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Effect of nicotine and nicotinic receptors on anxiety and depression

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Nicotine has been shown to have effects on anxiety and depression in both human and animal studies. These studies suggest that nicotinic acetylcholine receptors (nAChRs) can modulate the function of pathways involved in stress response, anxiety and depression in the normal brain, and that smoking can result in alterations of anxiety level and mood. The effects of nicotine are complex however, and nicotine treatment can be either anxiolytic or anxiogenic depending on the anxiety model tested, the route of nicotine administration and the time course of administration. The paradoxical effects of nicotine on emotionality are likely due to the broad expression of nAChRs throughout the brain, the large number of nAChR subtypes that have been identified and the ability of nicotine treatment to both activate and desensitize nAChRs. Activation of nAChRs has been shown to modulate many systems associated with stress response including stress hormone pathways, monoaminergic transmission and release of classical neurotransmitters throughout the brain. Local administration studies in animals have identified brain areas that may be involved in the anxiogenic and anxiolytic actions of nicotine including the lateral septum, the dorsal raphe nuclei, the mesolimbic dopamine system and the hippocampus. The ensemble of studies to date suggest that under certain conditions nicotine can act as an anxiolytic and an antidepressant, but that following chronic use, adaptations to nicotine can occur resulting in increased anxiety and depression following withdrawal. *NeuroReport* 13:1097–1106 © 2002 Lippincott Williams & Wilkins.

INTRODUCTION

Tobacco smoking is one of the leading causes of death in both the developed and the developing world [1–3]. Since the primary addictive substance in tobacco is nicotine [4], it is of great interest to understand the behavioral effects of nicotine that might contribute to ongoing tobacco use. In addition to the reinforcing and dopamine-stimulating properties that nicotine shares with other psychostimulants [5], other properties of nicotine, including effects on anxiety, depression, stress and emotionality, may contribute to its abuse potential in humans. For example, studies of smokers have shown that nicotine can reduce anxiety and relieve stress [6,7], suggesting that smokers continue smoking to regulate anxiety state. Several studies have also shown that smokers with a history of major depressive episodes have an increased risk of relapse to smoking after a quit attempt than smokers with no history of depression [8,9], while spontaneous nicotine withdrawal leads to a significant decrease in brain reward threshold in rats [10]. These effects of nicotine suggest that the ability of the drug to affect emotionality may be critical to understanding ongoing nicotine use.

The primary targets for nicotine in the brain are the neuronal nicotinic acetylcholine receptors (nAChRs). These receptors are pentamers made up of α and β subunits which form several different nAChR subtypes in different brain areas (for review see [5]). Broadly, α4 and β2 make up the highest affinity nAChRs and are found in many brain areas [11]. α4 and β2 can co-assemble with α5 in areas such as cerebral cortex [12] and appear to combine with the α5, α6 and β3 subunits in catecholaminergic nuclei [13–15]. α3 and β4 are found at high levels in the autonomic ganglia and are more limited in expression in the brain with highest levels in the medial habenula, superior colliculus and interpeduncular nucleus [11]. α7 can form homopentamers *in vitro* and is critical for α-bungarotoxin binding in the brain [16]. The α7 subunit is expressed widely in the brain with highest levels in the hippocampus and cortex [17]. α7 subunit-containing nAChRs are highly permeable to calcium and have a lower affinity for nicotine and acetylcholine *in vitro* [17]. These classes of nAChRs are expressed in overlapping sets of neurons in the brain [11,18–22]. Nicotine can stimulate the release of many different neurotransmitters including glutamate, GABA, ACh, dopamine, norepinephrine and serotonin (5HT) by activating these nAChRs [23–29]. The varied properties of these nAChRs can result in different patterns of neurotransmitter release following acute and chronic nicotine administration, perhaps accounting for the complex behavioral effects of nicotine on anxiety and depression under different regimens of administration.

NICOTINE AND ANXIETY

The literature on the effects of nicotine on anxiety in humans and behavioral paradigms of emotionality in rodents is complex and difficult to interpret (Table 1, Table 2, Table 3). The ability of nicotine to act as an anxiolytic is dependent on the regimen of administration (acute, different chronic

Table 1. Anxiety models used in rodents.

Test	Anxiety measure	Benzodiazepine sensitive?	Potential human condition modeled?	References
Social interaction test	Decreased time in social interaction (sniffing, following grooming) and aggression (boxing, wrestling) with partner	Yes	Generalized anxiety disorder (GAD)	[48,154]
Elevated plus maze	Decreased number of entries into, and decreased time spent in, open arms	Trial 1: yes; Trial 2: No	Trial 1: panic disorder, GAD; Trial 2: simple, specific phobia	[155–158]
Fear-potentiated startle	Increased startle following shock	Yes	Post-traumatic stress disorder, GAD	[159]
Light–dark exploration	Decreased latency to enter dark and decreased time spent in brightly lit chamber	Yes	GAD	[160,161]
Mirrored chamber	Increased latency to enter and decreased time spent in mirrored chamber	Yes	Social anxiety, GAD	[162]

Table 2. Effects of nicotine on anxiety-like behaviors: social interaction, light–dark, mirrored chamber.

Paradigm	Route of administration	Drug dose	Anxiolytic or anxiogenic?	References
Social interaction test (rat)	Systemic (i.p.)	Acute nicotine (0.01 mg/kg)	Anxiogenic 5 min after injection; anxiolytic 30 min after injection; anxiogenic 60 min after injection	[39]
	Systemic (i.p.)	Acute nicotine (0.01–1 mg/kg)	Anxiolytic 0.01, 0.1 mg/kg; anxiogenic 0.5, 1 mg/kg	[32]
	Systemic (s.c.)	Acute mecamylamine (1 mg/kg)	Mecamylamine reversed the anxiolytic effect of aniracetam	[163]
	Systemic (s.c.)	Chronic nicotine (7 days; 0.1 mg/kg/day)	Nicotine pretreatment blocked anxiolytic effect of local midazolam infusions	[143]
	Local infusion: dorsal hippocampus	Acute nicotine infusion (1–8 μg)	Anxiogenic; WAY100635 reversed nicotine anxiogenisis	[32,118]
	Local infusion: dorsal hippocampus	2 nicotine infusions (50 nmol)	No anxiogenic effect following 2nd infusion	[118]
	Local infusion: dorsal hippocampus	Acute mecamylamine (30,100, 300 ng)	Anxiogenic	[55]
	Local infusion: lateral septum	Acute nicotine infusion (4–8 μg)	Anxiogenic	[46]
	Local infusion: dorsal raphe	Acute nicotine infusion (2.5 ng–4 μg)	2.5–10 ng: anxiolytic (reversed by WAY100635); 100–1000 ng: no effect; 4 μg: anxiogenic	[52]
	Systemic (s.c.) Local infusion: dorsal raphe	Chronic nicotine (0.1 mg/kg, 6 days) acute nicotine infusion challenge (2.5–7.5 ng)	Tolerance to anxiolytic action in DRN Anxiogenic response 72 h after withdrawal reversed by s.c. (0.1 mg/kg) or DRN (5 ng) infusion of nicotine	[52]
Light–dark chamber (mouse)	Systemic (i.p.); local infusion: dorsal raphe, amygdala	Chronic nicotine (14 days, 0.1 mg/kg, b.i.d.)	Anxiolytic 48 h withdrawal: anxiogenic, reversed by local 5-HT3 antagonism	[33,164]
Mirrored chamber (mouse)	Systemic (i.p.)	Acute nicotine (0.1–2 mg/kg)	Anxiolytic at 1, 1.5 mg/kg	[30]

regimens, withdrawal), the route of administration (i.p., s.c., i.v., smoked) and the behavioral state of the experimental subjects (relaxed, stressed, in nicotine withdrawal). It is likely that the large number of nAChRs with different time courses of activation and inactivation and the large number of neurotransmitter systems that can be regulated by those nAChRs is responsible for some of this complexity. That is, since nicotine can activate the release of stimulatory (glutamate), inhibitory (GABA) and modulatory (DA, NE, 5-HT) neurotransmitters in different brain regions with different time courses, as well as stress hormone levels in the periphery, the behavioral output following nicotine administration is a sum of the effect of all of these neurotransmitter systems taken together. Thus, if some nAChRs are more sensitive to inactivation following chronic nicotine administration, that will alter the balance between these neurotransmitter systems and result in a different behavioral output. In this review we will attempt to

Table 3. Effects of nicotine on anxiety-like behaviors: elevated plus maze.

Paradigm	Route of administration	Drug dose	Anxiolytic or anxiogenic?	References
Elevated plus maze: 1st trial (rat)	Systemic (s.c.)	Acute nicotine (0.35 mg/kg)	Anxiogenic	[42]
	Systemic (s.c.)	Chronic nicotine (15 days, 0.35 mg/kg)	Anxiolytic reversed by 8-OH-DPAT or citalopram treatment	[42,44,65]
	Systemic (i.p.)	Chronic (15 days) mecamylamine or hexamethonium	Anxiolytic when challenged with acute nicotine (0.35 mg/kg)	[65]
	Systemic (i.p.)	Acute nicotine low: (0.001–0.1 mg/kg), high: (0.5–1.0 mg/kg)	Anxiogenic at high doses, no effect at low doses	[41]
	Local infusion: dorsal hippocampus	0.1, 1, 4, 8 μg	No effect on trial 1	[41]
	Systemic (s.c.), local infusion: dorsal hippocampus	Chronic nicotine (0, 7, 14 days, 0.1 mg/kg); Acute nicotine challenge (0.1 mg/kg)	Acute + 30 min: anxiogenic; 7 days + acute: anxiolytic; 14 day: no effect (tolerance); 24 h withdrawal: anxiogenic reversed by nicotine into dorsal hippocampus	[64]
	Local infusion: dorsal hippocampus	Acute nicotine infusion (0.1–8 μg)	No effect	[41]
	Local infusion: lateral septum	Acute nicotine infusion (1–4 μg)	Anxiogenic	[46,47]
(Mouse)	Systemic (i.p.)	Acute nicotine (0.3 mg/kg)	Flumazenil blocked anxiolytic effects of nicotine	[141]
	Systemic (i.p.)	Acute nicotine (0.25, 0.5 mg/kg)	Anxiogenic reversed by prazosin	[37]
Elevated plus maze: 2nd trial (rat)	Local infusion: dorsal hippocampus	Acute nicotine (1 μg)	Anxiolytic	[41]
	Local infusion: lateral septum	Acute nicotine (4, 8 μg)	No effect	[48]
	Systemic (i.p.)	Acute nicotine low: (0.001–0.1 mg/kg), high: (0.5–1.0 mg/kg)	Anxiogenic at high doses No effect at low doses	[41]

correlate what is known about the effects of different nicotine treatments on measures of anxiety with what is known about the neurochemical effects of nicotine and the identity of nAChR subtypes involved in the different responses. There are still many pieces of information missing, so it is not yet possible to correlate the behavioral, neurochemical and molecular data with great precision.

Effects of acute, systemic administration of nicotine on anxiety-like behavior: In rodents nicotine administration has effects on several different behavioral tests including the mirrored chamber [30], the elevated plus maze [31], the social interaction test [32], the two-compartment light–dark transition test [33], fear conditioning [34,35] and fear-potentiated startle [36]. Each of these paradigms has been proposed to measure a different aspect of emotionality, and has been considered to represent, to a greater or lesser extent, an animal model of various human anxiety disorders (Table 1). These studies of anxiety-like behavior have been performed in rats and several strains of mice. For example, the CD-1 and NMRI outbred strains of mice have been used for elevated plus maze testing [31], the Bradford strain of BKW mice have been used to test the effects of nicotine in the light-dark exploration test [33], and long sleep (LS) and short sleep (SS) mice selectively bred for ethanol sensitivity have been tested in the mirrored chamber [30]. Across several different tests, anxiolytic-like effects of acute, systemic injection of nicotine have been reported in mice [30,33], although an anxiolytic effect of nicotine was seen in only in SS and not LS mice in the mirrored chamber [30] and nicotine was anxiogenic in NMRI mice in the elevated plus maze [37]. Taken together, these studies suggest that there are strain-dependent differences in the ability of nicotine to be anxiolytic in mice.

In rats, the effects of nicotine administration have been even more variable (Table 2, Table 3). This could be due to several factors including the different testing methods used, general species differences or variations in housing conditions (mice tend to be group housed while rats are generally singly housed [38]).

Social interaction test: The social interaction test has been proposed as a model of generalized anxiety disorder (Table 1). In rats the effects of acute, systemic nicotine on the social interaction test were dose dependent (low doses (0.01. and 0.1 mg/kg) were anxiolytic and high doses 0.5 and 1.0 mg/kg were anxiogenic) and dependent on the baseline level of anxiety (bidirectional effects were only seen under moderate anxiety conditions [32]). Further, the low dose produced opposing responses, depending on the time course; at 5 min low-dose nicotine was anxiogenic, at 30 min it was anxiolytic, and at 60 min it produced a second anxiogenic response [39].

Elevated plus maze: In the elevated plus maze, acute systemic nicotine has been reported to be anxiolytic in rats [40], anxiogenic [38,41,42] or to have no effect [41,43,44]. This variation does not appear to be influenced solely by dose because the variability is seen over a wide range of doses. Strain differences, variation in baseline anxiety, or some combination of these factors with dose may explain some of these discrepancies. In the CD1 strain of mice, the

anxiolytic effects of acute systemic nicotine injection in the plus maze were blocked by the central nicotinic antagonist mecamylamine but not the peripheral nicotinic antagonist hexamethonium, that does not cross the blood–brain barrier, suggesting a role for central nicotinic receptors in anxiolytic effects of nicotine on the plus maze [31].

Effects of local administration of nicotine on anxiety-like behavior: Local injection studies have also been used to identify the sites of action of nicotine in behavioral models of anxiety. The septo-hippocampal system has been identified as an anatomical locus mediating some effects of anxiolytic drugs on behavior [45], thus it is not surprising that injection of nicotine into either the dorsal hippocampus or the lateral septum can influence behavior in both the social interaction test and the elevated plus maze in rats. Acute injection of nicotine into lateral septum was anxiogenic in the social interaction test [46–48] and the elevated plus maze, but only upon first exposure to the apparatus (trial 1 but not trial 2) [47]. These studies suggest that some of the anxiogenic effects of nicotine are mediated through the lateral septum. In contrast, the dorsal hippocampus appears to be involved in mediating both anxiolytic and anxiogenic actions of nicotine. In the social interaction test, infusion of nicotine into the dorsal hippocampus was anxiogenic [49], whereas in the elevated plus maze, administration of nicotine into the dorsal hippocampus did not affect behavior on trial 1, but was anxiolytic on trial 2 [41]. Projections to the lateral septum arise from the dorsal raphe nucleus (DRN) [50,51]. Acute injection of nicotine into the DRN had differential effects on behavior in the social interaction test depending on the dose used. Low doses of nicotine were anxiolytic, intermediate doses had no effect, and high doses were anxiogenic [52].

Role of endogenous cholinergic tone in anxiety-like behavior: The role of intrinsic cholinergic tone in mediating anxiety can be tested by administering the nicotinic receptor antagonist mecamylamine. Acute, systemic injections of mecamylamine have been reported to be either anxiolytic (with lower doses being more effective than higher doses under anxiety provoking conditions [53]) or anxiogenic [37] in the elevated plus maze. Whether mecamylamine is anxiolytic or anxiogenic may reflect the basal state of anxiety of the animal which would in turn affect the endogenous levels of acetylcholine. For example, it has been suggested that threatening stimuli result in ACh release, and that this contributes to an animal's response to an aversive situation [54].

Like nicotine, injection of mecamylamine into the dorsal hippocampus can be anxiogenic in the social interaction test in rats [55]. This is most readily seen when there is high endogenous cholinergic tone in conditions of low anxiety [49]. Mecamylamine administered into dorsal hippocampus is also anxiogenic on trial 2 of the elevated plus maze [55]. These data together suggest that increased acetylcholine release in the dorsal hippocampus is anxiolytic. The effects of mecamylamine injected into the lateral septum on the social interaction test are more complex. At a low dose mecamylamine is anxiolytic, whereas at higher doses it has no effect [47], suggesting that the endogenous cholinergic tone in lateral septum is predominantly anxiogenic [48].

Effects of chronic administration of nicotine or withdrawal on anxiety-like behaviors: A longitudinal study of children in New Zealand has shown that anxiety disorders in adolescence are correlated with abuse of nicotine (and other illicit drugs) in young adulthood, suggesting that in some individuals, nicotine might be used to self-treat anxiety symptoms [56]. Extensive human studies on nicotine and anxiety have not been performed in nicotine-naive individuals, however, making it possible that nicotine can only relieve the anxiety caused by withdrawal in smokers, rather than having anxiolytic properties on its own. Thus, a loss of the stress-relieving effects of nicotine may contribute to the anxiety perceived during nicotine withdrawal. Nesbitt's paradox states that cigarette smoking generates incompatible physiological (sympathetic arousal) and psychological (smokers report themselves as calmer, more relaxed, less stressed after smoking) effects on emotionality [57]. The contrary states described in Nesbitt's paradox may be resolved if the calming effect of nicotine in smokers is primarily due to relief of a stressful withdrawal state between cigarettes [57]. This would also be compatible with the observation that, despite reports that nicotine can act as an anxiolytic [6,7], people who quit smoking have been shown to have fewer symptoms of stress than smokers [58].

The idea that the anxiolytic effects of smoking are primarily on relief of withdrawal is supported by studies of the startle reflex. Nicotine withdrawal does not appear to have an effect on baseline startle magnitude, however, smokers who underwent prolonged smoking deprivation showed an abrupt reduction in startle amplitude after smoking their first cigarette, but not the second. In contrast, heart rate remained elevated throughout the session [59]. These data are intriguing given the large number of studies that have shown that stress [60,61] and stress hormones [62] can increase drug self-administration and result in reinstatement even after long periods of abstinence [63].

Chronic nicotine administration in the rat can result in either sensitization (increase) or tolerance (decrease) of the acute effects of nicotine in the social interaction test and elevated plus maze. In the social interaction test, tolerance develops to both the anxiolytic and anxiogenic effects of nicotine following chronic treatment [39]. In contrast, in the elevated plus maze chronic nicotine treatment results in tolerance to its anxiogenic effects but sensitization to the anxiolytic/behavioral disinhibition effects, even at doses at which acute nicotine treatment had no effect [44,64–66]. The behavioral disinhibition following chronic nicotine treatment in the plus maze was not blocked by chronic co-treatment with the centrally acting nicotinic antagonist mecamylamine or the peripherally acting nicotinic antagonist hexamethonium [65]. In addition, rats treated chronically with either mecamylamine or hexamethonium and challenged with nicotine showed a similar behavioral disinhibition on the plus maze as animals treated chronically with nicotine [65]. These results suggest a role for peripheral nicotinic receptors in behavioral disinhibition following chronic nicotine treatment and may suggest that both chronic blockade of peripheral nicotinic receptors by

antagonists or desensitization of these receptors as a result of chronic nicotine treatment are responsible for the anxiolytic-like effects in this paradigm.

Studies in both rats and mice have shown only anxiogenic effects from nicotine following withdrawal. In the social interaction test, rats tested 72 h after 7 or 14 days nicotine injection showed an anxiogenic withdrawal response [39]. The increase in anxiety following withdrawal was not seen in self-administering rats, however, suggesting mode of administration may be an important factor in the anxiogenic response to nicotine following withdrawal [67]. Similarly, following nicotine withdrawal, animals showed increased anxiety on the plus maze [66,67] and anxiogenic responses were observed 8–96 h after withdrawal in the light–dark exploration test [33]. These data support human studies suggesting withdrawal from nicotine is anxiogenic.

Effects of nicotine on conditioned fear learning: In addition to its effects on anxiety-like behaviors, nicotine has been shown to alter emotional-based learning in tests of conditioned anxiety or fear. Like other tests of emotionality, the effects of nicotine on contextual and cued fear conditioning vary depending on dosage, method, length, and time of administration. Acute, systemic injection of nicotine selectively enhanced contextual, but not cued, fear conditioning, but only when given before both training and testing [34]. This effect, which appeared to be an effect on learning and not anxiety, was blocked by mecamylamine [34]. Acute nicotine treatment can also enhance passive avoidance learning, and this enhancement depends on activation of nAChRs containing the β2 subunit [68]. In contrast, fear conditioning was attenuated by acute, systemic administration of nicotine on the day of training [35]. Repeated nicotine administration appears to alter the behavioral response to conditioned fear stress. Chronic administration of nicotine resulted in a reduction of both cued and contextual conditioned fear, potentially an effect on anxiety state [35,69], suggesting that the cholinergic system may contribute to fear learning and that extended nicotine use might serve to reduce conditioned anxiety in smokers.

Receptor subtypes: specific agonists and knockout studies: It is of great interest to determine the specific nAChR subtypes that mediate the anxiolytic and anxiogenic responses to nicotine. Studies using nicotinic receptor agonists as well as experiments in genetically altered mice with mutations in specific nicotinic receptor subunits have suggested the involvement of many different nAChR subunits. Several studies have suggested that nicotine may modulate anxiety-like behavior through α4/β2-containing receptors. ABT 418, an agonist at α4/β2-containing receptors, has anxiolytic effects in the elevated plus maze [40,70] and attenuated the anxiogenic response elicited by nicotine withdrawal [40]. Anxiogenic effects of ABT 418 have not been reported. The anxiolytic actions of nicotine in the plus maze were blocked by erysodine, an antagonist with high affinity for α4/β2-containing receptors [71]. In contrast, GTS-21, another agonist with affinity for α4/β2-containing receptors, did not influence behavior in the elevated plus maze in mice [72].

Studies with mutant mice also support the role of nAChR subunits in behaviors related to anxiety. Knockout mice lacking the α4 subunit of the nAChR and mice with a knockin mutation of the α4 subunit, resulting in a hypersensitive channel, show increased anxiety in the elevated plus maze and the mirrored chamber, consistent with the idea that the α4 subunit might be involved in mediating both anxiolytic and anxiogenic effects of nicotine in different conditions [73,74]. Polymorphisms in the human α4 subunit gene are not associated with panic disorder, however [75]. In addition, knockout mice lacking the β2 subunit of the nicotinic receptor were not different than wild type mice in the elevated plus maze, light dark exploration test, and mirrored chamber [76]. Knockout mice lacking the α7 subunit of the nAChR spend more time in the center of an open field arena, consistent with the idea that α7 knockout mice have a lower level of anxiety than wild type mice, although no difference in behavior was seen in the light-dark box [77].

SMOKING AND DEPRESSION

Several studies have shown abnormalities of the cholinergic system associated with depression [78]. While many studies have focused on the muscarinic receptor system, strong links have also been shown between depression and nicotinic receptors. Connections between nicotine and depression have come in part from studies of smoking and depression. Depressed individuals are more likely to smoke than those with no history of depression (49% *vs* 22–30% in general population [79,80]. In addition, smoking cessation can precipitate depressive symptoms [81].

Several hypotheses can be proposed to explain these data. These hypotheses are not mutually exclusive, and indeed all may be important for interactions between nicotine and depression. First, nicotine may act as an antidepressant and thus smokers may be attempting to self-treat mood symptoms by smoking. This hypothesis is supported by both human and animal studies. One study with transdermal nicotine in depressed non-smokers showed that depressive symptoms improved as a result of treatment with the nicotine patch [82]. I.v. nicotine, inhaled nicotine, and the nicotine patch have all been shown to improve mood [81], although in one study of patients with Alzheimer's disease i.v. nicotine provoked symptoms of anxiety and depression [83]. In addition, tricyclic antidepressants [84], serotonin-selective re-uptake inhibitors (SSRIs) [85] and the atypical antidepressant bupropion [86] have all been shown to be non-competitive antagonists of nAChRS. The effects of nicotine on depressive symptoms can also be seen in two animal models of depression. Chronic nicotine treatment can decrease the learned helplessness response to repeated foot shock in rats [87,88], and both acute and chronic nicotine treatment can decrease immobility in the forced swim test [89–91].

A second hypothesis is that nicotine withdrawal can cause depression. In one study, three women with no history of depression developed significant depressive symptoms requiring psychiatric intervention following smoking cessation, supporting the idea that chronic nicotine intake can change the brain leading to depressive symptoms

upon nicotine withdrawal. Two days of nicotine withdrawal can also increase immobility time in the forced swim test in rats, providing support for the idea that nicotine withdrawal can increase depressive-like symptoms [89].

A third hypothesis is that shared genetic factors can predispose some individuals to vulnerability to both major depression and nicotine addiction. In a 5-year longitudinal study of adolescents, history of major depression at baseline increased the risk for progression to daily smoking, while a history of daily smoking at baseline significantly increased the risk for major depression [92]. In another study of female twins, average lifetime daily cigarette consumption was strongly related to prevalence of major depression [93]. In this study, family history of smoking predicted risk for major depression while family history of major depression predicted smoking, suggesting that lifetime smoking and lifetime incidence of major depression resulted from genes that predispose to both conditions. Animal models also support a potential shared genetic risk for nicotine use and depression. The Flinders Sensitive Line (FSL) of rats, a potential animal model of depression, was originally bred for hyper-responsiveness to cholinergic stimulation [91], suggesting a genetic link between the cholinergic system and vulnerability to depression.

Receptor subtypes: genetic and pharmacological studies of nAChRs: Genetic variability in the cholinergic system has been shown to influence baseline responsiveness in the forced swim test. FSL rats have been selectively bred for hyperresponsiveness of the cholinergic system and have been proposed as an animal model of depression. Compared with the Flinder's Resistant Line (FRL), FSL rats have lower body weight, lower locomotor activity, increased REM sleep, altered learning, anhedonia when exposed to chronic mild stress, and greater immobility in the forced swim test [89,91,94]. These baseline differences in behavior between FRL and FSL rats may be due to variations in number or function of nAChRs in the brain. FSL rats show no difference in α-bungarotoxin binding (α7-containing nAChRs) but have higher levels of cytisine binding (α4/β2-containing nAChRs) compared to FRL rats in the frontal cortex, striatum, midbrain and colliculi [91]. Pharmacological studies also support the role of α4/β2-containing nAChRs in the antidepressant effects of nicotine. SIB-1508Y, an agonist with high affinity for α4/β2-containing nAChRs, reverses learned helplessness behavior in rats [88]. However, a human study found a modest association between major depressive disorder and a polymorphism associated with a partial duplication of the α7 subunit gene, suggesting that α7-containing nAChRs may also be important in regulating affect [95].

NEUROCHEMICAL MECHANISMS UNDERLYING NICOTINE'S EFFECTS ON EMOTIONALITY

Stress hormones: To understand the interaction between nicotine, stress responsivity, anxiety and depression it is critical to understand the effects of nicotine on neurotransmitter systems and circulating stress hormones. Despite its ability to act as an anxiolytic under some circumstances, nicotine, like acute stress, stimulates the hypothalamic pituitary adrenal axis (HPA), leading to increases in levels of circulating corticosteroids (CORT), in both animals [96] and human smokers [97]. Nicotinic receptor blockade with mecamylamine also decreases circulating CORT levels in stressed rats [53]. Nicotine increases CORT mRNA in cell lines from the amygdala [98], a brain area critical for emotionality. Further, nicotine administration increases plasma levels of corticotrophin releasing factor (CRF) and adrenocorticotropic hormone (ACTH) in rodents [99] and humans [97,100]. CORT, in turn, can modulate the physiological and behavioral effects of nicotine in rodents. Administration of CORT or exposure to a stressor antagonizes the analgesic [101] and hypothermic [102] effects of nicotine. Conversely, adrenalectomy results in an increase in nicotine-dependent decreases in locomotor activity, heart rate and body temperature and an enhancement of the effect of nicotine on the startle response [103,104], that is reversed following administration of CORT [103,105]. These studies suggest that the HPA axis may be involved in tolerance to nicotine. Studies in mice also suggest that genetic background affects the interaction between nicotine and the HPA axis [103]. Interestingly, mice lacking the β2 subunit of the nAChR, have high circulating levels of CORT, but only after aging (20–24 months of age) [106].

Nicotine treatment also alters activity of the HPA axis, and this might predispose smokers to depressive episodes during withdrawal. Nicotine withdrawal leads to a reduction in CORT but not ACTH levels [107]. ACTH is known to facilitate, while CORT suppresses, fear behaviors in rodents, thus these responses to nicotine withdrawal might enhance anxiety during withdrawal [108].

It has been shown that the effects of nicotine on the HPA axis are centrally mediated. Systemic administration of mecamylamine, a nAChR antagonist that crosses the blood brain barrier, but not a peripheral antagonist, hexamethonium, blocks nicotine-induced increases in ACTH [109]. Neuronal nicotinic receptors are found throughout the brain and are known to modulate the release of many neurotransmitters [110], thus the effects of nicotine on circulating stress hormones are likely to occur through stimulation of the release of neurotransmitters centrally.

Norepinephrine: Some of the effects of nicotine on the HPA axis appear to be mediated by release of norepinephrine (NE) in the paraventricular nucleus of the hypothalamus (PVN). Nicotine administration into the cerebral aqueduct leads to a dose-dependent increase in NE levels in the PVN, as measured by microdialysis, resulting in release of CRF and ACTH and subsequently affecting release of CORT from the adrenal gland [111,112]. NE is also increased in the PVN of mice self-administering nicotine following active bar presses and this response is desensitized with repeated bar presses [113].

Systemic administration of nicotine also results in increased levels of NE in the amygdala and hippocampus [114], limbic structures known to regulate HPA axis responses [115,116]. Studies using a hippocampal synaptosome preparation suggest that the nAChRs that mediate norepinephrine release are pharmacologically distinct from those that mediate DA release [117]. The nAChR subtypes that are involved in NE release in the hippocampus have

been elucidated using a combination of molecular and phsyiological techniques. Two types of locus coeruleus (LC) neurons express different subtypes of nAChR [14]. The α3 and β4 subunits are detected by RT-PCR in a specific subpopulation of LC neurons, called Type A cells that are small in size but have large nicotinic currents [14]. Type B cells express β2, α4, α6 and β3 subunits and correspond to the predominant noradrenergic projection neurons to the hippocampus. The α5 and α7 subunits are also expressed in LC neurons [14]. These studies suggest that nicotine can activate Type A and B cells with differential kinetic and desensitization properties.

5-HT: The strongest evidence for the contribution of the serotonergic system to the effects of nicotine on fear and anxiety are data showing that systemic and local administration of 5-HT antagonists in rats can reverse anxiogenic effects of nicotine. Local administration of the 5HT1A antagonist, WAY100635, reversed the anxiogenic effects of acute hippocampal and lateral septal nicotine infusion on social interaction and plus maze behavior [46,118] (Table 2, Table 3). Behavioral dishibition in the elevated plus maze following chronic nicotine treatment, an anxiolytic effect, was blocked by increasing serotonergic transmission with the SSRI citalopram [44,65] and by the 5HT1A agonist 8-OH-DPAT [42]. In the dorsal raphe, WAY100635 blocked the anxiolytic effect of local nicotine infusion on the social interaction test, suggesting that the effects of nicotine on the 5-HT cell bodies are different from the effects on the 5-HT terminals [52]. Nicotine has also been shown to inhibit the firing of serotonergic neurons in the dorsal raphe which project to many areas of the CNS [119], suggesting that decreased serotoninergic firing as a result of nicotine treatment may be anxiolytic, whereas increased 5-HT levels induced by nicotine in the terminal regions of the hippocampus and lateral septum may be anxiogenic

Nicotine administration can also result in increased transcription of the 5HT1A receptor, suggesting that there are adaptations in 5-HT signaling as a result of nicotine exposure [120]. Behavioral sensitization to repeated nicotine treatment and the corresponding increase in DA levels in response to nicotine injection may also involve the 5HT2 subclass of receptors, suggesting that this is a site for plasticity in response to nicotine administration [42]. In contrast, activation of 5HT1A receptors was important for behavioral disinhibition in the elevated plus maze, suggesting that the 5HT1A receptors may be more critical for the effects of nicotine on emotionality [42].

Dopamine: In addition to their role in nicotine reinforcement [121,122], DA projections to the NAc and medial prefrontal cortex (mPFC) have also been shown to be involved in responsivity to stress [123,124]. DA turnover is increased in the NAc and mPFC as a result of acute, but not chronic nicotine injection [125]. However, in another study, 50 days of oral nicotine treatment led to a significant increase in the level of DA metabolites that was reversed following nicotine withdrawal [126].

The discovery that patients using the DA and NE reuptake blocker bupropion [127,128] reported decreases in cigarette craving [129] that resulted in spontaneous smoking cessation [130] supports the hypothesis that stimulation of the DA system might result in a reversal of the negative symptoms of nicotine withdrawal. It has been shown that treatment with chronic low-dose nicotine is able to attenuate the increase in DA utilization and attendant freezing behavior caused by footshock stress in rats [69], suggesting a role of the DA system in nicotine's anxiolytic effects. Nicotine receptor blockade with mecamylamine also attenuates footshock stress-induced DA utilization in the NAC [131], suggesting that chronic nicotine treatment may result in inactivation of nAChRs. Some studies have suggested that changes in central dopaminergic activity caused by stress may be more critical for susceptibility to drug self-administration than increases in stress hormone levels [132–134].

The ability of systemic nicotine administration to stimulate DA release [135] and the ability of nicotine to induce release of DA from a synaptosome preparation [29] require nAChRs containing the β2 subunit. A study combining RT-PCR and patch clamp physiology in wild type mice and mice lacking either the α4, β2 or α7 subunits showed that most DA neurons in the midbrain express two nAChR subtypes [15]. Both types contain the β2 subunit [11], and the second subtype is also sensitive to low concentrations of α-conotoxin MII and methyllycaconitine (MLA), although neither subtype contains the α7 subunit [15]. These two classes of nAChRs are thought to be made up of the α4/α5/β2 and the α4/α5/α6/β2 subunits, respectively [15]. An α7 subunit-containing nAChR is also expressed in somewhat less than half of the DA neurons [15]. In slices through the SN and VTA, a concentration-dependent increase in Ca^{2+} could be evoked with either nicotine or choline, suggesting that both β2 and α7 subunit-containing nAChRs can contribute to this effect [136]. The α6 subunit is also highly expressed in catecholaminergic nuclei [22], is likely to contribute to the observed α-conotoxin MII-sensitive nAChRs in DA neurons [15,137], and is co-localized with the β3 subunit [14,22]. The α6 and β3 subunits have been shown to combine with the β2 subunit *in vitro*, with or without the α4 subunit, to form functional nAChRs [138], suggesting that these subunits may contribute to the observed nicotine-sensitive currents in DA neurons [15]. These studies taken together suggest that nicotine can potently modulate the DA system through its action on many different nAChR subtypes.

Despite the focus of this review on nicotine and nAChRs, it should be noted that an unknown component of cigarette smoke is also able to act as a monoamine oxidase inhibitor, suggesting that components in smoke in addition to nicotine can also affect monoaminergic neurotransmission [139].

GABA: The benzodiazepines, co-agonists at GABA-A receptors, are potent anxiolytic compounds and have focused attention on the GABAergic system in modulation of anxiety state [140]. Nicotine's anxiolytic and anxiogenic properties may also be regulated, in part, by changes in GABAergic transmission. For example, the benzodiazepine receptor antagonist flumazanil blocked anxiolytic effects of nicotine on the plus maze [141]. Mecamylamine did not block the anxiolytic effects of diazepam in the same task, suggesting that nicotine treatment activates the GABAergic

system, while benzodiazepines may not activate the nicotinic system [141]. Nicotine treatment results in up-regulation of benzodiazepine receptor binding in cerebral cortex [142,143]. Nicotine can increase stimulation-evoked efflux of GABA in hippocampal slice [144] and modulate release of both GABA and glutamate in amygdala/olfactory bulb neurons in culture [145]. This ability of nicotine to stimulate GABA release may explain the cross-tolerance between nicotine and benzodiazepines.

Neuropeptides and neurotrophins: The neuropeptides arginine vasopressin (AVP) and neuropeptide Y (NPY), as well as the neurotrophin brain derived neurotrophic factor (BDNF), have been implicated in stress responses, anxiety and depression [146]. Administration of nicotine can enhance plasma levels of AVP [147]. AVP-positive neurons project from the bed nucleus of the stria terminalis to innervate the lateral septum, an area of the brain shown to be involved in anxiety that is responsive to local nicotine administration [46,47]. NPY-positive neurons in the LC and nucleus tractus solitarus are activated by nicotine administration as measured by cfos expression [148]. Nicotine treatment also increased NPY expression in the adrenal medulla, although nicotine infusion did not modify stress-induced NPY release [149]. Animal models of depression suggest that hippocampal levels of BDNF are negatively correlated with depressed behavior and reversed with antidepressant treatment [150]. Consistent with behavioral data showing antidepressant effects of chronic nicotine exposure, acute injections of nicotine attenuated and chronic systemic nicotine enhanced hippocampal BDNF expression in male rats [151], although in another study using a different rat strain, female animals did not show an increase in BDNF mRNA after chronic nicotine treatment [152]. Intra-hippocampal infusion of nicotine did not significantly alter levels of BDNF but did lead to a delayed and transient enhancement of its receptor the trkB protein [153].

CONCLUSIONS

Studies in both human and animals have shown that nicotine treatment can affect many aspects of emotionality. The large variety of nAChR subtypes expressed in areas of the brain involved in stress response suggest that cholinergic innervation of these pathways is critical in modulating mood and anxiety. Following nicotine administration, however, the route of administration, dose, and time course of administration can result in differential activation of pathways involved in emotionality, such that, depending on the behavioral study, nicotine can be either anxiolytic or anxiogenic. Local infusion and pharmacological studies have suggested that serotonergic pathways may be particularly important for the anxiogenic properties of nicotine, while peripheral stress hormones and GABAergic pathways have been identified as potential sites for nicotine's anxiolytic actions. In addition, the mesolimbic dopamine system may be critical for the effects of nicotine on stress-related behaviors. The ability of nicotine to affect the level of neurotrophic factors may also be critical in the antidepressant actions of nicotine. In addition to its acute effects, chronic nicotine treatment also results in molecular and cellular adaptations in the brain such that withdrawal from nicotine can result in both increased anxiety and the onset of depressive symptoms. One active area for future research will be the identification of the molecules and brain regions that are involved in these adaptations to nicotine. Ultimately, an understanding of the nAChR subtypes involved in the ability of nicotine to modulate mood and anxiety could be useful in drug development to combat depression, while identification of the sites where adaptation to nicotine use can result in anxiety and depression upon withdrawal could aid in development of therapies for smoking cessation.

REFERENCES

1. Novick LF. *J Public Health Manage Practice* **6**, 6 (2000).
2. Lopez AD. *BMJ* **317**, 1399–1400 (1998).
3. Peto R, Darby S, Deo H *et al. BMJ* **321**, 323–329 (2000).
4. US Department of Health and Human Services. *The Health Consequences of Smoking: Nicotine Addiction.* A report of the Surgeon General. Washington: US Government Printing Office 1988.
5. Picciotto MR. *Drug Alcohol Depend* **51**, 165–172 (1998).
6. Pomerleau OF. *Psychopharm Bull* **22**, 865–869 (1986).
7. Gilbert DG, Robinson JH, Chamberlin CL *et al. Psychophysiol* **26**, 311–319 (1989).
8. Fergusson DM, Lynskey MT and Horwood LJ. *Arch Gen Psychiatry* **53**, 1043–107 (1996).
9. Kinnunen T, Doherty K, Militello FS *et al. J Consult Clin Psychol* **64**, 791–798 (1996).
10. Epping-Jordan MP, Watkins SS, Koob GF *et al. Nature* **393**, 76–79 (1998).
11. Zoli M, Léna C, Picciotto MR *et al. J Neurosci* **18**, 4461–4472 (1998).
12. Ramirez-Latorre J, Yu CR, Qu X *et al. Nature* **380**, 347–351 (1996).
13. Le Novère N, Zoli M, Léna C *et al. Neuroreport* **10**, 2497–2501 (1999).
14. Léna C, de Kerchove D'Exaerde A, Cordero-Erausquin M *et al. Proc Natl Acad Sci USA* **96**, 12126–12131 (1999).
15. Klink R, de Kerchove d'Exaerde A, Zoli M *et al. J Neurosci* **21**, 1452–1463 (2001).
16. Orr-Urtreger A, Goldner F M, Saeki M *et al. J Neurosci* **17**, 9165–9171 (1997).
17. Seguela P, Wadiche J, Dineley-Miller K *et al. J Neurosci* **13**, 596–604 (1993).
18. Wada E, Wada K, Boulter J *et al. J Comp Neurol* **284**, 314–335 (1989).
19. Hill JA Jr, Zoli M, Bourgeois J-P *et al. J Neurosci* **13**, 1551–1568 (1993).
20. Zoli M, Le Novère N, Hill J A *et al. J Neurosci* **15**, 1912–1939 (1995).
21. Wada E, McKinnon D, Heinemann S *et al. Brain Res* **526**, 45–53 (1990).
22. Le Novère N, Zoli M and Changeux J-P. *Eur J Neurosci* **8**, 2428–2439 (1996).
23. Lu Y, Marks MJ and Collins AC. *J Pharmacol Exp Ther* **291**, 1127–1134 (1999).
24. Wonnacott S, Irons J, Rapier C *et al.* Presynaptic modulation of transmitter release by nicotinic receptors. In: Nordberg A *et al.* eds. *Progress in Brain Research.* Amsterdam: Elsevier; 1989, pp. 157–163.
25. Grady S, Marks MJ, Wonnacott S *et al. J Neurochem* **59**, 848–856 (1992).
26. McGehee DS, Heath MJ, Gelber S *et al. Science* **269**, 1692–1696 (1995).
27. Marshall DL, Redfern PH and Wonnacott S. *J Neurochem* **68**, 1511–1519 (1997).
28. Lu Y, Grady S, Marks MJ *et al. J Pharmacol Exp Ther* **287**, 648–657 (1998).
29. Grady SR, Meinerz N M, Cao J *et al. J Neurochem* **76**, 258–268 (2001).
30. Cao W, Burkholder T, Wilkins L *et al. Pharmacol Biochem Behav* **45**, 803–809 (1993).
31. Brioni JD, O'Neill AB, Kim DJB *et al. Eur J Pharmacol* **238**, 1–8 (1993).
32. File SE, Kenny PJ and Ouagazzal AM. *Behav Neurosci* **112**, 1423–1429 (1998).
33. Costall B, Kelly ME, Naylor RJ *et al. Pharmacol Biochem Behav* **33**, 197–203 (1989).
34. Gould TJ and Wehner JM. *Behav Brain Res* **102**, 31–39 (1999).
35. Szyndler J, Sienkiewicz-Jarosz H, Maciejak P *et al. Pharmacol Biochem Behav* **69**, 511–518 (2001).
36. Vale ALA and Green SS. *Behav Pharmacol* **7**, 138–143 (1996).
37. Zarrindast MR, Homayoun H, Babaie A *et al. Eur J Pharmacol* **407**, 145–158 (2000).

38. Cheeta S, Irvine E, File SE. *Br J Pharmacol* **132**, 1389–1395 (2001).
39. Irvine EE, Cheeta S, File SE. *Behav Pharmacol* **10**, 691–697 (1999).
40. Brioni JD, O'Neill AB, Kim DB *et al. J Pharm Exp Ther* **271**, 353–361 (1994).
41. Ouagazzal AM, Kenny PJ and File SE. *Psychopharmacology* **144**, 54–60 (1999).
42. Olausson P, Akesson P, Engel JA *et al. Eur J Pharmacology* **420**, 45–54 (2001).
43. Balfour DJ, Graham CA and Vale AL. *Psychopharmacology* **90**, 528–532 (1986).
44. Olausson P, Engel JA and Soderpalm B. *Psychopharmacologia* **142**, 111–119 (1999).
45. Gray JA. *Pharmacol Biochem Behav* **29**, 767–769 (1988).
46. Cheeta S, Kenny PJ and File SE. *Eur J Neurosci* **12**, 3797–3802 (2000).
47. Ouagazzal AM, Kenny PJ and File SE. *Eur J Neurosci* **11**, 3957–3962 (1999).
48. File SE, Cheeta S and Kenny PJ. *Eur J Pharmacol* **393**, 231–236 (2000).
49. File SE, Kenny PJ and Cheeta S. *Pharmacol Biochem Behav* **66**, 65–72 (2000).
50. Andersen E, Rigor B and Dafny N. *Int J Neurosci* 107–115 (1983).
51. Vertes RP. *J Comp Neurol* **313**, 643–668 (1991).
52. Cheeta S, Irvine EE, Kenny PJ *et al. Psychopharmacologia* **155**, 78–85 (2001).
53. Newman MB, Nazian SJ, Sanberg PR *et al. Prog Neuro-Psychopharmacol Biol Psychiatry* **25**, 609–620 (2001).
54. Smythe JW, Bhatnagar S, Murphy D *et al. Brain Res Bull* **45**, 89–93 (1998).
55. File SE, Gonzalez LE and Andrews N. *Behav Neurosci* **112**, 352–359 (1998).
56. Woodward LJ and Fergusson DM. *J Am Acad Child Adol Psychiatry* **40**, 1086–1093 (2001).
57. Parrott AC. *Addiction* **93**, 27–39 (1998).
58. Parrott AC. *Int J Addict* **30**, 1509–1516 (1995).
59. Mueller V, Mucha RF and Pauli P. *Pharmacol Biochem Behav* **59**, 1031–1038 (1998).
60. Miczek KA and Mutschler NH. *Psychopharmacology* **128**, 256–264 (1996).
61. Haney M, Maccari S, Le Moal M *et al. Brain Res* **698**, 46–52 (1995).
62. Goeders NE and Guerin GF. *Neuroendocrinology* **64**, 337–348 (1996).
63. Erb S, Shaham Y and Stewart J. *Psychopharmacology* **128**, 408–412 (1996).
64. Irvine EE, Cheeta S and File SE. *Pharmacol Biochem Behav* **68**, 319–325 (2001).
65. Ericson M, Olausson P, Engel JA *et al. Eur J Pharmacol* **397**, 103–111 (2000).
66. Bhattacharya SK, Chakrabarti A, Sandler M *et al. Neurosci Lett* **199**, 103–106 (1995).
67. Irvine EE, Bagnalasta M, Marcon C *et al. Psychopharmacology* **153**, 315–320 (2001).
68. Picciotto MR, Zoli M, Léna C *et al. Nature* **374**, 65–67 (1995).
69. George T, Verrico CD and Roth RH. *Brain Res* **801**, 36–49 (1998).
70. Decker MW, Brioni JD, Sullivan JP *et al. J Pharmacol Exp Ther* **270**, 319–328 (1994).
71. Decker MW, Anderson DJ, Brioni JD *et al. Eur J Pharmacol* **280**, 79–89 (1995).
72. Briggs CA, Anderson DJ, Brioni JD *et al. Pharmacol Biochem Behav* **57**, 231–241 (1997).
73. Ross SA, Wong JYF, Clifford JJ *et al. J Neurosci* **20**, 6431–6441 (2000).
74. Labarca C, Schwarz J, Deshpande P *et al. Proc Natl Acad Sci USA* **98**, 2786–2791 (2001).
75. Steinlein O, Sander T, Stoodt J *et al. Am J Med Genet* **74**, 445–449 (1997).
76. Picciotto MR, Zoli M, Zachariou V *et al. Biochem Soc Trans* **25**, 824–829 (1997).
77. Paylor R, Nguyen M, Crawley JN *et al. Learn Mem* **5**, 302–316 (1998).
78. Janowsky DS and Overstreet DH. *Pharmacol Toxicol* **3**, 100–111 (1990).
79. Diwan A, Castine M, Pomerleau CS *et al. Schizophrenia Res* **33**, 113–118 (1998).
80. Breslau N. *Behav Genet* **25**, 95–101 (1995).
81. Laje RP, Berman JA and Glassman AH. *Curr Psychiatry Rep* **3**, 470–476 (2001).
82. Salin-Pascual RJ, Rosas M, Jiminez Genchi A *et al. J Clin Psychiatry* **57**, 387–389 (1996).
83. Newhouse PA, Sunderland T, Tariot PN *et al. Psychopharmacology* **95**, 171–175 (1988).
84. Rana B, McMorn SO, Reeve HL *et al. Eur J Pharmacol* **250**, 247–251 (1993).
85. Fryer JD and Lukas RJ. *J Neurochem* **72**, 1117–1124 (1999).
86. Fryer JD and Lukas RJ. *J Pharmacol Exp Ther* **288**, 88–92 (1999).
87. Semba J, Mataki C, Yamada S *et al. Biol Psychiatry* **43**, 389–391 (1998).
88. Ferguson SM, Brodkin JD, Lloyd GK *et al. Psychopharmacologia* **152**, 295–303 (2000).
89. Djuric VJ, Dunn E, Overstreet DH *et al. Physiol Behav* **67**, 533–537 (1999).
90. Tizabi Y, Rezvani AH, Russell LT *et al. Pharmacol Biochem Behav* **66**, 73–77 (2000).
91. Tizabi Y, Overstreet DH, Rezvani AH *et al. Psychopharmacologia* **142**, 193–199 (1999).
92. Breslau N, Peterson EL, Schultz LR *et al. Arch Gen Psychiatry* **55**, 161–166 (1998).
93. Kendler KS, Neale MC, MacLean CJ *et al. Arch Gen Psychiatry* **50**, 36–43 (1993).
94. Overstreet DH. *Neurosci Biobehav Rev* **17**, 51–68 (1993).
95. Lai IC, Hong CJ and Tsai SJ. *J Affect Disorders* **66**, 79–82 (2001).
96. Caggiula AR, Epstein LH, Antelman SM *et al. Pharmacol Biochem Behav* **40**, 53–59 (1991).
97. Wilkins N, Carlson H, Van Vunakis H *et al. Psychopharmacology* **78**, 305–308 (1982).
98. Kasckow JW, Regmi A, Sheriff S *et al. Life Sci* **65**, 2709–2714 (1999).
99. Andersson K, Siegal R, Fuxe K *et al. Acta Physiol Scand* **118**, 35–40 (1983).
100. Pomerleau OF and Pomerleau CS. *Pharmacol Biochem Behav* **36**, 211–213 (1990).
101. Caggiula AR, Epstein LH, Antelman SM *et al. Psychopharmacology* **111**, 499–507 (1993).
102. Flemmer DD and Dilsaver SC. *Pharmacol Biochem Behav* **34**, 261–263 (1989).
103. Pauly J, Ullman E and Collins A. *Pharmacol Biochem Behav* **35**, 171–179 (1990).
104. Shoaib M and Shippenberg TS. *Psychopharmacology* **128**, 343–350 (1996).
105. Pauly JR, Ullman EA and Collins AC. *Physiol Behav* **44**, 109–116 (1988).
106. Zoli M, Picciotto MR, Ferrari R *et al. EMBO J* **18**, 1235–1244 (1999).
107. Andersson K, Fuxe K, Eneroth P *et al. Naunyn-Schm Arch Pharmacol* **339**, 387–396 (1989).
108. Fuxe K, Andersson K, Eneroth P *et al. Psychoneuroendocrinology* **14**, 19–41 (1989).
109. Matta S, Beyer H, McAllen K *et al. J Pharm Exp Ther* **243**, 217–226 (1987).
110. Wonnacott S, Drasdo A, Sanderson E *et al. Presynaptic nicotinic receptors and the modulation of transmission release.* In: Block G and Marsh J, eds. *The Biology of Nicotine Dependence*. Chichester: Wiley; 1990, pp. 87–105.
111. Sharp BM and Matta SG. *Endocrinology* **133**, 11–19 (1993).
112. Fu Y, Matta SG, Valentine JD *et al. Endocrinology* **138**, 1935–1943 (1997).
113. Fu Y, Matta SG, Brower VG *et al. J Neurosci* **21**, 8979–8989 (2001).
114. Fu Y, Matta SG, James TJ *et al. J Pharmacol Exp Ther* **284**, 1188–1196 (1998).
115. Feldman S, Conforti N and Weidenfeld J. *Neurosci Biobehav Rev* **19**, 235–240 (1995).
116. Feldman S and Weidenfeld J. *Neuroreport* **7**, 3055–3057 (1996).
117. Clarke PB and Reuben M. *Br J Pharmacol* **117**, 595–606 (1996).
118. Kenny PJ, Cheeta S and File SE. *Neuropharmacology* **39**, 300–307 (2000).
119. Engberg G, Erhardt S, Sharp T *et al. Naunyn Schmeiders Arch Pharmacol* **362**, 41–45 (2000).
120. Kenny PJ, File SE and Rattray M. *Eur J Neurosci* **13**, 1267–1271 (2001).
121. Corrigall WA, Franklin KB, Coen KM *et al. Psychopharmacology* **107**, 285–289 (1992).
122. Corrigall WA, Coen KM and Adamson KL. *Brain Res* **653**, 278–284 (1994).
123. George TP, Verrico CD, Xu L *et al. Neuropsychopharmacology* **23**, 79–88 (2000).
124. George TP, Verrico CD, Picciotto MR *et al. J Pharm Exp Ther* **295**, 58–66 (2000).
125. George TP, Picciotto MR, Verrico CD *et al. Biol Psychiatry* **49**, 300–306 (2001).
126. Gaddnas H, Pietila K adn Ahtee L. *Behav Brain Res* **113**, 65–72 (2000).
127. Cooper BR, Wang CM, Cox RF *et al. Neuropsychopharmacology* **11**, 133–141 (1994).
128. Canfield DR, Spealman RD, Kaufman MJ *et al. Synapse* **6**, 189–195 (1990).
129. Shiffman S, Johnston JA, Khayrallah M *et al. Psychopharmacology* **148**, 33–40 (2000).
130. Jorenby DE, Leischow SJ, Nides MA *et al. New Eng J Med* **340**, 685–691 (1999).
131. Yamanashi K, Miyamae T, Misu Y *et al. Eur J Pharmacol* **424**, 199–202 (2001).

132. Prasad BM, Sorg BA, Ulibarri C *et al. Ann NY Acad Sci* **771**, 617–625 (1995).
133. Meiergerd SM, Schenk JO and Sorg BA. *Brain Res* **773**, 203–207 (1997).
134. Tidey JW and Miczek KA. *Psychopharmacology* **130**, 203–212 (1997).
135. Picciotto MR, Zoli M, Rimondini R *et al. Nature* **391**, 173–177 (1998).
136. Tsuneki H, Klink R, Léna C *et al. Eur J Neurosci* **12**, 2475–2485 (2000).
137. Vailati S, Hanke W, Bejan A *et al. Mol Pharmacol* **56**, 11–19 (1999).
138. Kuryatov A, Olale F, Cooper J *et al. Neuropharmacology* **39**, 2570–2590 (2000).
139. Oreland L, Fowler CJ and Schalling D. *Life Sci* **29**, 2511–2518 (1981).
140. Green S. *Trends Neurosci* **14**, 101–104 (1991).
141. O'Neill AB and Brioni JD. *Pharmacol Biochem Behav* **49**, 755–757 (1994).
142. Magata Y, Kitano H, Shiozaki T *et al. Nucl Med Biol* **27**, 57–60 (2000).
143. Irvine E E, Cheeta S, Lovelock C *et al. Neuropharmacology* **40**, 710–716 (2001).
144. Kofalvi A, Sperlagh B, Zelles T *et al. J Pharmacol Exp Ther* **295**, 453–462 (2000).
145. Barazangi N and Role LW. *J Neurophysiol* **86**, 463–474 (2001).
146. Matta SG, Fu Y, Valentine JD *et al. Psychoneuroendocrinology* **23**, 103–113 (1998).
147. Rhodes ME, O'Toole SM, Czambel RK *et al. Brain Res Bull* **54**, 681–688 (2001).
148. Matta SG, Valentine JD and Sharp BM. *Brain Res* **759**, 259–269 (1997).
149. Serova L, Danailov E, Chamas F *et al. J Pharmacol Exp Ther* **291**, 884–892 (1999).
150. Vaidya VA and Duman RS. *Br Med Bull* **57**, 61–79 (2001).
151. Kenny PJ, File SE and Rattray M. *Mol Brain Res* **85**, 234–238 (2000).
152. Lapchak PA, Araujo DM and Hefti F. *Neuroscience* **52**, 575–85 (1993).
153. French SJ, Humby T, Horner CH *et al. Brain Res Mol Brain Res* **67**, 124–136 (1999).
154. File SE. *J Neurosci Methods* **2**, 219–238 (1980).
155. Pellow S, Chopin P, File SE *et al. J Neurosci Methods* **14**, 149–167 (1985).
156. Graeff FG, Viana MB and Tomaz C. *Braz J Med Biol Res* **26**, 67–70 (1993).
157. Graeff FG, Silveira MC, Nogueira RL *et al. Behav Brain Res* **58**, 123–131 (1993).
158. Treit D and Menard J. *Behav Neurosci* **111**, 653–658 (1997).
159. Davis M. *Trends Pharm Sci* **13**, 35–41 (1992).
160. Crawley J and Goodwin FK. *Pharmacol Biochem Behav* **13**, 167–170 (1980).
161. Crawley Jr MPJ, Paul SM, Skolnick P *et al. Science* **211**, 725–727 (1981).
162. Toubas P, Abla K, Cao W *et al. Pharmacol Biochem Behav* **35**, 121–126 (1990).
163. Nakamura K and Kurasawa M. *Eur J Pharmacol* **420**, 33–43 (2001).
164. Costall B, Jones BJ, Kelly ME *et al. Pharmacol Biochem Behav* **36**, 339–344 (1990).

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