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More than Smoke and Patches: The Quest for Pharmacotherapies to Treat Tobacco Use Disorder

M. J. Moerke, L. R. McMahon, and J. L. Wilkerson

Division of Preclinical Pharmacology, National Institute on Drug Abuse, National Institutes of Health, Baltimore, Maryland (M.J.M.) and Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, Florida (L.R.M., J.L.W.)

Address correspondence to: Dr. J. L. Wilkerson, Department of Pharmacodynamics, College of Pharmacy, University of Florida, 1345 Center Drive, JHMHSC P1-31 P.O. Box 100487, Gainesville, FL 32610. E-mail: jenny.wilkerson@cop.ufl.edu; orDr. M. J. Moerke, Biomedical Research Center, 251 Bayview Boulevard, Suite 200, 05A711, Baltimore, MD 21224. E-mail: megan.moerke@nih.gov

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***Abstract*——Tobacco use is a persistent public health issue. It kills up to half its users and is the cause of nearly 90% of all lung cancers. The main psychoactive component of tobacco is nicotine, primarily responsible for its abuse-related effects. Accordingly, most pharmacotherapies for smoking cessation target nicotinic acetylcholine receptors (nAChRs), nicotine's major site of action in the brain. The goal of the current review is twofold: first, to provide a brief overview of the most commonly used behavioral procedures for evaluating smoking cessation pharmacotherapies and an introduction to pharmacokinetic and pharmacodynamic properties of nicotine important for consideration in the development of new pharmacotherapies; and second, to discuss current and potential future pharmacological interventions aimed at decreasing tobacco use. Attention will focus on the potential for allosteric modulators of nAChRs to offer an improvement over currently approved pharmacotherapies. Additionally, given increasing public concern for the potential health consequences of using electronic nicotine delivery systems, which allow users to inhale aerosolized solutions as an alternative to smoking tobacco, an effort will be made throughout this review to address the implications of this relatively new form of nicotine delivery, specifically as it relates to smoking cessation.**

***Significance Statement*——Despite decades of research that have vastly improved our understanding of nicotine and its effects on the body, only a handful of pharmacotherapies have been successfully developed for use in smoking cessation. Thus, investigation of alternative pharmacological strategies for treating tobacco use disorder remains active; allosteric modulators of nicotinic acetylcholine receptors represent one class of compounds currently under development for this purpose.**

I. Introduction

The year 2014 marked the 50th anniversary of the first Surgeon General's report on tobacco in 1964, which officially linked lung cancer to cigarette smoking (U.S. Department of Health, Education, and Welfare, 1964). In 1964, 42% of Americans were cigarette smokers (U.S. Department of Health and Human Services, 2014), including the Surgeon General himself. Fifty years later, it is estimated that the number of Americans smoking cigarettes has dropped to about 20% (U.S. Department of Health and Human Services, 2014). This decline has generally been reflected in other high-income nations worldwide; meanwhile, the tobacco industry has redirected its efforts, and the numbers of cigarette smokers are increasing in low-income countries (World Health Organization, 2018). The World Health Organization (WHO) adopted a Framework Convention on Tobacco Control in 2005 with the purpose of collecting better data from global populations on tobacco smoking behavior; this was followed by an initiative in 2011 to reduce worldwide prevalence of smoking by 30% from 2010 to 2025 (World Health Organization, 2013). The most recent projections fall considerably short of that goal (World Health Organization, 2018); however, the WHO continues to focus on implementing strategies, specifically in low- and middle-income countries, that have successfully reduced the prevalence of cigarette smoking in America. These include increasing public awareness of the health consequences of smoking tobacco, enforcing bans on advertising and promotion of tobacco products, imposing higher taxes on tobacco and tobacco-related products, and providing resources that enable smokers to quit using tobacco. Still, estimates of global health care costs from tobacco use are upwards of 1.4 trillion dollars a year and second-hand smoke alone causes 1.2 million deaths annually (GBD 2017 Risk Factor Collaborators, 2018). Unequivocally, tobacco use remains a worldwide public health issue.

Cigarette smoking is the largest single cause of preventable death in the world, killing more than eight million people every year, or one person every 6 seconds (World Health Organization, 2012). Since 1964, it is estimated that 20 million Americans have died from cigarette smoking–related causes (U.S. Department of Health and Human Services, 2014). This includes not only cigarette smokers but also approximately 2.5 million nonsmokers from causes related to secondhand smoke and at least 100,000 infants from pregnancy complications and Sudden Infant Death Syndrome linked to parental smoking. Despite an overall national decline in the prevalence of cigarette smoking, it remains responsible for 480,000 deaths each year in the United States, a rate of mortality 10 times as high as the number of opioid overdose deaths in 2017 (U.S. Department of Health and Human Services, 2014; Scholl et al., 2018). Specifically, one of every three cancer deaths is linked to smoking, including nearly 90% of all lung cancer deaths. Smoking causes not only cancer of the mouth, throat, larynx, lungs, esophagus, pancreas, kidney, bladder, stomach, cervix, blood, liver, and colon, but it also causes diabetes mellitus, rheumatoid arthritis, inflammation, and impaired immune function. Globally, it is the cause of 14% of deaths from noncommunicable

ABBREVIATIONS: AChE, acetylcholinesterase; dFBr, desformylflustrabromine; DHβE, dihydro-β-erythroidine; ENDS, electronic nicotine delivery system; FDA, Food and Drug Administration; hcrt, hypocretin; 5HT, serotonin; ICSS, intracranial self-stimulation; mAChR, muscarinic acetylcholine receptor; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor; NAM, negative allosteric modulator; PAM, positive allosteric modulator; WHO, World Health Organization.

diseases in adults and at least 5% of deaths from communicable diseases (World Health Organization, 2012). Despite the risks of tobacco smoking, each day, nearly 2000 children in the United States under the age of 18 smoke their first cigarette (Lipari et al., 2017). Fifteen percent of those children will go on to be daily cigarette users; half will likely die of cigarette smoking–related causes (Lipari et al., 2017).

The word "addiction" is universally understood but difficult to define. Clinically, cigarette smokers may be diagnosed in one of two ways, depending on the classification system used. The International Classification of Diseases, currently in its 10th edition, refers to the cluster of symptoms typically recognized as addiction as "dependence syndrome" and, specifically, "nicotine dependence." The most recent update of the classification system developed by the American Psychiatric Association, the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition, prefers the terminology "substance use disorder" and, more specifically, "tobacco use disorder." For the sake of clarity and consistency, we will refer to substance use disorders or tobacco use disorder for the remainder of this review. Substance use disorders are characterized by compulsive use of a substance both to produce its subjective effects and to alleviate symptoms associated with its absence. Nicotine is the primary psychoactive component in tobacco, and although other chemicals that are either present in tobacco or added as adulterants also play a role, nicotine has been identified as the primary compound responsible for maintaining tobacco use in humans (Henningfield et al., 1985; Stolerman and Jarvis, 1995). Accordingly, most of the currently approved smoking-cessation therapeutics target nicotine's primary mechanism of action, nicotinic acetylcholine receptors (nAChRs).

The focus of this review is on the development of medications for tobacco use disorder. We summarize preclinical assays typically used to evaluate medications as well as pharmacokinetic and pharmacodynamic considerations required for the interpretation of preclinical results as they are likely to translate to humans. We discuss current pharmacotherapies approved by the U.S. Food and Drug Administration (FDA), experimental and emerging pharmacotherapies that target nAChRs, and other mechanisms to promote smoking cessation. Additionally, this review provides an updated overview of the preclinical literature relevant to allosteric modulators of nAChRs as pharmacotherapies for tobacco use disorder (Mohamed et al., 2015). Novel pharmacotherapies targeting nAChRs are also under development for the treatment of pain (see Bagdas et al., 2018b) and for neurodegenerative and psychiatric conditions (see Bertrand and Terry, 2018), but they are outside the scope of the current review. Finally, the expansion of electronic nicotine delivery systems (ENDS) in the consumer market has dramatically increased the consumption of nicotine independently of tobacco products. The long-term consequences of this practice, commonly referred to as vaping, are currently unknown. Nonetheless, an effort will be made throughout this review to address the implications of this growing trend of nicotine delivery, specifically as it relates to smoking cessation.

II. Preclinical Methods for Evaluating Potential Pharmacotherapies

A. Self-Administration

Self-administration is a behavioral assay that has traditionally served as the cornerstone for examining the abuse liability of drugs and can be used to evaluate potential pharmacotherapies for substance use disorders. In this assay, animals are trained to make an operant response, typically either a lever press or a nose poke, to receive a drug infusion. Typically, drugs in self-administration procedures are delivered via an intravenous injection, and the nicotine literature is no exception (Goodwin et al., 2015); however, vapor chambers have recently been used to deliver nicotine in animal studies (George et al., 2010), although no published studies have used this for nicotine self-administration to date. Nonetheless, nicotine can serve as a positive reinforcer and is self-administered by rodents (Collins et al., 1984; Corrigall and Coen, 1989; Donny et al., 1995; Valentine et al., 1997; Picciotto et al., 1998), nonhuman primates (Goldberg et al., 1981; Sannerud et al., 1994), and humans (Henningfield and Goldberg, 1983; Henningfield et al., 1983).

Early nicotine self-administration studies were successful in demonstrating that nicotine could serve as a reinforcer, but they were insufficient to characterize nicotine as a drug of abuse because nicotine did not maintain rates of behavior comparable to drugs such as cocaine (Deneau and Inoki, 1967). Although it has now been shown repeatedly and definitively that under certain conditions nicotine maintains rates of behavior equivalent to cocaine, the experimental variables that impact this behavior have been a consistent topic of study. For example, pretraining with food or a drug like cocaine is often used to facilitate acquisition of nicotine self-administration (Griffiths et al., 1979; Goldberg et al., 1981; Yanagita et al., 1983; Slifer and Balster, 1985). Also, because nicotine self-administration in animals is typically done intravenously, it has been argued that nicotine infusion rate is an important factor in nicotine self-administration, with slower rates supporting more robust self-administration in rats (Sorge and Clarke, 2009). However, it has also been shown that slower infusion rates of nicotine decrease self-administration (Wakasa et al., 1995; Wing and Shoaib, 2013). Furthermore, length of self-administration session has also been manipulated, and rats show signs of withdrawal when nicotine is removed from extended

access sessions of self-administration (O'Dell et al., 2007a); however, limited access sessions have been shown to produce similar levels of dependence when sessions are conducted 7 days a week (Paterson and Markou, 2004). Thus, the relative importance of some variables in nicotine self-administration is open to debate.

One experimental variable that clearly influences the reinforcing effectiveness of nicotine is the schedule of reinforcement. A second order schedule of reinforcement was the first to demonstrate intravenous self-administration of nicotine at high rates (Goldberg et al., 1981). Another commonly used schedule of reinforcement in self-administration is the progressive ratio schedule of reinforcement (Donny et al., 1999; Brunzell et al., 2010; Cohen et al., 2012; Le Foll et al., 2012; Weaver et al., 2012; Gamaleddin et al., 2013; Garcia et al., 2014); this is typically used to estimate the maximum reinforcing effectiveness of a drug. In progressive ratio experiments, the number of responses required for a single drug infusion increases by some amount with each successive infusion instead of remaining constant. This allows for determination of a "breakpoint," the point at which the response demand is high enough that the animal will no longer work to receive drug infusions. Thus, a favorable outcome for a potential pharmacotherapy for smoking cessation in a progressive ratio experiment would be to decrease the breakpoint or, in other words, make the animal less willing to work to receive an infusion of nicotine.

Another variable that is uniquely important to the study of nicotine self-administration is pairing of the nicotine infusion with some other unconditioned stimuli. In fact, some groups have specifically sought to better understand this relationahip and developed other variations on self-administration of nicotine that integrate both Pavlovian and operant conditioning components to study its effectiveness as a reinforcer. For example, it has been shown in rats that when a nonrewarding conditioned stimulus is paired with nicotine (the unconditioned stimulus), the nonrewarding conditioned stimulus is reinforced (Bevins and Palmatier, 2004). Furthermore, pairing this conditioned stimulus with access to nicotine increases the amount of nicotine that is self-administered. Among other benefits, studies like these can serve to help in our understanding of how other effects of cigarettes, such as the subjective feeling of holding a cigarette or drawing cigarette smoke into the lungs, might be related to smoking cessation and relapse.

Importantly, in the abovementioned self-administration procedures, the primary dependent variable is typically a measure of the rate of responding. One limitation of many traditional preclinical self-administration procedures when evaluating potential pharmacotherapies for tobacco use disorder has been the integration of control experiments that allow for a distinction to be made between drug effects that selectively decrease the rate of responding for a self-administered drug as opposed to effects that produce generalized suppression of behavior. However, self-administration studies in humans have a long history of using choice experiments to examine a variety of abused drugs, including nicotine (Johnson and Bickel, 2003; Bisaga et al., 2007; Odum and Baumann, 2007; Stoops et al., 2011; Green and Lawyer, 2014; Cassidy et al., 2015). In preclinical choice procedures, in addition to a rate-dependent measure of responding, rate-independent data about the allocation of responses for the drug as opposed to the nondrug reinforcer can also be collected (see Banks and Negus, 2017, for review). For this reason, drug versus nondrug choice experiments are becoming a more frequently used method for studying changes in preclinical self-administration behavior, although relatively few studies have used nicotine in choice paradigms to date (Lesage, 2009; Panlilio et al., 2015; Huynh et al., 2017; Bagdas et al., 2019). This may reflect a difference between nicotine and other drugs that has been noted in the human literature; although an alternative reinforcer (e.g., money) is effective for reducing cigarette smoking in humans (Bisaga et al., 2007), a meta-analysis revealed that the effectiveness of alternative options has a relatively weaker effect in studies with nicotine as compared with heroin or cocaine (Prendergast et al., 2006). However, it may also simply result from the nature of nicotine as a reinforcer that is typically not self-administered as robustly as other drugs of abuse in preclinical studies.

B. Intracranial Self-Stimulation

Intracranial self-stimulation (ICSS) is another operant procedure in which behavior is maintained by pulses of electrical brain stimulation (for review see Carlezon and Chartoff, 2007; Negus and Miller, 2014). When this procedure is used for evaluating the abuse potential of drugs, an electrode is most commonly implanted, targeting the medial forebrain bundle at the level of the hypothalamus. Following electrode implantation, the animal is trained to complete an operant response to produce an electrical stimulation that can be modified in terms of both amplitude and frequency. ICSS procedures have been performed in mice (Johnson et al., 2008; Fowler et al., 2013), rats (Schaefer and Michael, 1992; Panagis et al., 2000; Kenny et al., 2009), and nonhuman primates (Routtenberg et al., 1971) to study the ability of drugs (Negus and Miller, 2014; Freitas et al., 2016) and physiologic conditions (Freitas et al., 2015) to produce increases or decreases in baseline ICSS responding. Many drugs of abuse produce increases in measures of baseline ICSS responding; this is typically interpreted as an abuse-related effect (Bonano et al., 2014) and is correlated with alterations in dopamine signaling (Bauer et al., 2013). Furthermore, both drugs of abuse as well as drugs that do not produce abuse-related effects in animals are able to produce

decreases in measures of baseline ICSS responding given sufficiently large doses; this is typically interpreted as an abuse-limiting effect (Bauer et al., 2013). Nicotine produces dose-dependent biphasic effects in ICSS, increasing responding at lower doses of nicotine and decreasing responding at higher doses (Schaefer and Michael, 1986; Huston-Lyons and Kornetsky, 1992; Bauco and Wise, 1994; Spiller et al., 2009; Freitas et al., 2016), similar to effects seen in the self-administration assay (Lau et al., 1994; Valentine et al., 1997; Le Foll et al., 2007). Thus, a favorable outcome for a potential pharmacotherapy for smoking cessation might be to attenuate nicotine-induced increases in ICSS, as seen with the N-methyl-D-aspartate receptor antagonist LY235959 (Kenny et al., 2009); however, this type of ICSS procedure is not the most commonly used for evaluating pharmacotherapies for tobacco use disorder.

An alternative ICSS procedure uses discrete trials that vary the current intensity to determine a threshold amplitude that will maintain operant responding. In these types of procedures, the reward-enhancing effects of acute nicotine are observed in the form of decreases in brain reward threshold (Bespalov et al., 1999; Nakahara, 2004; Paterson, 2009). Furthermore, following a regimen of chronic nicotine administration, both spontaneous and precipitated withdrawal produce increases in brain reward threshold, an anhedonia-like effect (Epping-Jordan et al., 1998; Bruijnzeel et al., 2007; Johnson et al., 2008). This increase in brain reward threshold is typically interpreted as diminished sensitivity to reward and decreased motivation for previously rewarding stimuli under conditions of nicotine withdrawal, and it is considered to be relevant insofar as preventing withdrawal plays an important role in successfully maintaining abstinence from smoking (Bruijnzeel and Gold, 2005; Hughes, 2006; Koob, 2008).

C. Drug Discrimination

Drug discrimination is another behavioral assay that is often used to examine compounds for abuse potential and to evaluate potential pharmacotherapies. Commonly, subjects are trained to make some response (e.g., pressing a lever) when they receive vehicle and some other response (e.g., pressing a different lever) when they receive the training dose of a drug. The training dose of the training drug then sets the occasion for responding on the drug-paired lever, and, with training, animals accurately choose the appropriate response lever even though there may be no other observable measures to indicate that they have received the training drug. In humans, a drug can be trained as a discriminative stimulus; simultaneously, subjects can be asked to respond on a variety of standardized questionnaires and rating scales (e.g., measures of "good" or "bad" drug effect) to collect subjective effects and discriminative stimulus effects simultaneously, which can be dissociable (Lamb and Henningfield, 1989). However, in humans that have been trained to discriminate nicotine from saline, the discriminative stimulus effects of nicotine are directly correlated with its subjective effects (Perkins et al., 1999).

The nicotine discriminative stimulus was one of the first studied in the operant discrimination procedure that is most commonly used today (Morrison and Stephenson, 1969). Thus, it should be no surprise that nicotine has been trained as a discriminative stimulus in a variety of species, including mouse (Gommans et al., 2000), rat (Zaniewska et al., 2006), monkey (Takada et al., 1988), and human (Perkins et al., 1996). If a test compound shares discriminative stimulus effects with nicotine, then it might serve as an effective substitution pharmacotherapy; however, there is also the potential for the test compound to have abuse liability itself.

Drug discrimination is a pharmacologically selective bioassay that was used in the past for elucidating the receptor pharmacology of nicotine in vivo (Pratt et al., 1983; Stolerman et al., 1999; Rollema et al., 2007). The nicotine cue is thought to be mediated centrally, and this is supported by the fact that a peripherally restricted nicotinic agonist, methylcarbamylcholine, does not substitute for nicotine (Desai et al., 1999). Specific brain regions can also be implicated by targeted injections of nicotine into the brain; in rats, nicotine injected into the dorsal hippocampus, but not the nucleus accumbens, produces nicotine-like discriminative stimulus effects (Shoaib and Stolerman, 1996).

One feature of drug discrimination is that the dose of the drug that is selected for training as a discriminative stimulus is known to impact the pharmacological selectivity of the resulting discrimination. For example, the discrimination of a relatively small training dose can lack pharmacological selectivity because the magnitude of the difference between the presence of a "drug effect" versus its absence is relatively small and difficult to detect. Lack of pharmacological selectivity is evidenced by substitution of test drugs with mechanisms of action distinct from the training drug. In contrast, sufficiently large training doses can result in discriminations that are relatively selective for test drugs that share a mechanism of action with the training drug [for examples with nicotine as a training drug, see Smith and Stolerman (2009) and Cunningham and McMahon (2013)].

D. Place Conditioning

Place conditioning is different from the operant assays discussed previously because it uses classic conditioning to measure preference for or avoidance of a location that has been paired with a drug stimulus. Both two- and three-chamber variations are common, in which the third chamber is a neutral, unpaired chamber that connects the first and second chambers. One of the

chambers is typically paired with a dose of a drug, whereas a separate, distinct chamber is paired with the administration of the drug vehicle alone. After some number of pairings of the drug in one compartment and the absence of drug in the other, the animal is placed in the apparatus without an injection of drug or vehicle, and the amount of time spent in the two chambers previously paired with either drug or vehicle is measured. Most drugs of abuse produce a conditioned place preference. That is, animals will spend more time in the chamber previously paired with an injection of drug compared with the time they spend in the chamber previously paired with drug vehicle. Nicotine produces a place preference in both rats and mice at smaller doses (Fudala et al., 1985; Vastola et al., 2002; Walters et al., 2006) but an aversion to the place paired with larger doses of nicotine in mice, resulting in an inverted U-shaped dose-response curve (Risinger and Oakes, 1995).

One variation of this procedure is conditioned place aversion, in which instead of pairing one chamber with a drug, one chamber is paired with antagonist-precipitated withdrawal. Under these conditions, animals typically spend less time in the withdrawal-paired chamber (i.e., it is "avoided"). Conditioned place aversion studies of both mice and rats have found that adolescents, as compared with adults, have a smaller response in terms of avoidance of a chamber previously paired with nicotine withdrawal (O'Dell et al., 2007b; Jackson et al., 2009). For further review on conditioned place assays, please see Prus et al. (2009).

III. Pharmacokinetic Considerations for Evaluating Potential Pharmacotherapies

A. Absorption and Distribution

Once inhaled from a cigarette, nicotine reaches the brain within 10–20 seconds (Benowitz, 1990, 1996). This rapid rise in nicotine concentration, which allows for the titration of nicotine dose on a puff by puff basis, contributes to the high abuse liability inherent in this form of nicotine administration (Benowitz, 1990; Henningfield and Keenan, 1993).

Although nicotine is most commonly inhaled through cigarette smoke, translating this to preclinical studies has inherent difficulties. Monkeys can be taught to smoke cigarettes (Ando and Yanagita, 1981), but the variables described above limit the ability to deliver a specific, predetermined dose of nicotine via inhalation of tobacco smoke. Recent advances in technology have yielded vapor chambers for the reliable delivery of inhaled nicotine in preclinical studies. However, intravenous administration with chronic indwelling catheters remains the most common route for nicotine delivery in preclinical monkey, rat, and mouse administration procedures, in addition to extensive utilization in human studies (Goldberg et al., 1981; Spealman and Goldberg, 1982; Henningfield et al., 2016).

Nicotine delivered intravenously has 100% bioavailability compared with inhaled nicotine, 80%–90% of which is absorbed during smoking (Armitage et al., 1975). However, nicotine delivered by the intravenous route does not reach the brain as quickly as inhaled nicotine (Benowitz, 1990, 1996). Nevertheless, intravenous nicotine takes less than 60 seconds to reach the brain and provides the closest approximation of inhalation that allows for precise delivery of a specific dose. Humans report differences in the subjective effects of nicotine based on the route of administration (Henningfield and Keenan, 1993). However, of the routes of administration typically used in animal studies, only intravenous nicotine has been shown to share subjective effects with cigarette smoking in humans (Henningfield and Keenan, 1993); the subjective effects of subcutaneous nicotine in humans are modest at best (Le Houezec et al., 1993). Additionally, inhaled nicotine and intravenous nicotine follow a comparable time course in regard to onset and duration of action (Henningfield et al., 1985; Mello et al., 2013). This is opposed to subcutaneous administration of nicotine, which reaches a peak blood concentration between 20 and 25 minutes after injection in humans, although this route of administration also appears to offer 100% bioavailability (Le Houezec et al., 1993).

Solutions intended for use in ENDS that are currently available for consumers typically label nicotine content as a concentration of nicotine per total volume of liquid, and these concentrations range from 0 to 30 mg/ml. However, individual differences in inhalation variables, such as puff duration and velocity, that impact nicotine delivery from cigarettes also apply to nicotine delivered from ENDS, meaning that nicotine yield from ENDS can vary by more than 50-fold (Talih et al., 2015). Additional factors such as output voltage, other components of the nicotine solution (e.g., propylene glycol, vegetable glycerin, flavors), and the pH of the solution also impact nicotine exposure with ENDS, so it is not surprising that studies using different procedures often report different results. For example, some studies report that ENDS deliver less nicotine than a cigarette (Trehy et al., 2011; Farsalinos et al., 2013; Yingst et al., 2019) and increased latencies to reach peak nicotine concentration in blood (Farsalinos et al., 2014). Several important limitations of these studies are worth noting. First, many early studies of ENDS used experienced smokers that were relatively naïve to vaping (Schroeder and Hoffman, 2014), and it has been shown that different inhalation strategies used by naïve compared with experienced ENDS users may be responsible for lower nicotine delivery from ENDS, and once sufficiently experienced in the use of ENDS products, users may achieve higher concentrations of nicotine in blood (Farsalinos et al., 2014; Schroeder and Hoffman, 2014).

Furthermore, the increased latency to peak nicotine concentration in blood may be a result of significant buccal absorption in vaping-naïve ENDS users as opposed to primarily pulmonary absorption in experienced cigarette users (Schroeder and Hoffman, 2014). More recent studies suggest that experienced ENDS users alter their inhalation strategy to achieve similar peak levels of nicotine with ENDS use as they achieve with cigarette use, independent of the concentration of nicotine solution used (St Helen et al., 2016b), and that the time course of nicotine in blood is very similar to cigarette smoking, with peak nicotine concentrations within 2–5 minutes of vaping (St Helen et al., 2016a). However, even if ENDS users receive similar amounts of nicotine, the absence of toxins present in combusted smoke have led to the generally accepted conclusion that ENDS are less harmful than cigarettes (https://www.cdc.gov/tobacco/basic_information/e-cigarettes/about-e-cigarettes.html), although they are not approved pharmacotherapies for smoking cessation. Studies in rats have found that experimental vapor chambers reliably produce air-nicotine concentrations of 4–12 mg/m^3 and that within 60 minutes of exposure, animals have levels of nicotine in blood equivalent to the average concentration observed in human smokers (Gilpin et al., 2014).

All formulations of FDA-approved nicotine replacement therapy are absorbed more gradually than either inhaled or intravenous nicotine, resulting in slower increases in nicotine blood levels (Henningfield et al., 1985; West et al., 2000). This more gradual increase in nicotine concentration results in lower relative abuse liability, as slower absorption produces modest increases of dopamine over time in key areas of the brain related to substance use disorders in contrast to the corresponding quick spike of dopamine release and subsequent downstream signaling events produced by cigarette smoking (Dani and De Biasi, 2001; Nestler, 2005). Evidence suggests that simultaneous smoking may slow transdermal absorption, as was found to be the case when nicotine was administered intravenously to nicotine patch wearers (Benowitz et al., 1992). Thus, absorption kinetics, as opposed to simply absorption route, is a critical factor in determining the therapeutic potential of a nicotine replacement strategy.

Nicotine absorption is dependent on the pH of the vehicle used for administration as well as the environment it is administered into (e.g., liquid of the oral cavity for buccal absorption) (Le Houezec, 2003; Hukkanen et al., 2005; U.S. Department of Health and Human Services, 2010; Pickworth et al., 2014). However, once nicotine is in the bloodstream at a physiologic pH, it is distributed extensively to body tissues. In autopsies of smokers, the highest affinity for nicotine was found in the liver, kidney, spleen, and lung, and the lowest was found in adipose tissue (Hukkanen et al., 2005).

The time course of nicotine, its accumulation in various organs of the body, and its pharmacologic effects are highly dependent on the route of administration and rate of dosing. The concentration of nicotine in blood after smoking a cigarette can reach 100 ng/ml but is generally in the range of 20–60 ng/ml (Armitage et al., 1975; Henningfield and Keenan, 1993; Gourlay and Benowitz, 1997; Rose et al., 1999; Lunell et al., 2000). Blood levels of nicotine peak after smoking a cigarette and fall rapidly over the subsequent 20 minutes; the average distribution half-life of nicotine is about 8 minutes (Hukkanen et al., 2005). Over the course of a day, smokers typically demonstrate trough concentrations of nicotine in blood from 10 to 35 ng/ml and peak concentrations between 20 and 50 ng/ml (Schneider et al., 2001). The average elimination half-life of nicotine in plasma is the same for both inhaled and intravenous nicotine, approximately 100–150 minutes (Benowitz and Jacob, 1993, 1994). Thus, typical patterns of cigarette smoking result in considerable accumulation of nicotine over the course of a day, which then diminishes overnight, resulting in very low nicotine levels upon waking in the morning.

Nicotine present in saliva is often used as a convenient proxy for the amount of nicotine present in blood. However, in nicotine skin patch users, nicotine in saliva was a factor of 8.13-times greater than nicotine in plasma (Rose et al., 1993). This accumulation is likely due to ion trapping of nicotine in saliva when in ionized form (Hukkanen et al., 2005).

B. Metabolism and Elimination

Metabolism of nicotine takes place primarily in the liver, and nicotine has six primary metabolites, although numerous others have also been identified, including cotinine, *trans*-3-hydroxycotinine, nicotine *N*-oxide, nornicotine, norcotinine, and cotinine *N*-oxide (Hukkanen et al., 2005). Cotinine is the primary metabolite in both humans and nonhuman primates; 70%–80% of nicotine is metabolized to cotinine in the liver in humans (Benowitz and Jacob, 1994), whereas rhesus macaques metabolize 80% of nicotine to cotinine (Poole and Urwin, 1976). Mice, rabbits, and dogs also metabolize nicotine into cotinine at a rate similar to humans and nonhuman primates. However, rats and guinea pigs metabolize nicotine equally into nicotine-*N*-oxide, cotinine, and *trans*-3-hydroxycotinine (Matta et al., 2007). Cotinine and *trans*-3-hydroxycotinine are the primary metabolites identified in urine for all mammalian species studied to date (Jenner et al., 1973; Nwosu and Crooks, 1988; Kyerematen et al., 1990). Half-lives of nicotine and cotinine appear to be similar in humans and macaques (Seaton et al., 1991). The half-life of nicotine is generally 45 minutes in rats and between 6 and 7 minutes in mice. This is considerably shorter than the 2-hour half-life of nicotine observed in humans and nonhuman primates (Matta et al., 2007). Thus, an important consideration

for nicotine studies in rodents is that a higher dose of nicotine is needed to achieve equivalent human physiologic levels.

The enzyme responsible for both metabolism of nicotine to cotinine and cotinine to *trans*-3-hydroxycotinine in both humans and rhesus monkeys is CYP2A6 (Murphy et al., 1999; Hecht et al., 2000; Hukkanen et al., 2005). In mice, CYP2A5 is the functional homolog of human CYP2A6. In rats, CYP2A6 is inactive. Instead, CYP1B1/2 is the enzyme responsible for nicotine metabolism (Hammond et al., 1991; Nakayama et al., 1993). Additionally, cigarette smoking is known to accelerate the metabolism of some drugs (Zevin and Benowitz, 1999), although it appears to slow the metabolism of nicotine itself (Benowitz and Jacob, 1993). In humans, differences in metabolism based on both ethnicity and sex have been reported, including faster nicotine and cotinine clearance in women than in men (Pérez-Stable et al., 1998; Hukkanen et al., 2005; Benowitz et al., 2006, 2009; Tanner et al., 2015).

Cotinine has a longer elimination half-life than nicotine, averaging about 770–1130 minutes (Benowitz and Jacob, 1994), but the elimination half-life of *trans*-3-hydroxycotinine falls between nicotine and cotinine at about 400 minutes (Benowitz and Jacob, 2001). The longer elimination half-life of cotinine relative to nicotine results in less variability in cotinine concentrations measured over the course of the day. This has resulted in the wide-spread use of cotinine concentration as a biomarker for daily tobacco consumption (Benowitz et al., 1996), and several studies have demonstrated that experienced ENDS users achieve levels of cotinine similar to cigarette smokers (Etter and Bullen, 2011; Caponnetto et al., 2013). Furthermore, the ratio of *trans*-3-hydroxycotinine to cotinine present in plasma or saliva can be used as a marker of CYP2A6 activity (Dempsey et al., 2004). A genetic polymorphism in the CYP2A6 gene results in individuals who may be broadly categorized as fast or slow metabolizers, and this ratio is a predictor of cigarette consumption (Benowitz et al., 2003).

Nonrenal clearance accounts for the majority of nicotine elimination. Renal clearance is, on average, about 35–90 ml/min, which accounts for about 5% of total nicotine clearance (Hukkanen et al., 2005).

IV. Pharmacodynamic Considerations for Evaluating Potential Pharmacotherapies

A. Receptor Pharmacology

Acetylcholine is the endogenous neurotransmitter for acetylcholine receptors, which fall into two major groups: nAChRs and muscarinic acetylcholine receptors (mAChRs) (Albuquerque et al., 1995; Gotti and Clementi, 2004; Eglen, 2005; Dani and Bertrand, 2007). Muscarinic receptors are metabotropic, G protein-coupled seven transmembrane receptors that were originally defined with activation by muscarine, a product of the *Amanita muscaria* mushroom (Eugster et al., 1965). There are five subtypes, labeled M1 through M5 (Hulme et al., 1990; Fredriksson et al., 2003). Like nicotinic receptors, mAChRs are located both centrally and in the periphery on neuronal and nonneuronal cells. However, in comparison with nicotinic receptors, which are rapidly activated (i.e., microseconds), activation of mAChRs is generally slower (i.e., milliseconds). For a review of mAChR pharmacology, see Kruse et al. (2014). Although there is no evidence that nicotine binds to muscarinic receptors, effects mediated by muscarinic receptors may be an important consideration in the development of potential pharmacotherapies that target endogenous acetylcholine.

Nicotinic receptors are ionotropic, ligand-gated ion channels that were originally defined by activation with nicotine, an alkaloid produced by plants in the nightshade family, but traditionally associated with plants of the genus *Nicotiana*, otherwise known as tobacco plants. Nicotinic receptors are composed of five subunits (Cooper et al., 1991), which, together, form a pore in the cell membrane that allows for the passage of ions in and out of the cell.

Nicotinic receptors can be generally divided into two populations: muscle-type and neuronal. Muscle-type nAChRs were identified first and are found at the neuromuscular junction, where ion conductance through the channel produces excitatory postsynaptic potentials that are characteristic of muscle contraction. Neuronal nAChRs can be further subdivided into those that serve the autonomic nervous system (i.e., ganglionic) and those that are present in the brain (i.e., central). Like muscle-type nAChRs, ganglionic nAChRs are generally located postsynaptically and transmit fast excitatory postsynaptic potentials that are often the first signal in a serial circuit followed by slow excitatory postsynaptic potentials mediated by mAChRs. Compounds restricted to the periphery by poor penetration of the blood-brain barrier act selectively at these receptors, and ganglionic receptors may be responsible for some side effects of cholinergic drugs.

Nicotinic receptors in the brain are of primary interest for the study of tobacco use disorder. Like muscle-type and ganglionic receptors, nicotinic receptors in the brain are ligand-gated ion channels composed of five subunits (Fig. 1A). The subunits that have been identified in mammalian brain are notated as $\alpha 2$ through $\alpha 7$ and $\beta 2$ through $\beta 4$. Although in theory, many different possible combinations of subunits could come together to form an ion channel, there are apparent limitations. One of these limitations is that certain α subunits are required for a functional binding site; thus, the β subunits are sometimes referred to as accessory subunits. Both homomeric subtypes, which include five of the same α subunit and heteromeric subtypes, containing both α and β subunits, have been identified. In mammalian brain, homomeric receptors are thought to

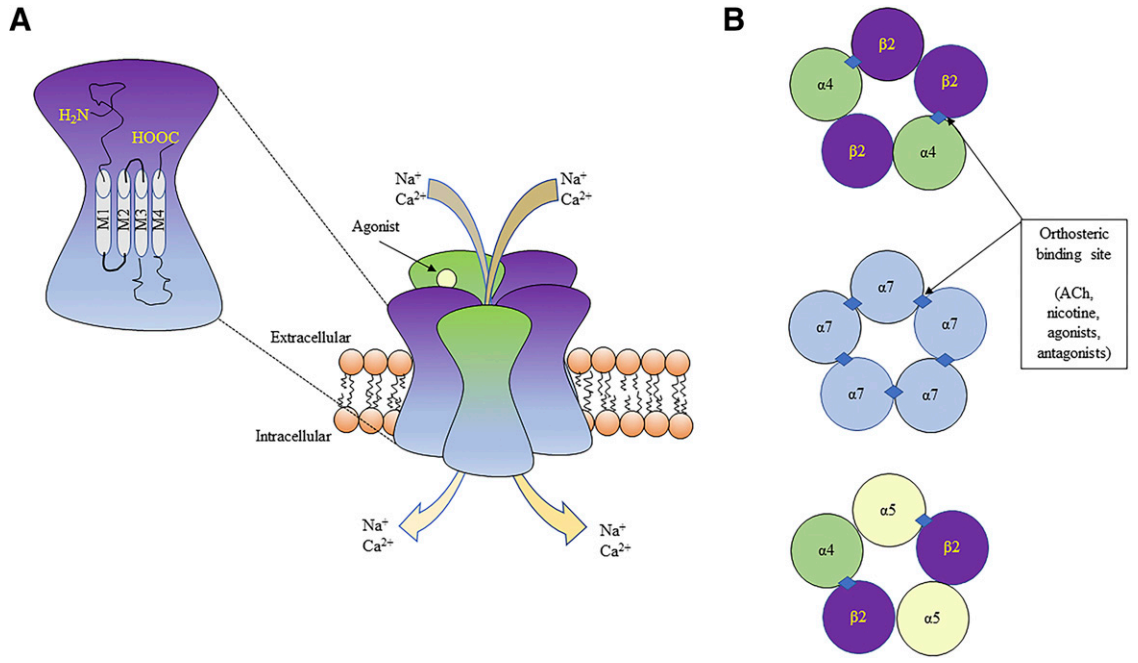

Fig. 1. Basic structure of neuronal nAChRs. (A) A nAChR is composed of five subunits. Each subunit contains four transmembrane domains (M1–M4). An amine functional group is located at the end of the M1 transmembrane domain, whereas a carboxyl group is located at the end of the M4 transmembrane domain (insert). When an agonist (e.g., acetylcholine, nicotine) binds an orthosteric site, extracellular sodium and calcium enter the cell. (B) Diagram of the pentameric structure of neuronal nAChRs, which can be heteromeric (e.g., $\alpha4\beta2$, $\alpha4\alpha5\beta2$) or homomeric (e.g., $\alpha7$) in composition.

be limited to those containing five $\alpha7$ subunits, whereas several distinct heteromeric subtypes have been identified, each with different pharmacological characteristics (Fig. 1B). Although they serve distinct functions, nicotine binds to all subtypes of nAChRs in the brain; however, the affinity of nicotine for the nAChR varies by subtype.

1. α4β2 Nicotinic Acetylcholine Receptors.* The most prevalent nAChR subtype in the mammalian brain is the heteromeric $\alpha4\beta2$* subtype (in which * denotes the possible involvement of additional subunits), which binds nicotine with high affinity (Whiting and Lindstrom, 1987; Flores et al., 1997; Zoli et al., 2002). There is abundant evidence that the $\alpha4\beta2$* subtype is of particular importance to the abuse potential of nicotine (Corrigall et al., 1992; Picciotto et al., 1998; Tapper et al., 2004; Maskos et al., 2005; Besson et al., 2006; Ikemoto et al., 2006; Gotti et al., 2010). For example, studies have shown that $\beta2$ knockout mice do not self-administer nicotine in the absence of the $\beta2$ subunit; however, when $\beta2$ subunit functionality is returned to these mice, they begin to self-administer nicotine (Picciotto et al., 1998). Also, $\alpha4$ knockout mice do not acquire nicotine self-administration (Pons et al., 2008) in addition to expressing fewer nicotine binding sites and a significant decrease of nicotine binding in the brain (Marubio et al., 1999; Ross et al., 2000). Furthermore, in rats, compounds that act as partial agonists at the $\alpha4\beta2$* subtype have been shown to decrease the acquisition, expression, and reinstatement of nicotine's effects in the place preference assay (Biala et al., 2010); to reverse nicotine-induced facilitation of intracranial self-stimulation (Vann et al., 2011); and to reduce nicotine withdrawal–induced increases in intracranial self-stimulation thresholds (Igari et al., 2014).

Individual assemblies of the $\alpha4\beta2$* subtype may or may not contain an accessory subunit (e.g., $\alpha5$). In those that do not contain an accessory subunit, combinations of $\alpha4$ and $\beta2$ subunits occur in ratios of 3:2 and 2:3. Both of these combinations are expressed in recombinant receptors, and this ratio determines receptor affinity and sensitivity to ligands (Bertrand and Terry, 2018). Of note, although different potencies and binding affinities are reported, both nicotine and varenicline bind to $\alpha4\beta2$* nAChRs with both subunit ratios (Moroni et al., 2006; Anderson et al., 2009). However, it has yet to be determined if targeting high [i.e., (2) $\alpha4$ plus (3) $\beta2$ subunit] or low [i.e., (3) $\alpha4$ plus (2) $\beta2$ subunit] nAChRs is more beneficial for the development of smoking-cessation pharmacotherapies.

Of $\alpha4\beta2$* nAChRs containing an accessory subunit, those containing the $\alpha5$ subunit [i.e., $(\alpha4\beta2)_2\alpha5$] are important in the brain and account for between 10% and 37% of total $\alpha4\beta2$* nAChRs, depending on the brain region (Brown et al., 2007; Mao et al., 2008). Interestingly, a single nucleotide polymorphism found in the human gene encoding the $\alpha5$ subunit results in decreased $(\alpha4\beta2)_2\alpha5$ function, and this has been linked to an increased vulnerability to tobacco use disorder (Bierut et al., 2008; Kuryatov et al., 2011). Studies in mice lacking functional $\alpha5$ subunits found that $\alpha5$ knockout mice show significant decreases of nicotine

binding in the brain as well as dysfunction of dopamine transmission regulated by $\alpha4\beta2^*$ nAChRs in the striatum (Exley et al., 2012; Besson et al., 2016). Furthermore, the self-administration of nicotine by $\alpha5$ knockout mice is increased compared with controls, and this effect can be reversed by re-expression of the $\alpha5$ subunit in medial habenula (Fowler et al., 2011). This increase was only apparent at high doses of nicotine, however, and given the role of the habenula in regulating avoidance of noxious substances (Donovick et al., 1970), further evidence supported a role for $(\alpha4\beta2)_2\alpha5$ nAChRs in the medial habenula-interpeduncular nucleus pathway mediating negative effects of nicotine that limit its intake (Fowler et al., 2011). Similar effects were seen in a nicotine conditioned place preference assay, in which low nicotine doses induced a preference in both wildtype and $\alpha5$ knockout mice, but high doses only induced a preference in knockout mice (Jackson et al., 2010). Furthermore, nAChRs containing the $\alpha5$ subunit, unlike other $\alpha4\beta2^*$ nAChRs, which are highly upregulated as a result of chronic nicotine treatment, show no such change in expression (Mao et al., 2008).

Based on our current understanding of nicotinic receptor subtypes and the accumulation of clinical and preclinical evidence, many experts believe that pharmacotherapies for smoking cessation are likely to be most effective if they selectively target the $\alpha4\beta2^*$ subtype of nAChR. However, as substance use disorders are highly complex, medications targeting other nAChR subtypes may also be relevant and have been explored for their potential utility in treating tobacco use disorder.

2. α7 Nicotinic Acetylcholine Receptors. The second most prevalent nAChR subtype in the brain is the homomeric $\alpha7$ subtype, which binds nicotine with low affinity (Wada et al., 1989; Anand et al., 1991; Flores et al., 1997). The $\alpha7$ nAChR subtype seems to play an important part in both cognitive function (Pichat et al., 2007; Roncarati et al., 2009; Wallace et al., 2011) and inflammation (Alsharari et al., 2013; Egea et al., 2015). There is also evidence that this receptor subtype plays some role in the reinforcing effects of nicotine, as rats self-administered significantly less nicotine after administration of an antagonist that prevented nicotine from interacting with $\alpha7$-containing nAChRs (Markou and Paterson, 2001) despite evidence that $\alpha7$ knockout mice self-administer nicotine to the same extent as controls (Pons et al., 2008). Furthermore, modulation of dopamine signaling by $\alpha7$-containing nAChRs may also play a role in tobacco use disorder (Kaiser and Wonnacott, 2000), and additional studies indicate that $\alpha7$-containing nAChRs may be important in the somatic signs of nicotine withdrawal (Jackson et al., 2018). Despite this, $\alpha7$ nAChRs do not appear to be necessary for the nicotine discriminative stimulus, as $\alpha7$ knockout mice can be readily trained to discriminate nicotine from saline (Stolerman et al., 2004). There are species differences in nAChR density and distribution that should be considered in interpreting these studies and their translational relevance. For example, the $\alpha7$ subtype is more widely distributed in the primate brain (Papke et al., 2005) than it is in the rodent brain (Papke and Porter Papke, 2002) and thus might be expected to differentially mediate the effects of nicotine in primates and rodents.

3. Other Nicotinic Acetylcholine Receptor Subtypes. With respect to behavioral effects of nicotine, there is evidence that other subtypes of nAChR may also play an important role. It has been found that nAChRs containing $\alpha3\beta4$ subunits mediate some effects of nicotine. Specifically, receptors containing these subunits can mediate seizure and hypolocomotor effects of nicotine in mice (Salas et al., 2004a). A partial agonist at nAChRs containing $\alpha3\beta4$ was found to decrease reinstatement of nicotine seeking in a rat model of stress-induced relapse (Yuan et al., 2017). Furthermore, mice lacking the $\beta4$ subunit display a decrease in behaviors associated with nicotine withdrawal (Salas et al., 2004b). It is likely that some of these other receptor subunits are part of heteromeric receptors containing the $\alpha4$ and $\beta2$ subunits, but the extent to which this may be the case is not clear. For example, small molecule antagonists have been developed that selectively reduce the activity of nAChRs containing the $\alpha6$ subunit; however, this includes both heteromeric receptors of the $\alpha4\beta2^*$ type as well as other receptors containing the $\alpha6$ subunit. In rats, this manipulation dose-dependently decreased nicotine self-administration, suggesting a potential role for the $\alpha6$ subunit in the reinforcing effects of nicotine (Dwoskin et al., 2009).

4. Antagonists as Pharmacological Tools. The use of nAChR antagonists as pharmacological tools has offered insight into the role of nAChR subunits and the pharmacological profile of nicotine's effects as well as provided further evidence for central mediation of the nicotine discriminative stimulus. Specifically, antagonists restricted to the periphery by poor blood-brain barrier penetration, such as hexamethonium and chlorisondamine, fail to antagonize the nicotine discriminative stimulus (Hazell et al., 1978; Stolerman et al., 1984, 1988; Besheer et al., 2004; Palmatier et al., 2004). Notably, when chlorisondamine is administered intracerebroventricularly (i.e., precluding the need to cross the blood-brain barrier), it produces persistent antagonism of the nicotine discriminative stimulus for several weeks (Kumar et al., 1987). Atropine, a muscarinic acetylcholine receptor antagonist, does not antagonize the discriminative stimulus effects of nicotine in rodents (Rosecrans, 1989), although it did antagonize the discriminative stimulus effects of a relatively large (1.78 mg/kg, s.c.) dose of nicotine in monkeys (Moerke and McMahon, 2019). Furthermore, several brain-penetrant nAChR antagonists with varying subunit selectivity are commonly employed in pharmacological studies of nicotine and nAChRs.

Mecamylamine is a relatively nonselective, noncompetitive antagonist of nAChRs (Papke et al., 2001; Cunningham et al., 2014). It was initially approved in humans for use in the treatment of hypertension, although it is now rarely used for this purpose (Shytle et al., 2002). It functions as a channel blocker of all nAChRs, including both the $\alpha4\beta2^*$ and the $\alpha7$ subtypes of nAChR (Varanda et al., 1985), which are the two most prevalent subtypes present in the brain. Mecamylamine blocked the discriminative stimulus effects of nicotine in mice (Stolerman et al., 1999), rats (Morrison and Stephenson, 1969; Jutkiewicz et al., 2011), and monkeys (Cunningham et al., 2016; Moerke et al., 2017). Although mecamylamine has been investigated as a stand-alone or adjunct treatment of smoking cessation (Rose et al., 1989, 1994, 1998), it is generally thought that compliance and compensatory smoking would limit its effectiveness in treating tobacco use disorder (Rose et al., 1989). More recently, evidence from rodent models of depression-like behavior have suggested its potential use for depression (Popik et al., 2003; Rabenstein et al., 2006; Andreasen et al., 2009), although phase III trials of a mecamylamine enantiomer did not support translation for use as an antidepressant medication in humans (Moller et al., 2015).

Pempidine (1:2:2:6:6-pentamethylpiperidine) is a brain-penetrant noncompetitive cholinergic receptor antagonist originally developed for the treatment of hypertension; however, it has largely been replaced by newer drugs with greater specificity and fewer side effects (Corne and Edge, 1958; Klowden et al., 1978). Pempidine is able to produce full antagonism of nicotine's physiologic effects (Haikala and Ahtee, 1988; Martin et al., 1990). Additionally, in both rats (Garcha and Stolerman, 1993) and monkeys (Cunningham et al., 2019) trained to discriminate mecamylamine, pempidine produced full substitution in the drug discrimination assay, further supporting the characterization of pempidine as a functional nonselective nAChR antagonist.

Dihydro-β-erythroidine (DHβE) is a competitive antagonist selective for nAChRs containing the $\beta2$ subunit in vitro (Williams and Robinson, 1984; Mansvelder et al., 2002), and can be used as a tool in vivo to examine effects mediated by these receptors. Antagonism of the discriminative stimulus effects of nicotine by DHβE has been demonstrated in mice, rats, and rhesus monkeys (Stolerman et al., 1997; Gommans et al., 2000; Shoaib et al., 2000; Moerke et al., 2017). However, there is also evidence from the literature for differential antagonism of nicotine by DHβE dependent on the size of the training dose (Stolerman et al., 1997; Jutkiewicz et al., 2011). Specifically, DHβE does not consistently antagonize the discriminative stimulus effects of nicotine in rodents (Shoaib et al., 2000; Jutkiewicz et al., 2011) or in monkeys (Cunningham et al., 2012). These results have been interpreted as evidence that, while the discriminative stimulus effects of a small training dose of nicotine are mediated by $\alpha4\beta2^*$ nAChRs, the discriminative stimulus effects of a larger training dose of nicotine recruit other nAChR subtypes in addition to $\alpha4\beta2^*$. As with mecamylamine, DHβE has been found to produce antidepressant-like effects in rodents (Popik et al., 2003; Rabenstein et al., 2006; Andreasen et al., 2009); however, to our knowledge, it is not currently under development for this purpose.

Methyllycaconitine (MLA) was originally isolated from *Delphinium brownie* and is a competitive antagonist selective for the $\alpha7$ nAChR subtype (Alkondon et al., 1992; Mogg et al., 2002; Stegelmeier et al., 2003). MLA does not antagonize the nicotine discriminative stimulus in rhesus monkeys (Moerke et al., 2017), rats (Zaniewska et al., 2006), or mice (Gommans et al., 2000), even when MLA is administered via the intracerebroventricular route (Brioni et al., 1996). It has been suggested for use in treating cannabis dependence (Weinstein and Gorelick, 2011) and cancer (Wu et al., 2011), and it did reduce self-administration of nicotine in rats (Markou and Paterson, 2001). However, it is unlikely to be used for any of these purposes in humans without further development, as it is highly toxic in sufficient doses (Nation et al., 1982). Results described above from preclinical studies using nicotine self-administration and nicotine discrimination assays are summarized in Table 1.

B. Tolerance

There are three different types of drug tolerance: pharmacokinetic, pharmacodynamic, and behavioral. Tolerance is said to occur when a larger dose of drug is required to achieve the same level of effect previously achieved by a smaller dose of the drug or when the same dose of drug produces a smaller magnitude of effect with subsequent administration. Tolerance to the effects of abused drugs often occurs in individuals with substance use disorders, but tolerance itself is not indicative of substance use disorder. In the context of the current review, the word "tolerance" will be used exclusively to describe pharmacodynamic tolerance to effects of nicotine. Importantly, nicotine produces at least two distinct types of tolerance: chronic tolerance, which develops over a period of days and can be observed in experienced smokers even following a period of abstinence, and acute tolerance, which develops over a period of minutes to hours. In clinical studies, it is generally presumed that chronic tolerance has developed in habitual smokers, allowing for comparisons to be made between groups both with (i.e., smokers) and without (i.e., nonsmokers) chronic tolerance to nicotine (Perkins et al., 1993). Acute tolerance, on the other hand, occurs as rapidly as after one dose of nicotine (Stolerman et al., 1973) and clinically can be observed in both experienced smokers as well as nicotine-naïve individuals (Perkins et al., 1993). Evidence suggests that both acute and chronic tolerance to nicotine are important considerations in

TABLE 1
Summary of results from nicotinic and nonnicotinic compounds studied in nicotine self-administration and drug discrimination

Drug	Mechanism of action	Nicotine self-administration	Nicotine discrimination	References
Varenicline	partial $\alpha4\beta2$* nAChR agonist full $\alpha7$ nAChR agonist	↓ Nicotine SA in rats ↓ cue-induced reinstatement of nicotine SA in rats	Full substitution in mice, rats, monkeys; partial substitution with no antagonism in mice; partial substitution with antagonism in rats	Rollema et al., 2007; LeSage et al., 2009; Jutkiewicz et al., 2011; Cunningham et al., 2012; Le Foll et al., 2012; Cunningham and McMahon, 2013; Moerke et al., 2017
Bupropion	DAT/NET reuptake inhibitor		No substitution in monkeys and rats; partial substitution in rats and mice; full substitution in rats	Wiley et al., 2002; Young and Glennon, 2002; Desai et al., 2003; Shoaib et al., 2003; Damaj et al., 2010; Cunningham et al., 2012
AT-1001	$\alpha3\beta4$ nAChR partial agonist	↓ Stress-induced reinstatement of nicotine SA in rats		Yuan et al., 2017
Clonidine	$\alpha2$ adrenergic agonist	↓ Footshock-induced reinstatement of nicotine SA in rats		Zislis et al., 2007; Yamada and Bruijnzeel, 2011
Nortriptyline	NET/SERT reuptake inhibitor	↓ Nicotine SA in rats (only at rate-suppressing doses)	No substitution in rats	Wing and Shoaib, 2012
Physostigmine	AChE inhibitor		No substitution in rats; partial substitution in rats	Rosecrans and Meltzer, 1981; Pratt et al., 1983; Rosecrans, 1989; Giarola et al., 2011
Donepezil	AChE inhibitor	↓ Nicotine SA in rats	Full substitution in monkeys	Ashare et al., 2012; Kimmey et al., 2014; Moerke and McMahon, 2019
Galantamine	AChE inhibitor	↓ Nicotine SA in rats	Full substitution in monkeys; partial substitution in rats	Giarola et al., 2011; Hopkins et al., 2012; Liu, 2013; Moerke and McMahon, 2019
Atropine	muscarinic AChR antagonist		No antagonism in rats; antagonism in monkeys (but very large training dose)	Rosecrans, 1989; Moerke and McMahon, 2019
Hexamethonium	Peripherally restricted nAChR antagonist		No antagonism in rats (including i.c.v.)	Hazell et al., 1978; Stolerman et al., 1984; Rosecrans, 1989; Besheer et al., 2004; Palmatier et al., 2004
Chlorisondamine	Peripherally restricted nAChR antagonist		No antagonism in rats (systemic) persistent antagonism in rats (i.c.v.)	Kumar et al., 1987; Stolerman et al., 1988
Mecamylamine	nAChR antagonist	.	Antagonism in mice, rats, monkeys	Morrison and Stephenson, 1969; Stolerman et al., 1984, 1988, 1999; Besheer et al., 2004; Palmatier et al., 2004; Jutkiewicz et al., 2011; Cunningham et al., 2016; Moerke et al., 2017
Pempidine	nAChR antagonist		Antagonism in rats	Stolerman et al., 1988
DHβE	$\beta2$* nAChR-selective antagonist		Antagonism in mice, rats, monkeys	Stolerman et al., 1997; Gommans et al., 2000; Shoaib et al., 2000; Moerke et al., 2017
MLA	$\alpha7$ nAChR antagonist	↓ Nicotine SA in rats	No antagonism in mice, rats, monkeys (including i.c.v.)	Brioni et al., 1996; Gommans et al., 2000; Markou and Paterson, 2001; Zaniewska et al., 2006; Moerke et al., 2017
SB-334867	hcrtR1 antagonist	↓ Nicotine SA in rats ↓ cue-induced reinstatement of nicotine SA in rats no effect on footshock-induced reinstatement of nicotine SA in rats		Hollander et al., 2008; LeSage et al., 2010; Plaza-Zabala et al., 2010, 2013
TCSOX229	hcrtR2 antagonist	No attenuation of cue-induced reinstatement of nicotine SA in rats		Plaza-Zabala et al., 2013
2-SORA	hcrtR2 antagonist	No effect nicotine SA in rats blocked cue-induced reinstatement of nicotine SA in rats no effect on nicotine-induced reinstatement of nicotine SA in rats		Uslaner et al., 2014
Almorexant	hcrtR1/hcrtR2 antagonist	↓ Nicotine SA in rats		LeSage et al., 2010
TCS1102	hcrtR1/hcrtR2 antagonist	No effect on cue-induced reinstatement of nicotine SA in rats		Khoo et al., 2017

DAT, dopamine transporter; i.c.v., intracerebroventricular; NET, norepinephrine transporter; SERT, serotonin transporter.

the development of pharmacotherapies for tobacco use disorder (Balfour, 1994; Benowitz, 2008).

Nicotine in the context of cigarette smoking is typically dosed repeatedly at semiregular intervals over the course of the day. This leads to cycles of receptor activation and desensitization, and accumulating evidence suggests that both states contribute to the reinforcing effects of cigarette smoking [see Picciotto et al. (2008) for review]. This pattern of behavior is generally disrupted overnight, when sleeping precludes continued nicotine dosing and leads to a period of nighttime abstinence, with subsequent withdrawal symptoms occurring when waking in the morning. Thus, the first cigarette is smoked on a baseline that differs from every other cigarette during the day.

Acute tolerance to the subjective effects of nicotine is thought to be responsible for the finding that smokers report the first cigarette of the day as the most pleasurable (Fant et al., 1995). Experimentally, acute tolerance to effects of nicotine has been examined in both monkeys and humans, and there is evidence for tolerance to both physiologic (e.g., cardiovascular) effects of nicotine (Perkins et al., 1991) as well as the discriminative stimulus effects of nicotine (Perkins et al., 1996; Moerke and McMahon, 2018) and the subjective effects of nicotine (Perkins et al., 1993). Furthermore, acute tolerance to the subjective effects of nicotine is reported both in smokers and subjects who have never smoked (Perkins et al., 1993), although these two groups rate the subjective effects of nicotine differently.

However, tolerance to effects of nicotine that develops over days and years has historically been of more interest for the purpose of developing pharmacotherapies for the treatment of tobacco use disorder (Kauer and Malenka, 2007; Kalivas et al., 2009). Thus, chronic tolerance to the effects of nicotine on locomotor activity has been studied extensively in rodents (Keenan and Johnson, 1972; Stolerman et al., 1973, 1974; Hubbard and Gohd, 1975; Hatchell and Collins, 1977; Clarke and Kumar, 1983a,b; Marks et al., 1983, 1985), and chronic tolerance to the subjective effects of nicotine has been studied in humans (Perkins et al., 1993, 1994, 2002). Although the precise mechanisms underlying tolerance to locomotor activity and subjective effects of nicotine remain unclear, it has long been known that nicotine receptor binding is increased in the brains of animals after chronic exposure to nicotine (Marks et al., 1983; Schwartz and Kellar, 1983; Sanderson et al., 1993) as well as in human smokers compared with nonsmokers (Benwell et al., 1988; Breese et al., 1997; Court et al., 1998; Perry et al., 1999). Indeed, receptor upregulation is considered one hallmark of chronic tolerance to nicotine (Benwell et al., 1988; Cairns and Wonnacott, 1988). This is in opposition to what is typically observed after chronic drug treatment, which is downregulation of receptors (Overstreet and Yamamura, 1979; Creese and Sibley, 1981). Currently, this exception to the general rule is thought to be related to nicotine's ability to inactivate nAChRs (Marks et al., 1983; Schwartz and Kellar, 1985).

In vitro studies suggest that upregulation of nicotinic receptors varies based on the subtype of receptor. Whereas the $\alpha4\beta2^*$ subtype is readily upregulated after nicotine exposure, the $\alpha3\beta4$ subtype is upregulated to a lesser degree. Furthermore, the $\alpha4\beta2^*$ subtype appears to recover more slowly from upregulation than does the $\alpha3\beta4$ subtype (Peng et al., 1994; Wang et al., 1998; Fenster et al., 1999; Meyer et al., 2001; Harkness and Millar, 2002). Evidence for this difference has been demonstrated in vivo as well. In rats treated with nicotine for 14 days, upregulation of $\alpha4\beta2^*$ binding sites was shown to be between 20% and 100%, depending on brain region; however, similar upregulation of $\alpha3\beta4$ receptors was not apparent (Nguyen et al., 2003).

Interestingly, altered transcription does not seem to play a role in this receptor upregulation, as mRNA levels remain constant over time with nicotine exposure (Marks et al., 1992; Peng et al., 1994; Ke et al., 1998). Furthermore, chronic treatment with nicotine does not appear to modify metabolism (Hatchell and Collins, 1977; Marks et al., 1983). In preclinical rodent studies of chronic tolerance, 1 mg/kg nicotine administered twice daily for 5 days is sufficient for receptor upregulation to reach its half-maximal state (Marks et al., 1985; Schwartz and Kellar, 1985).

The subjective effects of nicotine in cigarette smokers are influenced by both chronic and acute tolerance to nicotine. By extension, the subjective effects of nicotine differ as a function of duration of abstinence and thus are likely to change over the course of smoking-cessation treatments. For example, both chronic and acute nicotine tolerance can lead to decreases in some subjective measures of smoking. As mentioned above, nicotine acts as an array of nicotinic receptors and has a wide range of physiologic effects. Also, as mentioned above, selective targeting of specific nAChR subtypes is common in the development of pharmacotherapies for smoking cessation. Thus, an agonist at $\alpha4\beta2^*$ used as a smoking-cessation pharmacotherapy may produce tolerance to the effects of nicotine that are mediated by $\alpha4\beta2^*$ and may or may not alter nicotine's actions at other nAChR subtypes (de Moura and McMahon, 2017). Furthermore, both acute and chronic tolerance will alter the effectiveness of treatments for smoking cessation, insomuch as these treatments are or are not affected by cross-tolerance. Thus, these factors must be considered when evaluating potential pharmacotherapies for tobacco use disorder.

C. Physiologic Dependence and Withdrawal

Although substance use disorders are often associated with the positive subjective effects of the abused drug, continued drug use can also be motivated by the desire to avoid negative effects associated with drug

withdrawal. Discontinuation of nicotine use in dependent individuals leads to increases in stress, appetite, insomnia, anxiety, and irritability as well as disruptions in cognition (Hughes, 2007; American Psychiatric Association, 2013; Wesnes et al., 2013). Many pharmacotherapies for smoking cessation attenuate the withdrawal effects of nicotine discontinuation, and this is thought to be one important aspect of their effectiveness. Experimental paradigms in rodents and nonhuman primates can recapitulate many of the symptoms associated with nicotine withdrawal in humans, such as increases in stress-like, anhedonia-like, and anxiety-like behaviors and disruptions in learned memory tasks (Malin et al., 1994; De Biasi and Salas, 2008). For example, nicotine withdrawal in rats was found to increase corticotropin-releasing factor levels in the central nucleus of the amygdala, an area of the brain known to modulate stress response (George et al., 2007). Furthermore, nicotine withdrawal increased anxiety-like behavior through activation of a subset of corticotrophin-releasing factor receptors. Of note, repeated exposure to inhaled nicotine vapor (as with ENDS), upon abrupt discontinuation, produces signs of tobacco use disorder and withdrawal in both humans (Morean et al., 2018) and rodents (George et al., 2010, 2011). Clinically, the reported severity of nicotine withdrawal effects by smokers is a potential predictor of relapse (West et al., 1989). Thus, an additional consideration in developing potential pharmacotherapies for smoking cessation is the treatment of nicotine withdrawal–related effects.

V. U.S. Food and Drug Administration–Approved Pharmacotherapies for Smoking Cessation

It is estimated that 70%–80% of current cigarette smokers want to quit smoking, and many of them have made at least one quit attempt in the last year (Schuckit et al., 1994; U.S. Department of Health and Human Services, 2014; Babb et al., 2017). However, a recent study suggested that most current smokers will try to quit, on average, 30 times or more before being successful (Chaiton et al., 2016). In fact, most attempts to quit fail within the first week (Hughes et al., 2004). In the United States, at least 40% of smokers attempt to quit smoking each year (Hughes et al., 2004), but even with behavioral and pharmacotherapies, fewer than 5% remain abstinent for more than 3 months (U.S. Department of Health and Human Services, 2014). In the United States, there are three first-line pharmacotherapies approved by the FDA for smoking cessation: nicotine replacement therapy, varenicline, and bupropion.

A. Nicotine Replacement Therapies

In the United States, nicotine replacement is the most widely available and easily accessible pharmacotherapy for smoking cessation, as most formulations can be purchased over the counter and without a prescription. Nicotine replacement was added to the WHO List of Essential Medicines in 2009, and a wide variety of nicotine replacement products are marketed around the world. There are a number of legal and regulatory differences between countries as well as differences in attitude about nicotine replacement as a harm-reduction measure; thus, the availability (i.e., by prescription only or from a pharmacy) of different formulations varies from country to country. For example, nicotine gum (Nicorette), the first nicotine replacement therapy approved by the FDA for use in the United States in 1984, was originally available by prescription only. Similarly, the transdermal nicotine patch (NicoDerm CQ), approved by the FDA in 1991, originally required a prescription. These formulations only became available "over the counter" in the U.S. in 1996, when nicotine nasal spray (Nicotrol NS), followed by the nicotine inhaler (Nicotrol), became available by prescription. The nicotine lozenge (Commit), approved in 2002, was the only nicotine replacement therapy that never required a prescription in the United States. ENDS started appearing on the market around 2003, and use in the United States and elsewhere has grown dramatically in recent years; however, there is no current consensus on the safety or efficacy of these products, so regulations vary widely. In the United States, they are regulated by the FDA and are legal to buy for anyone 18 years of age or older; however, they are not considered a pharmacotherapy for smoking cessation.

One nicotine reduction strategy was part of an effort by tobacco companies to lower perceived harm of their product by marketing so-called "light" cigarettes. Although low-yield nicotine cigarettes are considered attractive measures for decreasing nicotine consumption (Benowitz and Henningfield, 1994), these products were primarily intended to reduce nicotine content with filter ventilation, not by changing the nicotine content of the tobacco used in cigarettes. Furthermore, for these types of cigarettes, evidence suggests that smokers will alter variables of nicotine intake, including puff volume, depth of inhalation, extent of dilution with room air, rate of puffing, and intensity of puffing, to compensate (U.S. Department of Health and Human Services, 1981). More recently, this approach has been revived by researchers studying low-yield nicotine cigarettes in clinical trials, indicating that cigarettes with reduced nicotine content do have potential for use in smoking-cessation treatments (Hatsukami et al., 2010; Donny et al., 2015; Pacek et al., 2016; Tidey et al., 2017), but this strategy is not currently approved by the FDA.

Nicotine replacement therapies, regardless of formulation, work on the same principle executed slightly differently; they promote smoking cessation because they are substitution therapies, continuing to provide nicotine by another route of administration and without

other tobacco constituents after an individual has quit using tobacco. These therapies are aimed at reducing tobacco cravings and withdrawal symptoms by delivering therapeutic doses of nicotine that generally have a slower onset and are thus less likely to be abused than inhaled nicotine. Furthermore, nicotine is delivered without the additional toxins that are inhaled along with tobacco smoke. With the exception of the nicotine patch, which delivers nicotine at a constant low rate, these formulations can be taken on an as-needed basis, although they do have recommended dosing regimens. Ideally, an individual will taper the magnitude of the dose of nicotine they are self-administering, as nicotine replacement is only intended to be used for 2–3 months. This is one potential drawback to nicotine replacement therapy, as many people find themselves unable or unwilling to gradually decrease their dose and continue long-term use (Hajek et al., 1988; Hughes et al., 1991; Johnson et al., 1991; Hurt et al., 1995). Despite this pattern of behavior, studies to date have not examined the effects of these pharmacotherapies over extended periods of time, so the relative safety of long-term use of nicotine replacement therapies is unknown. Additionally, even among patients receiving a prescription for a product available over the counter (i.e., nicotine patch, nicotine gum), only 67% are given any instructions for usage by their physician, and 77% receive no follow-up (Shiffman et al., 2007). Nicotine replacement therapies may also produce side effects, such as insomnia, dizziness, and headaches, that compromise compliance (Jorenby et al., 1995; Hurt et al., 1998; Hajek et al., 1999) as well as have the potential for nicotine toxicity at large doses (Dale et al., 1995).

Despite these potential disadvantages, nicotine replacement therapies generally appear to approximately double a smoker's chance of remaining abstinent 6 months after quitting over placebo, and there do not appear to be differences among the different formulations (Cahill et al., 2013). However, a conflicting report suggests that after properly adjusting for bias, there is no indication that use of any nicotine replacement formulation improves outcomes for smoking cessation over placebo (Stanley and Massey, 2016). This claim does not extend to findings that suggest that using two nicotine replacement therapies in combination appears to be about twice as effective as using any one of the formulations alone (Cahill et al., 2013, 2014). The benefit of combining two different forms of nicotine replacement therapy is similar to what has been reported for varenicline alone.

B. Varenicline (Chantix)

Varenicline is a novel compound developed specifically for use in smoking cessation. Approved by the FDA in 2006, varenicline is only available by prescription. The recommended course of treatment is to begin taking varenicline a week before planning to quit smoking. For the first 3 days, 0.5 mg is taken as a tablet once per day and then twice per day for the rest of that week. At this point, when no longer smoking, the dose is increased to 1 mg twice daily for an additional 12 weeks. This course of treatment can be repeated or extended for those who relapse. Varenicline was identified among a series of compounds synthesized based on the structure of cytisine, a natural compound and partial nAChR agonist marketed for smoking cessation in Europe as Tabex (Coe et al., 2005). Like cytisine, it is designated as a partial, or low-efficacy, agonist. Theoretically, as a partial agonist, varenicline is effective as a pharmacotherapy for smoking cessation in two complimentary ways: 1) it functions as a substitution therapy like nicotine replacement, reducing cravings and withdrawal effects; 2) it also functions as an antagonist therapy, reducing or preventing the reinforcing effects of tobacco if an individual continues to smoke while taking it (Coe et al., 2005).

Electrophysiological studies in transfected cells demonstrate that varenicline does not produce the same maximum effect as nicotine at $\alpha4\beta2^*$ nAChRs (Coe et al., 2005; Rollema et al., 2007). Furthermore, varenicline antagonizes nicotine's effects at this receptor subtype (Coe et al., 2005). Varenicline also demonstrates lower efficacy than nicotine to stimulate dopamine release from rat brain slices (Rollema et al., 2007). In vivo varenicline does not alter ICSS thresholds; however, varenicline does block nicotine-induced facilitation of ICSS in rats (Vann et al., 2011). In many drug discrimination studies, including those in mice (Cunningham and McMahon, 2013), rats (Rollema et al., 2007; Jutkiewicz et al., 2011), and monkeys (Cunningham et al., 2012; Moerke et al., 2017) discriminating nicotine, varenicline fully substitutes for the nicotine discriminative stimulus, although additional studies in rats found this was only the case at relatively short pretreatment times (i.e., 5–40 minutes), whereas longer pretreatment times (i.e., 2–4 hours) resulted in very low levels of generalization with the nicotine discriminative stimulus (Le Foll et al., 2012). Under conditions in which varenicline only partially substitutes for the nicotine discriminative stimulus, varenicline sometimes, but not always, antagonizes the discriminative-stimulus effects of nicotine (LeSage et al., 2009; Cunningham and McMahon, 2013). When varenicline fully substituted for the nicotine discriminative stimulus in monkeys, combinations of nicotine and varenicline were synergistic (Cunningham et al., 2012). These observations, including full substitution of varenicline for nicotine, do not exclude the possibility that varenicline has lower efficacy than nicotine. Instead, it may simply be that for the training doses of nicotine studied in these discrimination assays, the efficacy demand might be sufficiently low, such that even a low-efficacy agonist can mimic the effects of a higher-efficacy agonist. Another possibility is that varenicline pretreatment results in acute tolerance

through desensitization of the receptors where nicotine is acting to produce discriminative-stimulus effects; experimentally, acute cross-tolerance from varenicline to nicotine would not readily be distinguishable from antagonism of nicotine. These results are summarized in Table 1.

Although classified as a partial agonist at $\alpha4\beta2$* nAChRs, as described above, varenicline has also been characterized as a full agonist at homomeric $\alpha7$ nAChRs (Mihalak et al., 2006). In mice, varenicline dose-dependently blocks a conditioned place preference for nicotine (Bagdas et al., 2018a). Interestingly, $\alpha5$ but not $\alpha7$ nAChR knockout mice display an attenuation of varenicline's effects on nicotine conditioned place preference (Bagdas et al., 2018a). That is, although varenicline is classified as an $\alpha7$ nAChR full agonist, varenicline's actions at this receptor in vivo seem somewhat dispensable. Meanwhile, it appears that nAChRs containing the $\alpha5$ subunit may mediate at least some of varenicline's effects.

Early reports of the side effects of varenicline prompted the FDA to require a black box warning for depression, suicidal thoughts, and suicidal actions on varenicline in 2009. However, several meta-analyses have found no evidence for an increase in any adverse neuropsychiatric events beyond sleep disturbances, which have been well-documented, and the warning has since been removed (Harrison-Woolrych and Ashton, 2011; Thomas et al., 2015). Other side effects, predominantly nausea, are reported more frequently than similar side effects with nicotine replacement therapies (Cahill et al., 2013, 2016). In addition to being generally unpleasant, side effects can also compromise compliance as well as promote relapse (Williams et al., 2007; Faessel et al., 2009; Kasliwal et al., 2009; Harrison-Woolrych and Ashton, 2011; Jimenez-Ruiz et al., 2013). Varenicline appears to approximately double one's chances of remaining abstinent for a year over placebo alone (Gonzales et al., 2006), but it does not appear to be any more or less effective than multiple nicotine replacement therapies used in combination (Cahill et al., 2013, 2016; Baker et al., 2016).

C. Bupropion (Zyban)

Bupropion was approved for use in the United States as an antidepressant under the trade name Wellbutrin as early as 1985 but not as a pharmacotherapy for smoking cessation until 1997 (Zyban). In some countries, such as the United Kingdom, its only approved indication is as a smoking-cessation aid. It is considered an atypical antidepressant in the sense that it does not appear to function as a selective serotonin reuptake inhibitor, as is the case for many commonly prescribed antidepressants. Chemically, it is a substituted cathinone with a complex mechanism of action that is not completely understood. Thus, the exact mechanism(s) by which bupropion functions as a smoking-cessation aid is unknown, as it has effects at many targets in the central nervous system.

Bupropion shares many of its effects with other psychostimulants, and it is typically characterized as a dual norepinephrine and dopamine reuptake inhibitor (Stahl et al., 2004). In drug discrimination studies, both amphetamine, a structurally related compound, and cocaine, a structurally distinct compound, fully cross-generalize with bupropion in both rats and monkeys (Jones et al., 1980; Blitzer and Becker, 1985; Kamien and Woolverton, 1989; Kleven et al., 1990; Lamb and Griffiths, 1990; Terry and Katz, 1997; Bondarev et al., 2003). Furthermore, bupropion supports self-administration behavior in rats as well as monkeys (Bergman et al., 1989; Lamb and Griffiths, 1990; Tella et al., 1997). This does not appear to completely translate to humans, however, as human studies have shown that although bupropion produces some subjective effects similar to abused drugs like amphetamine, it does not have abuse liability different from placebo (Griffith et al., 1983; Miller and Griffith, 1983).

Other data from studies comparing bupropion and nicotine have also produced mixed results. For example, in animals trained to discriminate nicotine, the ability of bupropion to substitute for nicotine has varied widely between studies. In rhesus monkeys, bupropion did not substitute for nicotine (Cunningham et al., 2012), and in mice, it only partially substituted for nicotine (Damaj et al., 2010); however, in rats, bupropion fully substituted (Wiley et al., 2002; Young and Glennon, 2002), partially substituted (Desai et al., 2003), or did not substitute (Shoaib et al., 2003), depending on the study. These results are summarized in Table 1.

Understanding the mechanism that underlies the therapeutic effects of bupropion for smoking cessation is further complicated by other actions of bupropion itself in addition to its active metabolites at a variety of central nervous system targets. One possibility is that bupropion functions as a smoking-cessation aid through antagonism of nAChRs (Slemmer et al., 2000). However, as bupropion is predominately metabolized by the CYP2B6 enzyme into its active metabolites *R,R*-hydroxybupropion, *S,S*-hydroxybupropion, *threo*-hydrobupropion, and *erythro*-hydrobupropion, these metabolites may also play an important role in its therapeutic effects (Cooper et al., 1984). Preclinical studies in mice found that both *R,R*-hydroxybupropion and *S,S*-hydroxybupropion were similar to bupropion in reversal of antagonist-precipitated nicotine withdrawal symptoms (Damaj et al., 2010), and the clinical effectiveness of bupropion treatment has also been linked to its active metabolites (Zhu et al., 2012). Clinical trials suggest that although bupropion is not as effective for smoking cessation as varenicline, it does work better than placebo (Hughes et al., 2014; Anthenelli et al., 2016; Stead et al., 2016; Windle et al., 2016). It is, however, a potent inhibitor of the CYP2D6 enzyme (Güzey et al., 2002; Jefferson et al., 2005), which is

necessary for the metabolism of a variety compounds; thus, it may have adverse effects in combination with other drugs that rely on this mechanism for their clearance.

D. Off-Label Pharmacotherapies for Smoking Cessation

Clonidine and nortriptyline are considered "second line" medications for smoking cessation. Although these drugs appear to be more effective than placebo at maintaining abstinence at 6 months, they are associated with an increased risk of adverse side effects as compared with "first line" pharmacotherapies (Cahill et al., 2013). Furthermore, relatively little preclinical research has addressed the potential mechanism of action that these pharmacotherapies use to produce therapeutic effects in tobacco use disorder. Clonidine attenuates footshock-induced reinstatement of nicotine-seeking behavior in rats self-administering nicotine (Zislis et al., 2007; Yamada and Bruijnzeel, 2011) but, to our knowledge, has not been tested with nicotine in drug discrimination. Nortriptyline has no effect on nicotine discrimination and is only effective in decreasing nicotine self-administration in rats at doses sufficiently high to also depress responding for food (Wing and Shoaib, 2012). Mecamylamine, the nAChR antagonist, was originally approved for use in hypertension but has also been used both alone and in combination with nicotine as a potential therapy for tobacco use disorder. Alone, mecamylamine is ineffective for smoking cessation (Lancaster and Stead, 2000), but there is very limited evidence that it might be slightly more effective in combination with nicotine replacement therapy than nicotine replacement therapy alone (Rose et al., 1998). More recently, ENDS have been proposed for use in smoking cessation, as previously discussed. There is still a lack of evidence, but ENDS technologies might also help improve outcomes for smoking cessation, although the extent to which ENDS might be more effective than currently approved pharmacotherapies is unknown (Malas et al., 2016).

VI. Experimental Pharmacotherapies for Smoking Cessation

A. Allosteric Modulation of Nicotinic Acetylcholine Receptors

Allosteric modulation has long been recognized as the primary mechanism of action for FDA-approved drugs (e.g., benzodiazepines, barbiturates), but only more recently has it inspired widespread interest as an alternative strategy for targeting nAChRs, not just for smoking cessation but also for a variety of disorders characterized by dysfunction of the central nervous system. Allosteric interactions were originally proposed in the context of enzymatic reactions (Monod et al., 1965), but it was not long before this model was extended to include ligand binding at biologic membranes (e.g., nAChRs) (Changeux et al., 1967). Importantly, the word "allosteric" has been used somewhat ambiguously as a descriptor of ligands in the literature. For the sake of simplicity in the current review, we consider "orthosteric ligands" of nAChRs to include both acetylcholine and nicotine as well as other ligands that share the same canonical binding site as acetylcholine and nicotine; "allosteric ligands" will be used to refer to compounds that bind to nAChRs at sites that are distinct from the canonical "orthosteric" site. It has been theorized that one important feature of an allosteric modulator for use in smoking cessation might be that it does not produce effects alone in the absence of an orthosteric ligand. Instead, only when the orthosteric ligand is present would the allosteric modulator serve to produce a change in either the affinity and/or the efficacy of the orthosteric ligand (Uteshev, 2014) (Fig. 2). This is particularly promising in terms of a therapeutic strategy, as the effects of an allosteric modulator alone should be minimal, reducing the potential for unwanted side effects like those that often occur with approved substitution-type pharmacotherapies. For example, a negative allosteric modulator (NAM) might serve to reduce the reinforcing effects of nicotine, whereas a positive allosteric modulator (PAM) in combination with a more traditional orthosteric agonist therapy could reduce the dose of agonist required to produce a therapeutic effect. Decreasing the minimal effective therapeutic dose of an orthosteric ligand through combination with a positive allosteric ligand could both retain therapeutic effectiveness and decrease the potential for toxicity and other side effects, particularly when substitution pharmacotherapies are taken in larger quantities or more often than the recommended clinical indication. Furthermore, agonist activation of nAChRs also perpetuates cycles of activation and desensitization, which are also of importance in tobacco use disorder [see Picciotto et al. (2008) for review]. In this case, smaller doses of an orthosteric agonist in combination with a PAM should result in less receptor desensitization produced by the orthosteric ligand (Williams et al., 2011).

PAMs of nAChRs are proposed to increase the binding affinity and/or efficacy of an orthosteric agonist (Pandya and Yakel, 2013), whereas NAMs theoretically do the opposite by decreasing the binding affinity and/or efficacy of an orthosteric agonist. However, there are several different mechanisms by which allosteric modulators may accomplish these effects, which we will discuss briefly with a focus on heteromeric nAChRs. The prevailing consensus is that nAChRs are composed of dynamic proteins that are capable of multiple different states. However, for simplification, here we limit discussion to three possible states: closed, open, and desensitized. Furthermore, heteromeric nAChRs can potentially have 0, 1, or 2 agonist molecules bound. The likelihood of the receptor remaining stable in any one of the three possible states and the rates at which one state shifts to

another state depend on the level of agonist occupancy (Changeux and Edelstein, 1998; Auerbach, 2010). At baseline equilibrium, when exogenous and endogenous signaling does not occur and no agonist is bound, nAChRs remain preferentially in the closed state. However, with an extremely low probability of shifting to the open state, the receptor may exist with some equilibrium between the closed state and the desensitized state (Williams et al., 2011). If a high concentration of agonist sufficient to saturate all the possible binding sites is rapidly applied, $\alpha 4\beta 2$ nAChRs have an 80% probability of simultaneously shifting transiently into the open state before reaching a new equilibrium in the desensitized state (Li and Steinbach, 2010). Furthermore, it is known that once receptors are in the desensitized state, agonists bind with higher apparent affinity.

One way that a PAM of nAChRs may exert its effects would be to increase the agonist binding to the closed state of the receptor, which is experimentally represented by an increase in the potency of the agonist. This type of modulation might be most advantageous under a condition in which there is a low concentration of agonist, which would not otherwise produce a maximal response. By increasing the potency of the agonist, lower concentrations would be able to produce the maximum response. However, it would remain impossible to exceed the maximum response if the modulator is only changing the potency of the orthosteric ligand.

An alternative effect observed with PAMs is that they are often observed to increase the efficacy of an agonist. One way a PAM might accomplish this is via shifting the equilibrium between the open and closed states, making it easier to move from closed to open state. This would result in not only more receptors moving to the open state but also a greater likelihood that they might move from closed to open more than once before shifting to the desensitized state. Thus, this would yield a concurrent decrease in the rate of desensitization. Functionally, this could be observed as an overall increase in the time spent in the open state. Having this effect, a PAM can produce a transient increase in efficacy. The reverse of these conditions is hypothesized with the use of a NAM, leading to a transient decrease in agonist efficacy. However, neither a NAM nor a PAM in this scenario would alter how favored one state is over another, only the rate of change between states. Thus, receptors would eventually end up preferentially in the desensitized state, as under normal conditions.

Finally, if instead of altering the rate of change between states, as described above, a modulator functioned to shift equilibrium away from the desensitized state, this would result in yet another unique profile of observable effects. The result of this type of modulator would likely not be manifest as an increase in efficacy or the maximum effect, nor would it be observable under conditions in which the agonist interaction with the receptor pool is brief (e.g., acetylcholine broken down by acetylcholinesterase, rapid agonist application). Instead, it would be most apparent under equilibrium conditions, producing a significant amount of steady-state current (Williams et al., 2011).

The above is a simplified explanation of a theoretical model. Although there is experimental evidence that is consistent with these scenarios, it is not possible to prove or disprove them; they simply are not violated by what is currently known. Furthermore, it has been reported that, at least under some conditions, only a small percentage of available nAChRs are capable of being activated at once (McNerney et al., 2000; Li and Steinbach, 2010). Thus, the ability of a modulator to change receptors from the inactive to the active state is yet another possible mechanism by which it might enhance agonist activity. Finally, and perhaps most importantly, a similar but not identical model can be

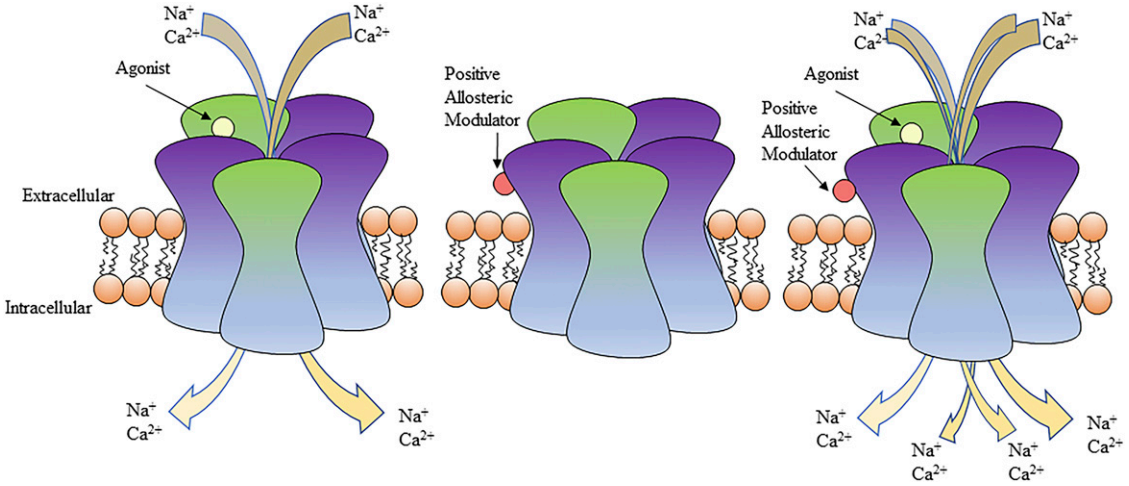

Fig. 2. Neuronal nAChR positive allosteric modulation. (Left) A neuronal nAChR with agonist bound at the orthosteric binding site allows for the influx of sodium and calcium into the cell. (Center) A positive allosteric modulator (PAM) bound to an allosteric site of a neuronal nAChR. By itself, it does not trigger the influx of ions into the cell. (Right) A neuronal nAChR PAM bound to an allosteric site, with an agonist at the orthosteric binding site, allows for an increased influx of sodium and calcium into the cell.

applied to homomeric nAChRs. Homomeric nAChRs are different from heteromeric nAChRs because they contain only one type of subunit; thus, they can potentially have 0, 1, 2, 3, 4, or 5 orthosteric agonist molecules bound to a single receptor (i.e., assemblies of five of the same subunit yield five essentially equal interfaces where ligand binding is possible). Heteromeric nAChRs, as described above, will have at the very most two similar interfaces for ligand binding, and it is likely that binding to one of these interfaces is not exactly interchangeable with binding to the other. Furthermore, an additional "fast" desensitization state has been described for homomeric $\alpha7$ nAChRs, which is concentration-dependent and thought to be possible under conditions in which more agonist molecules are bound to a single receptor than is possible for heteromeric receptors (Papke et al., 2000). However, regardless of the exact mechanism, both negative and positive allosteric modulation of nAChRs represents an attractive therapeutic strategy that may circumvent the limitations inherent in targeting the canonical orthosteric site. A list of current nAChR NAMs and PAMs are shown in Tables 2 and 3, respectively, and are discussed below with regard to potential application for the treatment of tobacco use disorder.

1. Negative Allosteric Modulators. Allosteric modulators that selectively bind one or a subset of nAChR subtypes have been developed in vitro. The compound UCI-30002 is classified as a NAM at several nAChR subtypes. Specifically, in vitro work performed in transfected *Xenopus* oocytes demonstrated that this compound produces complete blockade of $\alpha7$ and $\alpha3\beta4$ subtypes but only partial blockade (approximately 80%) of the $\alpha4\beta2$* subtype (Yoshimura et al., 2007). Further studies with this compound have demonstrated that it significantly diminishes nicotine self-administration in rats (Yoshimura et al., 2007). Other groups have discovered more selective nAChR NAMs, such as KAB-18, which has been characterized in vitro as a selective $\alpha4\beta2$* nAChR NAM (Henderson et al., 2010). Additional studies suggest that the compounds DB04763, DB08122, and pefloxacin may act in vitro as $\alpha7$ nAChR NAMs (Smelt et al., 2018). These nAChR NAMs are summarized in Table 2. However, more studies are needed to assess the selectivity of these compounds in vivo as well as their potential to be developed as pharmacotherapies for tobacco use disorder.

2. Positive Allosteric Modulators.

a. $\alpha4\beta2$ subtype selectivity.* Desformylflustrabromine (dFBr) is classified both in vitro and in vivo as an $\alpha4\beta2$* nAChR-selective PAM. It is a novel bromotryptamine derivative first isolated from *Flustra foliacea*, a marine bryozoan (Lysek et al., 2002; Peters et al., 2002), that was observed to selectively increase the current recorded in voltage clamp experiments conducted in oocytes transfected with human $\alpha4$ and $\beta2$ nAChR subunits when coapplied with acetylcholine (Sala et al., 2005). Subsequently, it was successfully synthesized in the laboratory, where similar voltage clamp experiments in transfected oocytes reproduced the previous finding with the natural product dFBr; coapplication with acetylcholine increased ionic current through $\alpha4\beta2$ nAChRs but not $\alpha7$ nAChRs (Kim et al., 2007). Additionally, these experiments revealed a bell-shaped dose-response curve, with dFBr at concentrations in excess of 10 μM causing inhibition.

Further study of dFBr in vitro has increased our understanding of the possible mechanism(s) responsible for both its potentiating and inhibiting effects. In combination with different orthosteric nAChR agonists, dFBr increases the maximum effect of acetylcholine, nicotine, cytisine, and choline but does not change the potency of these agonists. Furthermore, dFBr increases the maximum effect of low efficacy agonists (i.e., cytisine, choline) more than it increases the maximum effect of high efficacy agonists (i.e., acetylcholine, nicotine). Additional experiments examined modulation by dFBr of the effects of three nAChR antagonists, DHβE, DMAB-anabaseine, and tropisetron, to determine if dFBr produced effects in the presence of the antagonist-bound receptor, but neither alone nor in combination with acetylcholine were any significant changes apparent with the addition of dFBr (Weltzin and Schulte, 2010). One interesting finding from these experiments was the ability of dFBr to reactivate desensitized receptors; that is, receptors in the desensitized state from previous saturating applications of acetylcholine did not respond to further addition of acetylcholine until dFBr was also applied to the preparation (Weltzin and Schulte, 2010). Altogether, these data suggest that the potentiating effect of dFBr can likely be attributed to an ability to change the equilibrium between receptor states (e.g., open) and that inhibition with higher concentrations of

TABLE 2
Negative allosteric modulators of neuronal nAChRs and studies investigating them as pharmacotherapies for tobacco use disorder

Drug	nAChR subtype activity	Characterized in vitro?	In vivo findings	References
UCI-30002	Full activity at $\alpha7$ and $\alpha3\beta4$, partial activity at $\alpha4\beta2$	Yes	Diminishes nicotine self-administration in rats	Yoshimura et al., 2007
KAB-18	Selective activity at $\alpha4\beta2$	Yes	N.A.	Henderson et al., 2010
DB04763	Selective activity at $\alpha7$	Yes	N.A.	Smelt et al., 2018
DB08122	Selective activity at $\alpha7$	Yes	N.A.	Smelt et al., 2018
Pefloxacin	Selective activity at $\alpha7$	Yes	N.A.	Smelt et al., 2018

N.A., not applicable.

TABLE 3
Positive allosteric modulators of neuronal nAChRs and studies investigating them as pharmacotherapies for tobacco use disorder

Drug	nAChR subtype activity	Characterized in vitro?	In vivo findings	References
Desformylflustrabromine (dFBr)	Full activity at $\alpha4\beta2$, partial activity at $\alpha7$	Yes	Reduces nicotine self-administration in rats; blocks behavioral signs of nicotine withdrawal in mice	Sala et al., 2005; Kim et al., 2007; Liu, 2013; Hamouda et al., 2018
NS9283	Selective activity at $\alpha4\beta2$	Yes	Potentiates the effect of nicotine in the rat drug discrimination assay; acute and repeated dosing reduces nicotine self-administration in rats	Grupe et al., 2013; Mohler et al., 2014; Maurer et al., 2017
CMPI	Selective activity at $\alpha4\beta2$	Yes	N.A.	Albrecht et al., 2008; Hamouda et al., 2016
LY 2087101	Full activity at $\alpha4\beta2$, $\alpha4\beta4$, $\alpha7$	Yes	Does not potentiate the effect of nicotine in the mouse drug discrimination assay	Broad et al., 2006; Moerke et al., 2016
NS1738	Selective type I activity at $\alpha7$	Yes	Blocks behavioral signs of nicotine withdrawal in mice	Timmermann et al., 2007; Jackson et al., 2018
CCMI	Selective type I activity at $\alpha7$	Yes	N.A.	Ng et al., 2007
AVL-3288	Selective type I activity at $\alpha7$	Yes	N.A.	Bortz et al., 2016; Gee et al., 2017
PNU-120596	Selective type II activity at $\alpha7$	Yes	Enhances the hypothermic effects of nicotine; blocks behavioral signs of nicotine withdrawal in mice; does not potentiate the effect of nicotine in the mouse drug discrimination assay	Hurst et al., 2005; Barron et al., 2009; Moerke et al., 2016; Jackson et al., 2018
TQS	Selective type II activity at $\alpha7$	Yes	N.A.	Gronlien et al., 2007; Thomsen and Mikkelsen, 2012
A-867744	Selective type II activity at $\alpha7$	Yes	N.A.	Faghih et al., 2009
TBS-345, TBS-346, TBS-516, TBS-546, TBS-556	Selective type II activity at $\alpha7$	Yes	N.A.	Chatzidaki et al., 2015
RO5126946	Selective type II activity at $\alpha7$	Yes	N.A.	Sahdeo et al., 2014
GAT107	Selective ago-PAM at $\alpha7$	Yes	N.A.	Thakur et al., 2013; Papke et al., 2014, 2018
B-973	Selective ago-PAM at $\alpha7$	Yes	N.A.	Post-Munson et al., 2017
JNJ-39393406	Selective activity at $\alpha7$	Yes	Produces positive outcomes in preclinical rat and mouse models of schizophrenia-induced cognitive impairment; does not reduce cigarette craving or total smoking and does not increase number of quit days in humans	Winterer et al., 2013; Perkins et al., 2018

N.A., not applicable.

dFBr engages a different mechanism likely related to block of the ion channel (Weltzin and Schulte, 2010). Further voltage clamp experiments suggest that dFBr might have differential effects at $\alpha4\beta2$* nAChRs dependent on the stoichiometry of the receptor (Weltzin et al., 2014). Other studies have found that dFBr also has inhibitory (but not potentiating) effects at muscle-type nAChRs, which also appears to be a result of dFBr binding within the ion channel, consistent with earlier studies suggesting that dFBr's inhibitory effects were a result of channel blockade (Hamouda et al., 2015). Recent evidence from mutated receptors suggests that the magnitude of effect that dFBr produces may be dependent on the stoichiometry of the $\alpha4\beta2$* nAChR in question (Weltzin and Schulte, 2015). However, the extent to which the details of the mechanism of dFBr observed in vitro are relevant in vivo is currently unknown. These findings are summarized in Table 3.

Desformylflustrabromine has also been studied extensively in vivo. In rats trained to self-administer nicotine (0.03 mg/kg per infusion), lower doses of dFBr (0.1 and 1 mg/kg) had no effect on nicotine self-administration, but larger doses (3 and 6 mg/kg) reduced the number of nicotine infusions earned (Liu, 2013). The largest dose of dFBr reduced the number of infusions earned by about half, from approximately 14 infusions under control conditions to seven infusions in combination with 6 mg/kg dFBr. Furthermore, in a separate group of rats, no dose of dFBr changed the number of responses made on either the active or inactive levers, indicating that there was not a general depression of behavior (Liu, 2013). To test the extent to which dFBr was acting via a central mechanism, cerebrospinal fluid was collected along with plasma after subcutaneous administration of dFBr. The elimination half-life of dFBr was estimated to be 8.6 hours, and it was present in the cerebrospinal fluid at about 30% of the concentration seen in plasma, indicating that it was crossing the blood-brain barrier and having actions in the central nervous system (Liu, 2013). Recently, in vivo studies with dFBr have demonstrated that this compound can reverse behavioral signs of nicotine withdrawal in nicotine-dependent mice (Hamouda et al., 2018). The effects of the nAChR PAM dFBr are summarized in Table 3.

Additional PAMs of $\alpha4\beta2$* nAChRs have been identified. In vitro, the selective PAM NS9283 increased the potency of currents evoked with acetylcholine in human embryonic kidney 293 cells transfected with human

$\alpha4\beta2$ nAChR subunits. Furthermore, it was found that NS9283 did not alter the rate of desensitization of currents evoked with acetylcholine (Grupe et al., 2013). In the rat drug discrimination assay, NS9283 failed to produce substitution for 0.4 mg/kg nicotine at any dose tested (Mohler et al., 2014). When NS9283 was paired with doses of nicotine that did not produce significant substitution for 0.4 mg/kg nicotine, full substitution was observed. In the rat self-administration assay, NS9283 was not readily self-administered. However, both acute and repeated administration of NS9283 dose-dependently reduced nicotine self-administration in rats (Maurer et al., 2017). As $\alpha4\beta2$* nAChRs are pentameric, it has been found that NS9283 selectively and preferentially acts on nAChRs with the combination of (3) $\alpha4$ plus (2) $\beta2$ subunits (Timmermann et al., 2012; Grupe et al., 2013). This combination of subunits has been found to possess low sensitivity to acetylcholine (i.e., EC_{50} = 100 μM) and is in contrast to the combination of (2) $\alpha4$ plus (3) $\beta2$ subunit ratio, which has been found to have high sensitivity to acetylcholine (i.e., EC_{50} = 1 μM) (Nelson et al., 2003; Moroni et al., 2006).

The compound (3-(2-chlorophenyl)-5-(5-methyl-1-(piperidin-4-yl)-1H-pyrrazol-4-yl)isoxazole) (CMPI) is structurally distinct from NS9283 and is also classified in vitro as a selective and preferential PAM at (3) $\alpha4$ (2) $\beta2$ nAChRs (Albrecht et al., 2008; Hamouda et al., 2016). Interestingly, it appears that different binding sites on $\alpha4\beta2$* nAChRs may be responsible for the actions of CMPI compared with NS9283 (Wang et al., 2017).

LY 2087101 is classified in vitro as a PAM at $\alpha4\beta2$*, $\alpha4\beta4$, and $\alpha7$ nAChRs (Broad et al., 2006). Additionally, in vitro LY 2087101 produces an increase in both potency and magnitude of nicotine-induced currents (Broad et al., 2006). In vivo, this compound fails to produce substitution for 1 mg/kg nicotine at any dose tested in the mouse drug discrimination assay, even at doses that produce significant reduction of schedule-controlled responding (Moerke et al., 2016). Furthermore, when LY 2087101 is paired with doses of nicotine that do not produce significant substitution for 1 mg/kg nicotine, no potentiation is observed (Moerke et al., 2016). Thus, there appears to be a disconnect between the in vitro and in vivo literature regarding whether LY 2087101 is a true functional nAChR PAM. This discussion of $\alpha4\beta2$* nAChR PAMs is summarized in Table 3.

b. α7 subtype selectivity. The $\alpha7$ nAChR has garnered a lot of interest in its potential as a target for numerous cognitive diseases, including schizophrenia, Alzheimer disease, and inflammation-driven diseases, as well as smoking cessation. Because of the homomeric structure of the $\alpha7$ nAChR, two distinctive types of PAMs have been developed to selectively leverage the pharmacology of this receptor. Specifically, type I PAMs increase the cholinergic activation of $\alpha7$ nAChRs without altering either the receptor's spatiotemporal features of synaptic transmission or the receptor's desensitization kinetics, whereas type II $\alpha7$ nAChR PAMs not only increase the duration of the open state of the receptor, leading to greater ion influx, but also decrease the time a receptor spends in a desensitized state. For further review of the mechanisms of the two distinctive types of $\alpha7$ nAChR PAMs, please see the work of King et al. (2018).

NS1738 is a type I $\alpha7$ nAChR PAM. In vitro, NS1738 has been found to neither displace nor alter radioligand binding to the nicotinic receptor agonist binding sites and did not produce a functional current at nAChRs. However, when NS1738 was combined with subthreshold doses of acetylcholine, a significant increase in peak currents in oocytes transfected with $\alpha7$ nAChRs was observed. NS1738 was determined to be a type I PAM, as the compound produced no significant change in the desensitization kinetics of $\alpha7$ nAChRs (Timmermann et al., 2007). More recent studies have examined the theoretical binding of NS1738 at $\alpha7$ nAChRs, with a computer model examining the molecular docking, molecular dynamics stimulation, and free energy calculation. In this study, it was found that NS1738 has three theoretical binding sites (Kuang et al., 2016). In a mouse in vivo study, this compound successfully blocked somatic behavioral signs of antagonist-precipitated nicotine withdrawal (Jackson et al., 2018). However, NS1738 did not block increased anxiety-related behaviors in the same mice (Jackson et al., 2018). Meanwhile, in the same study, the $\alpha7$ nAChR orthosteric agonist PNU282986 significantly reduced both the somatic signs and anxiety-related behavior associated with antagonist-precipitated nicotine withdrawal. Given wide interest in the $\alpha7$ nAChR as a therapeutic target, other type I $\alpha7$ nAChRs PAMs, such as the compound [N-(4-chlorophenyl)]-α-[(4-chlorophenyl)-aminomethylene]-3-methyl-5-isoxazoleacetamide (CCMI, also known as Compound 6) (Ng et al., 2007), and AVL-3288 (Bortz et al., 2016; Gee et al., 2017), have been developed but have not been investigated for their utility as pharmacotherapies in smoking cessation.

As opposed to the abovementioned type I PAM, type II $\alpha7$ nAChR PAMs both increase the duration of the open state of the receptor and decrease the time a receptor spends in a desensitized state. PNU-120596 was first discovered via a high-throughput screen and has been characterized in vitro as a type II $\alpha7$ nAChR PAM (Hurst et al., 2005; Barron et al., 2009). In mice, PNU-120596 enhanced the hypothermic effects of nicotine (Moerke et al., 2016). In a different study, this compound blocked some of the behavioral effects associated with nicotine withdrawal in mice (Jackson et al., 2018). However, PNU-120596 did not block increased anxiety-related behaviors in the same mice (Jackson et al., 2018). Additionally, PNU-120596 did not increase the substitution of subthreshold doses of nicotine to the

discriminative stimulus of 1 mg/kg nicotine in the mouse drug discrimination assay (Moerke et al., 2016). Other type II α7 nAChR PAMs, such as the compound 3a,4,5,9b-tetrahydro-4-(1-naphthalenyl)-3H-cyclopentan[c]quinoline-8-sulfonamide (TQS) (Grønlien et al., 2007; Thomsen and Mikkelsen, 2012), A-867744 (Faghih et al., 2009), TBS-345, TBS-346, TBS-516, TBS-546, and TBS-556 (Chatzidaki et al., 2015), have been developed but have not been investigated for their utility in preclinical models typically used for evaluating potential pharmacotherapies for tobacco use disorder.

The in vivo effects of other α7 nAChR PAMs have been examined. Specifically, RO5126946 acts selectively as a PAM at human α7 nAChRs, as it was found to increase acetylcholine-induced currents and postpone current decay (Sahdeo et al., 2014). However, the application of RO5126946 did not alter receptor desensitization kinetics. In vivo, this compound increased the effects of nicotine in a rat footshock model of memory (Sahdeo et al., 2014), demonstrating potential as a cognitive enhancer.

In addition to type I and type II α7 nAChR PAMs, another class of α7 nAChR compounds are dual orthosteric agonists and PAMs, also known as ago-PAMs. GAT107 is the enantiomer of 4BP-TQS, derived from the parent compound TQS described above, and acts as both an orthosteric agonist and PAM at α7 nAChRs (Thakur et al., 2013; Papke et al., 2014). This dual effect allows GAT107 to produce a long-lasting activation of the α7 nAChR (Papke et al., 2018), which is likely a critical mediator in its in vivo anti-inflammatory and antipathologic pain effects (Bagdas et al., 2016). Additionally, the compound B-973 has been characterized in vitro as a functional ago-PAM at α7 nAChRs (Post-Munson et al., 2017). However, it remains to be seen if RO5126946, GAT107, or B-973 have potential relevance as smoking-cessation pharmacotherapies. Meanwhile, the compound JNJ-39393406, classified as an α7 nAChR PAM, was recently investigated in humans as a possible smoking-cessation pharmacotherapy. Prior to human trials, Johnson & Johnson stated that this compound produced positive outcomes in preclinical (i.e., rat and mouse) studies typically used to test for cognitive impairment seen in schizophrenia (Winterer et al., 2013). However, it is notable that Johnson & Johnson did not report examining JNJ-39393406 in combination with nicotine in preclinical animal models that are commonly used to evaluate potential pharmacotherapies for tobacco use disorder. It was reported that this compound failed to reduce cigarette craving or total smoking and did not increase number of quit days in study participants. Furthermore, this compound did not meet study criteria, and Johnson & Johnson reported that they would not be moving forward with the development of this compound as a smoking-cessation pharmacotherapy (Perkins et al., 2018). The above-discussed α7 nAChR PAMs are summarized in Table 3.

Current pharmacotherapies associated with the best outcomes for smoking cessation (i.e., nicotinic agonists) are active at all subtypes of nAChR, albeit with differing affinities for the various receptor subtypes. Although it appears that $\alpha4\beta2$* nAChRs are important targets for smoking-cessation therapeutics based on preclinical data, the contribution of other nAChRs should not be discounted. Thus, using a more selective positive allosteric modulator could provide several advantages from a therapeutic standpoint. Greater selectivity, to the extent that only one receptor subtype mediates all effects targeted for smoking cessation, allows for less side effects resulting from actions at other receptor subtypes. Conversely, it may be that a polypharmacological approach (i.e., targeting multiple receptors or signaling pathways) may be the most advantageous approach for the development of new pharmacological treatments for smoking cessation. That relapse is relatively common even among individuals receiving nicotine replacement therapies (which are arguably the most effective pharmacotherapies currently available) may be one indication that polypharmacological approaches are a strategy worth pursuing.

B. Acetylcholinesterase Inhibitors

Identifying novel targets for smoking cessation and developing drugs that produce a desirable profile of action at these targets is a process that can take more than 10 years and an investment of several billion dollars before a new drug ever reaches the market. One way to circumvent this process is to consider repurposing drugs that have already been approved by the FDA and examining their potential to produce therapeutic effects outside of their current indications.

Acetylcholinesterase (AChE) inhibitors represent one such class of drugs, as three are currently approved by the FDA for the treatment of cognitive deficits associated with Alzheimer disease: donepezil, rivastigmine, and galantamine. AChE inhibitors produce their effects by preventing AChE, an endogenous enzyme that helps regulate cholinergic neurotransmission, from breaking down acetylcholine. In theory, this would lead to increases in receptor activation by endogenous acetylcholine and might produce nicotine-like effects. Early experiments that attempted to use this strategy in rats found that the nicotine discriminative stimulus could not be mimicked or potentiated by the AChE inhibitor physostigmine (Rosecrans and Meltzer, 1981; Pratt et al., 1983). However, when physostigmine was trained as a discriminative stimulus, although nicotine did not substitute, oxotremorine and arecoline, both mAChR agonists, did (Jung et al., 1988). Thus, AChE inhibitors were not studied further as potential treatments for smoking cessation.

Following FDA approval for Alzheimer disease, however, a small study of patients undergoing treatment of alcohol dependence indicated that galantamine was effective in reducing smoking in this population (Diehl et al., 2006), renewing interest in AChE inhibitors as pharmacotherapies for smoking cessation. Discrimination studies in rats (Giarola et al., 2011) and monkeys (Moerke and McMahon, 2019) provided evidence that donepezil and galantamine partially substituted for the nicotine discriminative stimulus, and several groups found that both donepezil and galantamine were effective at reducing nicotine self-administration in rats (Hopkins et al., 2012; Liu, 2013; Kimmey et al., 2014; Ashare et al., 2016). Results from preclinical studies using self-administration and drug discrimination assays are summarized in Table 1.

All three FDA-approved AChE inhibitors have been examined in smokers, and thus far galantamine has shown the most potential for smoking cessation. Rivastigmine, like galantamine, reduced tobacco consumption in alcohol-dependent individuals (Diehl et al., 2009) but had no effect on cigarette smoking in methamphetamine-dependent individuals (De la Garza and Yoon, 2011). Furthermore, donepezil did not effectively decrease cigarette smoking in a pilot study (Ashare et al., 2012). In comparison, only one clinical study using galantamine found no decrease in smoking among individuals with schizophrenia (Kelly et al., 2008). Several other clinical studies have shown that galantamine can attenuate cigarette craving and reduce smoking satisfaction in addition to reducing overall tobacco consumption (Sofuoglu et al., 2012; Ashare et al., 2016; MacLean et al., 2018).

One point of interest is that of the three AChE inhibitors approved for Alzheimer disease and discussed here, only galantamine has been shown to also have activity as a positive allosteric modulator of nAChRs (Maelicke et al., 2001; Farlow, 2003). However, recent studies suggest that galantamine does not functionally act at human $\alpha4\beta2^*$ or $\alpha7$ nAChRs as a PAM (Kowal et al., 2018). Thus, although it seems unlikely that action as a PAM is responsible for the relative success of galantamine to decrease smoking in some populations, it cannot be ruled out entirely. Clearly, much more work needs to be done both to evaluate AChE inhibitors in general as potential smoking-cessation pharmacotherapies as well as to determine if galantamine offers any benefits in comparison with existing pharmacotherapies for smoking cessation.

C. Psilocybin

Psychedelics, including psilocybin, were actively studied for their potential in treating substance use disorders from the 1950s into the early 1970s. Passage of the Controlled Substances Act in 1970, however, which classified psilocybin as a Schedule I compound, effectively halted any further development as a pharmacotherapy for decades.

Psilocybin is a prodrug that in vivo is rapidly metabolized to psilocin, an agonist at serotonin (5HT) receptors, and there is overwhelming evidence to support that 5HT2A is the most important 5HT receptor subtype for mediating the effects of classic psychedelics [see Nichols (2016) for review]. Recent study of psilocybin in the preclinical literature, however, suggests that 5HT2A receptors are only partially responsible for mediating the discriminative stimulus effects of psilocybin; in rats trained to discriminate 0.5 mg/kg psilocybin, M100907, a 5HT2A receptor antagonist, did not fully antagonize the discriminative stimulus effects of psilocybin up to doses that suppressed responding (Winter et al., 2007). In humans, however, there is abundant evidence that 5HT2A receptors mediate the subjective effects of psilocybin (Vollenweider et al., 1998; Kometer et al., 2012, 2013; Quednow et al., 2012).

A recent pilot study administered psilocybin over the course of a 15-week program of cognitive behavior therapy for current smokers. Participants received either two or three administrations of psilocybin under guided supervision in the treatment setting, and 12 of the 15 (80%) participants were confirmed abstinent by measure of urine cotinine levels at a 6-month follow-up; this is a notably higher percentage than is typically seen for smoking interventions, which is generally less than 35% (Johnson et al., 2014). Furthermore, follow-up at 2.5 years revealed that 60% of the participants had remained abstinent (Johnson et al., 2017). Such positive results should be interpreted with caution given the small sample size of the study and the fact that there was no comparison group; nonetheless, further trials are underway with psilocybin for not only tobacco use disorder but also alcohol use disorder, cocaine use disorder, depression, anorexia, and obsessive-compulsive disorder.

D. Hypocretin Receptor Antagonists

Hypocretins (Hcrt), also referred to as orexins, are neuropeptides produced within a small population of neurons located in the hypothalamus with projections to many other regions of the brain, including the limbic system (Peyron et al., 1998). Two G-protein coupled receptors, hcrtR1 and hcrtR2, have been identified, and the hypocretin system plays an important role in the homeostatic regulation of adaptive behaviors associated with arousal. Hypocretin signaling is necessary for the regulation of sleep cycles (Nishino et al., 2000; Thannickal et al., 2000) in addition to playing an important role in feeding (Haynes et al., 2000; Inutsuka et al., 2014) and mating (Muschamp et al., 2007) behaviors. Furthermore, hypocretin has also been implicated in behaviors of overconsumption, including substance use disorders (Barson and Leibowitz, 2017). For a current review on hypocretin receptors, please see Wang et al. (2018).

Accumulating preclinical evidence focuses on hypocretin receptor antagonists in reducing the reinforcing effects of nicotine. For example, both the hcrtR1 antagonist

SB-334867 as well as a dual hcrtR1/hcrtR2 antagonist, almorexant, dose-dependently reduced nicotine self-administration in rats (Hollander et al., 2008; LeSage et al., 2010). However, 2-SORA, a hcrtR2 selective antagonist, had no effect on nicotine self-administration in rats, suggesting hcrtR1-mediated activity was responsible for decreases in self-administration (Uslaner et al., 2014). In tests of nicotine reinstatement, on the other hand, both hcrtR1 and hcrtR2 have been implicated but different groups have reported results that make interpretation difficult. Specifically, one group has reported attenuation of cue-induced reinstatement of nicotine-seeking behavior with hcrtR1 antagonist SB-334867 but not hcrtR2 antagonist TCSOX229 (Plaza-Zabala et al., 2013), whereas a second group has reported that cue-induced reinstatement of nicotine-seeking behavior is blocked by hcrtR2 antagonist 2-SORA (Uslaner et al., 2014), and a third group has reported no effect on cue-induced reinstatement of nicotine-seeking behavior with dual hcrtR1/hcrtR2 antagonist TCS1102 (Khoo et al., 2017). Furthermore, SB-334867 did not block footshock-induced reinstatement of nicotine seeking (Plaza-Zabala et al., 2010), and 2-SORA did not block nicotine-induced reinstatement of nicotine-seeking behavior (Uslaner et al., 2014). Results from preclinical studies of hcrtR compounds in nicotine self-administration and nicotine discrimination assays are summarized in Table 1. Nonetheless, a clinical trial is currently planned to evaluate the effects of suvorexant, a dual hcrtR1/hcrtR2 antagonist currently approved for the treatment of insomnia, in treatment of tobacco use disorder.

VII. Conclusion

A major challenge in the development of pharmacotherapies for tobacco use disorder lies in the apparent probability that several different nAChR subtypes play important roles in the behavioral effects of nicotine. Furthermore, these receptor subtypes can be dynamically regulated by nicotine and other nAChR ligands through both orthosteric and allosteric action, which adds to the complexity of designing therapeutic interventions, including options with limited side effects. In the case of varenicline, it appears that polypharmacology at nAChR subtypes, with some differences from nicotine, including lower efficacy at some subtypes, is important for the compound's mechanisms of action. However, the limited therapeutic utility of varenicline, albeit at least equal to nicotine replacement, leaves room for improvement. Thus, in developing the next generation of pharmacotherapies for tobacco use disorder, it may be critical to design compounds that interact with more than one nAChR subtype and/or other receptor systems. Additionally, the pharmacokinetics of potential smoking-cessation pharmacotherapies, including a suitable long duration of action and bioavailability through the oral, nasal, and inhalation roues, are critical in ongoing development strategies. Most of the experimental compounds listed in the second half of this review were administered preclinically via a systemic injection, leaving other routes of administration unexplored thus far. We hope to have highlighted here the untapped therapeutic potential of nAChR allosteric modulators as smoking-cessation aids, which is a relatively new area of drug discovery. It may be that varying the route of administration may enhance the therapeutic window of a compound developed as a medication for smoking cessation. For example, one intriguing development in the battle against tobacco use disorder is ENDS. Work on the delivery of compounds in vapor form has only just begun, and there are other intriguing formulations and routes, including nasal mist. Although 55 years have passed since the first Surgeon General's report on tobacco in 1964, which officially linked lung cancer to cigarette smoking (U.S. Department of Health, Education, and Welfare, 1964), there is still much work to be done in the development of pharmacotherapies to treat tobacco use disorder.

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References

Albrecht BK, Berry V, Boezio AA, Cao L, Clarkin K, Guo W, Harmange JC, Hierl M, Huang L, Janosky B, et al. (2008) Discovery and optimization of substituted piperidines as potent, selective, CNS-penetrant alpha4beta2 nicotinic acetylcholine receptor potentiators. *Bioorg Med Chem Lett* **18**:5209–5212.

Albuquerque EX, Pereira EF, Castro NG, Alkondon M, Reinhardt S, Schröder H, and Maelicke A (1995) Nicotinic receptor function in the mammalian central nervous system. *Ann N Y Acad Sci* **757**:48–72.

Alkondon M, Pereira EF, Wonnacott S, and Albuquerque EX (1992) Blockade of nicotinic currents in hippocampal neurons defines methyllycaconitine as a potent and specific receptor antagonist. *Mol Pharmacol* **41**:802–808.

Alsharari SD, Freitas K, and Damaj MI (2013) Functional role of alpha7 nicotinic receptor in chronic neuropathic and inflammatory pain: studies in transgenic mice. *Biochem Pharmacol* **86**:1201–1207.

American Psychiatric Association (2013) *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed, American Psychiatric Association, Arlington, VA.

Anand R, Conroy WG, Schoepfer R, Whiting P, and Lindstrom J (1991) Neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes have a pentameric quaternary structure. *J Biol Chem* **266**:11192–11198.

Anderson DJ, Malysz J, Grønlien JH, El Kouhen R, Håkerud M, Wetterstrand C, Briggs CA, and Gopalakrishnan M (2009) Stimulation of dopamine release by nicotinic acetylcholine receptor ligands in rat brain slices correlates with the profile of high, but not low, sensitivity alpha4beta2 subunit combination. *Biochem Pharmacol* **78**:844–851.

Ando K and Yanagita T (1981) Cigarette smoking in rhesus monkeys. *Psychopharmacology (Berl)* **72**:117–127.

Andreasen JT, Olsen GM, Wiborg O, and Redrobe JP (2009) Antidepressant-like effects of nicotinic acetylcholine receptor antagonists, but not agonists, in the mouse forced swim and mouse tail suspension tests. *J Psychopharmacol* **23**: 797–804.

Anthenelli RM, Benowitz NL, West R, St Aubin L, McRae T, Lawrence D, Ascher J, Russ C, Krishen A, and Evins AE (2016) Neuropsychiatric safety and efficacy of varenicline, bupropion, and nicotine patch in smokers with and without psychiatric disorders (EAGLES): a double-blind, randomised, placebo-controlled clinical trial. *Lancet* **387**:2507–2520.

Armitage AK, Dollery CT, George CF, Houseman TH, Lewis PJ, and Turner DM (1975) Absorption and metabolism of nicotine from cigarettes. *BMJ* **4**:313–316.

Ashare RL, Kimmey BA, Rupprecht LE, Bowers ME, Hayes MR, and Schmidt HD (2016) Repeated administration of an acetylcholinesterase inhibitor attenuates nicotine taking in rats and smoking behavior in human smokers. *Transl Psychiatry* **6**:e713.

Ashare RL, Ray R, Lerman C, and Strasser AA (2012) Cognitive effects of the acetylcholinesterase inhibitor, donepezil, in healthy, non-treatment seeking smokers: a pilot feasibility study. *Drug Alcohol Depend* **126**:263–267.
Auerbach A (2010) The gating isomerization of neuromuscular acetylcholine receptors. *J Physiol* **588**:573–586.
Babb S, Malarcher A, Schauer G, Asman K, and Jamal A (2017) Quitting smoking among adults - United States, 2000-2015. *MMWR Morb Mortal Wkly Rep* **65**: 1457–1464.
Bagdas D, Alkhlaif Y, Jackson A, Carroll FI, Ditre JW, and Damaj MI (2018a) New insights on the effects of varenicline on nicotine reward, withdrawal and hyperalgesia in mice. *Neuropharmacology* **138**:72–79.
Bagdas D, Diester CM, Riley J, Carper M, Alkhlaif Y, AlOmari D, Alayoubi H, Poklis JL, and Damaj MI (2019) Assessing nicotine dependence using an oral nicotine free-choice paradigm in mice. *Neuropharmacology* **157**:107669.
Bagdas D, Gurun MS, Flood P, Papke RL, and Damaj MI (2018b) New insights on neuronal nicotinic acetylcholine receptors as targets for pain and inflammation: a focus on α7 nAChRs. *Curr Neuropharmacol* **16**:415–425.
Bagdas D, Wilkerson JL, Kulkarni A, Toma W, AlSharari S, Gul Z, Lichtman AH, Papke RL, Thakur GA, and Damaj MI (2016) The α7 nicotinic receptor dual allosteric agonist and positive allosteric modulator GAT107 reverses nociception in mouse models of inflammatory and neuropathic pain. *Br J Pharmacol* **173**: 2506–2520.
Baker TB, Piper ME, Stein JH, Smith SS, Bolt DM, Fraser DL, and Fiore MC (2016) Effects of nicotine patch vs varenicline vs combination nicotine replacement therapy on smoking cessation at 26 weeks: a randomized clinical trial. *JAMA* **315**: 371–379.
Balfour DJ (1994) Neural mechanisms underlying nicotine dependence. *Addiction* **89**: 1419–1423.
Banks ML and Negus SS (2017) Insights from preclinical choice models on treating drug addiction. *Trends Pharmacol Sci* **38**:181–194.
Barron SC, McLaughlin JT, See JA, Richards VL, and Rosenberg RL (2009) An allosteric modulator of alpha7 nicotinic receptors, N-(5-Chloro-2,4-dimethoxyphenyl)-N′-(5-methyl-3-isoxazolyl)-urea (PNU-120596), causes conformational changes in the extracellular ligand binding domain similar to those caused by acetylcholine. *Mol Pharmacol* **76**:253–263.
Barson JR and Leibowitz SF (2017) Orexin/hypocretin system: role in food and drug overconsumption. *Int Rev Neurobiol* **136**:199–237.
Bauco P and Wise RA (1994) Potentiation of lateral hypothalamic and midline mesencephalic brain stimulation reinforcement by nicotine: examination of repeated treatment. *J Pharmacol Exp Ther* **271**:294–301.
Bauer CT, Banks ML, Blough BE, and Negus SS (2013) Use of intracranial self-stimulation to evaluate abuse-related and abuse-limiting effects of monoamine releasers in rats. *Br J Pharmacol* **168**:850–862.
Benowitz NL (1990) Clinical pharmacology of inhaled drugs of abuse: implications in understanding nicotine dependence. *NIDA Res Monogr* **99**:12–29.
Benowitz NL (1996) Pharmacology of nicotine: addiction and therapeutics. *Annu Rev Pharmacol Toxicol* **36**:597–613.
Benowitz NL (2008) Neurobiology of nicotine addiction: implications for smoking cessation treatment. *Am J Med* **121** (4 Suppl 1):S3–S10.
Benowitz NL and Henningfield JE (1994) Establishing a nicotine threshold for addiction. The implications for tobacco regulation. *N Engl J Med* **331**:123–125.
Benowitz NL, Hukkanen J, and Jacob P III (2009) Nicotine chemistry, metabolism, kinetics and biomarkers. *Handb Exp Pharmacol* 29–60.
Benowitz NL and Jacob P III (1993) Nicotine and cotinine elimination pharmacokinetics in smokers and nonsmokers. *Clin Pharmacol Ther* **53**:316–323.
Benowitz NL and Jacob P III (1994) Metabolism of nicotine to cotinine studied by a dual stable isotope method. *Clin Pharmacol Ther* **56**:483–493.
Benowitz NL and Jacob P III (2001) Trans-3′-hydroxycotinine: disposition kinetics, effects and plasma levels during cigarette smoking. *Br J Clin Pharmacol* **51**:53–59.
Benowitz NL, Jacob P III, Olsson P, and Johansson CL (1992) Intravenous nicotine retards transdermal absorption of nicotine: evidence of blood flow--limited percutaneous absorption. *Clin Pharmacol Ther* **52**:223–230.
Benowitz NL, Jacob P III, and Perez-Stable E (1996) CYP2D6 phenotype and the metabolism of nicotine and cotinine. *Pharmacogenetics* **6**:239–242.
Benowitz NL, Lessov-Schlaggar CN, Swan GE, and Jacob P III (2006) Female sex and oral contraceptive use accelerate nicotine metabolism. *Clin Pharmacol Ther* **79**:480–488.
Benowitz NL, Pomerleau OF, Pomerleau CS, and Jacob P III (2003) Nicotine metabolite ratio as a predictor of cigarette consumption. *Nicotine Tob Res* **5**:621–624.
Benwell ME, Balfour DJ, and Anderson JM (1988) Evidence that tobacco smoking increases the density of (-)-[3H]nicotine binding sites in human brain. *J Neurochem* **50**:1243–1247.
Bergman J, Madras BK, Johnson SE, and Spealman RD (1989) Effects of cocaine and related drugs in nonhuman primates. III. Self-administration by squirrel monkeys. *J Pharmacol Exp Ther* **251**:150–155.
Bertrand D and Terry AV Jr. (2018) The wonderland of neuronal nicotinic acetylcholine receptors. *Biochem Pharmacol* **151**:214–225.
Besheer J, Palmatier MI, Metschke DM, and Bevins RA (2004) Nicotine as a signal for the presence or absence of sucrose reward: a Pavlovian drug appetitive conditioning preparation in rats. *Psychopharmacology (Berl)* **172**:108–117.
Bespalov A, Lebedev A, Panchenko G, and Zvartau E (1999) Effects of abused drugs on thresholds and breaking points of intracranial self-stimulation in rats. *Eur Neuropsychopharmacol* **9**:377–383.
Besson M, David V, Suarez S, Cormier A, Cazala P, Changeux JP, and Granon S (2006) Genetic dissociation of two behaviors associated with nicotine addiction: beta-2 containing nicotinic receptors are involved in nicotine reinforcement but not in withdrawal syndrome. *Psychopharmacology (Berl)* **187**:189–199.
Besson M, Guiducci S, Granon S, Guilloux JP, Guiard B, Repérant C, Faure P, Pons S, Cannazza G, Zoli M, et al. (2016) Alterations in alpha5* nicotinic acetylcholine receptors result in midbrain- and hippocampus-dependent behavioural and neural impairments. *Psychopharmacology (Berl)* **233**:3297–3314.
Bevins RA and Palmatier MI (2004) Extending the role of associative learning processes in nicotine addiction. *Behav Cogn Neurosci Rev* **3**:143–158.
Biala G, Staniak N, and Budzynska B (2010) Effects of varenicline and mecamylamine on the acquisition, expression, and reinstatement of nicotine-conditioned place preference by drug priming in rats. *Naunyn Schmiedebergs Arch Pharmacol* **381**:361–370.
Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Grucza RA, Xuei X, Saccone NL, Saccone SF, Bertelsen S, Fox L, et al. (2008) Variants in nicotinic receptors and risk for nicotine dependence. *Am J Psychiatry* **165**:1163–1171.
Bisaga A, Padilla M, Garawi F, Sullivan MA, and Haney M (2007) Effects of alternative reinforcer and craving on the choice to smoke cigarettes in the laboratory. *Hum Psychopharmacol* **22**:41–47.
Blitzer RD and Becker RE (1985) Characterization of the bupropion cue in the rat: lack of evidence for a dopaminergic mechanism. *Psychopharmacology (Berl)* **85**: 173–177.
Bonano JS, Glennon RA, De Felice LJ, Banks ML, and Negus SS (2014) Abuse-related and abuse-limiting effects of methcathinone and the synthetic "bath salts" cathinone analogs methylenedioxypyrovalerone (MDPV), methylone and mephedrone on intracranial self-stimulation in rats. *Psychopharmacology (Berl)* **231**: 199–207.
Bondarev ML, Bondareva TS, Young R, and Glennon RA (2003) Behavioral and biochemical investigations of bupropion metabolites. *Eur J Pharmacol* **474**: 85–93.
Bortz DM, Upton BA, Mikkelsen JD, and Bruno JP (2016) Positive allosteric modulators of the α7 nicotinic acetylcholine receptor potentiate glutamate release in the prefrontal cortex of freely-moving rats. *Neuropharmacology* **111**:78–91.
Breese CR, Marks MJ, Logel J, Adams CE, Sullivan B, Collins AC, and Leonard S (1997) Effect of smoking history on [3H]nicotine binding in human postmortem brain. *J Pharmacol Exp Ther* **282**:7–13.
Brioni JD, Kim DJ, and O'Neill AB (1996) Nicotine cue: lack of effect of the alpha 7 nicotinic receptor antagonist methyllycaconitine. *Eur J Pharmacol* **301**:1–5.
Broad LM, Zwart R, Pearson KH, Lee M, Wallace L, McPhie GI, Emkey R, Hollinshead SP, Dell CP, Baker SR, et al. (2006) Identification and pharmacological profile of a new class of selective nicotinic acetylcholine receptor potentiators. *J Pharmacol Exp Ther* **318**:1108–1117.
Brown RW, Collins AC, Lindstrom JM, and Whiteaker P (2007) Nicotinic alpha5 subunit deletion locally reduces high-affinity agonist activation without altering nicotinic receptor numbers. *J Neurochem* **103**:204–215.
Bruijnzeel AW and Gold MS (2005) The role of corticotropin-releasing factor-like peptides in cannabis, nicotine, and alcohol dependence. *Brain Res Brain Res Rev* **49**:505–528.
Bruijnzeel AW, Zislis G, Wilson C, and Gold MS (2007) Antagonism of CRF receptors prevents the deficit in brain reward function associated with precipitated nicotine withdrawal in rats. *Neuropsychopharmacology* **32**:955–963.
Brunzell DH, Boschen KE, Hendrick ES, Beardsley PM, and McIntosh JM (2010) Alpha-conotoxin MII-sensitive nicotinic acetylcholine receptors in the nucleus accumbens shell regulate progressive ratio responding maintained by nicotine. *Neuropsychopharmacology* **35**:665–673.
Cahill K, Lindson-Hawley N, Thomas KH, Fanshawe TR, and Lancaster T (2016) Nicotine receptor partial agonists for smoking cessation. *Cochrane Database Syst Rev* **5**:CD006103.
Cahill K, Stevens S, and Lancaster T (2014) Pharmacological treatments for smoking cessation. *JAMA* **311**:193–194.
Cahill K, Stevens S, Perera R, and Lancaster T (2013) Pharmacological interventions for smoking cessation: an overview and network meta-analysis. *Cochrane Database Syst Rev* **5**:CD009329.
Cairns NJ and Wonnacott S (1988) [3H](-)nicotine binding sites in fetal human brain. *Brain Res* **475**:1–7.
Caponnetto P, Campagna D, Cibella F, Morjaria JB, Caruso M, Russo C, and Polosa R (2013) EffiCiency and Safety of an eLectronic cigAreTte (ECLAT) as tobacco cigarettes substitute: a prospective 12-month randomized control design study. *PLoS One* **8**:e66317.
Carlezon WA Jr. and Chartoff EH (2007) Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. *Nat Protoc* **2**:2987–2995.
Cassidy RN, Tidey JW, Kahler CW, Wray TB, and Colby SM (2015) Increasing the value of an alternative monetary reinforcer reduces cigarette choice in adolescents. *Nicotine Tob Res* **17**:1449–1455.
Chaiton M, Diemert L, Cohen JE, Bondy SJ, Selby P, Philipneri A, and Schwartz R (2016) Estimating the number of quit attempts it takes to quit smoking successfully in a longitudinal cohort of smokers. *BMJ Open* **6**:e011045.
Changeux JP and Edelstein SJ (1998) Allosteric receptors after 30 years. *Neuron* **21**: 959–980.
Changeux JP, Thiéry J, Tung Y, and Kittel C (1967) On the cooperativity of biological membranes. *Proc Natl Acad Sci USA* **57**:335–341.
Chatzidaki A, D'Oyley JM, Gill-Thind JK, Sheppard TD, and Millar NS (2015) The influence of allosteric modulators and transmembrane mutations on desensitisation and activation of α7 nicotinic acetylcholine receptors. *Neuropharmacology* **97**: 75–85.
Clarke PB and Kumar R (1983a) Characterization of the locomotor stimulant action of nicotine in tolerant rats. *Br J Pharmacol* **80**:587–594.
Clarke PB and Kumar R (1983b) The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. *Br J Pharmacol* **78**:329–337.
Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, Sands SB, Davis TI, Lebel LA, Fox CB, et al. (2005) Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation. *J Med Chem* **48**:3474–3477.
Cohen A, Koob GF, and George O (2012) Robust escalation of nicotine intake with extended access to nicotine self-administration and intermittent periods of abstinence. *Neuropsychopharmacology* **37**:2153–2160.
Collins RJ, Weeks JR, Cooper MM, Good PI, and Russell RR (1984) Prediction of abuse liability of drugs using IV self-administration by rats. *Psychopharmacology (Berl)* **82**:6–13.

Cooper E, Couturier S, and Ballivet M (1991) Pentameric structure and subunit stoichiometry of a neuronal nicotinic acetylcholine receptor. *Nature* **350**:235–238.
Cooper TB, Suckow RF, and Glassman A (1984) Determination of bupropion and its major basic metabolites in plasma by liquid chromatography with dual-wavelength ultraviolet detection. *J Pharm Sci* **73**:1104–1107.
Corne SJ and Edge ND (1958) Pharmacological properties of pempidine (1:2:2:6:6-pentamethylpiperidine), a new ganglion-blocking compound. *Br J Pharmacol Chemother* **13**:339–349.
Corrigall WA and Coen KM (1989) Nicotine maintains robust self-administration in rats on a limited-access schedule. *Psychopharmacology (Berl)* **99**:473–478.
Corrigall WA, Franklin KB, Coen KM, and Clarke PB (1992) The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacology (Berl)* **107**:285–289.
Court JA, Lloyd S, Thomas N, Piggott MA, Marshall EF, Morris CM, Lamb H, Perry RH, Johnson M, and Perry EK (1998) Dopamine and nicotinic receptor binding and the levels of dopamine and homovanillic acid in human brain related to tobacco use. *Neuroscience* **87**:63–78.
Creese I and Sibley DR (1981) Receptor adaptations to centrally acting drugs. *Annu Rev Pharmacol Toxicol* **21**:357–391.
Cunningham CS, Javors MA, and McMahon LR (2012) Pharmacologic characterization of a nicotine-discriminative stimulus in rhesus monkeys. *J Pharmacol Exp Ther* **341**:840–849.
Cunningham CS and McMahon LR (2013) Multiple nicotine training doses in mice as a basis for differentiating the effects of smoking cessation aids. *Psychopharmacology (Berl)* **228**:321–333.
Cunningham CS, Moerke MJ, Javors MA, Carroll FI, and McMahon LR (2016) Attenuated nicotine-like effects of varenicline but not other nicotinic ACh receptor agonists in monkeys receiving nicotine daily. *Br J Pharmacol* **173**:3454–3466.
Cunningham CS, Moerke MJ, and McMahon LR (2014) The discriminative stimulus effects of mecamylamine in nicotine-treated and untreated rhesus monkeys. *Behav Pharmacol* **25**:296–305.
Cunningham CS, Moerke MJ, and McMahon LR (2019) Discriminative stimulus effects of mecamylamine and nicotine in rhesus monkeys: central and peripheral mechanisms. *Pharmacol Biochem Behav* **179**:27–33.
Dale LC, Hurt RD, Offord KP, Lawson GM, Croghan IT, and Schroeder DR (1995) High-dose nicotine patch therapy. Percentage of replacement and smoking cessation. *JAMA* **274**:1353–1358.
Damaj MI, Grabus SD, Navarro HA, Vann RE, Warner JA, King LS, Wiley JL, Blough BE, Lukas RJ, and Carroll FI (2010) Effects of hydroxymetabolites of bupropion on nicotine dependence behavior in mice. *J Pharmacol Exp Ther* **334**:1087–1095.
Dani JA and Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol* **47**:699–729.
Dani JA and De Biasi M (2001) Cellular mechanisms of nicotine addiction. *Pharmacol Biochem Behav* **70**:439–446.
De Biasi M and Salas R (2008) Influence of neuronal nicotinic receptors over nicotine addiction and withdrawal. *Exp Biol Med (Maywood)* **233**:917–929.
De la Garza R II and Yoon JH (2011) Evaluation of the effects of rivastigmine on cigarette smoking by methamphetamine-dependent volunteers. *Prog Neuropsychopharmacol Biol Psychiatry* **35**:1827–1830.
de Moura FB and McMahon LR (2017) The contribution of α4β2 and non-α4β2 nicotinic acetylcholine receptors to the discriminative stimulus effects of nicotine and varenicline in mice. *Psychopharmacology (Berl)* **234**:781–792.
Dempsey D, Tutka P, Jacob P III, Allen F, Schoedel K, Tyndale RF, and Benowitz NL (2004) Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clin Pharmacol Ther* **76**:64–72.
Deneau G and Inoki R (1967) Nicotine self-administration in monkeys. *Ann N Y Acad Sci* **142**:277–279.
Desai RI, Barber DJ, and Terry P (1999) Asymmetric generalization between the discriminative stimulus effects of nicotine and cocaine. *Behav Pharmacol* **10**:647–656.
Desai RI, Barber DJ, and Terry P (2003) Dopaminergic and cholinergic involvement in the discriminative stimulus effects of nicotine and cocaine in rats. *Psychopharmacology (Berl)* **167**:335–343.
Diehl A, Nakovics H, Croissant B, Smolka MN, Batra A, and Mann K (2006) Galantamine reduces smoking in alcohol-dependent patients: a randomized, placebo-controlled trial. *Int J Clin Pharmacol Ther* **44**:614–622.
Diehl A, Nakovics H, Mutschler J, Hermann D, and Kiefer F (2009) Rivastigmine reduces tobacco craving in alcohol-dependent smokers. *Pharmacopsychiatry* **42**:89–94.
Donny EC, Caggiula AR, Knopf S, and Brown C (1995) Nicotine self-administration in rats. *Psychopharmacology (Berl)* **122**:390–394.
Donny EC, Caggiula AR, Mielke MM, Booth S, Gharib MA, Hoffman A, Maldovan V, Shupenko C, and McCallum SE (1999) Nicotine self-administration in rats on a progressive ratio schedule of reinforcement. *Psychopharmacology (Berl)* **147**:135–142.
Donny EC, Denlinger RL, Tidey JW, Koopmeiners JS, Benowitz NL, Vandrey RG, al'Absi M, Carmella SG, Cinciripini PM, Dermody SS, et al. (2015) Randomized trial of reduced-nicotine standards for cigarettes. *N Engl J Med* **373**:1340–1349.
Donovick PJ, Burright RG, and Zuromski E (1970) Localization of quinine aversion within the septum, habenula, and interpeduncular nucleus of the rat. *J Comp Physiol Psychol* **71**:376–383.
Dwoskin LP, Smith AM, Wooters TE, Zhang Z, Crooks PA, and Bardo MT (2009) Nicotinic receptor-based therapeutics and candidates for smoking cessation. *Biochem Pharmacol* **78**:732–743.
Egea J, Buendia I, Parada E, Navarro E, León R, and Lopez MG (2015) Anti-inflammatory role of microglial alpha7 nAChRs and its role in neuroprotection. *Biochem Pharmacol* **97**:463–472.
Eglen RM (2005) Muscarinic receptor subtype pharmacology and physiology. *Prog Med Chem* **43**:105–136.
Epping-Jordan MP, Watkins SS, Koob GF, and Markou A (1998) Dramatic decreases in brain reward function during nicotine withdrawal. *Nature* **393**:76–79.
Etter JF and Bullen C (2011) Saliva cotinine levels in users of electronic cigarettes. *Eur Respir J* **38**:1219–1220.
Eugster CH, Müller GF, and Good R (1965) [The active ingredients from Amanita muscaria: ibotenic acid and muscazone]. *Tetrahedron Lett* **23**:1813–1815.
Exley R, McIntosh JM, Marks MJ, Maskos U, and Cragg SJ (2012) Striatal α5 nicotinic receptor subunit regulates dopamine transmission in dorsal striatum. *J Neurosci* **32**:2352–2356.
Faessel H, Ravva P, and Williams K (2009) Pharmacokinetics, safety, and tolerability of varenicline in healthy adolescent smokers: a multicenter, randomized, double-blind, placebo-controlled, parallel-group study. *Clin Ther* **31**:177–189.
Faghih R, Gopalakrishnan SM, Gronlien JH, Malysz J, Briggs CA, Wetterstrand C, Ween H, Curtis MP, Sarris KA, Gfesser GA, et al. (2009) Discovery of 4-(5-(4-chlorophenyl)-2-methyl-3-propionyl-1H-pyrrol-1-yl)benzenesulfonamide (A-867744) as a novel positive allosteric modulator of the alpha7 nicotinic acetylcholine receptor. *J Med Chem* **52**:3377–3384.
Fant RV, Schuh KJ, and Stitzer ML (1995) Response to smoking as a function of prior smoking amounts. *Psychopharmacology (Berl)* **119**:385–390.
Farlow MR (2003) Clinical pharmacokinetics of galantamine. *Clin Pharmacokinet* **42**:1383–1392.
Farsalinos KE, Romagna G, Tsiapras D, Kyrzopoulos S, and Voudris V (2013) Evaluating nicotine levels selection and patterns of electronic cigarette use in a group of "vapers" who had achieved complete substitution of smoking. *Subst Abuse* **7**:139–146.
Farsalinos KE, Spyrou A, Tsimopoulou K, Stefopoulos C, Romagna G, and Voudris V (2014) Nicotine absorption from electronic cigarette use: comparison between first and new-generation devices. *Sci Rep* **4**:4133.
Fenster CP, Whitworth TL, Sheffield EB, Quick MW, and Lester RA (1999) Upregulation of surface alpha4beta2 nicotinic receptors is initiated by receptor desensitization after chronic exposure to nicotine. *J Neurosci* **19**:4804–4814.
Flores CM, Dávila-García MI, Ulrich YM, and Kellar KJ (1997) Differential regulation of neuronal nicotinic receptor binding sites following chronic nicotine administration. *J Neurochem* **69**:2216–2219.
Fowler CD, Lu Q, Johnson PM, Marks MJ, and Kenny PJ (2011) Habenular α5 nicotinic receptor subunit signalling controls nicotine intake. *Nature* **471**:597–601.
Fowler CD, Tuesta L, and Kenny PJ (2013) Role of α5* nicotinic acetylcholine receptors in the effects of acute and chronic nicotine treatment on brain reward function in mice. *Psychopharmacology (Berl)*, doi: 10.1007/s00213-013-3235-1 [published ahead of print].
Fredriksson R, Lagerström MC, Lundin LG, and Schiöth HB (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol* **63**:1256–1272.
Freitas K, Carroll FI, and Negus SS (2016) Comparison of effects produced by nicotine and the α4β2-selective agonist 5-I-A-85380 on intracranial self-stimulation in rats. *Exp Clin Psychopharmacol* **24**:65–75.
Freitas KC, Carroll FI, and Negus SS (2015) Effects of nicotinic acetylcholine receptor agonists in assays of acute pain-stimulated and pain-depressed behaviors in rats. *J Pharmacol Exp Ther* **355**:341–350.
Fudala PJ, Teoh KW, and Iwamoto ET (1985) Pharmacologic characterization of nicotine-induced conditioned place preference. *Pharmacol Biochem Behav* **22**:237–241.
Gamaleddin I, Guranda M, Scherma M, Fratta W, Makriyannis A, Vadivel SK, Goldberg SR, and Le Foll B (2013) AM404 attenuates reinstatement of nicotine seeking induced by nicotine-associated cues and nicotine priming but does not affect nicotine- and food-taking. *J Psychopharmacol* **27**:564–571.
Garcha HS and Stolerman IP (1993) Discriminative stimulus effects of the nicotine antagonist mecamylamine in rats. *J Psychopharmacol* **7** (1 Suppl):43–51.
Garcia KL, Lê AD, and Tyndale RF (2014) Effect of food training and training dose on nicotine self-administration in rats. *Behav Brain Res* **274**:10–18.
GBD 2017 Risk Factor Collaborators (2018) Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **392**:1923–1994.
Gee KW, Olincy A, Kanner R, Johnson L, Hogenkamp D, Harris J, Tran M, Edmonds SA, Sauer W, Yoshimura R, et al. (2017) First in human trial of a type I positive allosteric modulator of alpha7-nicotinic acetylcholine receptors: pharmacokinetics, safety, and evidence for neurocognitive effect of AVL-3288. *J Psychopharmacol* **31**:434–441.
George O, Ghozland S, Azar MR, Cottone P, Zorrilla EP, Parsons LH, O'Dell LE, Richardson HN, and Koob GF (2007) CRF-CRF1 system activation mediates withdrawal-induced increases in nicotine self-administration in nicotine-dependent rats. *Proc Natl Acad Sci USA* **104**:17198–17203.
George O, Grieder TE, Cole M, and Koob GF (2010) Exposure to chronic intermittent nicotine vapor induces nicotine dependence. *Pharmacol Biochem Behav* **96**:104–107.
George O, Lloyd A, Carroll FI, Damaj MI, and Koob GF (2011) Varenicline blocks nicotine intake in rats with extended access to nicotine self-administration. *Psychopharmacology (Berl)* **213**:715–722.
Giarola A, Auber A, and Chiamulera C (2011) Acetylcholinesterase inhibitors partially generalize to nicotine discriminative stimulus effect in rats. *Behav Pharmacol* **22**:1–6.
Gilpin NW, Whitaker AM, Baynes B, Abdel AY, Weil MT, and George O (2014) Nicotine vapor inhalation escalates nicotine self-administration. *Addict Biol* **19**:587–592.
Goldberg SR, Spealman RD, and Goldberg DM (1981) Persistent behavior at high rates maintained by intravenous self-administration of nicotine. *Science* **214**:573–575.
Gommans J, Stolerman IP, and Shoaib M (2000) Antagonism of the discriminative and aversive stimulus properties of nicotine in C57BL/6J mice. *Neuropharmacology* **39**:2840–2847.

Gonzales D, Rennard SI, Nides M, Oncken C, Azoulay S, Billing CB, Watsky EJ, Gong J, Williams KE, and Reeves KR; Varenicline Phase 3 Study Group (2006) Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. *JAMA* **296**:47–55.

Goodwin AK, Hiranita T, and Paule MG (2015) The reinforcing effects of nicotine in humans and nonhuman primates: a review of intravenous self-administration evidence and future directions for research. *Nicotine Tob Res* **17**:1297–1310.

Gotti C and Clementi F (2004) Neuronal nicotinic receptors: from structure to pathology. *Prog Neurobiol* **74**:363–396.

Gotti C, Guiducci S, Tedesco V, Corbioli S, Zanetti L, Moretti M, Zanardi A, Rimondini R, Mugnaini M, Clementi F, et al. (2010) Nicotinic acetylcholine receptors in the mesolimbic pathway: primary role of ventral tegmental area alpha6beta2* receptors in mediating systemic nicotine effects on dopamine release, locomotion, and reinforcement. *J Neurosci* **30**:5311–5325.

Gourlay SG and Benowitz NL (1997) Arteriovenous differences in plasma concentration of nicotine and catecholamines and related cardiovascular effects after smoking, nicotine nasal spray, and intravenous nicotine. *Clin Pharmacol Ther* **62**: 453–463.

Green RM and Lawyer SR (2014) Steeper delay and probability discounting of potentially real versus hypothetical cigarettes (but not money) among smokers. *Behav Processes* **108**:50–56.

Griffith JD, Carranza J, Griffith C, and Miller LL (1983) Bupropion: clinical assay for amphetamine-like abuse potential. *J Clin Psychiatry* **44**:206–208.

Griffiths R, Brady J, and Bradford L (1979) Predicting the abuse liability of drugs with animal drug self-administration procedures: psychomotor stimulants and hallucinogens. *Adv Behav Pharmacol* **2**:163–208.

Grønlien JH, Håkerud M, Ween H, Thorin-Hagene K, Briggs CA, Gopalakrishnan M, and Malysz J (2007) Distinct profiles of alpha7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes. *Mol Pharmacol* **72**:715–724.

Grupe M, Jensen AA, Ahring PK, Christensen JK, and Grunnet M (2013) Unravelling the mechanism of action of NS9283, a positive allosteric modulator of (α4)3(β2) 2 nicotinic ACh receptors. *Br J Pharmacol* **168**:2000–2010.

Güzey C, Norström A, and Spigset O (2002) Change from the CYP2D6 extensive metabolizer to the poor metabolizer phenotype during treatment with bupropion. *Ther Drug Monit* **24**:436–437.

Haikala H and Ahtee L (1988) Antagonism of the nicotine-induced changes of the striatal dopamine metabolism in mice by mecamylamine and pempidine. *Naunyn Schmiedebergs Arch Pharmacol* **338**:169–173.

Hajek P, Jackson P, and Belcher M (1988) Long-term use of nicotine chewing gum. Occurrence, determinants, and effect on weight gain. *JAMA* **260**:1593–1596.

Hajek P, West R, Foulds J, Nilsson F, Burrows S, and Meadow A (1999) Randomized comparative trial of nicotine polacrilex, a transdermal patch, nasal spray, and an inhaler. *Arch Intern Med* **159**:2033–2038.

Hammond DK, Bjercke RJ, Langone JJ, and Strobel HW (1991) Metabolism of nicotine by rat liver cytochromes P-450. Assessment utilizing monoclonal antibodies to nicotine and cotinine. *Drug Metab Dispos* **19**:804–808.

Hamouda AK, Deba F, Wang ZJ, and Cohen JB (2016) Photolabeling a nicotinic acetylcholine receptor (nAChR) with an (α4)3(β2)2 nAChR-selective positive allosteric modulator. *Mol Pharmacol* **89**:575–584.

Hamouda AK, Jackson A, Bagdas D, and Imad Damaj M (2018) Reversal of nicotine withdrawal signs through positive allosteric modulation of α4β2 nicotinic acetylcholine receptors in male mice. *Nicotine Tob Res* **20**:903–907.

Hamouda AK, Wang ZJ, Stewart DS, Jain AD, Glennon RA, and Cohen JB (2015) Desformylflustrabromine (dFBr) and [3H]dFBr-labeled binding sites in a nicotinic acetylcholine receptor. *Mol Pharmacol* **88**:1–11.

Harkness PC and Millar NS (2002) Changes in conformation and subcellular distribution of alpha4beta2 nicotinic acetylcholine receptors revealed by chronic nicotine treatment and expression of subunit chimeras. *J Neurosci* **22**: 10172–10181.

Harrison-Woolrych M and Ashton J (2011) Psychiatric adverse events associated with varenicline: an intensive postmarketing prospective cohort study in New Zealand. *Drug Saf* **34**:763–772.

Hatchell PC and Collins AC (1977) Influences of genotype and sex on behavioral tolerance to nicotine in mice. *Pharmacol Biochem Behav* **6**:25–30.

Hatsukami DK, Kotlyar M, Hertsgaard LA, Zhang Y, Carmella SG, Jensen JA, Allen SS, Shields PG, Murphy SE, Stepanov I, et al. (2010) Reduced nicotine content cigarettes: effects on toxicant exposure, dependence and cessation. *Addiction* **105**: 343–355.

Haynes AC, Jackson B, Chapman H, Tadayyon M, Johns A, Porter RA, and Arch JR (2000) A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul Pept* **96**:45–51.

Hazell P, Peterson DW, and Laverty R (1978) Brief communication. Inability of hexamethonium to block the discriminative stimulus (SD) property of nicotine. *Pharmacol Biochem Behav* **9**:137–140.

Hecht SS, Hochalter JB, Villalta PW, and Murphy SE (2000) 2′-Hydroxylation of nicotine by cytochrome P450 2A6 and human liver microsomes: formation of a lung carcinogen precursor. *Proc Natl Acad Sci USA* **97**:12493–12497.

Henderson BJ, Pavlovicz RE, Allen JD, González-Cestari TF, Orac CM, Bonnell AB, Zhu MX, Boyd RT, Li C, Bergmeier SC, et al. (2010) Negative allosteric modulators that target human alpha4beta2 neuronal nicotinic receptors. *J Pharmacol Exp Ther* **334**:761–774.

Henningfield JE and Goldberg SR (1983) Control of behavior by intravenous nicotine injections in human subjects. *Pharmacol Biochem Behav* **19**:1021–1026.

Henningfield JE and Keenan RM (1993) Nicotine delivery kinetics and abuse liability. *J Consult Clin Psychol* **61**:743–750.

Henningfield JE, Miyasato K, and Jasinski DR (1983) Cigarette smokers self-administer intravenous nicotine. *Pharmacol Biochem Behav* **19**:887–890.

Henningfield JE, Miyasato K, and Jasinski DR (1985) Abuse liability and pharmacodynamic characteristics of intravenous and inhaled nicotine. *J Pharmacol Exp Ther* **234**:1–12.

Henningfield JE, Smith TT, Kleykamp BA, Fant RV, and Donny EC (2016) Nicotine self-administration research: the legacy of Steven R. Goldberg and implications for regulation, health policy, and research. *Psychopharmacology (Berl)* **233**:3829–3848.

Hollander JA, Lu Q, Cameron MD, Kamenecka TM, and Kenny PJ (2008) Insular hypocretin transmission regulates nicotine reward. *Proc Natl Acad Sci USA* **105**: 19480–19485.

Hopkins TJ, Rupprecht LE, Hayes MR, Blendy JA, and Schmidt HD (2012) Galantamine, an acetylcholinesterase inhibitor and positive allosteric modulator of nicotinic acetylcholine receptors, attenuates nicotine taking and seeking in rats. *Neuropsychopharmacology* **37**:2310–2321.

Hubbard JE and Gohd RS (1975) Tolerance development to the arousal effects of nicotine. *Pharmacol Biochem Behav* **3**:471–476.

Hughes JR (2006) Clinical significance of tobacco withdrawal. *Nicotine Tob Res* **8**: 153–156.

Hughes JR (2007) Effects of abstinence from tobacco: valid symptoms and time course. *Nicotine Tob Res* **9**:315–327.

Hughes JR, Gust SW, Keenan R, Fenwick JW, Skoog K, and Higgins ST (1991) Long-term use of nicotine vs placebo gum. *Arch Intern Med* **151**:1993–1998.

Hughes JR, Keely J, and Naud S (2004) Shape of the relapse curve and long-term abstinence among untreated smokers. *Addiction* **99**:29–38.

Hughes JR, Stead LF, Hartmann-Boyce J, Cahill K, and Lancaster T (2014) Antidepressants for smoking cessation. *Cochrane Database Syst Rev* CD000031.

Hukkanen J, Jacob P III, and Benowitz NL (2005) Metabolism and disposition kinetics of nicotine. *Pharmacol Rev* **57**:79–115.

Hulme EC, Birdsall NJ, and Buckley NJ (1990) Muscarinic receptor subtypes. *Annu Rev Pharmacol Toxicol* **30**:633–673.

Hurst RS, Hajós M, Raggenbass M, Wall TM, Higdon NR, Lawson JA, Rutherford-Root KL, Berkenpas MB, Hoffmann WE, Piotrowski DW, et al. (2005) A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. *J Neurosci* **25**:4396–4405.

Hurt RD, Dale LC, Croghan GA, Croghan IT, Gomez-Dahl LC, and Offord KP (1998) Nicotine nasal spray for smoking cessation: pattern of use, side effects, relief of withdrawal symptoms, and cotinine levels. *Mayo Clin Proc* **73**:118–125.

Hurt RD, Offord KP, Lauger GG, Marusić Z, Fagerström KO, Enright PL, and Scanlon PD (1995) Cessation of long-term nicotine gum use--a prospective, randomized trial. *Addiction* **90**:407–413.

Huston-Lyons D and Kornetsky C (1992) Effects of nicotine on the threshold for rewarding brain stimulation in rats. *Pharmacol Biochem Behav* **41**:755–759.

Huynh C, Fam J, Ahmed SH, and Clemens KJ (2017) Rats quit nicotine for a sweet reward following an extensive history of nicotine use. *Addict Biol* **22**:142–151.

Igari M, Alexander JC, Ji Y, Qi X, Papke RL, and Bruijnzeel AW (2014) Varenicline and cytisine diminish the dysphoric-like state associated with spontaneous nicotine withdrawal in rats. *Neuropsychopharmacology* **39**:455–465.

Ikemoto S, Qin M, and Liu ZH (2006) Primary reinforcing effects of nicotine are triggered from multiple regions both inside and outside the ventral tegmental area. *J Neurosci* **26**:723–730.

Inutsuka A, Inui A, Tabuchi S, Tsunematsu T, Lazarus M, and Yamanaka A (2014) Concurrent and robust regulation of feeding behaviors and metabolism by orexin neurons. *Neuropharmacology* **85**:451–460.

Jackson A, Papke RL, and Damaj MI (2018) Pharmacological modulation of the α7 nicotinic acetylcholine receptor in a mouse model of mecamylamine-precipitated nicotine withdrawal. *Psychopharmacology (Berl)* **235**:1897–1905.

Jackson KJ, Marks MJ, Vann RE, Chen X, Gamage TF, Warner JA, and Damaj MI (2010) Role of alpha5 nicotinic acetylcholine receptors in pharmacological and behavioral effects of nicotine in mice. *J Pharmacol Exp Ther* **334**:137–146.

Jackson KJ, McIntosh JM, Brunzell DH, Sanjakdar SS, and Damaj MI (2009) The role of alpha6-containing nicotinic acetylcholine receptors in nicotine reward and withdrawal. *J Pharmacol Exp Ther* **331**:547–554.

Jefferson JW, Pradko JF, and Muir KT (2005) Bupropion for major depressive disorder: pharmacokinetic and formulation considerations. *Clin Ther* **27**:1685–1695.

Jenner P, Gorrod JW, and Beckett AH (1973) Species variation in the metabolism of R-(+)- and S-(-)-nicotine by alpha-C- and N-oxidation in vitro. *Xenobiotica* **3**:573–580.

Jiménez-Ruiz CA, Barrios M, Peña S, Cicero A, Mayayo M, Cristóbal M, and Perera L (2013) Increasing the dose of varenicline in patients who do not respond to the standard dose. *Mayo Clin Proc* **88**:1443–1445.

Johnson MW and Bickel WK (2003) The behavioral economics of cigarette smoking: the concurrent presence of a substitute and an independent reinforcer. *Behav Pharmacol* **14**:137–144.

Johnson MW, Garcia-Romeu A, Cosimano MP, and Griffiths RR (2014) Pilot study of the 5-HT2AR agonist psilocybin in the treatment of tobacco addiction. *J Psychopharmacol* **28**:983–992.

Johnson MW, Garcia-Romeu A, and Griffiths RR (2017) Long-term follow-up of psilocybin-facilitated smoking cessation. *Am J Drug Alcohol Abuse* **43**:55–60.

Johnson PM, Hollander JA, and Kenny PJ (2008) Decreased brain reward function during nicotine withdrawal in C57BL6 mice: evidence from intracranial self-stimulation (ICSS) studies. *Pharmacol Biochem Behav* **90**:409–415.

Johnson RE, Hollis JF, Stevens VJ, and Woodson GT (1991) Patterns of nicotine gum use in a health maintenance organization. *DICP* **25**:730–735.

Jones CN, Howard JL, and McBennett ST (1980) Stimulus properties of antidepressants in the rat. *Psychopharmacology (Berl)* **67**:111–118.

Jorenby DE, Smith SS, Fiore MC, Hurt RD, Offord KP, Croghan IT, Hays JT, Lewis SF, and Baker TB (1995) Varying nicotine patch dose and type of smoking cessation counseling. *JAMA* **274**:1347–1352.

Jung M, Perio A, Worms P, and Soubrie P (1988) Pharmacological characterization of the physostigmine stimulus in rats. *Psychopharmacology (Berl)* **95**:553–555.

Jutkiewicz EM, Brooks EA, Kynaston AD, Rice KC, and Woods JH (2011) Patterns of nicotinic receptor antagonism: nicotine discrimination studies. *J Pharmacol Exp Ther* **339**:194–202.

Kaiser S and Wonnacott S (2000) alpha-bungarotoxin-sensitive nicotinic receptors indirectly modulate [(3)H]dopamine release in rat striatal slices via glutamate release. *Mol Pharmacol* **58**:312–318.

Kalivas PW, Lalumiere RT, Knackstedt L, and Shen H (2009) Glutamate transmission in addiction. *Neuropharmacology* **56** (Suppl 1):169–173.
Kamien JB and Woolverton WL (1989) A pharmacological analysis of the discriminative stimulus properties of d-amphetamine in rhesus monkeys. *J Pharmacol Exp Ther* **248**:938–946.
Kasliwal R, Wilton LV, and Shakir SA (2009) Safety and drug utilization profile of varenicline as used in general practice in England: interim results from a prescription-event monitoring study. *Drug Saf* **32**:499–507.
Kauer JA and Malenka RC (2007) Synaptic plasticity and addiction. *Nat Rev Neurosci* **8**:844–858.
Ke L, Eisenhour CM, Bencherif M, and Lukas RJ (1998) Effects of chronic nicotine treatment on expression of diverse nicotinic acetylcholine receptor subtypes. I. Dose- and time-dependent effects of nicotine treatment. *J Pharmacol Exp Ther* **286**:825–840.
Keenan A and Johnson FN (1972) Development of behavioural tolerance to nicotine in the rat. *Experientia* **28**:428–429.
Kelly DL, McMahon RP, Weiner E, Boggs DL, Dickinson D, Conley RR, and Buchanan RW (2008) Lack of beneficial galantamine effect for smoking behavior: a double-blind randomized trial in people with schizophrenia. *Schizophr Res* **103**:161–168.
Kenny PJ, Chartoff E, Roberto M, Carlezon WA Jr., and Markou A (2009) NMDA receptors regulate nicotine-enhanced brain reward function and intravenous nicotine self-administration: role of the ventral tegmental area and central nucleus of the amygdala. *Neuropsychopharmacology* **34**:266–281.
Khoo SY, McNally GP, and Clemens KJ (2017) The dual orexin receptor antagonist TCS1102 does not affect reinstatement of nicotine-seeking. *PLoS One* **12**:e0173967.
Kim JS, Padnya A, Weltzin M, Edmonds BW, Schulte MK, and Glennon RA (2007) Synthesis of desformylflustrabromine and its evaluation as an alpha4beta2 and alpha7 nACh receptor modulator. *Bioorg Med Chem Lett* **17**:4855–4860.
Kimmey BA, Rupprecht LE, Hayes MR, and Schmidt HD (2014) Donepezil, an acetylcholinesterase inhibitor, attenuates nicotine self-administration and reinstatement of nicotine seeking in rats. *Addict Biol* **19**:539–551.
King JR, Ullah A, Bak E, Jafri MS, and Kabbani N (2018) Ionotropic and metabotropic mechanisms of allosteric modulation of α7 nicotinic receptor intracellular calcium. *Mol Pharmacol* **93**:601–611.
Kleven MS, Anthony EW, and Woolverton WL (1990) Pharmacological characterization of the discriminative stimulus effects of cocaine in rhesus monkeys. *J Pharmacol Exp Ther* **254**:312–317.
Klowden AJ, Ivankovich AD, and Miletich DJ (1978) Ganglionic blocking drugs: general considerations and metabolism. *Int Anesthesiol Clin* **16**:113–150.
Kometer M, Schmidt A, Bachmann R, Studerus E, Seifritz E, and Vollenweider FX (2012) Psilocybin biases facial recognition, goal-directed behavior, and mood state toward positive relative to negative emotions through different serotonergic subreceptors. *Biol Psychiatry* **72**:898–906.
Kometer M, Schmidt A, Jäncke L, and Vollenweider FX (2013) Activation of serotonin 2A receptors underlies the psilocybin-induced effects on α oscillations, N170 visual-evoked potentials, and visual hallucinations. *J Neurosci* **33**:10544–10551.
Koob GF (2008) A role for brain stress systems in addiction. *Neuron* **59**:11–34.
Kowal NM, Ahring PK, Liao VWY, Indurti DC, Harvey BS, O'Connor SM, Chebib M, Olafsdottir ES, and Balle T (2018) Galantamine is not a positive allosteric modulator of human $\alpha4\beta2$ or $\alpha7$ nicotinic acetylcholine receptors. *Br J Pharmacol* **175**: 2911–2925.
Kruse AC, Kobilka BK, Gautam D, Sexton PM, Christopoulos A, and Wess J (2014) Muscarinic acetylcholine receptors: novel opportunities for drug development. *Nat Rev Drug Discov* **13**:549–560.
Kuang G, Wang X, Halldin C, Nordberg A, Långström B, Ågren H, and Tu Y (2016) Theoretical study of the binding profile of an allosteric modulator NS-1738 with a chimera structure of the α7 nicotinic acetylcholine receptor. *Phys Chem Chem Phys* **18**:28003–28009.
Kumar R, Reavill C, and Stolerman IP (1987) Nicotine cue in rats: effects of central administration of ganglion-blocking drugs. *Br J Pharmacol* **90**:239–246.
Kuryatov A, Berrettini W, and Lindstrom J (2011) Acetylcholine receptor (AChR) $\alpha5$ subunit variant associated with risk for nicotine dependence and lung cancer reduces $(\alpha4\beta2)_2\alpha5$ AChR function. *Mol Pharmacol* **79**:119–125.
Kyerematen GA, Morgan ML, Chattopadhyay B, deBethizy JD, and Vesell ES (1990) Disposition of nicotine and eight metabolites in smokers and nonsmokers: identification in smokers of two metabolites that are longer lived than cotinine. *Clin Pharmacol Ther* **48**:641–651.
Lamb RJ and Griffiths RR (1990) Self-administration in baboons and the discriminative stimulus effects in rats of bupropion, nomifensine, diclofensine and imipramine. *Psychopharmacology (Berl)* **102**:183–190.
Lamb RJ and Henningfield JE (1989) Human d-amphetamine drug discrimination: testing with d-amphetamine and hydromorphone. *NIDA Res Monogr* **95**:423–424.
Lancaster T and Stead LF (2000) Mecamylamine (a nicotine antagonist) for smoking cessation. *Cochrane Database Syst Rev* **2**:CD001009.
Lau CE, Spear DJ, and Falk JL (1994) Acute and chronic nicotine effects on multiple-schedule behavior: oral and SC routes. *Pharmacol Biochem Behav* **48**:209–215.
Le Foll B, Chakraborty-Chatterjee M, Lev-Ran S, Barnes C, Pushparaj A, Gamaleddin I, Yan Y, Khaled M, and Goldberg SR (2012) Varenicline decreases nicotine self-administration and cue-induced reinstatement of nicotine-seeking behaviour in rats when a long pretreatment time is used. *Int J Neuropsychopharmacol* **15**: 1265–1274.
Le Foll B, Wertheim C, and Goldberg SR (2007) High reinforcing efficacy of nicotine in non-human primates. *PLoS One* **2**:e230.
Le Houezec J (2003) Role of nicotine pharmacokinetics in nicotine addiction and nicotine replacement therapy: a review. *Int J Tuberc Lung Dis* **7**:811–819.
Le Houezec J, Jacob P III, and Benowitz NL (1993) A clinical pharmacological study of subcutaneous nicotine. *Eur J Clin Pharmacol* **44**:225–230.
Lesage MG (2009) Toward a nonhuman model of contingency management: effects of reinforcing abstinence from nicotine self-administration in rats with an alternative nondrug reinforcer. *Psychopharmacology (Berl)* **203**:13–22.
LeSage MG, Perry JL, Kotz CM, Shelley D, and Corrigall WA (2010) Nicotine self-administration in the rat: effects of hypocretin antagonists and changes in hypocretin mRNA. *Psychopharmacology (Berl)* **209**:203–212.
LeSage MG, Shelley D, Ross JT, Carroll FI, and Corrigall WA (2009) Effects of the nicotinic receptor partial agonists varenicline and cytisine on the discriminative stimulus effects of nicotine in rats. *Pharmacol Biochem Behav* **91**:461–467.
Li P and Steinbach JH (2010) The neuronal nicotinic alpha4beta2 receptor has a high maximal probability of being open. *Br J Pharmacol* **160**:1906–1915.
Lipari RN, Ahrnsbrak RD, Pemberton MR, and Porter JD (2017) Risk and protective factors and estimates of substance use initiation: results from the 2016 National Survey on Drug Use and Health, *CBHSQ Data Review* pp 1–32, Substance Abuse and Mental Health Services Administration (US), Rockville, MD.
Liu X (2013) Positive allosteric modulation of $\alpha4\beta2$ nicotinic acetylcholine receptors as a new approach to smoking reduction: evidence from a rat model of nicotine self-administration. *Psychopharmacology (Berl)* **230**:203–213.
Lunell E, Molander L, Ekberg K, and Wahren J (2000) Site of nicotine absorption from a vapour inhaler--comparison with cigarette smoking. *Eur J Clin Pharmacol* **55**:737–741.
Lysek N, Rachor E, and Lindel T (2002) Isolation and structure elucidation of deformylflustrabromine from the North Sea bryozoan Flustra foliacea. *Z Natforsch C J Biosci* **57**:1056–1061.
MacLean RR, Waters AJ, Brede E, and Sofuoglu M (2018) Effects of galantamine on smoking behavior and cognitive performance in treatment-seeking smokers prior to a quit attempt. *Hum Psychopharmacol* **33**:e2665.
Maelicke A, Samochocki M, Jostock R, Fehrenbacher A, Ludwig J, Albuquerque EX, and Zerlin M (2001) Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer's disease. *Biol Psychiatry* **49**:279–288.
Malas M, van der Tempel J, Schwartz R, Minichiello A, Lightfoot C, Noormohamed A, Andrews J, Zawertailo L, and Ferrence R (2016) Electronic cigarettes for smoking cessation: a systematic review. *Nicotine Tob Res* **18**:1926–1936.
Malin DH, Lake JR, Carter VA, Cunningham JS, Hebert KM, Conrad DL, and Wilson OB (1994) The nicotinic antagonist mecamylamine precipitates nicotine abstinence syndrome in the rat. *Psychopharmacology (Berl)* **115**:180–184.
Mansvelder HD, Keath JR, and McGehee DS (2002) Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. *Neuron* **33**:905–919.
Mao D, Perry DC, Yasuda RP, Wolfe BB, and Kellar KJ (2008) The alpha4beta2alpha5 nicotinic cholinergic receptor in rat brain is resistant to up-regulation by nicotine in vivo. *J Neurochem* **104**:446–456.
Markou A and Paterson NE (2001) The nicotinic antagonist methyllycaconitine has differential effects on nicotine self-administration and nicotine withdrawal in the rat. *Nicotine Tob Res* **3**:361–373.
Marks MJ, Burch JB, and Collins AC (1983) Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. *J Pharmacol Exp Ther* **226**:817–825.
Marks MJ, Pauly JR, Gross SD, Deneris ES, Hermans-Borgmeyer I, Heinemann SF, and Collins AC (1992) Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. *J Neurosci* **12**:2765–2784.
Marks MJ, Stitzel JA, and Collins AC (1985) Time course study of the effects of chronic nicotine infusion on drug response and brain receptors. *J Pharmacol Exp Ther* **235**:619–628.
Martin TJ, Suchocki J, May EL, and Martin BR (1990) Pharmacological evaluation of the antagonism of nicotine's central effects by mecamylamine and pempidine. *J Pharmacol Exp Ther* **254**:45–51.
Marubio LM, del Mar Arroyo-Jimenez M, Cordero-Erausquin M, Léna C, Le Novère N, de Kerchove d'Exaerde A, Huchet M, Damaj MI, and Changeux JP (1999) Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. *Nature* **398**:805–810.
Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, Evrard A, Cazala P, Cormier A, Mameli-Engvall M, et al. (2005) Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature* **436**:103–107.
Matta SG, Balfour DJ, Benowitz NL, Boyd RT, Buccafusco JJ, Caggiula AR, Craig CR, Collins AC, Damaj MI, Donny EC, et al. (2007) Guidelines on nicotine dose selection for in vivo research. *Psychopharmacology (Berl)* **190**:269–319.
Maurer JJ, Sandager-Nielsen K, and Schmidt HD (2017) Attenuation of nicotine taking and seeking in rats by the stoichiometry-selective alpha4beta2 nicotinic acetylcholine receptor positive allosteric modulator NS9283. *Psychopharmacology (Berl)* **234**:475–484.
McNerney ME, Pardi D, Pugh PC, Nai Q, and Margiotta JF (2000) Expression and channel properties of alpha-bungarotoxin-sensitive acetylcholine receptors on chick ciliary and choroid neurons. *J Neurophysiol* **84**:1314–1329.
Mello NK, Peltier MR, and Duncanson H (2013) Nicotine levels after IV nicotine and cigarette smoking in men. *Exp Clin Psychopharmacol* **21**:188–195.
Meyer EL, Xiao Y, and Kellar KJ (2001) Agonist regulation of rat alpha 3 beta 4 nicotinic acetylcholine receptors stably expressed in human embryonic kidney 293 cells. *Mol Pharmacol* **60**:568–576.
Mihalak KB, Carroll FI, and Luetje CW (2006) Varenicline is a partial agonist at alpha4beta2 and a full agonist at alpha7 neuronal nicotinic receptors. *Mol Pharmacol* **70**:801–805.
Miller L and Griffith J (1983) A comparison of bupropion, dextroamphetamine, and placebo in mixed-substance abusers. *Psychopharmacology (Berl)* **80**:199–205.
Moerke MJ, de Moura FB, Koek W, and McMahon LR (2016) Effects of nicotine in combination with drugs described as positive allosteric nicotinic acetylcholine receptor modulators in vitro: discriminative stimulus and hypothermic effects in mice. *Eur J Pharmacol* **786**:169–178.
Moerke MJ and McMahon LR (2018) Rapid nicotine tolerance and cross-tolerance to varenicline in rhesus monkeys: drug discrimination. *Exp Clin Psychopharmacol* **26**:541–548.
Moerke MJ and McMahon LR (2019) Nicotine-like discriminative stimulus effects of acetylcholinesterase inhibitors and a muscarinic receptor agonist in Rhesus monkeys. *Drug Dev Ind Pharm* **45**:861–867.
Moerke MJ, Zhu AZ, Tyndale RF, Javors MA, and McMahon LR (2017) The discriminative stimulus effects of i.v. nicotine in rhesus monkeys: pharmacokinetics

and apparent pA_2 analysis with dihydro-β-erythroidine. *Neuropharmacology* **116**: 9–17.
Mogg AJ, Whiteaker P, McIntosh JM, Marks M, Collins AC, and Wonnacott S (2002) Methyllycaconitine is a potent antagonist of alpha-conotoxin-MII-sensitive presynaptic nicotinic acetylcholine receptors in rat striatum. *J Pharmacol Exp Ther* **302**:197–204.
Mohamed TS, Jayakar SS, and Hamouda AK (2015) Orthosteric and allosteric ligands of nicotinic acetylcholine receptors for smoking cessation. *Front Mol Neurosci* **8**:71.
Mohler EG, Franklin SR, and Rueter LE (2014) Discriminative-stimulus effects of NS9283, a nicotinic α4β2* positive allosteric modulator, in nicotine-discriminating rats. *Psychopharmacology (Berl)* **231**:67–74.
Möller HJ, Demyttenaere K, Olausson B, Szamosi J, Wilson E, Hosford D, Dunbar G, Tummala R, and Eriksson H (2015) Two phase III randomised double-blind studies of fixed-dose TC-5214 (dexmecamylamine) adjunct to ongoing antidepressant therapy in patients with major depressive disorder and an inadequate response to prior antidepressant therapy. *World J Biol Psychiatry* **16**:483–501.
Monod J, Wyman J, and Changeux JP (1965) On the nature of allosteric transitions: a plausible model. *J Mol Biol* **12**:88–118.
Morean M, Krishnan-Sarin S, and O'Malley SS (2018) Comparing cigarette and e-cigarette dependence and predicting frequency of smoking and e-cigarette use in dual-users of cigarettes and e-cigarettes. *Addict Behav* **87**:92–96.
Moroni M, Zwart R, Sher E, Cassels BK, and Bermudez I (2006) alpha4beta2 nicotinic receptors with high and low acetylcholine sensitivity: pharmacology, stoichiometry, and sensitivity to long-term exposure to nicotine. *Mol Pharmacol* **70**: 755–768.
Morrison CF and Stephenson JA (1969) Nicotine injections as the conditioned stimulus in discrimination learning. *Psychopharmacology (Berl)* **15**:351–360.
Murphy SE, Johnson LM, and Pullo DA (1999) Characterization of multiple products of cytochrome P450 2A6-catalyzed cotinine metabolism. *Chem Res Toxicol* **12**: 639–645.
Muschamp JW, Dominguez JM, Sato SM, Shen RY, and Hull EM (2007) A role for hypocretin (orexin) in male sexual behavior. *J Neurosci* **27**:2837–2845.
Nakahara D (2004) Influence of nicotine on brain reward systems: study of intracranial self-stimulation. *Ann N Y Acad Sci* **1025**:489–490.
Nakayama H, Okuda H, Nakashima T, Imaoka S, and Funae Y (1993) Nicotine metabolism by rat hepatic cytochrome P450s. *Biochem Pharmacol* **45**:2554–2556.
Nation PN, Benn MH, Roth SH, and Wilkens JL (1982) Clinical signs and studies of the site of action of purified larkspur alkaloid, methyllycaconitine, administered parenterally to calves. *Can Vet J* **23**:264–266.
Negus SS and Miller LL (2014) Intracranial self-stimulation to evaluate abuse potential of drugs. *Pharmacol Rev* **66**:869–917.
Nelson ME, Kuryatov A, Choi CH, Zhou Y, and Lindstrom J (2003) Alternate stoichiometries of alpha4beta2 nicotinic acetylcholine receptors. *Mol Pharmacol* **63**: 332–341.
Nestler EJ (2005) Is there a common molecular pathway for addiction? *Nat Neurosci* **8**:1445–1449.
Ng HJ, Whittemore ER, Tran MB, Hogenkamp DJ, Broide RS, Johnstone TB, Zheng L, Stevens KE, and Gee KW (2007) Nootropic alpha7 nicotinic receptor allosteric modulator derived from GABAA receptor modulators. *Proc Natl Acad Sci USA* **104**: 8059–8064.
Nguyen HN, Rasmussen BA, and Perry DC (2003) Subtype-selective up-regulation by chronic nicotine of high-affinity nicotinic receptors in rat brain demonstrated by receptor autoradiography. *J Pharmacol Exp Ther* **307**:1090–1097.
Nichols DE (2016) Psychedelics. *Pharmacol Rev* **68**:264–355.
Nishino S, Ripley B, Overeem S, Lammers GJ, and Mignot E (2000) Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* **355**:39–40.
Nwosu CG and Crooks PA (1988) Species variation and stereoselectivity in the metabolism of nicotine enantiomers. *Xenobiotica* **18**:1361–1372.
O'Dell LE, Chen SA, Smith RT, Specio SE, Balster RL, Paterson NE, Markou A, Zorrilla EP, and Koob GF (2007a) Extended access to nicotine self-administration leads to dependence: circadian measures, withdrawal measures, and extinction behavior in rats. *J Pharmacol Exp Ther* **320**:180–193.
O'Dell LE, Torres OV, Natividad LA, and Tejeda HA (2007b) Adolescent nicotine exposure produces less affective measures of withdrawal relative to adult nicotine exposure in male rats. *Neurotoxicol Teratol* **29**:17–22.
Odum AL and Baumann AA (2007) Cigarette smokers show steeper discounting of both food and cigarettes than money. *Drug Alcohol Depend* **91**:293–296.
Overstreet DH and Yamamura HI (1979) Receptor alterations and drug tolerance. *Life Sci* **25**:1865–1877.
Pacek LR, Vandrey R, Dermody SS, Denlinger-Apte RL, Lemieux A, Tidey JW, McClernon FJ, Bangdiwala AS, Drobes DJ, al'Absi M, et al. (2016) Evaluation of a reduced nicotine product standard: moderating effects of and impact on cannabis use. *Drug Alcohol Depend* **167**:228–232.
Palmatier MI, Peterson JL, Wilkinson JL, and Bevins RA (2004) Nicotine serves as a feature-positive modulator of Pavlovian appetitive conditioning in rats. *Behav Pharmacol* **15**:183–194.
Panagis G, Kastellakis A, Spyraki C, and Nomikos G (2000) Effects of methyllycaconitine (MLA), an alpha 7 nicotinic receptor antagonist, on nicotine- and cocaine-induced potentiation of brain stimulation reward. *Psychopharmacology (Berl)* **149**:388–396.
Pandya AA and Yakel JL (2013) Effects of neuronal nicotinic acetylcholine receptor allosteric modulators in animal behavior studies. *Biochem Pharmacol* **86**: 1054–1062.
Panlilio LV, Hogarth L, and Shoaib M (2015) Concurrent access to nicotine and sucrose in rats. *Psychopharmacology (Berl)* **232**:1451–1460.
Papke RL, Horenstein NA, Kulkarni AR, Stokes C, Corrie LW, Maeng CY, and Thakur GA (2014) The activity of GAT107, an allosteric activator and positive modulator of α7 nicotinic acetylcholine receptors (nAChR), is regulated by aromatic amino acids that span the subunit interface. *J Biol Chem* **289**: 4515–4531.
Papke RL, McCormack TJ, Jack BA, Wang D, Bugaj-Gaweda B, Schiff HC, Buhr JD, Waber AJ, and Stokes C (2005) Rhesus monkey alpha7 nicotinic acetylcholine receptors: comparisons to human alpha7 receptors expressed in Xenopus oocytes. *Eur J Pharmacol* **524**:11–18.
Papke RL, Meyer E, Nutter T, and Uteshev VV (2000) alpha7 receptor-selective agonists and modes of alpha7 receptor activation. *Eur J Pharmacol* **393**:179–195.
Papke RL and Porter Papke JK (2002) Comparative pharmacology of rat and human alpha7 nAChR conducted with net charge analysis. *Br J Pharmacol* **137**:49–61.
Papke RL, Sanberg PR, and Shytle RD (2001) Analysis of mecamylamine stereoisomers on human nicotinic receptor subtypes. *J Pharmacol Exp Ther* **297**: 646–656.
Papke RL, Stokes C, Damaj MI, Thakur GA, Manther K, Treinin M, Bagdas D, Kulkarni AR, and Horenstein NA (2018) Persistent activation of α7 nicotinic ACh receptors associated with stable induction of different desensitized states. *Br J Pharmacol* **175**:1838–1854.
Paterson NE (2009) The neuropharmacological substrates of nicotine reward: reinforcing versus reinforcement-enhancing effects of nicotine. *Behav Pharmacol* **20**: 211–225.
Paterson NE and Markou A (2004) Prolonged nicotine dependence associated with extended access to nicotine self-administration in rats. *Psychopharmacology (Berl)* **173**:64–72.
Peng X, Gerzanich V, Anand R, Whiting PJ, and Lindstrom J (1994) Nicotine-induced increase in neuronal nicotinic receptors results from a decrease in the rate of receptor turnover. *Mol Pharmacol* **46**:523–530.
Pérez-Stable EJ, Herrera B, Jacob P III, and Benowitz NL (1998) Nicotine metabolism and intake in black and white smokers. *JAMA* **280**:152–156.
Perkins KA, Broge M, Gerlach D, Sanders M, Grobe JE, Cherry C, and Wilson AS (2002) Acute nicotine reinforcement, but not chronic tolerance, predicts withdrawal and relapse after quitting smoking. *Health Psychol* **21**:332–339.
Perkins KA, D'Amico D, Sanders M, Grobe JE, Wilson A, and Stiller RL (1996) Influence of training dose on nicotine discrimination in humans. *Psychopharmacology (Berl)* **126**:132–139.
Perkins KA, Fonte C, Sanders M, White W, and Wilson A (1999) Effects of training dose and two- versus three-choice testing procedure on nicotine discrimination responding in humans. *Psychopharmacology (Berl)* **145**:418–425.
Perkins KA, Grobe JE, Epstein LH, Caggiula A, Stiller RL, and Jacob RG (1993) Chronic and acute tolerance to subjective effects of nicotine. *Pharmacol Biochem Behav* **45**:375–381.
Perkins KA, Grobe JE, Fonte C, Goettler J, Caggiula AR, Reynolds WA, Stiller RL, Scierka A, and Jacob RG (1994) Chronic and acute tolerance to subjective, behavioral and cardiovascular effects of nicotine in humans. *J Pharmacol Exp Ther* **270**:628–638.
Perkins KA, Roy Chengappa KN, Karelitz JL, Boldry MC, Michael V, Herb T, Gannon J, Brar J, Ford L, Rassnick S, et al. (2018) Initial cross-over test of a positive allosteric modulator of alpha-7 nicotinic receptors to aid cessation in smokers with or without schizophrenia. *Neuropsychopharmacology* **43**:1334–1342.
Perkins KA, Stiller RL, and Jennings JR (1991) Acute tolerance to the cardiovascular effects of nicotine. *Drug Alcohol Depend* **29**:77–85.
Perry DC, Dávila-García MI, Stockmeier CA, and Kellar KJ (1999) Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies. *J Pharmacol Exp Ther* **289**:1545–1552.
Peters L, König GM, Terlau H, and Wright AD (2002) Four new bromotryptamine derivatives from the marine bryozoan Flustra foliacea. *J Nat Prod* **65**:1633–1637.
Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, and Kilduff TS (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* **18**:9996–10015.
Picciotto MR, Addy NA, Mineur YS, and Brunzell DH (2008) It is not "either/or": activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. *Prog Neurobiol* **84**:329–342.
Picciotto MR, Zoli M, Rimondini R, Léna C, Marubio LM, Pich EM, Fuxe K, and Changeux JP (1998) Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature* **391**:173–177.
Pichat P, Bergis OE, Terranova JP, Urani A, Duarte C, Santucci V, Gueudet C, Voltz C, Steinberg R, Stemmelin J, et al. (2007) SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (II) efficacy in experimental models predictive of activity against cognitive symptoms of schizophrenia. *Neuropsychopharmacology* **32**:17–34.
Pickworth WB, Rosenberry ZR, Gold W, and Koszowski B (2014) Nicotine absorption from smokeless tobacco modified to adjust pH. *J Addict Res Ther* **5**:1000184.
Plaza-Zabala A, Flores Á, Martín-García E, Saravia R, Maldonado R, and Berrendero F (2013) A role for hypocretin/orexin receptor-1 in cue-induced reinstatement of nicotine-seeking behavior. *Neuropsychopharmacology* **38**:1724–1736.
Plaza-Zabala A, Martín-García E, de Lecea L, Maldonado R, and Berrendero F (2010) Hypocretins regulate the anxiogenic-like effects of nicotine and induce reinstatement of nicotine-seeking behavior. *J Neurosci* **30**:2300–2310.
Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM, Changeux JP, Maskos U, and Fratta W (2008) Crucial role of alpha4 and alpha6 nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. *J Neurosci* **28**:12318–12327.
Poole A and Urwin C (1976) The metabolism of (14C)nicotine by isolated rhesus monkey hepatocytes in vitro. *Biochem Pharmacol* **25**:281–283.
Popik P, Kozela E, and Krawczyk M (2003) Nicotine and nicotinic receptor antagonists potentiate the antidepressant-like effects of imipramine and citalopram. *Br J Pharmacol* **139**:1196–1202.
Post-Munson DJ, Pieschl RL, Molski TF, Graef JD, Hendricson AW, Knox RJ, McDonald IM, Olson RE, Macor JE, Weed MR, et al. (2017) B-973, a novel piperazine positive allosteric modulator of the α7 nicotinic acetylcholine receptor. *Eur J Pharmacol* **799**:16–25.
Pratt JA, Stolerman IP, Garcha HS, Giardini V, and Feyerabend C (1983) Discriminative stimulus properties of nicotine: further evidence for mediation at a cholinergic receptor. *Psychopharmacology (Berl)* **81**:54–60.

Prendergast M, Podus D, Finney J, Greenwell L, and Roll J (2006) Contingency management for treatment of substance use disorders: a meta-analysis. *Addiction* **101**:1546–1560.

Prus AJ, James JR, and Rosecrans JA (2009) Conditioned place preference, in *Methods of Behavior Analysis in Neuroscience* (Buccafusco JJ, CRC Press/Taylor & Francis, Boca Raton, FL.

Quednow BB, Kometer M, Geyer MA, and Vollenweider FX (2012) Psilocybin-induced deficits in automatic and controlled inhibition are attenuated by ketanserin in healthy human volunteers. *Neuropsychopharmacology* **37**:630–640.

Rabenstein RL, Caldarone BJ, and Picciotto MR (2006) The nicotinic antagonist mecamylamine has antidepressant-like effects in wild-type but not beta2- or alpha7-nicotinic acetylcholine receptor subunit knockout mice. *Psychopharmacology (Berl)* **189**:395–401.

Risinger FO and Oakes RA (1995) Nicotine-induced conditioned place preference and conditioned place aversion in mice. *Pharmacol Biochem Behav* **51**:457–461.

Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, Lu Y, Mansbach RS, Mather RJ, Rovetti CC, et al. (2007) Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. *Neuropharmacology* **52**:985–994.

Roncarati R, Scali C, Comery TA, Grauer SM, Aschmi S, Bothmann H, Jow B, Kowal D, Gianfriddo M, Kelley C, et al. (2009) Procognitive and neuroprotective activity of a novel alpha7 nicotinic acetylcholine receptor agonist for treatment of neurodegenerative and cognitive disorders. *J Pharmacol Exp Ther* **329**:459–468.

Rose JE, Behm FM, and Westman EC (1998) Nicotine-mecamylamine treatment for smoking cessation: the role of pre-cessation therapy. *Exp Clin Psychopharmacol* **6**: 331–343.

Rose JE, Behm FM, Westman EC, and Coleman RE (1999) Arterial nicotine kinetics during cigarette smoking and intravenous nicotine administration: implications for addiction. *Drug Alcohol Depend* **56**:99–107.

Rose JE, Behm FM, Westman EC, Levin ED, Stein RM, and Ripka GV (1994) Mecamylamine combined with nicotine skin patch facilitates smoking cessation beyond nicotine patch treatment alone. *Clin Pharmacol Ther* **56**:86–99.

Rose JE, Levin ED, and Benowitz N (1993) Saliva nicotine as an index of plasma levels in nicotine skin patch users. *Ther Drug Monit* **15**:431–435.

Rose JE, Sampson A, Levin ED, and Henningfield JE (1989) Mecamylamine increases nicotine preference and attenuates nicotine discrimination. *Pharmacol Biochem Behav* **32**:933–938.

Rosecrans JA (1989) Nicotine as a discriminative stimulus: a neurobehavioral approach to studying central cholinergic mechanisms. *J Subst Abuse* **1**:287–300.

Rosecrans JA and Meltzer LT (1981) Central sites and mechanisms of action of nicotine. *Neurosci Biobehav Rev* **5**:497–501.

Ross SA, Wong JY, Clifford JJ, Kinsella A, Massalas JS, Horne MK, Scheffer IE, Kola I, Waddington JL, Berkovic SF, et al. (2000) Phenotypic characterization of an alpha 4 neuronal nicotinic acetylcholine receptor subunit knock-out mouse. *J Neurosci* **20**:6431–6441.

Routtenberg A, Gardner EL, and Huang YH (1971) Self-stimulation pathways in the monkey, Macaca mulatta. *Exp Neurol* **33**:213–224.

Sahdeo S, Wallace T, Hirakawa R, Knoflach F, Bertrand D, Maag H, Misner D, Tombaugh GC, Santarelli L, Brameld K, et al. (2014) Characterization of RO5126946, a novel α7 nicotinic acetylcholine receptor-positive allosteric modulator. *J Pharmacol Exp Ther* **350**:455–468.

Sala F, Mulet J, Reddy KP, Bernal JA, Wikman P, Valor LM, Peters L, König GM, Criado M, and Sala S (2005) Potentiation of human alpha4beta2 neuronal nicotinic receptors by a Flustra foliacea metabolite. *Neurosci Lett* **373**:144–149.

Salas R, Cook KD, Bassetto L, and De Biasi M (2004a) The alpha3 and beta4 nicotinic acetylcholine receptor subunits are necessary for nicotine-induced seizures and hypolocomotion in mice. *Neuropharmacology* **47**:401–407.

Salas R, Pieri F, and De Biasi M (2004b) Decreased signs of nicotine withdrawal in mice null for the beta4 nicotinic acetylcholine receptor subunit. *J Neurosci* **24**: 10035–10039.

Sanderson EM, Drasdo AL, McCrea K, and Wonnacott S (1993) Upregulation of nicotinic receptors following continuous infusion of nicotine is brain-region-specific. *Brain Res* **617**:349–352.

Sannerud CA, Prada J, Goldberg DM, and Goldberg SR (1994) The effects of sertraline on nicotine self-administration and food-maintained responding in squirrel monkeys. *Eur J Pharmacol* **271**:461–469.

Schaefer GJ and Michael RP (1986) Task-specific effects of nicotine in rats. Intracranial self-stimulation and locomotor activity. *Neuropharmacology* **25**:125–131.

Schaefer GJ and Michael RP (1992) Interactions between alcohol and nicotine on intracranial self-stimulation and locomotor activity in rats. *Drug Alcohol Depend* **30**:37–47.

Schneider NG, Olmstead RE, Franzon MA, and Lunell E (2001) The nicotine inhaler: clinical pharmacokinetics and comparison with other nicotine treatments. *Clin Pharmacokinet* **40**:661–684.

Scholl L, Seth P, Kariisa M, Wilson N, and Baldwin G (2018) Drug and opioid-involved overdose deaths - United States, 2013-2017. *MMWR Morb Mortal Wkly Rep* **67**:1419–1427.

Schroeder MJ and Hoffman AC (2014) Electronic cigarettes and nicotine clinical pharmacology. *Tob Control* **23** (Suppl 2):ii30-ii35.

Schuckit MA, Helzer JE, Cottler LB, Crowley T, Nathan PE, and Woody GE (1994) Nicotine use fisorder: nicotine dependence, *Diagnostic and Statistical Manual of Mental Disorders* pp 243–247, American Psychiatric Association, Washington, D.C.

Schwartz RD and Kellar KJ (1983) Nicotinic cholinergic receptor binding sites in the brain: regulation in vivo. *Science* **220**:214–216.

Schwartz RD and Kellar KJ (1985) In vivo regulation of [3H]acetylcholine recognition sites in brain by nicotinic cholinergic drugs. *J Neurochem* **45**:427–433.

Seaton M, Kyerematen GA, Morgan M, Jeszenka EV, and Vesell ES (1991) Nicotine metabolism in stumptailed macaques, Macaca arctoides. *Drug Metab Dispos* **19**: 946–954.

Shiffman S, Ferguson SG, and Hellebusch SJ (2007) Physicians' counseling of patients when prescribing nicotine replacement therapy. *Addict Behav* **32**: 728–739.

Shoaib M, Sidhpura N, and Shafait S (2003) Investigating the actions of bupropion on dependence-related effects of nicotine in rats. *Psychopharmacology (Berl)* **165**: 405–412.

Shoaib M and Stolerman IP (1996) Brain sites mediating the discriminative stimulus effects of nicotine in rats. *Behav Brain Res* **78**:183–188.

Shoaib M, Zubaran C, and Stolerman IP (2000) Antagonism of stimulus properties of nicotine by dihydro-beta-erythroidine (DHbetaE) in rats. *Psychopharmacology (Berl)* **149**:140–146.

Shytle RD, Penny E, Silver AA, Goldman J, and Sanberg PR (2002) Mecamylamine (Inversine): an old antihypertensive with new research directions. *J Hum Hypertens* **16**:453–457.

Slemmer JE, Martin BR, and Damaj MI (2000) Bupropion is a nicotinic antagonist. *J Pharmacol Exp Ther* **295**:321–327.

Slifer BL and Balster RL (1985) Intravenous self-administration of nicotine: with and without schedule-induction. *Pharmacol Biochem Behav* **22**:61–69.

Smelt CLC, Sanders VR, Newcombe J, Burt RP, Sheppard TD, Topf M, and Millar NS (2018) Identification by virtual screening and functional characterisation of novel positive and negative allosteric modulators of the α7 nicotinic acetylcholine receptor. *Neuropharmacology* **139**:194–204.

Smith JW and Stolerman IP (2009) Recognising nicotine: the neurobiological basis of nicotine discrimination. *Handb Exp Pharmacol* 295–333.

Sofuoglu M, Herman AI, Li Y, and Waters AJ (2012) Galantamine attenuates some of the subjective effects of intravenous nicotine and improves performance on a Go No-Go task in abstinent cigarette smokers: a preliminary report. *Psychopharmacology (Berl)* **224**:413–420.

Sorge RE and Clarke PB (2009) Rats self-administer intravenous nicotine delivered in a novel smoking-relevant procedure: effects of dopamine antagonists. *J Pharmacol Exp Ther* **330**:633–640.

Spealman RD and Goldberg SR (1982) Maintenance of schedule-controlled behavior by intravenous injections of nicotine in squirrel monkeys. *J Pharmacol Exp Ther* **223**:402–408.

Spiller K, Xi ZX, Li X, Ashby CR Jr., Callahan PM, Tehim A, and Gardner EL (2009) Varenicline attenuates nicotine-enhanced brain-stimulation reward by activation of alpha4beta2 nicotinic receptors in rats. *Neuropharmacology* **57**:60–66.

St Helen G, Havel C, Dempsey DA, Jacob P III, and Benowitz NL (2016a) Nicotine delivery, retention and pharmacokinetics from various electronic cigarettes. *Addiction* **111**:535–544.

St Helen G, Ross KC, Dempsey DA, Havel CM, Jacob P III, and Benowitz NL (2016b) Nicotine delivery and vaping behavior during *ad libitum* E-cigarette access. *Tob Regul Sci* **2**:363–376.

Stahl SM, Pradko JF, Haight BR, Modell JG, Rockett CB, and Learned-Coughlin S (2004) A review of the neuropharmacology of bupropion, a dual norepinephrine and dopamine reuptake inhibitor. *Prim Care Companion J Clin Psychiatry* **6**:159–166.

Stanley TD and Massey S (2016) Evidence of nicotine replacement's effectiveness dissolves when meta-regression accommodates multiple sources of bias. *J Clin Epidemiol* **79**:41–45.

Stead LF, Koilpillai P, Fanshawe TR, and Lancaster T (2016) Combined pharmacotherapy and behavioural interventions for smoking cessation. *Cochrane Database Syst Rev* **3**:CD008286.

Stegelmeier BL, Hall JO, Gardner DR, and Panter KE (2003) The toxicity and kinetics of larkspur alkaloid, methyllycaconitine, in mice. *J Anim Sci* **81**:1237–1241.

Stolerman IP, Bunker P, and Jarvik ME (1974) Nicotine tolerance in rats; role of dose and dose interval. *Psychopharmacology (Berl)* **34**:317–324.

Stolerman IP, Chamberlain S, Bizarro L, Fernandes C, and Schalkwyk L (2004) The role of nicotinic receptor alpha 7 subunits in nicotine discrimination. *Neuropharmacology* **46**:363–371.

Stolerman IP, Chandler CJ, Garcha HS, and Newton JM (1997) Selective antagonism of behavioural effects of nicotine by dihydro-beta-erythroidine in rats. *Psychopharmacology (Berl)* **129**:390–397.

Stolerman IP, Fink R, and Jarvik ME (1973) Acute and chronic tolerance to nicotine measured by activity in rats. *Psychopharmacology (Berl)* **30**:329–342.

Stolerman IP, Garcha HS, Pratt JA, and Kumar R (1984) Role of training dose in discrimination of nicotine and related compounds by rats. *Psychopharmacology (Berl)* **84**:413–419.

Stolerman IP and Jarvis MJ (1995) The scientific case that nicotine is addictive. *Psychopharmacology (Berl)* **117**:2–10, NaN–20.

Stolerman IP, Kumar R, and Reavill C (1988) Discriminative stimulus effects of cholinergic agonists and the actions of their antagonists. *Psychopharmacol Ser* **4**:32–43.

Stolerman IP, Naylor C, Elmer GI, and Goldberg SR (1999) Discrimination and self-administration of nicotine by inbred strains of mice. *Psychopharmacology (Berl)* **141**:297–306.

Stoops WW, Poole MM, Vansickel AR, Hays KA, Glaser PE, and Rush CR (2011) Methylphenidate increases choice of cigarettes over money. *Nicotine Tob Res* **13**:29–33.

Takada K, Hagen TJ, Cook JM, Goldberg SR, and Katz JL (1988) Discriminative stimulus effects of intravenous nicotine in squirrel monkeys. *Pharmacol Biochem Behav* **30**:243–247.

Talih S, Balhas Z, Eissenberg T, Salman R, Karaoghlanian N, El Hellani A, Baalbaki R, Saliba N, and Shihadeh A (2015) Effects of user puff topography, device voltage, and liquid nicotine concentration on electronic cigarette nicotine yield: measurements and model predictions. *Nicotine Tob Res* **17**:150–157.

Tanner JA, Chenoweth MJ, and Tyndale RF (2015) Pharmacogenetics of nicotine and associated smoking behaviors. *Curr Top Behav Neurosci* **23**:37–86.

Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, and Lester HA (2004) Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. *Science* **306**:1029–1032.

Tella SR, Ladenheim B, and Cadet JL (1997) Differential regulation of dopamine transporter after chronic self-administration of bupropion and nomifensine. *J Pharmacol Exp Ther* **281**:508–513.

Terry P and Katz JL (1997) Dopaminergic mediation of the discriminative stimulus effects of bupropion in rats. *Psychopharmacology (Berl)* **134**:201–212.

Thakur GA, Kulkarni AR, Deschamps JR, and Papke RL (2013) Expeditious synthesis, enantiomeric resolution, and enantiomer functional characterization of (4-(4-bromophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide (4BP-TQS): an allosteric agonist-positive allosteric modulator of α7 nicotinic acetylcholine receptors. *J Med Chem* **56**:8943–8947.

Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, and Siegel JM (2000) Reduced number of hypocretin neurons in human narcolepsy. *Neuron* **27**:469–474.

Thomas KH, Martin RM, Knipe DW, Higgins JP, and Gunnell D (2015) Risk of neuropsychiatric adverse events associated with varenicline: systematic review and meta-analysis. *BMJ* **350**:h1109.

Thomsen MS and Mikkelsen JD (2012) Type I and II positive allosteric modulators differentially modulate agonist-induced up-regulation of α7 nicotinic acetylcholine receptors. *J Neurochem* **123**:73–83.

Tidey JW, Pacek LR, Koopmeiners JS, Vandrey R, Nardone N, Drobes DJ, Benowitz NL, Dermody SS, Lemieux A, Denlinger RL, et al. (2017) Effects of 6-week use of reduced-nicotine content cigarettes in smokers with and without elevated depressive symptoms. *Nicotine Tob Res* **19**:59–67.

Timmermann DB, Grønlien JH, Kohlhaas KL, Nielsen EO, Dam E, Jørgensen TD, Ahring PK, Peters D, Holst D, Christensen JK, et al. (2007) An allosteric modulator of the alpha7 nicotinic acetylcholine receptor possessing cognition-enhancing properties in vivo [published correction appears in *J Pharmacol Exp Ther* (2009) 331:1146]. *J Pharmacol Exp Ther* **323**:294–307.

Timmermann DB, Sandager-Nielsen K, Dyhring T, Smith M, Jacobsen AM, Nielsen EO, Grunnet M, Christensen JK, Peters D, Kohlhaas K, et al. (2012) Augmentation of cognitive function by NS9283, a stoichiometry-dependent positive allosteric modulator of α2- and α4-containing nicotinic acetylcholine receptors. *Br J Pharmacol* **167**:164–182.

Trehy ML, Ye W, Hadwiger ME, Moore TW, Allgire JF, Woodruff JT, Ahadi SS, Black JC, and Westenberger BJ (2011) Analysis of electronic cigarette cartridges, refill solutions, and smoke for nicotine and nicotine related impurities. *J Liq Chromatogr Relat Technol* **34**:1442–1458.

U.S. Department of Health, Education, and Welfare (1964) *Smoking and Health: Report of the Advisory Committee to the Surgeon General of the Public Health Service*, U.S. Department of Health, Education, and Welfare, Public Health Service, Washington, DC.

U.S. Department of Health and Human Services (1981) *The Health Consequences of Smoking: The Changing Cigarette A Report of the Surgeon General*, U.S. Department of Health and Human Services, Public Health Service, Office on Smoking and Health, Rockville, MD.

U.S. Department of Health and Human Services (2010) *How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General*, U.S. Department of Health and Human Services, Public Health Service, Office of Surgeon General, Rockville, MD.

U.S. Department of Health and Human Services (2014) *The Health Consequences of Smoking: 50 Years of Progress. A Report of the Surgeon General*, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, Atlanta, GA.

Uslaner JM, Winrow CJ, Gotter AL, Roecker AJ, Coleman PJ, Hutson PH, Le AD, and Renger JJ (2014) Selective orexin 2 receptor antagonism blocks cue-induced reinstatement, but not nicotine self-administration or nicotine-induced reinstatement. *Behav Brain Res* **269**:61–65.

Uteshev VV (2014) The therapeutic promise of positive allosteric modulation of nicotinic receptors. *Eur J Pharmacol* **727**:181–185.

Valentine JD, Hokanson JS, Matta SG, and Sharp BM (1997) Self-administration in rats allowed unlimited access to nicotine. *Psychopharmacology (Berl)* **133**:300–304.

Vann R, Tobey K, Lobe S, Kipps B, Kwilasz A, Aceto M, and Harris L (2011) Varenicline does not alter brain stimulation reward thresholds and reverses nicotine-facilitated thresholds in rats. *Drug Dev Res* **72**:310–314.

Varanda WA, Aracava Y, Sherby SM, VanMeter WG, Eldefrawi ME, and Albuquerque EX (1985) The acetylcholine receptor of the neuromuscular junction recognizes mecamylamine as a noncompetitive antagonist. *Mol Pharmacol* **28**:128–137.

Vastola BJ, Douglas LA, Varlinskaya EI, and Spear LP (2002) Nicotine-induced conditioned place preference in adolescent and adult rats. *Physiol Behav* **77**:107–114.

Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Bäbler A, Vogel H, and Hell D (1998) Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *Neuroreport* **9**:3897–3902.

Wada E, Wada K, Boulter J, Deneris E, Heinemann S, Patrick J, and Swanson LW (1989) Distribution of alpha 2, alpha 3, alpha 4, and beta 2 neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat. *J Comp Neurol* **284**:314–335.

Wakasa Y, Takada K, and Yanagita T (1995) Reinforcing effect as a function of infusion speed in intravenous self-administration of nicotine in rhesus monkeys. *Nihon Shinkei Seishin Yakurigaku Zasshi* **15**:53–59.

Wallace TL, Callahan PM, Tehim A, Bertrand D, Tombaugh G, Wang S, Xie W, Rowe WB, Ong V, Graham E, et al. (2011) RG3487, a novel nicotinic α7 receptor partial agonist, improves cognition and sensorimotor gating in rodents. *J Pharmacol Exp Ther* **336**:242–253.

Walters CL, Brown S, Changeux JP, Martin B, and Damaj MI (2006) The beta2 but not alpha7 subunit of the nicotinic acetylcholine receptor is required for nicotine-conditioned place preference in mice. *Psychopharmacology (Berl)* **184**:339–344.

Wang C, Wang Q, Ji B, Pan Y, Xu C, Cheng B, Bai B, and Chen J (2018) The orexin/receptor system: molecular mechanism and therapeutic potential for neurological diseases. *Front Mol Neurosci* **11**:220.

Wang F, Nelson ME, Kuryatov A, Olale F, Cooper J, Keyser K, and Lindstrom J (1998) Chronic nicotine treatment up-regulates human alpha3 beta2 but not alpha3 beta4 acetylcholine receptors stably transfected in human embryonic kidney cells. *J Biol Chem* **273**:28721–28732.

Wang ZJ, Deba F, Mohamed TS, Chiara DC, Ramos K, and Hamouda AK (2017) Unraveling amino acid residues critical for allosteric potentiation of (α4)3(β2)2-type nicotinic acetylcholine receptor responses. *J Biol Chem* **292**:9988–10001.

Weaver MT, Geier CF, Levin ME, Caggiula AR, Sved AF, and Donny EC (2012) Adolescent exposure to nicotine results in reinforcement enhancement but does not affect adult responding in rats. *Drug Alcohol Depend* **125**:307–312.

Weinstein AM and Gorelick DA (2011) Pharmacological treatment of cannabis dependence. *Curr Pharm Des* **17**:1351–1358.

Weltzin MM, Huang Y, and Schulte MK (2014) Allosteric modulation of alpha4beta2 nicotinic acetylcholine receptors by HEPES. *Eur J Pharmacol* **732**:159–168.

Weltzin MM and Schulte MK (2010) Pharmacological characterization of the allosteric modulator desformylflustrabromine and its interaction with alpha4beta2 neuronal nicotinic acetylcholine receptor orthosteric ligands. *J Pharmacol Exp Ther* **334**:917–926.

Weltzin MM and Schulte MK (2015) Desformylflustrabromine modulates α4β2 neuronal nicotinic acetylcholine receptor high- and low-sensitivity isoforms at allosteric clefts containing the β2 subunit. *J Pharmacol Exp Ther* **354**:184–194.

Wesnes KA, Edgar CJ, Kezic I, Salih HM, and de Boer P (2013) Effects of nicotine withdrawal on cognition in a clinical trial setting. *Psychopharmacology (Berl)* **229**:133–140.

West R, Hajek P, Foulds J, Nilsson F, May S, and Meadows A (2000) A comparison of the abuse liability and dependence potential of nicotine patch, gum, spray and inhaler. *Psychopharmacology (Berl)* **149**:198–202.

West RJ, Hajek P, and Belcher M (1989) Severity of withdrawal symptoms as a predictor of outcome of an attempt to quit smoking. *Psychol Med* **19**:981–985.

Whiting P and Lindstrom J (1987) Purification and characterization of a nicotinic acetylcholine receptor from rat brain. *Proc Natl Acad Sci USA* **84**:595–599.

Wiley JL, Lavecchia KL, Martin BR, and Damaj MI (2002) Nicotine-like discriminative stimulus effects of bupropion in rats. *Exp Clin Psychopharmacol* **10**:129–135.

Williams DK, Wang J, and Papke RL (2011) Positive allosteric modulators as an approach to nicotinic acetylcholine receptor-targeted therapeutics: advantages and limitations. *Biochem Pharmacol* **82**:915–930.

Williams KE, Reeves KR, Billing CB Jr., Pennington AM, and Gong J (2007) A double-blind study evaluating the long-term safety of varenicline for smoking cessation. *Curr Med Res Opin* **23**:793–801.

Williams M and Robinson JL (1984) Binding of the nicotinic cholinergic antagonist, dihydro-beta-erythroidine, to rat brain tissue. *J Neurosci* **4**:2906–2911.

Windle SB, Filion KB, Mancini JG, Adye-White L, Joseph L, Gore GC, Habib B, Grad R, Pilote L, and Eisenberg MJ (2016) Combination therapies for smoking cessation: a hierarchical Bayesian meta-analysis. *Am J Prev Med* **51**:1060–1071.

Wing VC and Shoaib M (2012) Translating the smoking cessation properties of the antidepressant nortriptyline using reinforcing, discriminative and aversive stimulus effects of nicotine in rats. *Psychopharmacology (Berl)* **219**:847–857.

Wing VC and Shoaib M (2013) Effect of infusion rate on intravenous nicotine self-administration in rats. *Behav Pharmacol* **24**:517–522.

Winter JC, Rice KC, Amorosi DJ, and Rabin RA (2007) Psilocybin-induced stimulus control in the rat. *Pharmacol Biochem Behav* **87**:472–480.

Winterer G, Gallinat J, Brinkmeyer J, Musso F, Kornhuber J, Thuerauf N, Rujescu D, Favis R, Sun Y, Franc MA, et al. (2013) Allosteric alpha-7 nicotinic receptor modulation and P50 sensory gating in schizophrenia: a proof-of-mechanism study. *Neuropharmacology* **64**:197–204.

World Health Organization (2012) *WHO Global Report: Mortality Attributable To Tobacco*, World Health Organization, Geneva, Switzerland.

World Health Organization (2013) *Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013-2020*, World Health Organization, Geneva, Switzerland.

World Health Organization (2018) *WHO Global Report on Trends in Prevalence of Tobacco Smoking 2000-2025*, 2nd ed, World Health Organization, Geneva, Switzerland.

Wu CH, Lee CH, and Ho YS (2011) Nicotinic acetylcholine receptor-based blockade: applications of molecular targets for cancer therapy. *Clin Cancer Res* **17**:3533–3541.

Yamada H and Bruijnzeel AW (2011) Stimulation of α2-adrenergic receptors in the central nucleus of the amygdala attenuates stress-induced reinstatement of nicotine seeking in rats. *Neuropharmacology* **60**:303–311.

Yanagita T, Ando K, Kato S, and Takada K (1983) Psychopharmacological studies on nicotine and tobacco smoking in rhesus monkeys. *Psychopharmacol Bull* **19**:409–412.

Yingst JM, Foulds J, Veldheer S, Hrabovsky S, Trushin N, Eissenberg TT, Williams J, Richie JP, Nichols TT, Wilson SJ, et al. (2019) Nicotine absorption during electronic cigarette use among regular users. *PLoS One* **14**:e0220300.

Yoshimura RF, Hogenkamp DJ, Li WY, Tran MB, Belluzzi JD, Whittemore ER, Leslie FM, and Gee KW (2007) Negative allosteric modulation of nicotinic acetylcholine receptors blocks nicotine self-administration in rats. *J Pharmacol Exp Ther* **323**:907–915.

Young R and Glennon RA (2002) Nicotine and bupropion share a similar discriminative stimulus effect. *Eur J Pharmacol* **443**:113–118.

Yuan M, Malagon AM, Yasuda D, Belluzzi JD, Leslie FM, and Zaveri NT (2017) The α3β4 nAChR partial agonist AT-1001 attenuates stress-induced reinstatement of nicotine seeking in a rat model of relapse and induces minimal withdrawal in dependent rats. *Behav Brain Res* **333**:251–257.

Zaniewska M, McCreary AC, Przegaliński E, and Filip M (2006) Evaluation of the role of nicotinic acetylcholine receptor subtypes and cannabinoid system in the discriminative stimulus effects of nicotine in rats. *Eur J Pharmacol* **540**:96–106.

Zevin S and Benowitz NL (1999) Drug interactions with tobacco smoking. An update. *Clin Pharmacokinet* **36**:425–438.

Zhu AZ, Cox LS, Nollen N, Faseru B, Okuyemi KS, Ahluwalia JS, Benowitz NL, and Tyndale RF (2012) CYP2B6 and bupropion's smoking-cessation pharmacology: the role of hydroxybupropion. *Clin Pharmacol Ther* **92**:771–777.

Zislis G, Desai TV, Prado M, Shah HP, and Bruijnzeel AW (2007) Effects of the CRF receptor antagonist D-Phe CRF(12-41) and the alpha2-adrenergic receptor agonist clonidine on stress-induced reinstatement of nicotine-seeking behavior in rats. *Neuropharmacology* **53**:958–966.

Zoli M, Moretti M, Zanardi A, McIntosh JM, Clementi F, and Gotti C (2002) Identification of the nicotinic receptor subtypes expressed on dopaminergic terminals in the rat striatum. *J Neurosci* **22**:8785–8789.