRs, with dense distribution in regions responsible for associative learning and memory, such as the nucleus accumbens, amygdala, hippocampus, ventral tegmental area and cortex (Clarke et al., 1985; Fu et al., 2000; Jones and Wonnacott, 2004; Quik et al., 2000). Specifically, in addition to postsynaptic regions, α7 nAChRs are located at presynaptic and perisynaptic sites and implicated in the regulation of the release of several neurotransmitters, including dopamine, acetylcholine, norepinephrine.
An increasing number of animal studies, including our own work, have identified some of the neuropharmacological substrates responsible for the cue-induced reinstatement of nicotine seeking. The behavioural motivational effect of nicotine cues was suppressed by antagonists selective for dopamine D1, D2 (Liu et al., 2010) and D3 receptors (Khaled et al., 2010), noradrenergic α1 (Forget et al., 2010) and β receptors (Chiamulera et al., 2010), cannabinoid CB1 receptors (Cohen et al., 2005; De Vries et al., 2005; Shoab, 2008), metabotropic glutamate receptor (mGluR) 1 (Dravolina et al., 2007) and mGluR5 (Bespalov et al., 2005), ionotropic glutamate N-methyl-D-aspartate receptors (NMDAR) (Pechnick et al., 2011) and T-type Ca2+ channels (Uslaner et al., 2010). The behavioural motivational effect of nicotine cues was also suppressed by a nonselective opioid receptor antagonist (Liu et al., 2009), mGluR2/3 agonist (Liechti et al., 2007), GABAβ receptor agonist (Paterson et al., 2005) and α-type peroxisome proliferator-activated receptor agonist (Panlilio et al., 2012). Interestingly, Li et al. (2012) found that interruption of α7 nAChR-NMDAR complex formation blocked cue-induced reinstatement of nicotine-seeking responses. These studies, together with the present demonstration of α7 nAChR involvement, highlight an array of biological signaling pathways that are responsible for the mediation of the cue-induced reinstatement of nicotine-seeking behaviour and provide insights into the mechanisms that underlie the conditioned incentive properties of nicotine cues. Building on our own work that demonstrated the involvement of both dopamine D1/D2 receptors and α7 nAChRs in the effects of nicotine-related cues (Liu et al., 2010 and this study) and evidence of the significance of ventral striatal (especially the nucleus accumbens core) dopaminergic neurotransmission in the conditioned motivational processes associated with other drugs of abuse (e.g. cocaine, heroin and alcohol) and natural rewards (e.g. food) (Alvarez-Jaimes et al., 2008; Bossert et al., 2007; Cacciapaglia et al., 2012; Chaudhri et al., 2010; Floresco et al., 2008; Fuchs et al., 2004; Hutcheson et al., 2001), a signaling cascade from α7 nAChRs to dopamine receptor activation in the nucleus accumbens region may be hypothesized to mediate the motivational effect of nicotine cues. This hypothesis remains to be tested.

Acknowledgments

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Statement of Interest

None.

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