MINIREVIEW

Influence of Neuronal Nicotinic Receptors over Nicotine Addiction and Withdrawal

MARIELLA DE BIASI[1] AND RAMIRO SALAS

Department of Neuroscience, Baylor College of Medicine, Houston, Texas 77030

Cigarette smoking represents an enormous, global public health threat. Nearly five million premature deaths during a single year are attributable to smoking. Despite the resounding message of risks associated with smoking and numerous public health initiatives, cigarette smoking remains the most common preventable cause of disease in the United States. Fortunately, even in an adult smoker, smoking cessation can reverse many of the potential harmful effects. The symptoms associated with nicotine withdrawal represent the major obstacle to smoking cessation. This minireview examines the roles of various nicotinic receptors in the mechanisms of nicotine dependence, discusses the potential role of the habenula-interpeduncular nucleus axis in nicotine withdrawal, and highlights nicotinic receptors containing the β4 subunit as a potential pharmacological target for smoking cessation strategies. Exp Biol Med 233:917–929, 2008

Key words: nicotinic acetylcholine receptor; subunits; tobacco; addiction; withdrawal; bupropion; MC-18; mecamylamine; rimonabant; varenicline; habenula; interpeduncular nucleus; knockout mice

Introduction

About 10 million cigarettes are sold every minute in the world and every eight seconds someone dies from tobacco use (1). If current trends continue, smoking will kill one in six people by 2030 (2). Cessation is the only effective measure to prevent and limit the long-term negative effects of smoke (3).

Several phenomena participate in the initiation, maintenance and escalation of drug use that underlie nicotine dependence. The neuronal adaptations produced by repeated exposure to nicotine include a greater expression of neuronal nicotinic acetylcholine receptors (nAChRs), the need of progressively higher doses of nicotine to obtain the same effect (tolerance), and the increased ability of the drug to activate dopaminergic neurotransmission and trigger appetitive behaviors (sensitization) (4, 5). Most smokers recognize the negative impact of smoking on health and would prefer to quit, if possible. However, very few actually succeed (6). This happens mainly because of the withdrawal symptoms that appear upon smoking cessation. In fact, withdrawal symptoms are a better predictor of unsuccessful quit attempts than smoke intake or dependence (7). While current therapies for smoking cessation are helpful, none can claim a very high rate of success (8). Therefore, there is a great need to better understand the interacting behavioral and biological factors that lead to the physical and psychological manifestations of nicotine dependence.

This brief review examines the roles of each nAChR subunit in nicotine addiction, with special emphasis on nicotine withdrawal. In addition, we present the view that nAChRs in the medial habenula (MHb) and interpeduncular nucleus (IPN) have great influence on the symptoms of nicotine abstinence and represent a novel target for smoking cessation therapies.

Nicotinic Acetylcholine Receptors Bind the Nicotine Contained in Tobacco

Neuronal nAChRs are pentameric ligand gated ion channels formed by either α subunits (α7, α9, α10) or combinations of α and β subunits (α2-α6 and β2-β4) (9). They bind the acetylcholine (ACh) released mainly by neurons located in the pedunculopontine tegmentum and the

This work was supported by the National Institute of Drug Addiction (NIDA) grant DA17173 to MDB.

[1] To whom correspondence should be addressed at Department of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. E-mail: debiasi@bcm.tmc.edu

DOI: 10.3181/0712-MR-355
1535-3702/08/2338-0917$15.00

laterodorsal pontine tegmentum, several basal forebrain nuclei, or the striatum (9). Upon opening, nAChRs are permeable to monovalent and divalent cations, mainly Na^+ and Ca^{2+} (9). The membrane depolarization that follows ion permeation can trigger a variety of intracellular events, including the activation of signal transduction cascades and the transcription of genes (10). Besides undergoing transitions between the open and closed states nAChRs can also exist in the desensitized state (11). The kinetics of each conformational state are influenced by the subunit composition of the channel (12) and might be relevant for the mechanisms of nicotine dependence (13).

Several studies on mRNA and protein expression levels have shown that different areas of the brain express specific subsets of nAChR subunits. This anatomical knowledge, together with studies in genetically modified mice, has shed light over the possible functions of several nAChR subunits. Experimental evidence from several groups has confirmed the role of α4β2 nAChRs in addiction (14, 15). Those receptors are necessary and sufficient for nicotine reward, tolerance and sensitization (16). Another subunit that may play a key role in nicotine addiction is α6, because α6-containing receptors seem to dominate nicotine control of dopamine neurotransmission in the nucleus accumbens (17). Interestingly, we showed that β4-containing (β4*) (19), but not β2-containing (β2*) nAChRs (18, 19) are necessary for the expression of the somatic signs of nicotine withdrawal. More recent data indicate that α7* nAChRs can also influence the somatic signs of withdrawal (20). Table 1 summarizes the main expression patterns and functions of each nAChR subunit.

Withdrawal Symptoms in Humans

In humans, cessation of tobacco intake precipitates both somatic and affective symptoms of withdrawal which may include severe craving for nicotine, irritability, anxiety, loss of concentration, restlessness, decreased heart rate, depressed mood, impatience, insomnia, and increased appetite and weight gain (21, 22). These withdrawal symptoms cause enough distress to become a deterrent to abstinence and a drive to relapse (7). The negative affective symptoms usually start 4 to 24 h after the last cigarette, peak in about three days and may not recede even after a month of tobacco abstinence (8, 21, 23–27).

Anxiety and stress have a complex influence on all aspects of nicotine dependence, including the withdrawal syndrome. While many smokers use cigarettes as a tool to attenuate stress and anxiety and maintain that smoking has a calming effect (28–30), anxiety increases during withdrawal. Indeed, several studies suggest that smokers continue to smoke to avoid that particular symptom of nicotine deprivation (31–38). Besides being a product of nicotine withdrawal, stress and anxiety have the ability to exacerbate its symptoms, which results in increased craving and relapse (36, 39–46).

Similarly to anxiety, depression may both promote smoking and be a symptom of nicotine withdrawal (47–49). Subjects with a history of depression seem more sensitive to the effects of nicotine and display elevated craving and withdrawal scores upon nicotine cessation (50). Finally, another stress-related disorder, PTSD (post-traumatic stress disorder) is also associated with increased smoking behavior and enhanced withdrawal symptoms (51, 52), purporting mood regulation as one of the factors prompting smoking and preventing smoking cessation.

Withdrawal Symptoms in Rodents

Withdrawal symptoms can be assessed in animals by the sudden discontinuation of chronic nicotine administration and recording of withdrawal signs, either through observation of behavioral signs or through monitoring of disruptions in operant behavior. Alternatively, withdrawal can be precipitated on chronic nicotine treated rodents by systemically administering nAChR antagonists. Nicotine withdrawal signs are both physical, or somatic, and affective, or non-somatic.

Somatic Signs. Somatic signs of withdrawal upon interruption of chronic nicotine treatment were first documented in the rat (53). These signs included teeth chattering, chews, gasps, palpebral ptosis, tremors, shakes, and yawns. The number of signs depended on the amount of nicotine infused, and were relieved by acute nicotine treatment (53). Another way of precipitating withdrawal in rats chronically treated with nicotine is the systemic injection of nicotinic antagonists such as mecamylamine, di-hydro-beta-erythroidine, or methyllycaconitine (54). Mecamylamine, which has a slightly higher affinity for α3β4* nAChRs than for β2* receptors, is the best antagonist at precipitating nicotine withdrawal (54).

The advent of genetic engineering techniques has prompted the study of nicotine withdrawal symptoms in the mouse. The symptoms of nicotine withdrawal are qualitatively similar to those observed in rats and consist of a sharp increase in certain normal behaviors that become repetitive and more frequent. Among those behaviors there are shaking, grooming and scratching. In addition, behaviors such as jumping, which are not normally observed, also appear in the mouse undergoing nicotine withdrawal (54, 55). Our lab has shown that mice null for the β4 subunit show no somatic signs when nicotine withdrawal is precipitated with systemic mecamylamine, and no spontaneous withdrawal-induced hyperalgesia (19). In the same report we also showed that β2 -/- mice show normal mecamylamine-precipitated somatic signs of behavior (19), a finding that was later confirmed by an independent group (18). In addition, we have shown that the α7 subunit also plays a role in mecamylamine-precipitated somatic signs of withdrawal. We showed that α7 -/- mice show an intermediate withdrawal phenotype on that experiment (20). Interestingly, it was reported by another group that

Table 1. nAChR Subunits, Their RNA Expression Patterns and the Major Phenotypes of Mutant Mice for Each Subunit[a]

Subunit	Expression	Major mutant mouse phenotypes	References
α2	High: IPN, amygdala Low: olf, basal ganglia, hippo, cx	α2 -/- mice show impaired hippocampal LTP, suggesting an effect on learning and memory.	Ishii et al., 2005 (193); Nakauchi et al., 2007 (194)
α3	High: olf, MHb, IPN, pineal, #10 Low: thal, hypothal, SN/VTA, Sup col, cx	α3 -/- mice die days after birth. α3 +/- mice show decreased nicotine-induced seizures.	Salas et al., 2004a (19); Xu et al., 1999 (195)
α4	High: cx, VTA, striatum, thal, hypothal, MHb, etc.	α4 -/- mice show altered nicotine-induced DA levels in the striatum. Activation of receptors in α4 gain of function mice is sufficient for reward, tolerance and sensitization.	Marubio et al., 2003 (196); Tapper et al., 2004 (16)
α5	High: hippo (CA1), IPN, SN/VTA, #10 Low: Cx	Decreased nicotine-induced hypolocomotion and seizures. Impaired autonomic function under certain conditions.	Salas et al., 2003 (185); Wang et al., 2002 (197)
α6	High: SN/VTA	α6 is a partner of the β3 and β2 subunits. It dominates the nicotine control of dopamine neurotransmission in nucleus accumbens.	Champtiaux et al., 2002 (198); Exley et al., 2007 (17)
α7	High: cx, hippo, thal, VTA, striatum, etc.	No major behavioral phenotype. Normal sensitivity to acute nicotine. Decreased nicotine withdrawal somatic signs and hypernociception. α7 gain of function mice show increased sensitivity to nicotine-induced seizures.	Broide et al., 2002 (199); Franceschini et al., 2002 (200); Grabus et al., 2005 (56); Paylor et al., 1998 (201); Salas et al., 2007 (20)
α9/α10	High: vestibular and cochlear hair cells	Related to hearing function. No known involvement with nicotine addiction.	Elgoyhen et al., 1994 (202); Elgoyhen et al., 2001 (203)
β2	High: cx, VTA, striatum, thal, hypothal, MHb, etc.	Implicated in nicotine reinforcement and self-administration. These phenotypes were rescued by viral re-expression of β2 in the VTA of β2 -/- mice. β2 -/- mice show normal somatic signs of nicotine withdrawal.	Maskos et al., 2005 (14); Picciotto et al., 1998 (15); Salas et al., 2004b (186)
β3	High: MHb, SN/VTA Low: Sup Col, LC	The α-conotoxin MII-sensitive component of nicotine-induced DA release is lost in β3 -/- mice, which also show less anxiety-like behavior.	Booker et al., 2007 (204); Cui et al., 2003 (205)
β4	High: olf, MHb, IPN, pineal, #10	β4 -/- mice show decreased anxiety-like behavior, are insensitive to nicotine-induced seizures and hypolocomotion. Do not show somatic signs of nicotine withdrawal.	Salas et al., 2004a (19); Salas et al., 2004b (186); Salas et al., 2003 (206)

[a] IPN, interpeduncular nucleus; olf, olfactory bulb; hippo, hippocampus; cx, cortex; MHb, medial habenula; #10, cranial nerve #10; thal, thalamus; hypothal, hypothalamus; SN/VTA substantia nigra/ventral tegmental area; Sup Col, superior colliculus; LC, locus coeruleus.

α7 -/- mice show normal somatic signs of spontaneous withdrawal, but decreased hyperalgesia upon spontaneous nicotine withdrawal (56). The apparent differences between these two reports may be due to the different protocols followed.

Non-Somatic Signs. There are four main measures of affective signs of withdrawal that have consistently been reported on rodents: anhedonia, conditioned place aversion, anxiety-related behavior and conditioned fear.

A major affective symptom of nicotine withdrawal is a diminished interest in rewarding stimuli, or anhedonia (57). Interestingly, depression, which is highly correlated to nicotine withdrawal, also produces anhedonia (58). In rats, anhedonia is measured as an increase in brain-stimulation reward thresholds (57). Withdrawal from several drugs of abuse, including nicotine, significantly elevates brain-stimulation reward thresholds, reflecting lower interest in the electrical stimuli that seem rewarding in basal conditions (58). Both spontaneous (59, 60) and mecamylamine-precipitated (61) nicotine withdrawal induce increases in self-stimulation thresholds.

Conditioned place aversion is another paradigm used to reveal the affective signs of withdrawal (58). After exposure to a two-chamber apparatus, chronic nicotine-treated animals are injected with an antagonist to precipitate withdrawal, and immediately confined in one of the chambers. Following injection of saline, animals are confined in the other compartment. When tested without drug and with access to both chambers, animals prefer to stay in the chamber that has been paired with saline and not

in the chamber paired with antagonist and therefore, withdrawal symptoms. Different antagonists and rat strains have been used in this paradigm, revealing an effect of both strain and type of drug on the amount of antagonist needed to observe place aversion (61, 62).

The third non-somatic manifestation of nicotine withdrawal is increased anxiety-like behavior in the elevated plus maze (EPM). In the EPM the animal explores 4 corridors arranged in a plus sign shape. Two of the corridors have tall walls while the other 2 are without walls, and the maze is elevated 50 cm from the floor. Rodent behavior on this maze has been repeatedly used as a model for anxiety (63). Mice and rats undergoing nicotine withdrawal showed increased anxiety-like behavior in the EPM (54, 64). This phenomenon mirrors the increase in anxiety reported by humans experiencing nicotine abstinence and suggests that the sensitivity to anxiety might influence the degree of withdrawal signs.

Cognitive symptoms of withdrawal can be explored in rodents with the conditioned fear paradigm (65). In this task, animals are trained in a context by pairing an auditory conditioned stimulus (CS) with a foot shock unconditioned stimulus (US). The association formed between the training context and the US (contextual fear conditioning) requires the hippocampus, while the association between the CS and the US (cued fear conditioning) does not require the hippocampus (66, 67). Nicotine withdrawal produces deficits in contextual fear conditioning and seems to selectively affect the acquisition of but not the recall or expression of the learned response (68). β2* nAChR are involved in this aspect of the withdrawal syndrome (68).

Current Pharmacotherapies for Smoking Cessation

Nicotine Replacement Therapy. Nicotine replacement therapy (NRT) is usually the first choice for those smokers that want to quit. NRT provides an alternate source of nicotine without the tars and poisonous gases found in cigarettes. It promotes smoking cessation by allowing smokers to control cravings while they gradually decrease nicotine intake. NRT is available as transdermal patches, chewing gum, nasal sprays, inhalers, sublingual tablets, and lozenges. Except for the nasal spray, all other forms of NRT deliver nicotine more slowly than cigarettes. NRT is effective at reducing craving and withdrawal associated with quitting (69). However, given the rapid rise in nicotine levels during smoking, NRT users may still be able to obtain additional reinforcement from cigarettes during treatment (70). This phenomenon, coupled with the sensory cues that further maintain tobacco dependence (71) make the success rate of NRT much lower than desirable.

Partial Agonists. A second approach to smoking cessation is the treatment with a combination of nicotine and a non-selective nAChR antagonist to achieve partial receptor agonism. Mechanistically, this combination of agents is expected to target the nAChRs that mediate the reinforcing effects of nicotine. Due to the presence of the antagonist, the effects of nicotine are attenuated so that its reinforcing effects are diminished but are still sufficient to prevent craving. The administration of the non-selective nAChR antagonist mecamylamine alone was one of the earliest suggestions for smoking cessation pharmacotherapy (72). Mecamylamine dose-dependently reduced the subjective effects of nicotine in some smokers (73). The results, however, were inconsistent in that mecamylamine increased cigarette consumption in some studies. In addition, side effects compromised compliance. Rose and Levin (74) were the first to propose the co-administration of nicotine and mecamylamine. Small clinical trials using transdermal nicotine and oral mecamylamine showed that this approach might be valuable, as the combination achieved higher abstinence rates than did transdermal nicotine alone (75). Despite the potential benefits of this pharmacological strategy, administering two separate compounds with different pharmacokinetic and metabolic profiles under the condition of maintaining a narrow agonist-to-antagonist ratio can be extremely challenging. Nevertheless, this attempt introduced the concept of partial nAChR agonism, especially at α4β2* nAChRs, as a strategy for smoking cessation. The use of cytisine further supported the concept. Cytisine, a nAChR partial agonist (76), is a plant alkaloid that has been used for over 40 years in Eastern Europe, but not in the US, as a smoking cessation agent (77).

Varenicline, the first partial nicotinic agonist approved in the USA as a therapeutic aid to smoking cessation, was developed by merging structural elements of nicotinic and opioid ligands (78, 79). The rationale behind varenicline's clinical efficacy is the one previously discussed. When nicotine is not present it acts as a partial agonist, providing at least part of the reinforcing effects of nicotine a smoker is used to receiving through nicotine. This effect would reduce cravings and withdrawal when nicotine is not present. In contrast, when nicotine is present, varenicline would act as an antagonist, preventing the rewarding effects of nicotine (80). In fact, varenicline has effects on dopamine release that are similar to those of nicotine, but at a smaller scale (80). In a study comparing varenicline with bupropion (brand name Zyban, see below) and placebo, 23% of smokers taking varenicline, 15% taking bupropion and 10% taking placebo were able to quit and remained abstinent for one year (81). It should be noted that although varenicline was conceived and marketed as a α4/β2-specific partial agonist, it is also a full agonist at α7*, and a partial agonist at α3β4* nAChRs. In fact, the efficacy of varenicline as a partial agonist is much higher for α3β4* and α7* nAChRs than it is for α4β2* receptors (82). Therefore, it is possible that nAChR subtypes other than those containing α4 and β2 are also implicated in the effects of varenicline on nicotine withdrawal.

Bupropion. Bupropion is an atypical antidepressant (83) marketed as Wellbutrin for its antidepressing effects,

and as Zyban (Glaxo Wellcome) for its anti-tobacco properties. It was the first non-nicotine-based therapy approved by the Food and Drug Administration (FDA) for smoking cessation (84). The mechanisms of bupropion's effects on depression and tobacco smoke seem independent from one another, i.e. the efficacy as anti-tobacco drug does not depend on the presence of current or past history of depression (85). Bupropion can double long-term abstinence rates compared to placebo (21) by decreasing both cue-induced tobacco craving (86) and withdrawal symptoms such as depression, difficulty concentrating and irritability (87).

Animal studies have shown that bupropion alters brain reward circuits influenced by nicotine, reversing the elevated intracranial self-stimulation thresholds resulting from nicotine abstinence (88), thus impairing the negative reinforcing effects of nicotine (59). In rodents, bupropion can alter nicotine reinforcement with some doses causing a decrease in nicotine self-administration behavior (89–91). However, a biphasic dose-response pattern to nicotine has also been reported, with low doses of bupropion increasing nicotine infusions and high doses decreasing responding non-specifically (89, 91). In addition, bupropion has been shown to attenuate the nicotine abstinence syndrome in rats. Acute and chronic bupropion exposures alleviate the expression of somatic signs associated with spontaneous and precipitated withdrawal, respectively, and chronic exposure also reduces the place aversion conditioned to mecamylamine-precipitated nicotine abstinence (88, 92).

Although the mechanisms of action of bupropion on nicotine addiction remain uncertain, bupropion is known to affect several systems involved in addiction. First, bupropion decreases DA reuptake in the mesolimbic system (93). Bupropion also inhibits noradrenaline (NE) reuptake in the locus coeruleus (93, 94), which is thought to be involved in nicotine withdrawal (95). Finally, bupropion also acts as an antagonist for nAChRs, including the $\alpha3\beta4^*$ subtype (96–98).

Cannabinoid Receptor Antagonists. Central cannabinoid (CB) receptors, especially the CB1 subtype, have been recently implicated in brain reward function due to the ability of endocannabinoids to increase dopamine (DA) levels in the mesolimbic system (99–101). Interestingly, $\Delta9$ tetrahydrocannabinol (THC), a CB receptor antagonist, potentiates the effects of non-pharmacologically active doses of nicotine (102), suggesting an interaction between the nicotinic and the cannabinoid systems. Therefore, CB1 receptor antagonists represent potential aids for smoking cessation. Indeed, the CB1 receptor antagonist rimonabant (SR 141716, trade name Acomplia) has been shown to reduce the motivational effects of nicotine in the conditioned place preference and the nicotine self-administration paradigms (103, 104). Rimonabant was the first CB1 receptor antagonist to be clinically tested in the European Union and it is currently under Phase III clinical trails in the US. The drug was initially developed as a possible treatment for obesity as CB1 receptors participate in the control of food consumption and energy expenditure. It has also been proposed as a smoking cessation aid and may protect successful quitters from significant post-cessation weight gain (105, 106). The beneficial effects of rimonabant on weight loss have been demonstrated in a recent Cochrane review (107). Since weight gain is one reason why some smokers avoid quit attempts, the dual action of rimonabant on decreasing weight gain and on smoking cessation may be particularly useful, especially when cardiac risk factors (obesity and tobacco smoke being two critical factors) are taken into account (108).

Mechanisms of Nicotine Withdrawal

nAChRs are expressed throughout the CNS and can influence a number of brain areas and functions. The nicotine contained in tobacco produces neuroadaptations that may explain the alteration in brain reward systems involved in the addiction process (109). Such neuroadaptations reflect nicotine's influences on several neurotransmitter systems, including acetylcholine, dopamine (DA), opioid peptides, serotonin (5-HT), and glutamate. Abrupt cessation of nicotine is likely to alter the neurochemistry of the addicted brain, thus triggering the affective and somatic signs of withdrawal. Neuroadaptations that affect multiple neurotransmitter systems are common to other drugs of abuse. One example is given by opiate addiction in which a number of non-opioid transmitters have been postulated to be involved in the development of both dependence and abstinence (110–113). The symptoms of nicotine abstinence may therefore reflect the activation of several brain circuits. How each brain circuit is altered during both nicotine exposure and withdrawal is likely to depend on the pharmacological and biophysical properties of the nAChR subtypes expressed in the brain areas that are important for that circuit.

Dopamine and Nicotine Withdrawal. The mesolimbic dopaminergic system serves a fundamental role in the acquisition of behaviors that are inappropriately reinforced by addictive drugs, and is very likely to also participate in the mechanisms of withdrawal (114). In the nucleus accumbens (NAcc), spontaneous or mecamylamine-precipitated nicotine withdrawal is associated with a decrease in extracellular DA levels (115–117). Whether altered DA levels participate in both the somatic and motivational aversive aspects of withdrawal is not clear (58, 118). In fact, the somatic signs of withdrawal seem to appear earlier than the decreases in accumbal DA output (115). This temporal dissociation between the two phenomena suggests that accumbal DA may not necessarily be involved in mediating the somatic aspects of nicotine withdrawal. Interestingly, the opioid receptor antagonist naloxone can increase somatic withdrawal signs in nicotine-dependent rats without affecting accumbal DA release (119).

In addition to the NAcc, DA fibers that arise within the

ventral tegmental area (VTA) also project to the prefrontal cortex (PFC). In contrast to the deficits in DA transmission observed in the NAcc, nicotine withdrawal increases DA output in the PFC of rats (119). Such increases in PFC DA release may be important in mediating aversive aspects of nicotine withdrawal, as enhanced DA transmission in the PFC has been observed during exposure to stressful and aversive stimuli (120–122), and has been implicated in mediating anxiety-related behaviors (123, 124). As previously discussed, anxiety is one of the manifestations of nicotine withdrawal in humans as well as in animal models of addiction.

Norepinephrine and Nicotine Withdrawal. Nicotine enhances the release of norepinephrine (NE) in various CNS regions (125, 126), and NE mechanisms can modulate midbrain DA function (127). In addition, the NE reuptake inhibitor reboxetine attenuates nicotine self-administration (128), and bupropion, which has an NE component to its action, is used in smoking cessation treatments (129). The noradrenergic tricyclic antidepressant nortriptyline, which inhibits serotonin and noradrenaline reuptake, could also work as second-line therapy for smoking cessation (130). Despite this evidence suggesting that noradrenergic mechanisms might be important for nicotine abuse, not much is known on the role of NE in the mechanisms of nicotine withdrawal. One report indicates that nicotine withdrawal alters NE levels in the hypothalamus and cortex of mice exposed to chronic nicotine in the drinking water (131). $\alpha 7^*$ nAChRs might be involved in this phenomenon (132). The paucity of data on nicotine contrasts with the abundant literature implicating noradrenergic mechanisms in opioid withdrawal. For example, withdrawal from chronic morphine increases NE release in the cortex and the bed nucleus of the stria terminalis (BNST) (133–135). NE release in the BNST may underlie anxiety associated with protracted withdrawal.

Serotonin and Nicotine Withdrawal. 5-HT is also expected to influence nicotine reward, as nicotine increases 5-HT release in the cortex, striatum, hippocampus, dorsal raphe nucleus, hypothalamus, and spinal cord (136, 137). In addition, DA neurons are influenced by 5-HT mechanisms (138). As for the role of 5-HT in withdrawal, it has been proposed that reduced serotonergic neurotransmission may contribute to the anhedonia observed during both amphetamine and nicotine withdrawal in humans (60, 139, 140). The role of the various 5-HT receptors and the anatomical localization of the 5-HT/withdrawal interaction are less clear. Some investigators suggested that during nicotine withdrawal, 5-HT activates inhibitory somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei leading to a decrease in 5-HT release into forebrain and limbic sites (141, 142). This conclusion is supported by the observation that a serotonergic antidepressant treatment that combines the 5-HT-selective re-uptake inhibitor fluoxetine and a 5-HT_{1A} receptor antagonist rapidly reverses the elevation in brain-stimulation reward thresholds observed in rats undergoing nicotine withdrawal (60, 143). Contrary to the view that reduced serotonergic transmission contributes to nicotine withdrawal, Cheeta and colleagues showed that administration of nicotine directly into the dorsal raphe nucleus, at a concentration that activates somatodendritic 5-HT_{1A} receptors, reverses the increase in anxiety observed in rats undergoing nicotine withdrawal as measured in the social interaction test (144). Other studies have implicated the activation of 5-HT_3 receptors in the amygdala in the heightened anxiety observed during nicotine withdrawal (145).

Opioids and Nicotine Withdrawal. The first model for nicotine withdrawal in rodents was developed by modification of a previous model used in opioid research (53). The behavioral similarities between these two models prompted a follow-up study, where rats treated chronically with nicotine were injected with the opioid receptor antagonist, naloxone, and nicotine withdrawal symptoms became apparent (146). In a separate experiment, acute morphine injection diminished spontaneous nicotine withdrawal, consistent with a major role for the opioid system in nicotine withdrawal (146). Genetic approaches have also demonstrated the impact of the opioid system in nicotine withdrawal in mice. A role for endogenous enkephalins on nicotine withdrawal was investigated by using preproenkephalin knock-out mice (147). In these mice the somatic expression of mecamylamine-precipitated nicotine withdrawal was significantly attenuated. Other effects of nicotine such as nicotine-induced antinociception, conditioned place preference, and enhancement in DA extracellular levels in the nucleus accumbens induced by nicotine were also reduced in preproenkephalin-deficient mice, demonstrating an important role for the endogenous opioid system not only in nicotine withdrawal but also in nicotine rewarding properties (147). Another interesting observation is that 18-Methoxycoronaridine (18-MC), a potent antagonist of $\alpha 3\beta 4$ nicotinic receptors, inhibits systemic morphine-induced increases in DA levels when injected into the rat habenula or IPN (148).

Glutamate and Nicotine Withdrawal. Nicotine, acting at presynaptic receptors, enhances glutamate release in several areas of the brain including the VTA, PFC and NAcc (149, 150). The glutamate released upon nicotine exposure binds to metabotropic and ionotropic glutamate receptors on postsynaptic dopaminergic neurons thereby increasing their bursting activity and increasing dopamine release. These actions may partly mediate the reinforcing effects of acute nicotine (150) as antagonists of the mGluR5 receptor subtype have been shown to decrease both intravenous nicotine self-administration (151) and cue-induced reinstatement of nicotine seeking (152). Other metabotropic glutamate receptor subtypes may be involved in the nicotine withdrawal syndrome, in particular in the negative affective symptoms. As previously discussed, nicotine withdrawal produces anhedonia, or diminished interest in pleasure. In rodents, anhedonia is believed to

produce an increase in the threshold for stimulation in the intracranial self-stimulation procedure (60). This elevation in threshold is blocked by mGluR2/3 antagonists (153). Therefore, mGluR5 affects nicotine self-administration while mGluR2/3 affects nicotine withdrawal. This parallels the roles of the β2 and β4 nAChR subunits in rodents. Interestingly, mGluR5 is expressed in several areas of the brain, but not in the MHb, while mGluR3 is expressed in a more restricted pattern, with high levels in the MHb, among other areas (154).

Monoamino Oxidase Inhibitors and Nicotine Withdrawal. Although it is known that nicotine is the main addictive component of tobacco, tobacco smoke contains many other compounds including monoamino oxidase inhibitors (MAOIs). It has been shown that whereas behavioral sensitization to D-amphetamine stayed constant following up to 30 days of withdrawal, similar conditions abolished behavioral sensitization to nicotine. Following 30 days of withdrawal, locomotor responses to nicotine were identical to those in naïve mice. However, when the MAOIs tranylcypromine or pargyline were co-injected with nicotine, behavioral sensitization was maintained even after long-term withdrawal (155). In a similar report, it was shown that in nicotine-infused rats, mecamylamine induced a place aversion that lasted 6 weeks. When nicotine-infused rats were also treated with a MAOI, mecamylamine-induced conditioned place aversion persisted for at least 8 months of abstinence. In addition, the MAOI treatment slightly decreased ratings of somatic signs induced by mecamylamine administration (156). These data suggest that nicotine may not be the only psychoactive substance in tobacco, and that MAOIs may potentiate the effects of nicotine.

Brain Regions Associated to Drug Withdrawal. A widely accepted view of the mechanisms of drug addiction is that the reinforcing effects of most drugs of abuse depend on induction of increases in DA levels in the nucleus accumbens. DA has been implicated in motor and cognitive function, and in regulation of reward, saliency and motivation (157–159). The NAcc receives dopaminergic innervation from the VTA, and it has been shown that β2* nAChRs in the VTA are necessary for the rewarding effects of nicotine (14). The PFC, which has connections to both NAcc and VTA, is usually considered as the third region in this circuit (157). Other areas of the brain have also been shown to be important for the effect of drugs of abuse, such as the amygdala (160) and the hypothalamus (160). The involvement of the VTA/NAcc/PFC in addiction has been extensively reviewed elsewhere (157, 159). We will focus on the role of the habenula and interpeduncular nucleus on nicotine withdrawal.

The Role of the Habenula in Drug Withdrawal. The role of DA in reward has been best defined in the concept of 'incentive salience', in which DA levels are involved in reward prediction for the purpose of reward seeking (161). In monkeys, dopaminergic cells were shown to fire in response to reward. After training with a cue that predicts reward, dopaminergic cells fired in response to the prediction of reward, and not to the reward itself. Interestingly, dopaminergic cells fired less than normal if reward was not delivered when expected (162). A recent report (163), as well as a body of literature from the 1980s (164–166) points to the habenula as the region that controls the decrease in firing of dopaminergic cells in the NAcc.

The habenula has been implicated in withdrawal to drugs of addiction by the use of ibogaine and its derivative, 18-Methoxycoronaridine (MC-18). Ibogaine is an alkaloid extracted from the African shrub Tabemanthe Iboga (167). Several chemical addictions such as opiate, cocaine, nicotine and alcohol might be sensitive to the effects of ibogaine (168). The effects of ibogaine in rats include a decrease in self-administration, a decrease in withdrawal signs and a block of drug induced DA release. Ibogaine's effects might reflect the interaction with multiple neurotransmitter systems as the alkaloid acts at several neurotransmitter receptors such as sigma opioid, NMDA and nAChRs (169). More recently, a derivative of ibogaine, 18-Methoxycoronaridine (18-MC), was synthesized and used in similar experiments (170). In rodents, 18-MC decreased morphine, cocaine, methamphetamine, alcohol and nicotine self-administration, and opioid withdrawal signs. 18-MC also blocked the sensitization of morphine- and cocaine-induced increase in DA levels in the nucleus accumbens (171). Since 18-MC is a specific blocker of α3*β4* nACRs (171), the possible role of this block as the main mechanism of action of 18-MC was studied. Two lines of evidence pointed to α3*β4* nACRs as the primary target for 18-MC. First, combinations of sub-effective doses of 18-MC and other α3β4* nACRs antagonist such as mecamylamine or bupropion also decreased morphine, methamphetamine, and nicotine self-administration in rats (171). Second, microinjection of 18-MC in the MHb or the IPN, two areas dominated by β4* nACRs, is sufficient to attenuate DA sensitization to morphine in the nucleus accumbens (148). The fact that β4 nAChR null mice show no somatic signs of nicotine withdrawal (19) is in agreement with a possible major role of the habenular/interpeduncular system in drug addiction.

Anatomical Connections Between the Habenula and Mesencephalic Dopaminergic Areas. The habenular complex is an epithalamic area composed of both medial and lateral compartments that receive massive afferents from several DA-rich areas (medial frontal cortex, nucleus accumbens, olfactory bulb, septum and striatum) via the stria medullaris thalami. The main efferent pathway is the fasciculus retroflexus, which projects to IPN, VTA, substantia nigra (SN), medial raphe complex, locus coeruleus, and central gray (172–176). These anatomical connections support the notion that dopaminergic transmission in the midbrain might be regulated by inputs traveling along the fasciculus retroflexus and suggest an important role of the habenular complex as a modulatory relay between limbic forebrain structures and the midbrain.

Modulation of Dopaminergic Activity and Relay of Negative Feedback. Animal studies provide evidence that the VTA and SN receive inhibitory input from the habenula. Electrical stimulation of the habenular nuclei causes inhibition of ~85–90% of the DA neurons in the VTA and SN in rats (164). In contrast, habenular lesions increase DA turnover in the NAcc and prefrontal cortex, reflecting an activation of the dopaminergic system (166, 177). The interest in this brain area has been increased by recent data in both human and non-human primates. Experiments conducted in behaving monkeys have shown that the habenula is a source of negative reward signals in DA neurons and that it plays an important role in determining the reward-related activity of DA neurons (163). Those data are corroborated by functional MRI (fMRI) studies in humans showing that when a decision-making error is made and negative feedback is received (when expected reward fails to occur), the habenular nuclei are strongly activated (178, 179). Based on this information, we hypothesize that the activity of the habenula is increased during withdrawal, possibly through the activation of β4* nAChRs. While the lateral habenula sends direct projections to the midbrain, including the DA neurons in the SN pars compacta and VTA, and the 5-HT neurons in the dorsal raphe (175), the MHb sends most projections to the IPN (180). The IPN in turn sends projections to the raphé nuclei and the VTA (180–182). Therefore, the MHb likely influences monoaminergic transmission via its connections to the IPN.

Expression Patterns of nAChRs in the Habenula and IPN. nAChRs are found on the soma of MHb cells, which express particularly high levels of mRNA for β4 and α3 and, to a lesser extent, for α5 (183–186). A combination of whole-cell recording and single cell RT-PCR techniques showed that, at least in the habenular areas studied, 95–100% of MHb cells express α3, α4, α5, β2 and β4, whereas approximately 40% of the cells express α6, α7 and β3 (187). Recent work using β4-selective monoclonal antibodies confirmed prominent immunoreactivity in the MHb (188). The MHb provides dense cholinergic innervation to the IPN with projections that run ipsilaterally in the fasciculus retroflexus (180, 189). The presynaptic terminals that regulate ACh release onto the IPN may also express α3*β4* nAChRs, as shown by experiments using α-conotoxin AuIB and β2 null mutants (190). IPN neurons express nAChR complexes containing both β2 and β4 (191, 192) along with α2. Based on the literature, most MHb neurons express α3*β4* nAChRs, while in the IPN α2 would be the most likely partner for β4, possibly in combination with α5.

Concluding Remarks

The nicotinic system and its roles in physiology and disease, including addiction, are actively being studied by different techniques. One of the most prolific approaches has been the study of subunit-specific mutant mice. From an assessment of the literature, a major role for α4*β2* receptors in reward seems apparent. This effect is mediated by the VTA/NAcc dopaminergic connection. In contrast, nicotine withdrawal, especially the somatic signs, seem to be mediated by β4* nAChRs in the MHb and IPN.

To design improved anti-tobacco therapies we must focus on the withdrawal symptoms that appear upon smoking cessation. The wealth of information on the nicotinic system and its roles on tobacco addiction is moving us closer to that goal.

1. WHO. http://www.who.int/mediacentre/news/releases/pr82/en. 2002.
2. WHO. http://www.who.int/tobacco/health_priority/en/print.html. 2007.
3. IARC. Tobacco smoke and involuntary smoking. IARC Monogr Eval Carcinog Risks Hum 83:1–1438, 2004.
4. Carlezon WA Jr, Duman RS, Nestler EJ. The many faces of CREB. Trends Neurosci 28(8):436–445, 2005.
5. Vezina P, McGehee DS, Green WN. Exposure to nicotine and sensitization of nicotine-induced behaviors. Prog Neuropsychopharmacol Biol Psychiatry 31(8):1625–1638, 2007.
6. Hughes JR, Keely J, Naud S. Shape of the relapse curve and long-term abstinence among untreated smokers. Addiction (Abingdon, England) 99(1):29–38, 2004.
7. West RJ, Hajek P, Belcher M. Severity of withdrawal symptoms as a predictor of outcome of an attempt to quit smoking. Psychol Med 19(4):981–985, 1989.
8. Piasecki TM, Jorenby DE, Smith SS, Fiore MC, Baker TB. Smoking withdrawal dynamics: III. Correlates of withdrawal heterogeneity. Exp Clin Psychopharmacol 11(4):276–285, 2003.
9. Dani JA, Bertrand D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol 47(1):699–729, 2007.
10. Dajas-Bailador F, Wonnacott S. Nicotinic acetylcholine receptors and the regulation of neuronal signalling. Trends Pharmacol Sci 25(6): 317–324, 2004.
11. Giniatullin R, Nistri A, Yakel JL. Desensitization of nicotinic ACh receptors: shaping cholinergic signaling. Trends Neurosci 28(7):371–378, 2005.
12. Quick MW, Lester RA. Desensitization of neuronal nicotinic receptors. J Neurobiol 53(4):457–478, 2002.
13. Dani JA, Heinemann S. Molecular and cellular aspects of nicotine abuse. Neuron 16(5):905–908, 1996.
14. Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, Evrard A, Cazala P, Cormier A, Mameli-Engvall M, Dufour N, Cloez-Tayarani I, Bemelmans AP, Mallet J, Gardier AM, David V, Faure P, Granon S, Changeux JP. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. Nature 436(7047):103–107, 2005.
15. Picciotto 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 391(6663):173–177, 1998.
16. Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, Lester HA. Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. Science 306(5698):1029–1032, 2004.
17. Exley R, Clements MA, Hartung H, McIntosh JM, Cragg SJ. Alpha6-containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine neurotransmission in nucleus accumbens. Neuropsychopharmacology, epub ahead of print, 2007.
18. Besson M, David V, Suarez S, Cormier A, Cazala P, Changeux JP,

Granon S. Genetic dissociation of two behaviors associated with nicotine addiction: beta-2 containing nicotinic receptors are involved in nicotine reinforcement but not in withdrawal syndrome. Psychopharmacology 187(2):189–199, 2006.
19. Salas R, Pieri F, De Biasi M. Decreased signs of nicotine withdrawal in mice null for the β4 nicotinic acetylcholine receptor subunit. J Neurosci 24(45):10035–10039, 2004.
20. Salas R, Main A, Gangitano DA, De Biasi M. Decreased withdrawal symptoms but normal tolerance to nicotine in mice null for the alpha 7 nicotinic acetylcholine receptor subunit. Neuropharmacology 53(7): 863–869, 2007.
21. Hughes JR. Effects of abstinence from tobacco: valid symptoms and time course. Nicotine Tob Res 9(3):315–327, 2007.
22. Hughes JR, Hatsukami D. Signs and symptoms of tobacco withdrawal. Arch Gen Psychiatry 43(3):289–294, 1986.
23. al'Absi M, Amunrud T, Wittmers LE. Psychophysiological effects of nicotine abstinence and behavioral challenges in habitual smokers. Pharmacol Biochem Behav 72(3):707–716, 2002.
24. Gilbert DG, McClernon FJ, Rabinovich NE, Plath LC, Masson CL, Anderson AE, Sly KF. Mood disturbance fails to resolve across 31 days of cigarette abstinence in women. J Consult Clin Psychol 70(1): 142–152, 2002.
25. Gulliver SB, Hughes JR, Solomon LJ, Dey AN. An investigation of self-efficacy, partner support and daily stresses as predictors of relapse to smoking in self-quitters. Addiction 90(6):767–772, 1995.
26. Swan GE, Ward MM, Jack LM. Abstinence effects as predictors of 28-day relapse in smokers. Addict Behav 21(4):481–490, 1996.
27. Ward MM, Swan GE, Jack LM. Self-reported abstinence effects in the first month after smoking cessation. Addict Behav 26(3):311–327, 2001.
28. Parrott AC. Stress modulation over the day in cigarette smokers. Addiction 90(2):233–244, 1995.
29. Cohen S, Lichtenstein E. Perceived stress, quitting smoking, and smoking relapse. Health Psychol 9(4):466–478, 1990.
30. Pomerleau OF, Pomerleau CS. Neuroregulators and the reinforcement of smoking: towards a biobehavioral explanation. Neurosci Biobehav Rev 8(4):503–513, 1984.
31. Giannakoulas G, Katramados A, Melas N, Diamantopoulos I, Chimonas E. Acute effects of nicotine withdrawal syndrome in pilots during flight. Aviat Space Environ Med 74(3):247–251, 2003.
32. Hughes JR, Gust SW, Skoog K, Keenan RM, Fenwick JW. Symptoms of tobacco withdrawal. A replication and extension. Arch Gen Psychiatry 48(1):52–59, 1991.
33. Jorenby DE, Hatsukami DK, Smith SS, Fiore MC, Allen S, Jensen J, Baker TB. Characterization of tobacco withdrawal symptoms: transdermal nicotine reduces hunger and weight gain. Psychopharmacology (Berl) 128(2):130–138, 1996.
34. Tate JC, Pomerleau OF, Pomerleau CS. Temporal stability and within-subject consistency of nicotine withdrawal symptoms. J Subst Abuse 5(4):355–363, 1993.
35. Lewis G. DSM-IV: Diagnostic and statistical manual of mental disorders. Arlington, VA: American Psychiatric Association, pp651–652, 1996.
36. Brown RA, Kahler CW, Zvolensky MJ, Lejuez CW, Ramsey SE. Anxiety sensitivity: relationship to negative affect smoking and smoking cessation in smokers with past major depressive disorder. Addict Behav 26(6):887–899, 2001.
37. Doherty K, Kinnunen T, Militello FS, Garvey AJ. Urges to smoke during the first month of abstinence: relationship to relapse and predictors. Psychopharmacology (Berl) 119(2):171–178, 1995.
38. Pomerleau CS, Marks JL, Pomerleau OF. Who gets what symptom? Effects of psychiatric cofactors and nicotine dependence on patterns of smoking withdrawal symptomatology. Nicotine Tob Res 2(3):275–280, 2000.
39. Carey MP, Kalra DL, Carey KB, Halperin S, Richards CS. Stress and unaided smoking cessation: a prospective investigation. J Consult Clin Psychol 61(5):831–838, 1993.
40. Daughton DM, Roberts D, Patil KD, Rennard SI. Smoking cessation in the workplace: evaluation of relapse factors. Prev Med 19(2):227–230, 1990.
41. Koval JJ, Pederson LL. Stress-coping and other psychosocial risk factors: a model for smoking in grade 6 students. Addict Behav 24(2): 207–218, 1999.
42. Morissette SB, Tull MT, Gulliver SB, Kamholz BW, Zimering RT. Anxiety, anxiety disorders, tobacco use, and nicotine: a critical review of interrelationships. Psychol Bull 133(2):245–272, 2007.
43. Perkins KA, Epstein LH, Grobe J, Fonte C. Tobacco abstinence, smoking cues, and the reinforcing value of smoking. Pharmacol Biochem Behav 47(1):107–112, 1994.
44. Perkins KA, Grobe JE. Increased desire to smoke during acute stress. Br J Addict 87(7):1037–1040, 1992.
45. Shiffman S, Hickcox M, Paty JA, Gnys M, Kassel JD, Richards TJ. Progression from a smoking lapse to relapse: prediction from abstinence violation effects, nicotine dependence, and lapse characteristics. J Consult Clin Psychol 64(5):993-1002, 1996.
46. Sinha R. How does stress increase risk of drug abuse and relapse? Psychopharmacology (Berl) 158(4):343–359, 2001.
47. Breslau N, Schultz L, Peterson E. Sex differences in depression: a role for preexisting anxiety. Psychiatry Res 58(1):1–12, 1995.
48. Japuntich SJ, Smith SS, Jorenby DE, Piper ME, Fiore MC, Baker TB. Depression predicts smoking early but not late in a quit attempt. Nicotine Tob Res 9(6):677–686, 2007.
49. Markou A. Metabotropic glutamate receptor antagonists: novel therapeutics for nicotine dependence and depression? Biol Psychiatry 61(1):17–22, 2007.
50. Pomerleau OF, Pomerleau CS, Mehringer AM, Snedecor SM, Ninowski R, Sen A. Nicotine dependence, depression, and gender: characterizing phenotypes based on withdrawal discomfort, response to smoking, and ability to abstain. Nicotine Tob Res 7(1):91–102, 2005.
51. Saladin ME, Brady KT, Dansky BS, Kilpatrick DG. Understanding comorbidity between ptsd and substance use disorders: two preliminary investigations. Addict Behav 20(5):643–655, 1995.
52. Erb S, Shaham Y, Stewart J. The role of corticotropin-releasing factor and corticosterone in stress- and cocaine-induced relapse to cocaine seeking in rats. J Neurosci 18(14):5529–5536, 1998.
53. Malin DH, Lake JR, Newlin-Maultsby P, Roberts LK, Lanier JG, Carter VA, Cunningham JS, Wilson OB. Rodent model of nicotine abstinence syndrome. Pharmacol Biochem Behav 43(3):779–784, 1992.
54. Damaj IM, Kao W, Martin BR. Characterization of spontaneous and precipitated nicotine withdrawal in the mouse. J Pharmacol Exp Ther, 307(2):526–534, 2003.
55. Isola R, Vogelsberg V, Wemlinger TA, Neff NH, Hadjiconstantinou M. Nicotine abstinence in the mouse. Brain Res 850(1–2):189–196, 1999.
56. Grabus SD, Martin BR, Imad Damaj M. Nicotine physical dependence in the mouse: involvement of the alpha7 nicotinic receptor subtype. Eur J Pharmacol 515(1–3):90–93, 2005.
57. Paterson NE, Markou A. Animal models and treatments for addiction and depression co-morbidity. Neurotox Res 11(1):1–32, 2007.
58. Kenny PJ, Markou A. Neurobiology of the nicotine withdrawal syndrome. Pharmacol Biochem Behav 70(4):531–549, 2001.
59. Epping-Jordan MP, Watkins SS, Koob GF, Markou A. Dramatic decreases in brain reward function during nicotine withdrawal. Nature 393(6680):76–79, 1998.
60. Harrison AA, Liem YT, Markou A. Fluoxetine combined with a serotonin-1A receptor antagonist reversed reward deficits observed during nicotine and amphetamine withdrawal in rats. Neuropsychopharmacology 25(1):55–71, 2001.

61. Watkins SS, Stinus L, Koob GF, Markou A. Reward and somatic changes during precipitated nicotine withdrawal in rats: centrally and peripherally mediated effects. J Pharmacol Exp Ther 292(3):1053–1064, 2000.
62. Suzuki T, Ise Y, Mori T, Misawa M. Attenuation of mecamylamine-precipitated nicotine-withdrawal aversion by the 5-HT3 receptor antagonist ondansetron. Life Sciences 61(16):PL249–254, 1997.
63. Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14(3):149–167, 1985.
64. Irvine EE, Cheeta S, File SE. Tolerance to nicotine's effects in the elevated plus-maze and increased anxiety during withdrawal. Pharmacol Biochem Behav 68(2):319–325, 2001.
65. Davis JA, James JR, Siegel SJ, Gould TJ. Withdrawal from chronic nicotine administration impairs contextual fear conditioning in C57BL/6 mice. J Neurosci 25(38):8708–8713, 2005.
66. Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106(2):274–285, 1992.
67. Logue SF, Paylor R, Wehner JM. Hippocampal lesions cause learning deficits in inbred mice in the Morris water maze and conditioned-fear task. Behav Neurosci 111(1):104–113, 1997.
68. Portugal GS, Kenney JW, Gould TJ. Beta2 subunit containing acetylcholine receptors mediate nicotine withdrawal deficits in the acquisition of contextual fear conditioning. Neurobiol Learn Mem 89(2):106–113, 2008.
69. Silagy C, Lancaster T, Stead L, Mant D, Fowler G. Nicotine replacement therapy for smoking cessation. Cochrane Database Syst Rev (3):CD000146, 2004.
70. Benowitz NL, Porchet H, Sheiner L, Jacob PD. Nicotine absorption and cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine gum. Clin Pharmacol Ther 44(1):23–28, 1988.
71. Rose JE, Behm FM. Extinguishing the rewarding value of smoke cues: pharmacological and behavioral treatments. Nicotine Tob Res 6(3):523–32, 2004.
72. Henningfield JE. Pharmacologic basis and treatment of cigarette smoking. J Clin Psychiatry 45(12 Pt 2):24–34, 1984.
73. Rose JE, Sampson A, Levin ED, Henningfield JE. Mecamylamine increases nicotine preference and attenuates nicotine discrimination. Pharmacol Biochem Behav 32(4):933–938, 1989.
74. Rose JE, Levin ED, Behm FM, Westman EC, Stein RM, Lane JD, Ripka GV. Combined administration of agonist-antagonist as a method of regulating receptor activation. Ann N Y Acad Sci 757: 218–221, 1995.
75. Lancaster T, Stead LF. Mecamylamine (a nicotine antagonist) for smoking cessation. Cochrane Database of Systematic Reviews (2): CD001009, 2000.
76. Papke RL, Heinemann SF. Partial agonist properties of cytisine on neuronal nicotinic receptors containing the beta 2 subunit. Mol Pharmacol 45(1):142–149, 1994.
77. Etter JF. Cytisine for smoking cessation: a literature review and a meta-analysis. Arch Intern Med 166(15):1553–1559, 2006.
78. Coe JW, Vetelino MG, Bashore CG, Wirtz MC, Brooks PR, Arnold EP, Lebel LA, Fox CB, Sands SB, Davis TI, Schulz DW, Rollema H, Tingley FD 3rd, O'Neill BT. In pursuit of alpha4beta2 nicotinic receptor partial agonists for smoking cessation: carbon analogs of (-)-cytisine. Bioorg Med Chem Lett 15(12):2974–2979, 2005.
79. Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, Lu Y, Mansbach RS, Mather RJ, Rovetti CC, Sands SB, Schaeffer E, Schulz DW, Tingley FD 3rd, Williams KE. Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. Neuropharmacology 52(3):985–994, 2007.
80. Rollema H, Coe JW, Chambers LK, Hurst RS, Stahl SM, Williams KE. Rationale, pharmacology and clinical efficacy of partial agonists of alpha4beta2 nACh receptors for smoking cessation. Trends Pharmacol Sci 28(7):316–325, 2007.
81. Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, Billing CB, Gong J, Reeves KR, Varenicline Phase 3 Study G. Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. [erratum appears in JAMA. 2006 Sep 20; 296(11):1355.] JAMA 296(1):56–63, 2006.
82. Mihalak KB, Carroll FI, Luetje CW. Varenicline is a partial agonist at alpha4beta2 and a full agonist at alpha7 neuronal nicotinic receptors. Mol Pharmacol 70(3):801–805, 2006.
83. Ascher JA, Cole JO, Colin JN, Feighner JP, Ferris RM, Fibiger HC, Golden RN, Martin P, Potter WZ, Richelson E. Bupropion: a review of its mechanism of antidepressant activity. J Clin Psychiatry 56(9): 395–401, 1995.
84. Siu EC, Tyndale RF. Non-nicotinic therapies for smoking cessation. Ann Rev Pharmacol Toxicol 47:541–564, 2007.
85. Hurt RD, Sachs DP, Glover ED, Offord KP, Johnston JA, Dale LC, Khayrallah MA, Schroeder DR, Glover PN, Sullivan CR, Croghan IT, Sullivan PM. A comparison of sustained-release bupropion and placebo for smoking cessation. N Engl J Med 337(17):1195–1202, 1997.
86. Brody AL, Mandelkern MA, Lee G, Smith E, Sadeghi M, Saxena S, Jarvik ME, London ED. Attenuation of cue-induced cigarette craving and anterior cingulate cortex activation in bupropion-treated smokers: a preliminary study. [erratum appears in Psychiatry Res. 2004 Dec 15; 132(2):183–184.] Psychiatry Res 130(3):269–281, 2004.
87. Perkins KA, Levine M, Marcus M, Shiffman S, D'Amico D, Miller A, Keins A, Ashcom J, Broge M. Tobacco withdrawal in women and menstrual cycle phase. J Consult Clin Psychol 68(1):176–180, 2000.
88. Cryan JF, Bruijnzeel AW, Skjei KL, Markou A. Bupropion enhances brain reward function and reverses the affective and somatic aspects of nicotine withdrawal in the rat. Psychopharmacology 168(3):347–358, 2003.
89. Bruijnzeel AW, Markou A. Characterization of the effects of bupropion on the reinforcing properties of nicotine and food in rats. Synapse 50(1):20–28, 2003.
90. Glick SD, Maisonneuve IM, Kitchen BA. Modulation of nicotine self-administration in rats by combination therapy with agents blocking alpha 3 beta 4 nicotinic receptors. Eur J Pharmacol 448(2–3):185–191, 2002.
91. Rauhut AS, Neugebauer N, Dwoskin LP, Bardo MT. Effect of bupropion on nicotine self-administration in rats. Psychopharmacology 169(1):1–9, 2003.
92. Malin DH, Lake JR, Smith TD, Khambati HN, Meyers-Paal RL, Montellano AL, Jennings RE, Erwin DS, Presley SE, Perales BA. Bupropion attenuates nicotine abstinence syndrome in the rat. Psychopharmacology 184(3–4):494–503, 2006.
93. Horst WD, Preskorn SH. Mechanisms of action and clinical characteristics of three atypical antidepressants: venlafaxine, nefazodone, bupropion. J Affect Disord 51(3):237–254, 1998.
94. Ferry LH. Non-nicotine pharmacotherapy for smoking cessation. Prim Care 26(3):653–669, 1999.
95. Leshner AI. Understanding drug addiction: implications for treatment. Hosp Pract (Minneapolis) 31(10):47–54, 1996.
96. Slemmer JE, Martin BR, Damaj MI. Bupropion is a nicotinic antagonist. J Pharmacol Exp Ther 295(1):321–327, 2000.
97. Fryer JD, Lukas RJ. Noncompetitive functional inhibition at diverse, human nicotinic acetylcholine receptor subtypes by bupropion, phencyclidine, and ibogaine. J Pharmacol Exp Ther 288(1):88–92, 1999.
98. Mansvelder HD, Fagen ZM, Chang B, Mitchum R, McGehee DS. Bupropion inhibits the cellular effects of nicotine in the ventral tegmental area. Biochem Pharmacol 74(8):1283–1291, 2007.

99. Diana M, Muntoni AL, Pistis M, Melis M, Gessa GL. Lasting reduction in mesolimbic dopamine neuronal activity after morphine withdrawal. Eur J Neurosci 11(3):1037–1041, 1999.
100. Bassareo V, Tanda G, Di Chiara G. Increase of extracellular dopamine in the medial prefrontal cortex during spontaneous and naloxone-precipitated opiate abstinence. Psychopharmacology (Berl) 122(2): 202–205, 1995.
101. Maldonado R, Valverde O, Berrendero F. Involvement of the endocannabinoid system in drug addiction. Trends Neurosci 29(4): 225–232, 2006.
102. Valjent E, Mitchell JM, Besson M-J, Caboche J, Maldonado R. Behavioural and biochemical evidence for interactions between [Delta]9-tetrahydrocannabinol and nicotine. Br J Pharmacol 135(2): 564–578, 2002.
103. Svedberg MM, Svensson AL, Johnson M, Lee M, Cohen O, Court J, Soreq H, Perry E, Nordberg A. Upregulation of neuronal nicotinic receptor subunits alpha4, beta2, and alpha7 in transgenic mice overexpressing human acetylcholinesterase. J Mol Neurosci 18(3): 211–222, 2002.
104. Le Foll B, Goldberg SR. Rimonabant, a CB1 antagonist, blocks nicotine-conditioned place preferences. Neuroreport 15(13):2139–2143, 2004.
105. EurekAlert!. Data shows new drug, rimonabant, helps smokers quit while limiting post cessation weight gain. Available at: http://www.eurekalert.org/pub_releases/2004-03/k-dsn030904.php. 2004.
106. Sanofi-Synth. New study confirms benefits of rimonabant in weight loss, waist circumference reduction and metabolic risk factor improvement. [cited 2004; Available from: http://es.sanofi-synthelabo.com/press/ppc_23804.asp]
107. Curioni C, Andre C. Rimonabant for overweight or obesity. Cochrane Database of Systematic Reviews (4):CD006162, 2006.
108. Pomerleau CS, Pomerleau OF, Namenek RJ, Mehringer AM. Short-term weight gain in abstaining women smokers. J Subst Abuse Treat 18(4):339–342, 2000.
109. Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24(2):97–129, 2001.
110. Aston-Jones G, Harris GC. Brain substrates for increased drug seeking during protracted withdrawal. Neuropharmacology 47(Suppl 1):167–179, 2004.
111. Harris GC, Aston-Jones G. Augmented accumbal serotonin levels decrease the preference for a morphine associated environment during withdrawal. Neuropsychopharmacology 24(1):75–85, 2001.
112. Koob GF, Nestler EJ. The neurobiology of drug addiction. J Neuropsychiatry Clin Neurosci 9(3):482–497, 1997.
113. Georges F, Aston-Jones G. Prolonged activation of mesolimbic dopaminergic neurons by morphine withdrawal following clonidine: participation of imidazoline and norepinephrine receptors. Neuropsychopharmacology 28(6):1140–1149, 2003.
114. Adriani W, Spijker S, Deroche-Gamonet V, Laviola G, Le Moal M, Smit AB, Piazza PV. Evidence for enhanced neurobehavioral vulnerability to nicotine during periadolescence in rats. J Neurosci 23(11):4712–4716, 2003.
115. Hildebrand BE, Nomikos GG, Hertel P, Schilstrom B, Svensson TH. Reduced dopamine output in the nucleus accumbens but not in the medial prefrontal cortex in rats displaying a mecamylamine-precipitated nicotine withdrawal syndrome. Brain Res 779(1–2): 214–225, 1998.
116. Rada P, Jensen K, Hoebel BG. Effects of nicotine and mecamylamine-induced withdrawal on extracellular dopamine and acetylcholine in the rat nucleus accumbens. Psychopharmacology (Berl) 157(1):105–110, 2001.
117. Rahman S, Zhang J, Engleman EA, Corrigall WA. Neuroadaptive changes in the mesoaccumbens dopamine system after chronic nicotine self-administration: a microdialysis study. Neuroscience 129(2):415–424, 2004.
118. Koob GF, Sanna PP, Bloom FE. Neuroscience of addiction. Neuron 21(3):467–476, 1998.
119. Carboni E, Bortone L, Giua C, Di Chiara G. Dissociation of physical abstinence signs from changes in extracellular dopamine in the nucleus accumbens and in the prefrontal cortex of nicotine dependent rats. Drug Alcohol Depend 58(1–2):93–102, 2000.
120. Thierry AM, Tassin JP, Blanc G, Glowinski J. Selective activation of mesocortical DA system by stress. Nature 263(5574):242–244, 1976.
121. Inglis FM, Moghaddam B. Dopaminergic innervation of the amygdala is highly responsive to stress. J Neurochem 72(3):1088–1094, 1999.
122. Kawasaki H, Kaufman O, Damasio H, Damasio AR, Granner M, Bakken H, Hori T, Howard MA 3rd, Adolphs R. Single-neuron responses to emotional visual stimuli recorded in human ventral prefrontal cortex. Nat Neurosci 4(1):15–16, 2001.
123. Bradberry CW, Lory JD, Roth RH. The anxiogenic beta-carboline FG 7142 selectively increases dopamine release in rat prefrontal cortex as measured by microdialysis. J Neurochem 56(3):748–752, 1991.
124. Broersen LM, Abbate F, Feenstra MG, de Bruin JP, Heinsbroek RP, Olivier B. Prefrontal dopamine is directly involved in the anxiogenic interoceptive cue of pentylenetetrazol but not in the interoceptive cue of chlordiazepoxide in the rat. Psychopharmacology (Berl) 149(4): 366–376, 2000.
125. Summers KL, Giacobini E. Effects of local and repeated systemic administration of (-)nicotine on extracellular levels of acetylcholine, norepinephrine, dopamine, and serotonin in rat cortex. Neurochem Res 20(6):753–759, 1995.
126. Fu Y, Matta SG, Brower VG, Sharp BM. Norepinephrine secretion in the hypothalamic paraventricular nucleus of rats during unlimited access to self-administered nicotine: an in vivo microdialysis study. J Neurosci 21(22):8979–8989, 2001.
127. Linner L, Endersz H, Ohman D, Bengtsson F, Schalling M, Svensson TH. Reboxetine modulates the firing pattern of dopamine cells in the ventral tegmental area and selectively increases dopamine availability in the prefrontal cortex. J Pharmacol Exp Ther 297(2):540–546, 2001.
128. Rauhut AS, Mullins SN, Dwoskin LP, Bardo MT. Reboxetine: attenuation of intravenous nicotine self-administration in rats. J Pharmacol Exp Ther 303(2):664–672, 2002.
129. Simon JA, Duncan C, Carmody TP, Hudes ES. Bupropion for smoking cessation: a randomized trial. Arch Intern Med 164(16): 1797–1803, 2004.
130. Hall SM, Reus VI, Munoz RF, Sees KL, Humfleet G, Hartz DT, Frederick S, Triffleman E. Nortriptyline and cognitive-behavioral therapy in the treatment of cigarette smoking. Arch Gen Psychiatry 55(8):683–690, 1998.
131. Gaddnas H, Pietila K, Ahtee L. Effects of chronic oral nicotine treatment and its withdrawal on locomotor activity and brain monoamines in mice. Behav Brain Res 113(1–2):65–72, 2000.
132. Jones IW, Barik J, O'Neill MJ, Wonnacott S. Alpha bungarotoxin-1.4 nm gold: a novel conjugate for visualising the precise subcellular distribution of alpha 7* nicotinic acetylcholine receptors. J Neurosci Methods 134(1):65–74, 2004.
133. Aghajanian GK. Tolerance of locus coeruleus neurones to morphine and suppression of withdrawal response by clonidine. Nature 276(5684):186–188, 1978.
134. Akaoka H, Aston-Jones G. Opiate withdrawal-induced hyperactivity of locus coeruleus neurons is substantially mediated by augmented excitatory amino acid input. J Neurosci 11(12):3830–3839, 1991.
135. Aston-Jones G, Delfs JM, Druhan J, Zhu Y. The bed nucleus of the stria terminalis. A target site for noradrenergic actions in opiate withdrawal. Ann N Y Acad Sci 877:486–498, 1999.
136. Hery F, Bourgoin S, Hamon M, Ternaux JP, Glowinski J. Control of the release of newly synthetized 3H-5-hydroxytryptamine by nicotinic and muscarinic receptors in rat hypothalamic slices. Naunyn Schmiedebergs Arch Pharmacol 296(2):91–97, 1977.
137. Ribeiro EB, Bettiker RL, Bogdanov M, Wurtman RJ. Effects of

systemic nicotine on serotonin release in rat brain. Brain Res 621(2): 311–318, 1993.
138. Kalivas PW. Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Res Brain Res Rev 18(1):75–113, 1993.
139. Hughes JR. Tobacco withdrawal in self-quitters. J Consult Clin Psychol 60(5):689–697, 1992.
140. Glassman AH. Cigarette smoking: implications for psychiatric illness. Am J Psychiatry 150(4):546–553, 1993.
141. Benwell ME, Balfour DJ. Effects of nicotine administration and its withdrawal on plasma corticosterone and brain 5-hydroxyindoles. Psychopharmacology (Berl) 63(1):7–11, 1979.
142. Benwell ME, Balfour DJ. The effects of nicotine administration on 5-HT uptake and biosynthesis in rat brain. Eur J Pharmacol 84(1–2):71–77, 1982.
143. Markou A, Paterson NE. The nicotinic antagonist methyllycaconitine has differential effects on nicotine self-administration and nicotine withdrawal in the rat. Nicotine Tob Res 3(4):361–373, 2001.
144. Cheeta S, Irvine EE, Kenny PJ, File SE. The dorsal raphe nucleus is a crucial structure mediating nicotine's anxiolytic effects and the development of tolerance and withdrawal responses. Psychopharmacology (Berl) 155(1):78–85, 2001.
145. Costall B, Jones BJ, Kelly ME, Naylor RJ, Onaivi ES, Tyers MB. Sites of action of ondansetron to inhibit withdrawal from drugs of abuse. Pharmacol Biochem Behav 36(1):97–104, 1990.
146. Malin DH, Lake JR, Carter VA, Cunningham JS, Wilson OB. Naloxone precipitates nicotine abstinence syndrome in the rat. Psychopharmacology (Berl) 112(2–3):339–342, 1993.
147. Berrendero F, Mendizabal V, Robledo P, Galeote L, Bilkei-Gorzo A, Zimmer A, Maldonado R. Nicotine-induced antinociception, rewarding effects, and physical dependence are decreased in mice lacking the preproenkephalin gene. J Neurosci 25(5):1103–1112, 2005.
148. Taraschenko OD, Shulan JM, Maisonneuve IM, Glick SD. 18-MC acts in the medial habenula and interpeduncular nucleus to attenuate dopamine sensitization to morphine in the nucleus accumbens. Synapse 61(7):547–560, 2007.
149. Mansvelder HD, McGehee DS. Long-term potentiation of excitatory inputs to brain reward areas by nicotine. Neuron 27(2):349–357, 2000.
150. Kenny PJ, Markou A. The ups and downs of addiction: role of metabotropic glutamate receptors. Trends Pharmacol Sci 25(5):265–272, 2004.
151. Paterson NE, Semenova S, Gasparini F, Markou A. The mGluR5 antagonist MPEP decreased nicotine self-administration in rats and mice. Psychopharmacology 167(3):257–264, 2003.
152. Bespalov AY, Dravolina OA, Sukhanov I, Zakharova E, Blokhina E, Zvartau E, Danysz W, van Heeke G, Markou A. Metabotropic glutamate receptor (mGluR5) antagonist MPEP attenuated cue- and schedule-induced reinstatement of nicotine self-administration behavior in rats. Neuropharmacology 49:167–178, 2005.
153. Kenny PJ, Gasparini F, Markou A. Group II metabotropic and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)/kainate glutamate receptors regulate the deficit in brain reward function associated with nicotine withdrawal in rats. J Pharmacol Exp Ther 306(3):1068–1076, 2003.
154. Mudo G, Trovato-Salinaro A, Caniglia G, Cheng Q, Condorelli DF. Cellular localization of mGluR3 and mGluR5 mRNAs in normal and injured rat brain. Brain Res 1149:1–13, 2007.
155. Villegier AS, Blanc G, Glowinski J, Tassin JP. Transient behavioral sensitization to nicotine becomes long-lasting with monoamine oxidases inhibitors. Pharmacol Biochem Behav 76(2):267–274, 2003.
156. Guillem K, Vouillac C, Azar MR, Parsons LH, Koob GF, Cador M, Stinus L. Monoamine oxidase inhibition dramatically increases the motivation to self-administer nicotine in rats. J Neurosci 25(38):8593–8600, 2005.
157. Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162(8):1403–1413, 2005.
158. Schultz W. Multiple dopamine functions at different time courses. Annu Rev Neurosci 30:259–288, 2007.
159. Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. Ann Rev Neurosci 29:565–598, 2006.
160. Koob G, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. Am J Psychiatry 164(8):1149–1159, 2007.
161. McClure SM, Daw ND, Montague PR. A computational substrate for incentive salience. Trends Neurosci 26(8):423–428, 2003.
162. Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. Science 275(5306):1593–1599, 1997.
163. Matsumoto M, Hikosaka O. Lateral habenula as a source of negative reward signals in dopamine neurons. Nature 447(7148):1111–1115, 2007.
164. Christoph GR, Leonzio RJ, Wilcox KS. Stimulation of the lateral habenula inhibits dopamine-containing neurons in the substantia nigra and ventral tegmental area of the rat. J Neurosci 6(3):613–619, 1986.
165. Lisoprawski A, Herve D, Blanc G, Glowinski J, Tassin JP. Selective activation of the mesocortico-frontal dopaminergic neurons induced by lesion of the habenula in the rat. Brain Res 183(1):229–234, 1980.
166. Nishikawa T, Fage D, Scatton B. Evidence for, and nature of, the tonic inhibitory influence of habenulointerpeduncular pathways upon cerebral dopaminergic transmission in the rat. Brain Res 373(1–2): 324–336, 1986.
167. Fernandez JW. Bwiti-An ethnography of the religious imagination in Africa. Princeton, NJ: Princeton University Press, pp470–493, 1982.
168. Alper KR, Lotsof HS, Kaplan CD. The ibogaine medical subculture. J Ethnopharmacol 115(1):9–24, 2008.
169. Glick SD, Maisonneuve IS. Mechanisms of antiaddictive actions of ibogaine. Ann N Y Acad Sci 844:214–226, 1998.
170. Glick SD, Maisonneuve IM, Szumlinski KK. Mechanisms of action of ibogaine: relevance to putative therapeutic effects and development of a safer iboga alkaloid congener. Alkaloids Chem Biol 56:39–53, 2001.
171. Maisonneuve IM, Glick SD. Anti-addictive actions of an iboga alkaloid congener: a novel mechanism for a novel treatment. Pharmacol Biochem Behav 75(3):607–618, 2003.
172. Greatrex RM, Phillipson OT. Demonstration of synaptic input from prefrontal cortex to the habenula in the rat. Brain Res 238(1):192–197, 1982.
173. Scheibel AB. The thalamus and neuropsychiatric illness. J Neuropsychiatry Clin Neurosci 9(3):342–353, 1997.
174. Sutherland RJ. The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex. Neurosci Biobehav Rev 6(1):1–13, 1982.
175. Herkenham M, Nauta WJ. Efferent connections of the habenular nuclei in the rat. J Comp Neurol 187(1):19–47, 1979.
176. Herkenham M, Nauta WJ. Afferent connections of the habenular nuclei in the rat. A horseradish peroxidase study, with a note on the fiber-of-passage problem. J Comp Neurol 173(1):123–146, 1977.
177. Blanc G, Herve D, Simon H, Lisoprawski A, Glowinski J, Tassin JP. Response to stress of mesocortico-frontal dopaminergic neurones in rats after long-term isolation. Nature 284(5753):265–267, 1980.
178. Ullsperger M, von Cramon DY. Error monitoring using external feedback: specific roles of the habenular complex, the reward system, and the cingulate motor area revealed by functional magnetic resonance imaging. J Neurosci 23(10):4308–4314, 2003.
179. Shepard PD, Holcomb HH, Gold JM. Schizophrenia in translation: the presence of absence: habenular regulation of dopamine neurons and the encoding of negative outcomes. Schizophr Bull 32(3):417–421, 2006.
180. Klemm WR. Habenular and interpeduncularis nuclei: shared components in multiple-function networks. Med Sci Monit 10(11):RA261–273, 2004.

181. Groenewegen HJ, Ahlenius S, Haber SN, Kowall NW, Nauta WJ. Cytoarchitecture, fiber connections, and some histochemical aspects of the interpeduncular nucleus in the rat. J Comp Neurol 249(1):65–102, 1986.
182. Montone KT, Fass B, Hamill GS. Serotonergic and nonserotonergic projections from the rat interpeduncular nucleus to the septum, hippocampal formation and raphe: a combined immunocytochemical and fluorescent retrograde labelling study of neurons in the apical subnucleus. Brain Res Bull 20(2):233–240, 1988.
183. Boulter J, O'Shea-Greenfield A, Duvoisin RM, Connolly JG, Wada E, Jensen A, Gardner PD, Ballivet M, Deneris ES, McKinnon D, et al. Alpha 3, alpha 5, and beta 4: three members of the rat neuronal nicotinic acetylcholine receptor-related gene family form a gene cluster. J Biol Chem 265(8):4472–4482, 1990.
184. Marks MJ, Smith KW, Collins AC. Differential agonist inhibition identifies multiple epibatidine binding sites in mouse brain. J Pharmacol Exp Ther 285(1):377–386, 1998.
185. Salas R, Orr-Urtreger A, Broide R, Beaudet AL, Paylor R, De Biasi M. The nicotinic acetylcholine receptor subunit alpha 5 mediates acute effects of nicotine in vivo. Mol Pharmacol 63:1059–1066, 2003.
186. Salas R, Cook KD, Bassetto L, De Biasi M. The alpha3 and beta4 nicotinic acetylcholine receptor subunits are necessary for nicotine-induced seizures and hypolocomotion in mice. Neuropharmacology 47(3):401–407, 2004.
187. Sheffield EB, Quick MW, Lester RA. Nicotinic acetylcholine receptor subunit mRNA expression and channel function in medial habenula neurons. Neuropharmacology 39(13):2591–2603, 2000.
188. Gahring LC, Persiyanov K, Rogers SW. Neuronal and astrocyte expression of nicotinic receptor subunit beta4 in the adult mouse brain. J Comp Neurol 468(3):322–333, 2004.
189. Sastry BR, Zialkowski SE, Hansen LM, Kavanagh JP, Evoy EM. Acetylcholine release in interpeduncular nucleus following the stimulation of habenula. Brain Res 164:334–337, 1979.
190. Grady SR, Meinerz NM, Cao J, Reynolds AM, Picciotto MR, Changeux JP, McIntosh JM, Marks MJ, Collins AC. Nicotinic agonists stimulate acetylcholine release from mouse interpeduncular nucleus: a function mediated by a different nAChR than dopamine release from striatum. J Neurochem 76(1):258–268, 2001.
191. Wada E, Wada K, Boulter J, Deneris E, Heinemann S, Patrick J, Swanson LW. Distribution of alpha 2, alpha 3, alpha 4, and beta 2 neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat. J Comp Neurol 284(2):314–335, 1989.
192. Whiteaker P, Drapeau J, Brown R, Marks M, Brennan R, Collins A, Lindstrom J, Boulter J. Pharmacological and immunochemical characterization of α2 nicotinic acetylcholine receptors (nAChRS) in mouse brain. 34th Society for Neuroscience Annual Meeting, San Diego, CA, 2004.
193. Ishii K, Wong JK, Sumikawa K. Comparison of alpha2 nicotinic acetylcholine receptor subunit mRNA expression in the central nervous system of rats and mice. J Comp Neurol 493(2):241–260, 2005.
194. Nakauchi S, Brennan RJ, Boulter J,Sumikawa K. Nicotine gates long-term potentiation in the hippocampal CA1 region via the activation of alpha2* nicotinic ACh receptors. Eur J Neurosci 25(9):2666–2681, 2007.
195. Xu W, Gelber S, Orr-Urtreger A, Armstrong D, Lewis RA, Ou C-N, Patrick J, Role LW, De Biasi M, Beaudet AL. Megacystis, mydriasis, and ion channel defect in mice lacking the α3 neuronal nicotinic acetylcholine receptor. Proc Natl Acad Sci U S A 96:5746–5751, 1999.
196. Marubio LM, Gardier AM, Durier S, David D, Klink R, Arroyo-Jimenez MM, McIntosh JM, Rossi F, Champtiaux N, Zoli M, Changeux JP. Effects of nicotine in the dopaminergic system of mice lacking the alpha4 subunit of neuronal nicotinic acetylcholine receptors. Eur J Neurosci 17(7):1329–1337, 2003.
197. Wang N, Orr-Urtreger A, Chapman J, Rabinowitz R, Nachman R, Korczyn AD. Autonomic function in mice lacking alpha5 neuronal nicotinic acetylcholine receptor subunit. J Physiol 542(Pt 2):347–354, 2002.
198. Champtiaux N, Han ZY, Bessis A, Rossi FM, Zoli M, Marubio L, McIntosh JM, Changeux JP. Distribution and pharmacology of alpha 6-containing nicotinic acetylcholine receptors analyzed with mutant mice. J Neurosci 22(4):1208–1217., 2002.
199. Broide RS, Salas R, Ji D, Paylor R, Patrick JW, Dani JA, De Biasi M. Increased sensitivity to nicotine-induced seizures in mice expressing the L250T alpha7 nicotinic acetylcholine receptor mutation. Mol Pharmacol 61(3):695–705, 2002.
200. Franceschini D, Paylor R, Broide R, Salas R, Bassetto L, Gotti C, De Biasi M. Absence of alpha7-containing neuronal nicotinic acetylcholine receptors does not prevent nicotine-induced seizures. Brain Res Mol Brain Res 98(1–2):29–40., 2002.
201. Paylor R, Nguyen M, Crawley JN, Patrick J, Beaudet A, Orr-Urtreger A. α7 nicotinic receptor subunits are not necessary for hippocampal-dependent learning or sensimotor gating: a behavioral characterization of Acra7-deficient mice. Learn Mem 5(4–5):302–316, 1998.
202. Elgoyhen AB, Johnson DS, Boulter J, Vetter DE, Heinemann S. α9: an acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. Cell 79:705–715, 1994.
203. Elgoyhen AB, Vetter DE, Katz E, Rothlin CV, Heinemann SF, Boulter J. Alpha10: a determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells. Proc Natl Acad Sci U S A 98(6):3501–3506., 2001.
204. Booker TK, Butt CM, Wehner JM, Heinemann SF, Collins AC. Decreased anxiety-like behavior in beta3 nicotinic receptor subunit knockout mice. Pharmacol Biochem Behav 87(1):146–157, 2007.
205. Cui C, Booker TK, Allen RS, Grady SR, Whiteaker P, Marks MJ, Salminen O, Tritto T, Butt CM, Allen WR, Stitzel JA, McIntosh JM, Boulter J, Collins AC, Heinemann SF. The beta3 nicotinic receptor subunit: a component of alpha-conotoxin MII-binding nicotinic acetylcholine receptors that modulate dopamine release and related behaviors. J Neurosci 23(35):11045–11053, 2003.
206. Salas R, Pieri F, Fung B, Dani JA, De Biasi M. Altered anxiety-related responses in mutantmice lacking the β4 Subunit of the nicotinic receptor. J Neurosci 23(15):6255–6263, 2003.