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Nicotinic acetylcholine receptors are required for the conditioned reinforcing properties of sucrose-associated cues

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Abstract

Rationale—We recently demonstrated that blocking specific nicotinic acetylcholine receptors (nAChRs) abolishes the conditioned reinforcing properties of ethanol-associated cues in rat, suggesting nAChRs as promising pharmacological targets for prevention of cue-induced relapse.

Objectives—The present study investigated the involvement of nAChR subtypes in the conditioned reinforcing properties of stimuli associated with a natural reward (sucrose).

Methods—Water-deprived rats were trained to associate a tone + light stimulus (CS) with the presentation of a 0.1 M sucrose solution for 10 consecutive days. On the subsequent day, the animals were tested on the stringent acquisition of a new instrumental response with conditioned reinforcement, following a systemic injection of the nonselective nAChR antagonist mecamylamine (MEC) or the selective $\alpha 7$ and $\alpha 6/\alpha 3\beta 2\beta 3^*$ nAChR antagonist methyllycaconitine (MLA). At testing, the rats were presented with two novel levers. Responding on the lever assigned as active (CR lever) resulted in a presentation of the CS alone, while pressing the inactive lever (NCR lever) had no programmed consequences.

Results—Control animals pressed the CR lever significantly more than the NCR lever, demonstrating that the CR had acquired conditioned reinforcing properties. Systemic MEC as well as MLA reduced the CR lever responses to the same level as for the NCR lever.

Conclusions—These results demonstrate a role for the $\alpha 7$ and/or $\alpha 6/\alpha 3\beta 2\beta 3^*$ nAChRs in conditioned reinforcement to a natural reward and suggest neuronal nAChRs as common mediators of the impact of cues on incentive processes.

Keywords

Nicotinic acetylcholine receptor; Conditioned reinforcement; Addiction; Reward; Rat

Introduction

Reward-associated stimuli or cues acting as conditioned reinforcers exert motivational control over natural and drug reinforcers and potentially enhance appetitive goal-directed behavior. In humans, cues associated with drugs or palatable foods can elicit craving and consumption and, for drug reinforcers, precipitate relapse (Childress et al. 1993; O'Brien et al. 1998; Weingarten 1983). In laboratory animals, such stimuli can reinstate drug seeking (Grimm et al. 2001; See 2002) and promote eating as well as other reward-motivated behaviors (Cardinal et al. 2002; Everitt et al. 1999; Holland et al. 2002; Petrovich et al. 2002). Thus, understanding the neurobiological mechanisms by which reward-associated stimuli come to act as conditioned reinforcers and stimulate appetitive behaviors has important clinical implications for the treatment of addiction and eating disorders.

Reward-related stimuli have the ability to promote firing of cholinergic mesopontine projections to the ventral tegmental area (VTA; Dormont et al. 1998; Pan and Hyland 2005). In this way, these projections are suggested to regulate the phasic activity of dopamine neurons in response to salient cues that promote reward-seeking behaviors (Brown et al. 1999; Inglis et al. 1994, 2000; Miller et al. 2002; Pan and Hyland 2005; Roitman et al. 2004; Schultz 1998; Yun et al. 2004). In line with these data, clinical as well as animal studies implicate nicotinic acetylcholine receptors (nAChRs) in incentive motivation and craving. Blocking nAChRs, by means of systemic administration of the nonselective nAChR antagonist mecamylamine, reduces cue-induced cocaine craving in humans (Reid et al. 1999) and responding with conditioned reinforcement in rats using stimuli associated with water (Olausson et al. 2004a) or ethanol (Löf et al. 2007) reinforcement. Conversely, stimulation of nAChRs with nicotine enhances cue-induced cocaine craving (Reid et al. 1998) and potentiates responding with conditioned reinforcement (Olausson et al. 2004a, b). Similarly, treatment with nicotine also enhances the reinforcing effects of stimuli with intrinsic or conditioned reinforcing value (Chaudhri et al. 2006a, b; Donny et al. 2003; Palmatier et al. 2006). Selective manipulation of nAChRs may thus reduce the impact of conditioned stimuli on reward-motivated behavior and could become a promising treatment strategy for a number of compulsive disorders.

Several functional neuronal nAChR subtypes, such as the $\alpha 7^*$, $\alpha 4\beta 2^*$, and $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChRs (Champtiaux et al. 2003; Charpentier et al. 1998; Klink et al. 2001; for recent review, see Jensen et al. 2005), have been identified in brain areas involved in reward and motivation. The high-affinity $\alpha 4\beta 2^*$ nAChRs are necessary for nicotine self-administration in rodents (Corrigall et al. 1994; Picciotto et al. 1998) and for the ability of prior chronic nicotine exposure to potentiate responding with conditioned reinforcement to water (Brunzell et al. 2005). Moreover, the $\beta 2^*$ nAChRs in the VTA were recently suggested to regulate the bursting activity of the mesolimbic dopamine neurons (Mameli-Engvall et al. 2006), i.e. the main activity induced by salient cues (Schultz 1998). However, in nicotine-naïve $\beta 2^{* -/-}$ animals, responding with conditioned reinforcement to water is intact (Brunzell et al. 2005). Blockade of $\alpha 4\beta 2^*$ nAChRs, by local VTA infusion of the selective $\alpha 4\beta 2^*$ nAChR antagonist dihydro- β -erythroiodine, neither affects the conditioned reinforcing nor the mesolimbic dopamine stimulatory effects of ethanol-associated stimuli in the rat (Löf et

al. 2007). Rather, these parameters were antagonized by local VTA infusion of the selective $\alpha 3\beta 2^*$ and $\alpha 6^*$ nAChR antagonist α -conotoxin MII (α -CtxMII), demonstrating that the $\alpha 3\beta 2^*$ and/or $\alpha 6^*$ nAChRs in the rat VTA are required for the conditioned reinforcing properties of such cues (Löff et al. 2007).

While the reinforcing effects of drug stimuli appear to require stimulation of nAChRs in the VTA, their role in the ability of cues associated with natural rewards to reinforce behavior is unknown. The present paper therefore investigated the involvement of specific nAChR subtypes on responding with conditioned reinforcement using stimuli associated with sucrose.

Materials and methods

Experimental design

Naïve rats were trained to associate a tone + light conditioned stimulus (CS) with the presentation of 0.1 M sucrose in a dipper elevated in the reinforcer magazine. Following this Pavlovian conditioning phase, all rats were subsequently tested on the stringent acquisition of a new instrumental response (lever pressing) with conditioned reinforcement. The testing was preceded by an acute systemic injection of mecamylamine or the more subtype-selective $\alpha 7$ and $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChR antagonist methyllycaconitine (MLA).

Animals

Sixty-four male Sprague-Dawley rats (Charles River, USA) weighing 225–275 g at the start of the experiments were housed in pairs under constant cage temperature (20–22°C) and humidity (40–50%) on a controlled light–dark cycle (light on at 7 a.m. and off at 7 p.m.). The rats had free access to rat chow at all times. Animals were restricted to a 1 h water access daily in their home cages starting 30 min after the Pavlovian training or the subsequent testing sessions. The experiments were approved by the Yale Animal Care and Use Committee and followed the NIH Guide for Care and Use of Laboratory Animals.

Drugs and experimental compounds

Sucrose (J.T. Baker, USA) was dissolved in tap water (0.1 M) and presented in plastic 250-ml bottles. Mecamylamine HCl (generously provided by the NIDA research substance supply program, NIDA, USA), a nonselective noncompetitive nAChR antagonist, was dissolved in phosphate-buffered saline (PBS; Invitrogen, USA) for systemic administration (2.0 ml/kg, i.p.). The plant alkaloid MLA (generously provided by the NIDA research substance supply program, NIDA, USA), a selective antagonist at $\alpha 7$ and $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChRs, was dissolved in PBS for systemic administration (2.0 ml/kg i.p.).

Experimental techniques

Equipment—Standard behavioral testing chambers (30×20×25 cm) with grid floors (Med Associates, USA) were used for behavioral testing. Each chamber was housed in a sound-attenuating outer box equipped with a white noise generator and a fan to reduce external noise. A liquid dipper (60 μ l) delivered a 0.1 M sucrose solution as the reinforcer into the magazine. This sucrose concentration was selected because, in a pilot study in our laboratory, free choice between tap water and 0.1 M sucrose in a 1 h limited access paradigm resulted in a close to 100% preference for the sucrose solution in all rats (unpublished data). Head entries were detected by a photocell mounted above the reinforcer receptacle. Above the magazine was a 2.5-W stimulus light, and the chamber was illuminated by a house light mounted on the back wall. A SonAlert tone (10 kHz) generator was mounted above the magazine, and retractable levers allowed for examination of

instrumental behaviors. The chambers were controlled by a PC using the MedPC software (Med Associates, USA).

Pavlovian training procedure—On the first day, a 5-s access to 60 μ l of 0.1 M sucrose [the unconditioned stimulus (US)] was available in the dipper on a fixed-time 15-s schedule (FT15; the US becomes available every 15 s); the session ended after delivery of 100 reinforcers. Beginning on the second day, the subjects received 30 pairings of a 5-s compound conditioned stimulus (light + tone) followed immediately by 5-s access to 60 μ l of 0.1 M sucrose; the CS+US pairings were delivered on a random-time 30-s schedule (RT30; the CS and the US are presented randomly on average following 30 s with no upper or lower limits). Head entries during the RT30 interval resulted in a 3-s delay during which time no reinforcement was given, and the schedule was restarted. Training on this schedule over a period of 10 days results in a discriminated pattern of approach of the magazine during CS and US, but not during inter-CS+US, periods (Burns et al. 1994; Taylor and Horger 1999; Taylor and Robbins 1984).

Conditioned reinforcement—After Pavlovian conditioning, all animals were tested in a conditioned reinforcement paradigm 10 min following acute administration of the nicotinic drugs (see “Antagonist administration” below). Testing utilized the behaviorally stringent acquisition of a new response with conditioned reinforcement (Taylor and Robbins 1984) and was performed in the absence of primary reinforcement. Here, two novel levers were introduced in the operant chambers. Responding on one lever (active or “CR lever”) resulted in the presentation of a 5-s CS and elevation of the liquid dipper (without sucrose). Responding on the other (inactive or “NCR lever”) had no programmed consequences and controlled for nonspecific alterations in responding. The first three responses on the active lever elicited presentation of the CS, following which the CS was presented on a variable ratio schedule (VR2; the number of lever presses required to get the CS is on average two and varies between one and three responses). The session lasted for 30 min following the first correct response on the CR lever. The position of the CR lever (left/right) was balanced for all treatment groups but remained the same for each rat in all testing sessions.

Antagonist administration—Experiment 1 tested the effect of two different doses of the nonselective nAChR antagonist mecamylamine (0.3 and 1.0 mg/kg i.p.) on responding with conditioned reinforcement. The low dose of mecamylamine (0.3 mg/kg i.p.) was tested in a balanced crossover design, i.e., during a single test, half of the animals were injected with mecamylamine (0.3 mg/kg i.p.) and the other half received PBS (2.0 ml/kg i.p.), 10 min prior to testing. The higher dose of mecamylamine (1.0 mg/kg i.p.) was analyzed in a different set of rats in a crossover counterbalanced design where each animal was tested on two consecutive days. Here, half of the animals were systemically injected with PBS the first day and with mecamylamine (1.0 mg/kg i.p.) the second day, and the other half of the animals received mecamylamine (1.0 mg/kg i.p.) the first day and PBS the second day.

Experiment 2 tested the effect of two different doses of systemically administered MLA (6.0 mg/kg i.p. and 3.0 mg/kg i.p.), a selective competitive antagonist at the homomeric $\alpha 7$ nAChR (Alkondon et al. 1992; Davies et al. 1999; Macallan et al. 1988; Ward et al. 1990) and the heteromeric $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChRs (Klink et al. 2001; Mogg et al. 2002; Salminen et al. 2004), on responding with conditioned reinforcement. First, half of the rats were injected with MLA (6.0 mg/kg i.p.) and the other half with PBS (2.0 ml/kg i.p.) 10 min prior to a single test. The animals were subsequently retrained for 5 days and retested after receiving 3.0 mg/kg i.p. (–10 min) MLA or PBS during a single test. In Experiment 2, the treatments were balanced so that half of the animals that received PBS during the first test received MLA after retraining and vice versa. The doses for all experiments were calculated based on consensus from published studies using systemic administration of these antagonists.

Statistics

The data obtained in all experiments were analyzed using two-factor analysis of variance (ANOVA) with treatment as the independent factor and lever (active/inactive) as a dependent factor. Paired *t* tests or Fisher's PLSD were used for post hoc comparisons of main effects. Due to the variation in the lever responses, a statistical test of variance homogeneity recommended that square root transformation of the data be used to fulfill the requirements for ANOVA analysis, as is normally done for analyses of conditioned reinforcement data (e.g., Taylor and Robbins 1984). A probability level (*p*) of less than 0.05 was considered significant. The results are presented as means $\pm$ SEM.

Results

Effect of systemic mecamylamine administration on the conditioned reinforcing effects of sucrose-associated stimuli

In Experiment 1, we first tested the effect of mecamylamine (0.3 mg/kg i.p.) during one experimental day. Here, a repeated-measures ANOVA revealed a main effect of lever ($F[1, 13]=13.603$; $p<0.0001$; Fig. 1). Subsequent paired *t* tests demonstrated the basic conditioned reinforcement effect in control animals (p 0.001, Fig. 1). However, there was no lever $\times$ treatment interaction ($F[1, 13]=0.643$; $p=0.31$; Fig. 1) under these conditions, and paired *t* tests revealed a significant difference between the CR and the NCR levers also following the mecamylamine injection (p 0.001, CR vs. NCR lever, Fig. 1). This demonstrates that a systemic mecamylamine injection at this low dose had no effect on responding with conditioned reinforcement.

We next tested the effect of a systemic injection of a moderate dose of mecamylamine (1.0 mg/kg i.p., -10 min) commonly used to examine the role of nAChRs. Here, a repeated-measures ANOVA revealed a significant lever $\times$ treatment interaction ($F[1, 12]=5.006$; p 0.05) and a main effect of treatment ($F[1, 12]=13.024$; p 0.01; Fig. 1). Subsequent paired *t* tests showed the basic conditioned reinforcement effect in control treated animals; animals made significantly more responses on the CR lever than the NCR lever (p 0.001). This demonstrates that the Pavlovian conditioning successfully established the sucrose-associated CS as a conditioned reinforcer. After pretreatment with 1.0 mg/kg mecamylamine, however, the responses on the CR and NCR levers were not statistically different (Fig. 1), demonstrating that blockade of nAChRs reduced or blocked the conditioned reinforcing properties of the sucrose-associated cue. However, an unpaired *t* test failed to demonstrate a significant reduction in responding on the active lever following mecamylamine (1.0 mg/kg) as compared to PBS treatment. The mecamylamine treatment (1.0 mg/kg) did not affect total lever responses (Fig. 2) or total magazine entry time (Fig. 3), demonstrating that systemic mecamylamine administration selectively reduced conditioned reinforcement and did not cause a general reduction in Pavlovian approach, general motivation, or motor function.

Effect of systemic MLA administration on the conditioned reinforcing effects of sucrose-associated stimuli

In an effort to identify the specific nAChR subtypes required for conditioned reinforcement, Experiment 2 examined the effects of MLA, a selective competitive antagonist at the homomeric $\alpha 7$ nAChR and the heteromeric $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChRs. Here, we employed a crossover treatment design, and the repeated-measures ANOVAs revealed a main effect of lever in both test 1 ($F[1, 20]=15.289$; p 0.001; Fig. 4) and test 2 ($F[1, 18]=13.436$; p 0.001; Fig. 4). Subsequent paired *t* tests demonstrated the basic conditioned reinforcement effect in control treated animals in both experiments (p 0.001 and p 0.01, Fig. 4).

Following systemic MLA administration of 6.0 mg/kg, a repeated-measures ANOVA revealed a main effect of treatment ($F[1, 20]=6.333$; $p < 0.05$) and a significant lever $\times$ treatment interaction ($F[1, 20]=6.650$; $p < 0.05$; Fig. 4). An unpaired t test demonstrated a significant reduction in responding on the active lever following MLA (6.0 mg/kg) as compared to PBS treatment ($p < 0.05$). After systemic MLA administration of 3.0 mg/kg, there was no effect of treatment or lever $\times$ treatment interaction (Fig. 4). A paired t test demonstrated no differences in responses on the CR lever compared to the NCR lever after MLA treatment of 6.0 or 3.0 mg/kg (Fig. 4). Thus, both doses of MLA reduced the conditioned reinforcing properties of the sucrose-associated cues.

MLA had no effect on the total magazine entry time (Fig. 3), but the higher dose significantly reduced the total number of lever responses ($p < 0.05$, unpaired t test, Fig. 2).

Discussion

The present series of experiments demonstrate that antagonism of nAChRs prevents the ability of sucrose-associated cues to act as a conditioned reinforcer in the stringent acquisition of a new response paradigm. These data are consistent with our previous demonstration of an important role for nAChRs in the conditioned reinforcing properties of ethanol cues (Löff et al. 2007) and the ability of nicotine administration to enhance conditioned reinforcement (Olausson et al. 2004b). Together, these findings suggest that nAChRs act as a common substrate that can modulate conditioned reinforcement processes.

Experiment 1 demonstrated that the nonselective nAChR antagonist mecamylamine blocked the conditioned reinforcing properties of a sucrose-associated stimulus that acted as a reinforcer for new learning in control rats in a dose-dependent manner. Indeed, the same dose of systemic mecamylamine (1.0 mg/kg i.p.) blocks the conditioned reinforcing effects also of alcohol-associated stimuli in the same experimental setup (Löff et al. 2007). While in vitro studies suggest that mecamylamine at higher doses also inhibits *N*-methyl-D-aspartate receptor complex-mediated currents (O'Dell and Christensen 1988) and blocks *N*-methyl-D-aspartate-induced noradrenaline release from hippocampal rat slices (Snell and Johnson 1989), pharmacological studies demonstrate that mecamylamine, at doses equivalent to that used in the present experiment, does not interact with these receptors in vivo (Ericson et al. 2003). Thus, the present observations suggest that mecamylamine (1.0 mg/kg i.p.) reduced or blocked the conditioned reinforcing effects of the sucrose-associated cues by antagonizing nAChRs. Importantly, this assumption was supported by data from the second experiment that confirmed a central role of these receptors in the neurobiological processes subserving responding with conditioned reinforcement.

Experiment 2 examined the effects of the selective competitive nAChR antagonist MLA at two different doses on responding with conditioned reinforcement for sucrose-associated conditioned stimuli. Here, a systemic MLA injection (6.0 or 3.0 mg/kg i.p., 10 min prior to testing) reduced the conditioned reinforcing properties of the sucrose-associated CS such that responses on the CR lever and NCR lever were not different following drug administration. These results suggest that the nAChR antagonist MLA blocks responding with conditioned reinforcement, consistent with the observations following mecamylamine administration in Experiment 1. MLA has generally been regarded as a selective $\alpha 7$ nAChR antagonist but was recently demonstrated to block also $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChRs at a similar concentration range (Klink et al. 2001; Mogg et al. 2002; Salminen et al. 2004). The $\alpha 3/\alpha 6\beta 2\beta 3^*$ receptors, but not the $\alpha 7$ subtype, are further antagonized by α -CtxMII, a compound that was recently demonstrated to block the conditioned reinforcing effects of ethanol-associated cues (Löff et al. 2007). This may indicate that the $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChRs are primarily responsible for the effects reported here. These converging effects of $\alpha 3/$

$\alpha 6\beta 2\beta 3^*$ nAChR antagonists support the notion of a common basis for the impact of reward-related cues on behavior (Kelley and Berridge 2002; Nie and Janak 2003) and suggest a role for the $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChR subtypes.

Although the mecamylamine (1.0 mg/kg) injection in Experiment 1 abolished the conditioned reinforcing effect observed following PBS treatment, an unpaired *t* test analysis of the number of responses on the active lever following mecamylamine (1.0 mg/kg) demonstrated a nonsignificant difference compared to PBS treatment. When the same statistical comparison was made in Experiment 2, there was a significantly lower response on the active lever following administration of the high MLA dose (6.0 mg/kg i.p., *p* 0.05), but not the low dose (3.0 mg/kg), as compared to PBS. This outcome is in line with the differences in the total number of lever presses between MLA dose (6.0 mg/kg i.p.) and PBS and may indicate an unspecific effect, such as suppression of locomotor activity, of the higher MLA dose. While both antagonists tested here should be specific for nAChRs at the present doses, MLA has been reported to be less selective for the specific nAChRs at higher concentrations. However, since it is difficult to precisely determine the achieved brain concentrations of these drugs, we cannot completely exclude the possibility that MLA also interferes with the function at additional nAChR receptor subtypes and configurations. Nevertheless, a pharmacokinetic study reported that an MLA dose of 5.4 mg/kg i.p. results in brain drug levels of approximately 50–100 nM (Turek et al. 1995), levels sufficient to displace α -CtxMII in binding studies (Mogg et al. 2002) and to inhibit $\alpha 7$ -nAChR-mediated responses in vitro (Alkondon and Albuquerque 1993; Yu and Role 1998).

While the present study investigated the effects of systemic administration of nAChR antagonists, the involvement of $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChRs in mediating the reinforcing effects of alcohol-associated cues was found to be localized to the VTA (Löff et al. 2007). We have therefore hypothesized that the presentation of an alcohol-associated cue results in the release of acetylcholine into the VTA and a subsequent release of dopamine in the nucleus accumbens (nAc; Löff et al. 2007). Subpopulations of nAc neurons that respond to sucrose cues require the dopaminergic projections from the VTA to promote reward-seeking behavior (Yun et al. 2004). Intermediate levels of MLA binding sites have been found in several of the brain areas involved in reward and motivation, such as the VTA (Mugnaini et al. 2002). We thus speculate that the effects of nAChR antagonism on conditioned reinforcement to sucrose observed in the present study could involve MLA-sensitive nAChRs on dopaminergic cell bodies in the VTA.

Increased consumption of sweet-tasting and high-caloric food is one consequence of withdrawal in human alcoholics (Junghanns et al. 2000; for review, see Kampov-Polevoy et al. 1999) and smokers (Grunberg 1982; Hall et al. 1989; Hatsukami et al. 1984, 1993) as well as in nicotine-dependent laboratory animals (Grunberg et al. 1985, 1988a, b). A suggested explanation for this phenomenon has been that nicotine as well as alcohol withdrawal increases plasma levels of insulin (Grunberg et al. 1985, 1988a; Passilta et al. 1999). The present study, however, also suggests that dysregulation of nAChR function in abstinent alcoholics and/or smokers may enhance the incentive or reinforcing properties of reward-associated cues, thereby increasing the motivation to consume natural rewards such as sucrose during the withdrawal phase.

In conclusion, the present set of experiments demonstrates that MLA-sensitive nAChRs are required for the conditioned reinforcing properties of cues associated with a palatable and rewarding food source. These receptors may be identical to the $\alpha 6^*$ and/or $\alpha 3\beta 2^*$ nAChRs that were recently demonstrated to be involved in the impact of alcohol cues on reward-related behaviors (Löff et al. 2007). Together, these observations suggest neuronal nAChRs as common mediators of the impact of cues on incentive processes and suggest that

antagonists of these nAChR subtypes should be explored as future medications for relapse prevention and overeating.

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Abbreviations

nAChR	Nicotinic acetylcholine receptor
CS	Conditioned stimulus
US	Unconditioned stimulus
MEC	Mecamylamine
MLA	Methyllycaconitine citrate
CR	Conditioned reinforcement
VTA	Ventral tegmental area
PBS	Phosphate-buffered saline
VR	Variable ratio
ANOVA	Analysis of variance
PLSD	Protected least-significant difference
α-CtxMII	α -Conotoxin MII

References

- Alkondon M, Albuquerque EX. Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes. *J Pharmacol Exp Ther.* 1993; 265:1455–1473. [PubMed: 8510022]
- Alkondon M, Pereira EF, Wonnacott S, Albuquerque EX. Blockade of nicotinic currents in hippocampal neurons defines methyllycaconitine as a potent and specific receptor antagonist. *Mol Pharmacol.* 1992; 41:802–808. [PubMed: 1569927]
- Brown J, Bullock D, Grossberg S. How the basal ganglia use parallel excitatory and inhibitory learning pathways to selectively respond to unexpected rewarding cues. *J Neurosci.* 1999; 19:10502–10511. [PubMed: 10575046]
- Brunzell DH, Chang JR, Schneider B, Olausson P, Taylor JR, Picciotto MR. Beta2-subunit-containing nicotinic acetylcholine receptors are involved in nicotine-induced increases in conditioned reinforcement but not progressive ratio responding for food in C57BL/6 mice. *Psychopharmacology (Berl).* 2005; 184:328–338. [PubMed: 16133126]
- Burns LH, Everitt BJ, Kelley AE, Robbins TW. Glutamate–dopamine interactions in the ventral striatum: role in locomotor activity and responding with conditioned reinforcement. *Psychopharmacology (Berl).* 1994; 115:516–528. [PubMed: 7871097]
- Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci Biobehav Rev.* 2002; 26:321–352. [PubMed: 12034134]

- Champtiaux N, Gotti C, Cordero-Erausquin M, David DJ, Przybylski C, Lena C, Clementi F, Moretti M, Rossi FM, Le Novere N, McIntosh JM, Gardier AM, Changeux JP. Subunit composition of functional nicotinic receptors in dopaminergic neurons investigated with knock-out mice. *J Neurosci.* 2003; 23:7820–7829. [PubMed: 12944511]
- Charpentier E, Barneoud P, Moser P, Besnard F, Sgard F. Nicotinic acetylcholine subunit mRNA expression in dopaminergic neurons of the rat substantia nigra and ventral tegmental area. *NeuroReport.* 1998; 9:3097–3101. [PubMed: 9804323]
- Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib M, Craven L, Palmatier MI, Liu X, Sved AF. Operant responding for conditioned and unconditioned reinforcers in rats is differentially enhanced by the primary reinforcing and reinforcement-enhancing effects of nicotine. *Psychopharmacology (Berl).* 2006a; 189:27–36. [PubMed: 17019569]
- Chaudhri N, Caggiula AR, Donny EC, Palmatier MI, Liu X, Sved AF. Complex interactions between nicotine and nonpharmacological stimuli reveal multiple roles for nicotine in reinforcement. *Psychopharmacology (Berl).* 2006b; 184:353–366. [PubMed: 16240165]
- Childress AR, Hole AV, Ehrman RN, Robbins SJ, McLellan AT, O'Brien CP. Cue reactivity and cue reactivity interventions in drug dependence. *NIDA Res Monogr.* 1993; 137:73–95. [PubMed: 8289929]
- Corrigall WA, Coen KM, Adamson KL. Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. *Brain Res.* 1994; 653:278–284. [PubMed: 7982062]
- Davies AR, Hardick DJ, Blagbrough IS, Potter BV, Wolstenholme AJ, Wonnacott S. Characterisation of the binding of [3H] methyllycaconitine: a new radioligand for labelling alpha 7-type neuronal nicotinic acetylcholine receptors. *Neuropharmacology.* 1999; 38:679–690. [PubMed: 10340305]
- Donny EC, Chaudhri N, Caggiula AR, Evans-Martin FF, Booth S, Gharib MA, Clements LA, Sved AF. Operant responding for a visual reinforcer in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. *Psychopharmacology (Berl).* 2003; 169:68–76. [PubMed: 12774186]
- Dormont JF, Conde H, Farin D. The role of the pedunculo-pontine tegmental nucleus in relation to conditioned motor performance in the cat. I. Context-dependent and reinforcement-related single unit activity. *Exp Brain Res.* 1998; 121:401–410. [PubMed: 9746146]
- Ericson M, Molander A, Löf E, Engel JA, Söderpalm B. Ethanol elevates accumbal dopamine levels via indirect activation of ventral tegmental nicotinic acetylcholine receptors. *Eur J Pharmacol.* 2003; 467:85–93. [PubMed: 12706460]
- Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW. Associative processes in addiction and reward. The role of amygdala-ventral striatal subsystems. *Ann N Y Acad Sci.* 1999; 877:412–438. [PubMed: 10415662]
- Grimm JW, Hope BT, Wise RA, Shaham Y. Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature.* 2001; 412:141–142. [PubMed: 11449260]
- Grunberg NE. The effects of nicotine and cigarette smoking on food consumption and taste preferences. *Addict Behav.* 1982; 7:317–331. [PubMed: 7183186]
- Grunberg NE, Bowen DJ, Maycock VA, Nespor SM. The importance of sweet taste and caloric content in the effects of nicotine on specific food consumption. *Psychopharmacology (Berl).* 1985; 87:198–203. [PubMed: 3931146]
- Grunberg NE, Popp KA, Bowen DJ, Nespor SM, Winders SE, Eury SE. Effects of chronic nicotine administration on insulin, glucose, epinephrine, and norepinephrine. *Life Sci.* 1988a; 42:161–170. [PubMed: 3275854]
- Grunberg NE, Popp KA, Winders SE. Effects of nicotine on body weight in rats with access to “junk” foods. *Psychopharmacology (Berl).* 1988b; 94:536–539. [PubMed: 3131800]
- Hall SM, McGee R, Tunstall C, Duffy J, Benowitz N. Changes in food intake and activity after quitting smoking. *J Consult Clin Psychol.* 1989; 57:81–86. [PubMed: 2925977]
- Hatsukami DK, Hughes JR, Pickens RW, Svikis D. Tobacco withdrawal symptoms: an experimental analysis. *Psychopharmacology (Berl).* 1984; 84:231–236. [PubMed: 6438682]
- Hatsukami D, LaBounty L, Hughes J, Laine D. Effects of tobacco abstinence on food intake among cigarette smokers. *Health Psychol.* 1993; 12:499–502. [PubMed: 8293734]

- Holland PC, Petrovich GD, Gallagher M. The effects of amygdala lesions on conditioned stimulus-potentiated eating in rats. *Physiol Behav.* 2002; 76:117–129. [PubMed: 12175595]
- Inglis WL, Dunbar JS, Winn P. Outflow from the nucleus accumbens to the pedunculo-pontine tegmental nucleus: a dissociation between locomotor activity and the acquisition of responding for conditioned reinforcement stimulated by *d*-amphetamine. *Neuroscience.* 1994; 62:51–64. [PubMed: 7816212]
- Inglis WL, Olmstead MC, Robbins TW. Pedunculo-pontine tegmental nucleus lesions impair stimulus-reward learning in autoshaping and conditioned reinforcement paradigms. *Behav Neurosci.* 2000; 114:285–294. [PubMed: 10832790]
- Jensen AA, Frolund B, Liljefors T, Krogsgaard-Larsen P. Neuronal nicotinic acetylcholine receptors: structural revelations, target identifications, and therapeutic inspirations. *J Med Chem.* 2005; 48:4705–4745. [PubMed: 16033252]
- Junghanns K, Veltrup C, Wetterling T. Craving shift in chronic alcoholics. *Eur Addict Res.* 2000; 6:64–70. [PubMed: 10899731]
- Kampov-Polevoy AB, Garbutt JC, Janowsky DS. Association between preference for sweets and excessive alcohol intake: a review of animal and human studies. *Alcohol Alcohol.* 1999; 34:386–395. [PubMed: 10414615]
- Kelley AE, Berridge KC. The neuroscience of natural rewards: relevance to addictive drugs. *J Neurosci.* 2002; 22:3306–3311. [PubMed: 11978804]
- Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. *J Neurosci.* 2001; 21:1452–1463. [PubMed: 11222635]
- Löf E, Olausson P, de Bejczy A, Stomberg R, McIntosh J, Taylor J, Söderpalm B. Nicotinic acetylcholine receptors in the ventral tegmental area mediate the dopamine activating and reinforcing properties of ethanol cues. *Psychopharmacology (Berl).* 2007; 195:333–343. [PubMed: 17703283]
- Macallan DRE, Lunt GG, Wonnacott S, Swanson KL, Rapoport H, Albuquerque EX. Methyllycaconitine and (+)-anatoxin-a differentiate between nicotinic receptors in vertebrate and invertebrate nervous systems. *FEBS Lett.* 1988; 226:357–363. [PubMed: 3338564]
- Mameli-Engvall M, Evrard A, Pons S, Maskos U, Svensson TH, Changeux JP, Faure P. Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. *Neuron.* 2006; 50:911–921. [PubMed: 16772172]
- Miller AD, Forster GL, Metcalf KM, Blaha CD. Excitotoxic lesions of the pedunculo-pontine differentially mediate morphine- and *d*-amphetamine-evoked striatal dopamine efflux and behaviors. *Neuroscience.* 2002; 111:351–362. [PubMed: 11983320]
- Mogg AJ, Whiteaker P, McIntosh JM, Marks M, Collins AC, Wonnacott S. Methyllycaconitine is a potent antagonist of alpha-conotoxin-MII-sensitive presynaptic nicotinic acetylcholine receptors in rat striatum. *J Pharmacol Exp Ther.* 2002; 302:197–204. [PubMed: 12065717]
- Mugnaini M, Tessari M, Tarter G, Merlo Pich E, Chiamulera C, Bunnemann B. Upregulation of [3H]methyllycaconitine binding sites following continuous infusion of nicotine, without changes of alpha7 or alpha6 subunit mRNA: an autoradiography and in situ hybridization study in rat brain. *Eur J Neurosci.* 2002; 16:1633–1646. [PubMed: 12431215]
- Nie H, Janak PH. Comparison of reinstatement of ethanol- and sucrose-seeking by conditioned stimuli and priming injections of allopregnanolone after extinction in rats. *Psychopharmacology.* 2003; 168:222–228. [PubMed: 12719962]
- O'Brien CP, Childress AR, Ehrman R, Robbins SJ. Conditioning factors in drug abuse: can they explain compulsion? *J Psychopharmacol.* 1998; 12:15–22. [PubMed: 9584964]
- O'Dell TJ, Christensen BN. Mecamylamine is a selective noncompetitive antagonist of *N*-methyl-D-aspartate- and aspartate-induced currents in horizontal cells dissociated from the catfish retina. *Neurosci Lett.* 1988; 94:93–98. [PubMed: 3071750]
- Olausson P, Jentsch JD, Taylor JR. Nicotine enhances responding with conditioned reinforcement. *Psychopharmacology (Berl).* 2004a; 171:173–178. [PubMed: 13680077]
- Olausson P, Jentsch JD, Taylor JR. Repeated nicotine exposure enhances responding with conditioned reinforcement. *Psychopharmacology (Berl).* 2004b; 173:98–104. [PubMed: 14727001]

- Palmatier MI, Evans-Martin FF, Hoffman A, Caggiula AR, Chaudhri N, Donny EC, Liu X, Booth S, Gharib M, Craven L, Sved AF. Dissociating the primary reinforcing and reinforcement-enhancing effects of nicotine using a rat self-administration paradigm with concurrently available drug and environmental reinforcers. *Psychopharmacology (Berl)*. 2006; 184:391–400. [PubMed: 16249908]
- Pan WX, Hyland BI. Pedunculo-pontine tegmental nucleus controls conditioned responses of midbrain dopamine neurons in behaving rats. *J Neurosci*. 2005; 25:4725–4732. [PubMed: 15888648]
- Passilta M, Kervinen K, Kesaniemi YA. Glucose metabolism, insulin-like growth factor-I, and insulin-like growth factor-binding protein-1 after alcohol withdrawal. *Alcohol Clin Exp Res*. 1999; 23:471–475. [PubMed: 10195820]
- Petrovich GD, Setlow B, Holland PC, Gallagher M. Amygdalohypothalamic circuit allows learned cues to override satiety and promote eating. *J Neurosci*. 2002; 22:8748–8753. [PubMed: 12351750]
- Piccio MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux JP. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature*. 1998; 391:173–177. [PubMed: 9428762]
- Reid MS, Mickalian JD, Delucchi KL, Hall SM, Berger SP. An acute dose of nicotine enhances cue-induced cocaine craving. *Drug Alcohol Depend*. 1998; 49:95–104. [PubMed: 9543646]
- Reid MS, Mickalian JD, Delucchi KL, Berger SP. A nicotine antagonist, mecamylamine, reduces cue-induced cocaine craving in cocaine-dependent subjects. *Neuropsychopharmacology*. 1999; 20:297–307. [PubMed: 10063490]
- Roitman MF, Stuber GD, Phillips PE, Wightman RM, Carelli RM. Dopamine operates as a subsecond modulator of food seeking. *J Neurosci*. 2004; 24:1265–1271. [PubMed: 14960596]
- Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC, Grady SR. Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol Pharmacol*. 2004; 65:1526–1535. [PubMed: 15155845]
- Schultz W. Predictive reward signal of dopamine neurons. *J Neurophysiol*. 1998; 80:1–27. [PubMed: 9658025]
- See RE. Neural substrates of conditioned-cued relapse to drug-seeking behavior. *Pharmacol Biochem Behav*. 2002; 71:517–529. [PubMed: 11830186]
- Snell LD, Johnson KM. Effects of nicotinic agonists and antagonists on *N*-methyl-D-aspartate-induced 3H-norepinephrine release and 3H-(1-[1-(2-thienyl)cyclohexyl]-piperidine) binding in rat hippocampus. *Synapse*. 1989; 3:129–135. [PubMed: 2564704]
- Taylor JR, Horger BA. Enhanced responding for conditioned reward produced by intra-accumbens amphetamine is potentiated after cocaine sensitization. *Psychopharmacology (Berl)*. 1999; 142:31–40. [PubMed: 10102780]
- Taylor JR, Robbins TW. Enhanced behavioural control by conditioned reinforcers following microinjections of *D*-amphetamine into the nucleus accumbens. *Psychopharmacology (Berl)*. 1984; 84:405–412. [PubMed: 6440188]
- Turek JW, Kang CH, Campbell JE, Arneric SP, Sullivan JP. A sensitive technique for the detection of the alpha 7 neuronal nicotinic acetylcholine receptor antagonist, methyllycaconitine, in rat plasma and brain. *J Neurosci Methods*. 1995; 61:113–118. [PubMed: 8618408]
- Ward JM, Cockcroft VB, Lunt GG, Smillie FS, Wonnacott S. Methyllycaconitine: a selective probe for neuronal [alpha]-bungarotoxin binding sites. *FEBS Lett*. 1990; 270:45–48. [PubMed: 2226787]
- Weingarten HP. Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. *Science*. 1983; 220:431–433. [PubMed: 6836286]
- Yu CR, Role LW. Functional contribution of the alpha7 subunit to multiple subtypes of nicotinic receptors in embryonic chick sympathetic neurones. *J Physiol*. 1998; 509(Pt 3):651–665. [PubMed: 9596789]
- Yun IA, Wakabayashi KT, Fields HL, Nicola SM. The ventral tegmental area is required for the behavioral and nucleus accumbens neuronal firing responses to incentive cues. *J Neurosci*. 2004; 24:2923–2933. [PubMed: 15044531]

Fig. 1.

Effects of systemic injections of the nAChR antagonist mecamylamine (*MEC*) at the dose 0.3 mg/kg (**a**; $n=14$) or 1.0 mg/kg (**b**; $n=13$) 10 min prior to testing on responding with conditioned reinforcement. The higher dose of *MEC* decreases responding with conditioned reinforcement using a sucrose-associated CS. Asterisks indicate effect of lever ($p<0.05$, paired t test). *CR* active lever, *NCR* inactive lever. Data are presented as square root of mean lever responses+SEM

Fig. 2.

Effects of systemic i.p. injections of the nonselective nAChR antagonist mecamylamine (*MEC*) at the dose 0.3 mg/kg ($n=14$) or 1.0 mg/kg ($n=13$) or the selective $\alpha 7$ and $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChR antagonist *MLA* at the dose 3.0 mg/kg ($n=10$) or 6.0 mg/kg ($n=11$) 10 min prior to testing on the total number of lever presses. The higher dose of *MLA* decreases the total number of lever presses. *Asterisk* indicates effect of drug ($p<0.05$, paired t test)

Fig. 3.

Effects of systemic i.p. injections of the nonselective nAChR antagonist mecamylamine (*MEC*) at the dose 0.3 mg/kg ($n=14$) or 1.0 mg/kg ($n=13$) or the selective $\alpha 7$ and $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChR antagonist *MLA* at the dose 3.0 mg/kg ($n=10$ in each group) or 6.0 mg/kg ($n=11$ in each group) 10 min prior to testing on the total magazine head entry time. There were no significant effects of the nAChR antagonists on the total magazine head entry time

Fig. 4.

Effects of systemic i.p. injections of the selective $\alpha 7$ and $\alpha 3/\alpha 6\beta 2\beta 3^*$ nAChR antagonist MLA at the dose 6.0 mg/kg (**a**; $n=11$ in each group) or 3.0 mg/kg (**b**; $n=10$ in each group), 10 min prior to testing on responding with conditioned reinforcement. MLA decreases responding with conditioned reinforcement using a sucrose-associated cue. *Asterisks* indicate effect of lever ($p<0.05$, paired t test). *CR* active lever, *NCR* inactive lever. Data are presented as square root of mean lever responses+SEM