The “Stop” and “Go” of Nicotine Dependence: Role of GABA and Glutamate

Manoranjan S. D’Souza and Athina Markou

Department of Psychiatry, School of Medicine, University of California San Diego, La Jolla, California 92093

Correspondence: amarkou@ucsd.edu

Nicotine plays an important role in the initiation and maintenance of tobacco smoking. Importantly, chronic nicotine exposure alters the function of brain reward systems, resulting in the development of a nicotine-dependent state. This nicotine-dependent state is associated with aversive affective and somatic signs upon abstinence from smoking, often leading to relapse in abstinent smokers. This article reviews the role of the major excitatory and inhibitory neurotransmitters glutamate and γ -aminobutyric acid (GABA), respectively, in both the reinforcing effects of nicotine and development of nicotine dependence. Evidence suggests that blockade of glutamatergic neurotransmission attenuates both nicotine intake and nicotine seeking. In contrast, both nicotine intake and nicotine seeking are attenuated when GABA neurotransmission is facilitated. In conclusion, medications that either attenuate/negatively modulate glutamatergic neurotransmission or facilitate/positively modulate GABA neurotransmission may be useful for promoting smoking cessation in humans.

Tobacco smoking is a chronic compulsive and relapsing disorder that results in significant morbidity and mortality worldwide (Giovino 2007; World Health Organization 2008; Centers for Disease Control and Prevention 2011). Although tobacco smoke contains thousands of chemicals, nicotine is the major psychoactive component that is largely responsible for the maintenance of the harmful tobacco smoking habit in humans (Henningfield and Goldberg 1983; Stolerman and Jarvis 1995). The central actions of nicotine are mediated by nicotinic acetylcholine (nACh) receptors that are distributed throughout the brain (Martin and Aceto 1981; Changeux 2010). The majority of the currently approved medications for smoking cessation focus on manipulating nicotine intake

by modulating these nicotinic receptors (Stack 2007; Benowitz 2010). The success rates of these nicotinic receptor-based medications are low, with much scope for improvement (Agboola et al. 2010). To accelerate the discovery and development of new medications for smoking cessation, understanding the neural substrates that mediate the development of nicotine dependence is essential.

The actions of nicotine in the brain are mediated by excitatory nACh receptors. These excitatory nACh receptors are present on several different types of neurons and regulate the release of a number of neurotransmitters, including the major excitatory and inhibitory neurotransmitters in the brain, namely glutamate and γ -aminobutyric acid (GABA) (Wonnacott

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1997). The present review focuses on the role of glutamate and GABA in nicotine dependence. More specifically, this review covers the following: (1) the effects of acute nicotine administration on glutamatergic and GABAergic neurotransmission; (2) alterations in glutamatergic and GABAergic neurotransmission after chronic nicotine exposure; and (3) the effects of pharmacological modulation of glutamatergic and GABAergic neurotransmission in animal models that assess different aspects of nicotine dependence. Identifying and targeting nonnicotinic neural substrates, such as those involved in glutamatergic and GABAergic neurotransmission, will help expand the repertoire of smoking cessation treatments and result in more efficacious medications for the treatment of nicotine dependence than those currently available.

NICOTINE DEPENDENCE

Nicotine dependence can be broadly divided into three phases. In humans, the initial phase of the acquisition and maintenance of nicotine seeking is mediated by the positive reinforcing effects of nicotine, such as mild euphoria, increased arousal, decreased fatigue, and relaxation (Henningfield et al. 1985). In addition, nicotine enhances the rewarding properties of environmental stimuli (Caggiula et al. 2001; Chaudhri et al. 2006). Chronic nicotine use leads to neuroadaptations in the function of brain reward systems, leading to the development of nicotine dependence and resulting in aversive withdrawal effects upon abstinence from smoking (i.e., the withdrawal phase). In humans, withdrawal from nicotine produces both affective and somatic adverse effects. Adverse affective symptoms in humans are more pronounced than adverse somatic symptoms and include depressed mood, dysphoria, anxiety, irritability, difficulty concentrating, and craving (Shiffman and Jarvik 1976). The “physical” or somatic withdrawal manifestations include bradycardia, insomnia, gastrointestinal discomfort, and weight gain (Hughes et al. 1991). Avoidance of these withdrawal symptoms can help sustain the motivation to seek nicotine. After this initial withdrawal phase, prolonged abstinence from

nicotine can result in a phase in which the individual is vulnerable to relapse (Brandon et al. 1998; Herd et al. 2009). Relapse can occur in response to cues/contexts associated with smoking, stressful events previously alleviated by smoking, and exposure to nicotine or tobacco smoke itself. Relapse to smoking in abstinent smokers is attributable to the learned associations between stimuli and the effects of nicotine.

GLUTAMATE AND NICOTINE DEPENDENCE

Glutamate is the major excitatory neurotransmitter in the mammalian brain, and its actions are mediated by glutamatergic receptors that can be broadly divided into fast-acting ionotropic receptors and slow-acting G-protein-coupled metabotropic receptors (Hollmann and Heinemann 1994; Conn and Pin 1997). The ionotropic receptors include *N*-methyl-D-aspartate (NMDA), amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainate receptors. Metabotropic glutamate (mGlu) receptors are classified into three groups (I, II, and III), depending on their signal transduction pathways, sequence homology, and pharmacological selectivity (Conn and Pin 1997). Group I (mGlu1 and mGlu5) receptors are predominantly located postsynaptically, and Group II (mGlu2 and mGlu3) and Group III (mGlu4, mGlu6, mGlu7, and mGlu8) receptors are primarily found on presynaptic terminals and on glial cells. The activation of Group II and III mGlu receptors negatively modulates glutamate release. Metabotropic glutamate receptors are critically important compared with ionotropic receptors because drugs that act on these receptors are hypothesized to subtly modulate glutamate transmission and produce fewer side effects. Furthermore, a significant body of literature has highlighted the role of mGlu receptors in drug dependence, including nicotine dependence (Kenny and Markou 2004; D'Souza and Markou 2011a). The levels of synaptic glutamate are closely regulated by transport proteins located on glial cells in the extrasynaptic space (McBean 2002; Huang and Bergles 2004). These transport proteins include the cystine-glutamate antiporter and glutamate uptake transporters (GLT1–4).

Glutamatergic neurotransmission can be modulated by pharmacological compounds that can either block or activate the various glutamatergic receptors and transporters.

Microdialysis studies suggest that nicotine increases glutamate release in both the ventral tegmental area (VTA) and nucleus accumbens (NAcc) (Schilstrom et al. 1998; Fu et al. 2000; Reid et al. 2000). Nicotine increases glutamate release by activating excitatory α_7 nACh receptors located on presynaptic glutamatergic terminals (Mansvelder and McGehee 2002). α_7 nACh receptors are highly permeable to calcium, and the binding of nicotine to these receptors leads to calcium-mediated glutamate release from presynaptic glutamatergic terminals (Seguela et al. 1993). Importantly, data from electrophysiological studies suggest that exposure to nicotine facilitates both glutamate release and postsynaptic AMPA/NMDA receptor-mediated neurotransmission at glutamatergic–dopaminergic synapses in the VTA (Gao et al. 2010; Mao et al. 2011). Finally, chronic nicotine self-administration increases the expression of the NR2A and NR2B NMDA receptor subunits in the prefrontal cortex and expression of the GluR2 and GluR3 AMPA receptor subunits in the VTA (Wang et al. 2007). In summary, acute nicotine administration increases the release of glutamate via excitatory nACh receptors located on presynaptic glutamatergic terminals. Additionally, nicotine exposure facilitates nicotine-induced presynaptic glutamate release, increases postsynaptic glutamate receptor-mediated neurotransmission, and alters the expression of ionotropic glutamate receptor expression. This nicotine-induced increase in glutamatergic neurotransmission is hypothesized to play an important role in the reinforcing effects of nicotine. The next few sections highlight the role of glutamate in nicotine-dependent behavior.

Glutamate and the Reinforcing Effects of Nicotine

Mesolimbic dopaminergic neurons that originate in the VTA are part of the neurocircuitry that mediates the reinforcing effects of most drugs of abuse, including nicotine (Fig. 1) (Wise 1987;

Koob and Volkow 2010). The activity of these dopaminergic neurons is regulated by glutamatergic inputs from different limbic, cortical, and subcortical nuclei, such as the amygdala, prefrontal cortex, lateral habenula, lateral and medial hypothalamus, ventral pallidum, medial septum, septofimbrial nucleus, and ventrolateral bed nucleus of the stria terminalis (Geisler and Zahm 2005; Geisler and Wise 2008). In addition, dopaminergic neurons in the VTA receive glutamatergic projections from brainstem structures, such as the mesopontine reticular formation, laterodorsal tegmental and pedunculopontine tegmental nucleus, cuneiform nucleus, median raphe, and superior colliculus (Geisler and Trimble 2008). Cortical and limbic nuclei play an important role in mediating motivational, emotional, and cognitive responses to rewarding stimuli.

Glutamate plays an important role in regulating nicotine-induced increases in mesolimbic dopamine. Nicotine-induced increases in dopamine in the NAcc were attenuated by systemic administration of the NMDA receptor antagonist CGP39551 and AMPA receptor antagonist ZK200775 in nicotine-naïve animals (i.e., animals with no prior exposure to nicotine) (Kosowski et al. 2004). Furthermore, microinjection of the competitive NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (AP5) into the VTA attenuated nicotine-induced increases in NAcc dopamine (Schilstrom et al. 1998; Fu et al. 2000). This decrease in nicotine-induced increase in NAcc dopamine after NMDA receptor blockade was possibly caused by decreased firing of mesolimbic dopaminergic neurons (Chergui et al. 1993). Administration of the mGlu5 receptor antagonist 2-methyl-6 (phenylethynyl) pyridine (MPEP) also attenuated nicotine-induced increases in NAcc dopamine in both nicotine-naïve and nicotine-experienced animals (Tronci and Balfour 2011). The mGlu2/3 receptor agonist LY379268 attenuated nicotine-induced increases in NAcc dopamine in nicotine-experienced animals only in the presence of a nicotine-associated context (Fig. 2A–C) (D'Souza et al. 2011). Interestingly, LY379268 did not attenuate the nicotine-induced increase in NAcc dopamine in nicotine-

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Figure 1. The figure shows a sagittal section of a rat brain that presents the regions involved in the neurochemical and behavioral effects of nicotine. Nicotine binds to nicotinic acetylcholine receptors that are located throughout the brain as auto- or heteroreceptors at presynaptic terminals that regulate the release of several neurotransmitters, including glutamate and γ -aminobutyric acid (GABA). The mesolimbic dopaminergic neurons that originate in the ventral tegmental area project to several limbic and cortical regions, including the nucleus accumbens, prefrontal cortex, amygdala, hippocampus, and habenula. The activity of these dopaminergic neurons is regulated by reciprocal glutamatergic (excitatory) and GABAergic (inhibitory) projections that originate from the aforementioned cortical and limbic brain regions. Major glutamatergic projections are depicted in blue. Dopaminergic projections that originate from the ventral tegmental area are depicted in yellow. Major GABAergic projections are depicted in red. AMY, amygdala; LHb, lateral habenula; HC, hippocampus; NAc, nucleus accumbens; PFC, prefrontal cortex; RN, raphe nucleus; VP, ventral pallidum; VTA, ventral tegmental area.

naive or nicotine-experienced animals in the absence of a nicotine-associated context. These intriguing findings are consistent with a large body of evidence that supports a role for mGlu2/3 receptors in the mediation of the context/cue-induced reinstatement of drug- and food-seeking behavior (Baptista et al. 2004; Bossert et al. 2006a,b; Liechti et al. 2007).

The most direct role for glutamate in the positive reinforcing effects of nicotine comes from intravenous nicotine self-administration studies in rats. Similar to humans, animals reliably self-administer nicotine (Corrigall and Coen 1989; Watkins et al. 1999) and provide the opportunity to assess the reinforcing and motivational effects of nicotine. The reinforcing effects of nicotine are assessed using a fixed-ratio (FR) schedule of reinforcement, and the motivational effects of nicotine are assessed using a progressive-ratio (PR) schedule (Markou 2008). In the FR schedule, animals have to press a lever a fixed number of times to obtain a single infusion of nicotine. The PR schedule involves an exponen-

tial increase in the number of lever responses required to obtain each subsequent infusion of nicotine and thus reflects the motivational effects of nicotine (Markou et al. 1993).

Blockade of glutamatergic neurotransmission either by systemic administration or site-specific microinjections of glutamate receptor antagonists into mesolimbic brain sites attenuated nicotine self-administration. Specifically, systemic administration or microinjection into the VTA of the competitive NMDA receptor antagonist LY235959 attenuated nicotine self-administration (FR5 schedule) in male Wistar rats (Kenny et al. 2009). Importantly, systemically administered LY235959 selectively decreased nicotine self-administration at low doses but did not affect food self-administration. At the highest doses tested, LY235959 attenuated both nicotine and food self-administration. Interestingly, D-cycloserine, an NMDA receptor partial agonist, also attenuated nicotine self-administration on an FR1 schedule in female Sprague-Dawley rats with low basal nicotine intake

Figure 2. The figure shows the effects of the mGlu2/3 agonist LY379268 on nicotine-induced increases in NAcc dopamine. Dopamine levels (mean $\pm$ SEM) are expressed as a percentage of baseline values. (A) Systemic administration of LY379268 (1 mg/kg) attenuated the increases in NAcc shell dopamine after self-administration of a single intravenous infusion of nicotine (0.06 mg/kg/infusion over 1 sec) in nicotine-experienced animals in a context associated with nicotine self-administration. $*p < 0.05$, $***p < 0.001$, saline-pretreated compared with their own baseline levels; $+p < 0.05$, $++p < 0.01$, $+++p < 0.001$, saline-pretreated compared with LY379268-pretreated animals. (Inset) Latency (mean $\pm$ SEM) in minutes to self-administer nicotine in saline- and LY379268-pretreated animals. $**p < 0.01$, LY379268-pretreated group compared with saline-pretreated group. The time period between -10 and 10 depicts the time taken by the animals to self-administer nicotine and the dead volume of the probe (no samples were collected during this period). The time taken to self-administer nicotine varied among LY379268- and saline-pretreated animals. Based on the dead volume of the tubing, sample collection was initiated 2 min after nicotine self-administration in all animals. (B) Systemic administration of LY379268 (1 mg/kg, s.c.) attenuated the increases in NAcc shell dopamine after a single experimenter-administered intravenous infusion of nicotine (0.06 mg/kg/infusion over 1 sec) in nicotine-experienced animals in a context associated with nicotine self-administration. $**p < 0.01$, saline-pretreated compared with their own baseline levels; $++p < 0.01$, saline-pretreated compared with LY379268-pretreated animals. The time period between -10 and 10 depicts the dead volume of the probe, which was 2 min. (C) Systemic administration of LY379268 (1 mg/kg, s.c.) had no effect on the increases in NAcc shell dopamine after a single experimenter-administered intravenous infusion of nicotine (0.06 mg/kg/infusion over 1 sec) in nicotine-experienced animals in a context never associated with nicotine self-administration. $*p < 0.05$, saline- and LY379268-pretreated compared with their respective baseline levels. The time period between -10 and 10 depicts the dead volume of the probe, which was 8 min. (From D'Souza et al. 2011; reprinted, with permission, from the author.)

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(Levin et al. 2011). The effects of D-cycloserine were evaluated only in female rats, and the effects of D-cycloserine on food self-administration have not yet been evaluated. Thus, the aforementioned findings indicate that either blockade of NMDA receptors with a NMDA receptor antagonist or activation of NMDA receptor activity using a coagonist, such as D-cycloserine, decreases nicotine intake. The similar effects of the competitive NMDA receptor coagonist D-cycloserine on nicotine self-administration can be attributed to the differences in gender (male vs. female), the strain of rats used (Wistar vs. Sprague-Dawley), and the nicotine self-administration protocols (FR5 vs. FR1) in these two studies. Taken together, the data support an important role for NMDA receptors in the regulation of the reinforcing effects of nicotine.

Similarly, the mGlu5 receptor antagonist MPEP selectively attenuated nicotine self-administration without affecting food self-administration (Paterson et al. 2003; Liechti and Markou 2007; Palmatier et al. 2008). In addition, MPEP decreased the motivation to self-administer nicotine under a PR schedule, suggesting a decrease in the motivation to self-administer nicotine (Paterson and Markou 2005). Furthermore, MPEP microinfusions directly into the NAcc shell or VTA decreased nicotine self-administration (D'Souza and Markou 2011b). Interestingly, microinjections of MPEP into the NAcc shell had no effect on food self-administration, but microinjections of MPEP into the VTA had similar effects on nicotine and food self-administration. Taken together, the data from these studies suggest that mGlu5 receptors play an important role in mediating the reinforcing and motivational effects of nicotine and that the effects of MPEP are partially mediated by mGlu5 receptors in the NAcc shell and VTA.

Decreases in glutamatergic neurotransmission by systemic administration of the mGlu2/3 receptor agonist LY379268 selectively decreased nicotine self-administration in rats without affecting food self-administration, except at the highest doses tested (Fig. 3A) (Liechti et al. 2007). Microinfusion of LY379268 directly into the VTA or NAcc shell also attenuated nicotine self-administration, thus highlighting an impor-

tant role for these mesolimbic nuclei in the reinforcing effects of nicotine (Fig. 3B,C). Repeated administration of LY379268 for 14 days selectively attenuated nicotine self-administration compared with food self-administration. However, rapid tolerance developed to the effects of LY379268 on nicotine self-administration. This rapid tolerance with the mGlu2/3 agonist suggests that alternative strategies to activate mGlu2/3 receptors, such as positive allosteric modulators, may be more effective than full agonists in the treatment of nicotine dependence.

Glutamate transmission can also be altered by activating the cystine-glutamate transporter (xCT) with *N*-acetylcysteine. *N*-acetylcysteine binds to the xCT and increases extrasynaptic glutamate levels in exchange for extracellular cystine (Baker et al. 2002). *N*-acetylcysteine administration attenuated nicotine self-administration, indicating that activation of the xCT decreased the reinforcing effects of nicotine (AM Ramirez-Niño, MS D'Souza, and A Markou, unpubl.). Importantly, the decrease in nicotine self-administration after *N*-acetylcysteine administration was seen at doses that did not affect food self-administration. Repeated administration of *N*-acetylcysteine for 14 days initially reduced both nicotine and food self-administration. Rapid tolerance developed to the effects of *N*-acetylcysteine on food-maintained responding, whereas the effects of *N*-acetylcysteine on nicotine self-administration persisted throughout the duration of treatment.

In summary, blockade of glutamatergic neurotransmission attenuated the reinforcing and motivational effects of nicotine. Several glutamatergic receptors, such as NMDA, mGlu2/3, and mGlu5 receptors, and the xCT mediate the reinforcing effects of nicotine. Therefore, pharmacological modulation of glutamatergic neurotransmission may be a promising strategy for the promotion of smoking cessation.

Glutamate and Nicotine-Induced Reward-Enhancing Effects

The stimulation of brain reward circuits using brief electrical pulses, also known as intracranial

Figure 3. The figure shows the effects of the mGlu2/3 agonist LY379268 on nicotine-self-administration and nicotine-seeking behavior. (A) Systemic administration (subcutaneous [s.c.]) of the mGlu2/3 receptor agonist LY379268 decreased nicotine (black bars; $n = 7$; timeout 20 sec) but not food (white bars; $n = 8$; timeout 210 sec) self-administration except at the highest LY379268 dose (3 mg/kg, s.c.). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, different from 0 mg/kg LY379268; $^+p < 0.05$, different from 1 mg/kg LY379268. (B) LY379268 microinjections into the nucleus accumbens (NAcc) shell ($n = 9$) attenuated nicotine self-administration. A control injection (Co; $n = 9$) of 1 μg LY379268 2 mm above the NAcc shell into the lateral septal nucleus had no effect on nicotine self-administration. $^{\#}p < 0.05$, $^{\#\#}p < 0.001$, different from control injection. (C) LY379268 microinjections into the ventral tegmental area (VTA; $n = 9$) also decreased nicotine self-administration. A control injection (Co; $n = 7$) of 0.6 μg LY379268 2 mm above the VTA into the red nucleus had no effect on nicotine self-administration. $^{\#}p < 0.05$, $^{\#\#}p < 0.001$, different from control injection. (D) Systemic administration of LY379268 attenuated the cue-induced reinstatement of nicotine seeking ($n = 32$; within-subject comparison). $^+p < 0.05$, $^{++}p < 0.01$, different from extinction; $^*p < 0.05$, $^{***}p < 0.001$, different from 0 mg/kg LY379268. EXT, extinction; Responses, total of active lever presses, including responses during the cue presentation. (E) Systemic administration of LY379268 also attenuated the cue-induced reinstatement of food seeking ($n = 10$ per dose; between-subject comparison). $^+p < 0.05$, $^{++}p < 0.01$, different from extinction; $^*p < 0.05$, $^{***}p < 0.001$, different from 0 mg/kg LY379268. EXT, extinction; Responses, total of active lever presses, including responses during the cue presentation. (From Liechti et al. 2007; reprinted, with permission, from *J Neurosci* © 2007.)

self-stimulation (ICSS), is a technique used to assess the reward-enhancing effects of drugs of abuse, such as nicotine (Markou and Koob 1993). In this technique, the animal is exposed to a series of brief electric pulses that vary in current intensity, and the minimal current that main-

tains self-stimulation behavior is determined. This minimal current is called the ICSS reward threshold. Psychostimulants, including nicotine, lower the ICSS threshold required to maintain self-stimulation behavior (Kornetsky and Espósito 1979; Harrison et al. 2002).

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Pharmacological blockade of glutamatergic neurotransmission attenuated the nicotine-induced lowering of ICSS thresholds. For example, the NMDA receptor antagonist LY235959 reversed the nicotine-induced lowering of reward thresholds (Kenny et al. 2009). Importantly, LY235959 did not affect brain reward thresholds when administered alone. These data suggest that NMDA receptors play an important role in the reward-enhancing effects of nicotine. The mGlu5 receptor antagonist MPEP and mGlu2/3 agonist LY314582 also attenuated the reward-enhancing effects of nicotine (Harrison et al. 2002). Interestingly, administration of either MPEP or LY314582 alone elevated brain reward thresholds, indicating that these compounds have an opposite effect to nicotine, rather than directly blocking or reversing the reward-enhancing effects of nicotine. Consistent with this interpretation, MPEP and another more selective mGlu5 receptor antagonist, MTEP, did not block the reward-enhancing effects of nicotine measured using an operant procedure in which animals received experimenter-administered nicotine in response to lever pressing for a visual stimulus (Palmatier et al. 2008). The ICSS findings suggest that NMDA-mediated neurotransmission attenuates the nicotine-induced facilitation of brain reward, whereas glutamatergic neurotransmission via mGlu5 and mGlu2/3 receptors appears to have an opposite effect in reward function compared with nicotine.

Glutamate and the Conditioned Rewarding Effects of Nicotine

Environmental cues associated with nicotine play an important role in the maintenance of nicotine intake in humans and animals (Caggiula et al. 2001; Le Foll and Goldberg 2005a). The positive rewarding effects of nicotine play an important role in the associative learning between nicotine and cues/contexts that predict nicotine administration. The conditioned rewarding effects of nicotine and underlying neural substrates can be studied using the conditioned place preference (CPP) procedure.

Most commonly, two chambers with distinct characteristics (e.g., color, texture, and flooring) are used in CPP studies. One of the two chambers is associated with nicotine during a training session and is called the drug-paired chamber. In another temporally distinct training session, the animals are treated with vehicle and placed in the other chamber, referred to as the saline-paired chamber. The repeated pairing of the drug-paired chamber with the rewarding effects of nicotine over time results in a preference for the drug-paired chamber over the saline-paired chamber during a test session conducted in the absence of the drug. Several studies have shown nicotine-induced CPP in rodents (Fudala et al. 1985; Shoaib et al. 1994; Risinger and Oakes 1995; McGeehan and Olive 2003; Le Foll and Goldberg 2005b; Grabus et al. 2006).

Glutamate plays an important role in the conditioned rewarding effects of nicotine. Selective knockout of NMDA receptors located on VTA dopaminergic neurons in mice attenuated the acquisition of nicotine-induced CPP (Wang et al. 2010). Consistent with these data, the glycine site partial agonist 1-aminocyclopropanecarboxylic acid (ACPC), which reduces NMDA receptor-mediated neurotransmission, attenuated the acquisition and expression of nicotine-induced CPP (Papp et al. 2002). These data support a role for NMDA receptors, particularly those in the VTA, in the conditioned rewarding effects of nicotine. The mGlu5 receptor antagonist MPEP also attenuated nicotine-induced CPP (Yararbas et al. 2010; but also see McGeehan and Olive 2003). Consistent with this finding, MPEP also decreased conditioned locomotor activity evoked by pairing nicotine with a specific environment (Tronci et al. 2010). Interestingly, one study reported that MPEP facilitated nicotine-induced CPP, resulting in a leftward shift of the minimum dose of nicotine required to induce CPP (Rutten et al. 2011). The facilitation of nicotine-induced CPP is surprising, considering that MPEP when administered alone elevated reward thresholds in the ICSS procedure. The conflicting data with regard to MPEP in nicotine-induced CPP could possibly be attributable to differences in the

strains of animals used, doses, and modes of MPEP administration and design used to show nicotine-induced CPP. In summary, the above data suggest that glutamate plays an important role in the conditioned rewarding effects of nicotine, and these effects are partially mediated by activation of postsynaptic NMDA and mGlu5 receptors.

Glutamate and Nicotine Seeking

As mentioned above, abstinent smokers are susceptible to relapse when exposed to either stimuli previously associated with smoking or stressful stimuli whose effects were previously alleviated by smoking or both. Similarly, experimental animals exposed to cues or contexts previously associated with nicotine self-administration show reinstatement of nicotine-seeking behavior even after the extinction of nicotine self-administration behavior (Paterson et al. 2005; Dravolina et al. 2007). This reinitiation of nicotine-seeking behavior in animals after a period of extinction training is a putative model of relapse in humans (Markou et al. 1993; Shaham et al. 2003). The reinstatement of nicotine-seeking behavior in animals is also seen after exposure to nicotine (Dravolina et al. 2007).

The mGlu5 receptor antagonist MPEP attenuated the cue-induced reinstatement of nicotine seeking but not food seeking (Bespalov et al. 2005). In contrast, the mGlu2/3 agonist LY379268 attenuated the cue-induced reinstatement of both nicotine and food seeking (Liechti et al. 2007). *N*-acetylcysteine also attenuated the cue-induced reinstatement of both nicotine and food seeking, supporting a role for the cystine-glutamate exchanger in cue-induced nicotine-seeking behavior (AM Ramirez-Niño, MS D'Souza, and A Markou, unpubl.). Altogether, these results show an important role for glutamate in the conditioned behavioral responses to cues and contexts associated with nicotine. However, the blockade of cue-induced food seeking suggests that the glutamatergic compounds tested so far attenuate conditioned behavioral responses, regardless of the reinforcer. Although this attenuation of cue-induced food-seeking behavior may be an undesirable effect in

most situations, it may be advantageous in the case of the medications being developed to prevent relapse in smokers. Because smokers who abstain from smoking often experience extreme food cravings that result in undesired weight gain (Friedman and Siegelau 1980), the ability to block cue-induced food seeking may be a useful property of medications intended to prevent relapse in abstinent smokers. Generally, the fact that these compounds block both nicotine and food seeking may indicate a critical role for these receptors in all conditioned behaviors.

Glutamate and Nicotine Withdrawal

The expression and function of glutamate receptors is altered during early withdrawal in animals chronically exposed to nicotine. Specifically, the expression of the NR2A, NR2B, and GluR2 subunits decreased in the prefrontal cortex (Kenny et al. 2009). However, the same study found increased expression of the NR1, NR2A, NR2B, GluR1, and GluR2 subunits in the amygdala. The expression of the NR2A and GluR1 subunits was also increased in the VTA and NAcc, respectively. Furthermore, down-regulation of mGlu2/3 receptor function in the VTA, NAcc, prefrontal cortex, amygdala, hippocampus, and hypothalamus was seen during early withdrawal after a period of chronic nicotine self-administration (Liechti et al. 2007). mGlu2/3 receptors are located on presynaptic glutamatergic terminals and negatively regulate synaptic glutamate levels. Therefore, the down-regulation of mGlu2/3 receptor function during early withdrawal suggests the development of a hypoglutamatergic state. Finally, down-regulation of the xCT and glutamate transporter (GLT-1) in the NAcc was also reported during early withdrawal after a period of chronic nicotine self-administration (Knackstedt et al. 2009). Both xCT and GLT-1 play an important role in maintaining extracellular glutamate levels, and down-regulation of these transporters suggests compensatory changes in response to decreased synaptic glutamate levels during early withdrawal. The above data suggest that withdrawal from nicotine after a period of nicotine self-administration results in the development

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of a hypoglutamatergic state that is characterized by: (1) the increased expression of postsynaptic glutamatergic receptors, such as NMDA and AMPA receptors; (2) down-regulation in the function of presynaptic autoreceptors, such as mGlu2/3 receptors; and (3) down-regulation in the function of extrasynaptic glutamatergic transporters, such as xCT and GLT-1.

Withdrawal from nicotine produces both somatic and affective disturbances in animals. The somatic withdrawal signs seen in rodents include rearing, jumping, shakes, abdominal constrictions, chewing, scratching, and facial tremors (Watkins et al. 2000; Malin et al. 2006). In animals, the affective depression-like aspects of nicotine withdrawal can be measured using the ICSS procedure and are reflected by elevations in reward thresholds (Epping-Jordan et al. 1998; Watkins et al. 2000). Importantly, the AMPA receptor antagonist NBQX elevated brain reward thresholds in nicotine-dependent rats, similar to the reward thresholds seen after administration of nACh receptor antagonists in nicotine-dependent animals (Kenny et al. 2003). This finding suggests that AMPA receptors are involved in the affective manifestations seen during nicotine withdrawal. Furthermore, the mGlu5 receptor antagonist MPEP exacerbated brain reward deficits and somatic signs associated with nicotine withdrawal (Liechti and Markou 2007). The same study also showed that administration of the mGlu2/3 receptor agonist LY379268 did not worsen brain reward thresholds during nicotine withdrawal. A possible reason for the lack of effects of the mGlu2/3 receptor agonist during nicotine withdrawal could be the down-regulation of mGlu2/3 receptor function. In summary, nicotine withdrawal is hypothesized to be associated with the development of a hypoglutamatergic state, and strategies that increase glutamate transmission may help reverse the affective and somatic signs of nicotine withdrawal.

GABA AND NICOTINE DEPENDENCE

GABA is the major inhibitory neurotransmitter in the brain. Endogenously released GABA acts either via ionotropic (GABA_A and GABA_C) or

metabotropic (GABA_B) receptors. The binding of GABA to GABA_A or GABA_C receptors allows the entry of Cl⁻ ions, resulting in neuron hyperpolarization and ultimately the decreased firing of these neurons. In addition to GABA, GABA_A but not GABA_C receptors have modulatory binding sites for barbiturates, benzodiazepines, neurosteroids, and ethanol. Apart from these pharmacological differences between GABA_A and GABA_C receptors, structural differences also exist that are beyond the scope of this review (see Bormann 2000). In contrast to GABA_A and GABA_C receptors, GABA_B receptors couple to Ca²⁺ and K⁺ ion channels via G-proteins and second messengers. Evidence suggests that the effects of nicotine on the mesocorticolimbic dopaminergic system are regulated primarily by GABA_B receptors in the VTA (Aman- tea and Bowery 2004). Endogenous GABA is metabolized by GABA transaminase. Therefore, GABA neurotransmission can be increased by compounds that enhance the activity of GABA receptors or inhibit the breakdown of GABA via inhibition of GABA transaminase.

Nicotine induces GABA release by binding to excitatory nACh receptors located on presynaptic GABA neurons. The cellular mechanism by which the activation of nACh receptors results in GABA release is not clearly understood. Recent work showed that activation of nACh receptors on GABAergic terminals depolarized cells, resulting in the opening of T-type Ca²⁺ channels and leading to GABA release (Tang et al. 2011). This study was conducted in hippocampal perisomatic GABA interneurons that contain $\alpha_3\beta_4$ -containing nACh receptors. It is not known whether the same mechanism is involved in the release of GABA from neurons located in the mesolimbic region.

The composition of the subtype of nACh receptors located on GABAergic neurons has not been fully identified. Existing evidence suggests the presence of nACh receptors composed of $\alpha_3\beta_4$ -, $\alpha_4\beta_2$ -, or $\alpha_6\beta_2$ -containing nACh receptors on GABAergic neurons that terminate on mesolimbic dopaminergic neurons (Mansvelder et al. 2002; Yang et al. 2011). Knowledge of the composition of nACh receptors on GABA neurons is essential because nAChR composition

determines the rate at which nACh receptors undergo desensitization or functional inactivation on exposure to nicotine (Fenster et al. 1999; Quick and Lester 2002). The desensitization of nACh receptors can result in decreased GABA release and consequently decreased inhibition of mesolimbic dopaminergic neurons, thus facilitating the nicotine-induced excitation of mesolimbic dopaminergic neurons. The desensitization of β_2 -containing nACh receptors on GABAergic interneurons may be involved in the decreased GABA-mediated inhibition of dopaminergic neurons (Mansvelder et al. 2002). A recent study reported that decreased GABA release on repeated nicotine exposure was mediated by $\alpha_6\beta_2$ -containing nACh receptors located on presynaptic GABA neurons in the VTA (Tang et al. 2011). Importantly, the latter study found decreased acetylcholine-induced GABA release after nicotine exposure. Nicotine-induced alterations in GABA_B receptor function or expression could also be a possible mechanism by which chronic nicotine exposure reduces GABAergic transmission. Evidence for this hypothesis, however, is mixed. For example, both the GABA structural analog γ -vinyl GABA (GVG) and GABA_B agonist CGP44532 did not affect brain reward thresholds in the ICSS procedure in saline- or nicotine-treated rats, whereas the highest dose of each drug elevated thresholds in both groups of rats (Paterson et al. 2005). Additionally, CGP44532 microinfusions into the VTA elevated thresholds equally in both saline- and nicotine-treated groups, indicating that prolonged nicotine exposure did not alter the GABA_B-mediated regulation of brain reward function. Altogether, these data suggest that nicotine exposure does not alter the GABA_B receptor-mediated regulation of brain reward function. Furthermore, changes in GABA_B receptor expression or GABA_B mRNA levels have not been observed after chronic nicotine treatment. No alterations in GABA_B-G-protein coupling have been reported in the VTA after chronic nicotine treatment. However, one study found reduced efficacy of the GABA_B receptor agonist baclofen to decrease electrically stimulated [³H]dopamine release in a slice preparation of VTA dopamine neurons

after chronic nicotine treatment (Amantea and Bowerly 2004). This finding suggests that chronic nicotine treatment alters the function of GABA_B receptors. GABA_B-G-protein coupling is also altered in other brain regions, such as the NAcc and prefrontal cortex, after chronic nicotine treatment (Amantea et al. 2004). However, these in vitro changes may not have functional significance, at least in terms of reward function (see above). These differences in the literature may be attributable to differences in nicotine exposure and the methods used to assess GABA_B receptor function. In summary, GABAergic neurons provide important inhibitory inputs to dopaminergic neurons. Acute nicotine administration increases GABA release by binding to excitatory presynaptic nACh receptors located on GABA neurons. Repeated nicotine administration decreases the GABA regulation of mesolimbic dopaminergic neurons by desensitizing nACh receptors or decreasing GABA_B receptor function or both, although the latter possibility is not supported by behavioral data (see above). The role of GABA in nicotine dependence has been studied in behavioral models using pharmacological compounds that either increase or decrease GABA neurotransmission. In the following sections, the role of GABA in nicotine-dependent behavior is discussed.

GABA and the Reinforcing Effects of Nicotine

GABA plays an important role in regulating mesolimbic dopaminergic neurons that partially mediate the reinforcing effects of nicotine and other drugs of abuse. These mesolimbic dopaminergic neurons receive extensive local and external GABAergic inputs. The local inputs are mainly derived from local interneurons, whereas the external inputs originate from distant nuclei, such as the NAcc, ventral pallidum, and pedunculopontine tegmental nucleus (Kavivas and O'Brien 2008).

Systemic administration of compounds that enhance GABA neurotransmission attenuate nicotine-induced increases in mesolimbic dopamine. For example, GVG, an inhibitor of GABA transaminase, dose-dependently attenuated the

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nicotine-induced increases in NAcc dopamine in both naive and nicotine-treated rats (Dewey et al. 1999). Baclofen, a GABA_B receptor agonist, dose-dependently decreased the nicotine-induced increases in NAcc shell (Fadda et al. 2003). Altogether, the data support a role for GABA and GABA_B receptors in the regulation of nicotine-induced increases in NAcc dopamine.

Consistent with the above effects on nicotine-induced increases in NAcc dopamine, GVG also attenuated nicotine self-administration (Paterson and Markou 2002). Furthermore, GABA_B receptor agonists, such as baclofen and CGP44532, attenuated nicotine self-administration (Paterson et al. 2004). Importantly, the decrease in nicotine self-administration was also seen after repeated administration of CGP44532 for 14 days, indicating little tolerance to this effect of the GABA_B receptor agonist (Paterson et al. 2005). Furthermore, microinjections of baclofen or CGP44532 into either the NAcc shell or VTA or pedunculopontine tegmental nucleus decreased nicotine self-administration (Corrigall et al. 2000, 2001). A preliminary clinical study reported reductions in the number of cigarettes smoked per day in smokers treated with baclofen (Franklin et al. 2009). In addition to GABA_B receptor agonists, GABA_B receptor positive allosteric modulators, such as CGP7930 and BHF177, decreased nicotine self-administration under both FR and PR schedules in rats at doses that did not affect behaviors reinforced by food (Fig. 4A) (Paterson et al. 2008). The decrease in nicotine self-administration under both FR and PR schedules suggests that these compounds attenuate both the reinforcing effects of nicotine and the motivation to self-administer nicotine. Moreover, repeated administration of BHF177 for 14 days selectively attenuated nicotine self-administration compared with food-maintained responding.

Overall, GABA_B receptor positive allosteric modulators have more subtle effects than GABA_B receptor agonists (Guery et al. 2007; Paterson et al. 2008). Importantly, GABA_B receptor positive allosteric modulators either do not induce or have minimal motor-impairing effects compared with GABA_B receptor agonists. Finally, GABA_B receptor positive allosteric modulators,

in contrast to GABA_B receptor agonists, do not affect responding for nondrug rewards, such as food, in animal models. Thus, GABA_B receptors play an important role in mediating the reinforcing effects of nicotine, and GABA_B receptor positive allosteric modulators may help promote smoking cessation and have a better side-effect profile than full GABA_B receptor agonists.

GABA and Nicotine-Induced Enhancement of Brain Reward Function

GABA transmission via GABA_B receptors partially mediates the nicotine-induced enhancement of the reinforcing effects of stimuli assessed using the ICSS procedure. GABA_B receptor activation via administration of either CGP44532 or BHF 177 attenuated the threshold-lowering effects of acute nicotine (Paterson et al. 2008). However, brain reward thresholds were elevated when CGP44532 or BHF177 was administered alone. Importantly, these threshold elevations were seen at doses that did not attenuate nicotine self-administration. These data suggest that attenuation of the nicotine-induced lowering of reward thresholds may simply be attributable to opposite effects of GABA_B receptor activation and nicotine on brain reward thresholds (i.e., these compounds may “neutralize” the effects of nicotine rather than reverse the rewarding effects of nicotine, similar to what is seen with an mGlu5 receptor antagonist and mGlu2/3 receptor agonist; see above). Notably, compared with CGP44532, BHF177-induced threshold elevations were seen at doses that were much higher than those required to block the nicotine-induced lowering of ICSS thresholds and nicotine self-administration. These data suggest that the positive modulation of GABA_B receptors may reduce the nicotine-induced enhancement of brain reward function, even if the effects do not involve a direct and specific pharmacodynamic interaction.

GABA and Nicotine-Induced Conditioned Behavioral Responses

GABA also plays a role in nicotine-induced CPP. 1R, 4S-4-amino-cyclopent-2-ene-carboxylic

Figure 4. The figure shows the effects of the GABA_B receptor positive modulator BHF177 on nicotine self-administration and the cue-induced reinstatement of nicotine-seeking behavior. (A) Systemic oral administration of BHF177 attenuated nicotine self-administration without affecting food-maintained responding assessed under a fixed-ratio schedule of reinforcement. The graph shows nicotine- and food-maintained responding expressed as a percentage of baseline number of rewards earned (mean $\pm$ SEM) and as the number of reinforcers earned (left ordinal axis). ** $p < 0.01$, significant differences compared with nicotine self-administration after vehicle administration; $^{\dagger}p < 0.05$, significant difference compared with 10 mg/kg BHF177. inf, infusion. (Panel A is from Paterson et al. 2008; reprinted, with permission, from the American Society for Pharmacology and Experimental Therapeutics © 2008.) (B) Administration of BHF177 attenuated the cue-induced reinstatement of nicotine-seeking behavior. The data are expressed as the number of active lever presses during the extinction session (i.e., the last extinction session of the three conducted between reinstatement sessions) that preceded each reinstatement session (white bars) and reinstatement session (black bars). * $p < 0.05$, ** $p < 0.01$, significant differences in responding between the reinstatement day and preceding extinction day; ### $p < 0.001$, significant differences in responding between the 10, 20, and 40 mg/kg doses compared with vehicle and the 2.5 and 5 mg/kg doses; ++ $p < 0.01$, significant difference between the 5 and 20 mg/kg doses. (Panel B is from Vlachou et al. 2011b; reprinted, with permission, from Springer © 2011.)

acid (ACC), an irreversible inhibitor of GABA transaminase, attenuated the expression of nicotine-induced CPP in rats (Ashby et al. 2002). In addition, GVG blocked both the acquisition and expression of nicotine-induced CPP (Dewey

et al. 1999). The GABA_B receptor agonist baclofen also attenuated the expression of nicotine-induced CPP (Fattore et al. 2009). The GABA_B receptor positive allosteric modulator GS39783 attenuated the development but not expression

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of nicotine-induced CPP (Mombereau et al. 2007). These data support a role for GABA_B receptors in nicotine-induced reward and conditioned behavioral responses.

GABA and Cue-Induced Reinstatement of Nicotine-Seeking Behavior

The GABA_B receptor agonists baclofen and CGP44532 also blocked the cue-induced reinstatement of nicotine-seeking behavior (Pateron et al. 2005). Importantly, baclofen did not block the cue-induced reinstatement of food seeking, suggesting that the effects of baclofen were specific for the nicotine-associated conditioned motivational behavioral responses. The GABA_B receptor positive allosteric modulator BHF177 also attenuated cue-induced nicotine seeking without affecting food seeking (Fig. 4B) (Vlachou et al. 2011a). These findings suggest that the activation of GABA_B receptors may be useful in attenuating relapse to nicotine seeking in abstinent human smokers.

GABA and Nicotine Withdrawal

The role of GABA in nicotine withdrawal is not entirely clear. The activation of GABA_B receptors by administering either the GABA_B receptor agonist CGP44532 or GABA_B receptor positive allosteric modulator BHF177 elevated ICSS thresholds during nicotine withdrawal in animals, indicating an exacerbation of the anhedonia-like aspects of nicotine withdrawal (Vlachou et al. 2011b). Interestingly, the GABA_B receptor antagonist CGP56433 also exacerbated the anhedonia-like effects of nicotine withdrawal (Vlachou et al. 2011b). It is not clear why both activation and blockade of GABA_B receptors resulted in a worsening of the anhedonia-like effects of nicotine withdrawal. The differential effects of these compounds at presynaptic hetero- and autoreceptors vs. postsynaptic GABA_B receptors may have contributed to these findings. Thus, GABA_B receptors are critically involved in the affective aspects of nicotine withdrawal, and the administration of compounds that modulate GABA_B-mediated transmission exacerbated the affective aspects of nicotine withdrawal.

CONCLUSION

Both glutamate and GABA play important roles in the reinforcing effects of nicotine and development of nicotine dependence. Chronic nicotine exposure facilitates excitatory glutamate neurotransmission, and repeated nicotine exposure attenuates inhibitory GABA neurotransmission. Overall, the preclinical data presented in this article strongly suggest that the substrates, such as receptors, transporters, or enzymes that regulate the actions of glutamate and GABA, may be useful targets for the treatment of nicotine dependence. Currently, medications that target either glutamate or GABA neurotransmission are being evaluated in human smokers. The next generation of glutamate- or GABA-based smoking cessation medications may have better efficacy and fewer adverse effects than the currently approved smoking cessation medications.

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