THE NEUROBIOLOGY OF NICOTINE ADDICTION: CLINICAL AND PUBLIC POLICY IMPLICATIONS

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Clinicians, social scientists, researchers, and policy makers appreciate the need to understand the neurobiology of nicotine addiction and how this information can lead to new treatments and provide support for public policy debates on parity and preventing adolescent tobacco use. In a "bench-to-bedside" manner, this review covers both clinical and basic science perspectives. Both the reward and sensitization-homeostasis theories of nicotine addiction are supported by new understanding of clinical issues of rapid tolerance, withdrawal, sensitization, and craving when examined by functional brain imaging, genetics, and basic science studies of nicotinic acetylcholine receptors. This review provides information to help shape public policy, fight stigma, and improve clinical treatment and research. The fight for parity in health care requires education about the neurobiological basis of addiction versus the stigmatized bad habit or simple socialization. Parity must support reimbursement for nicotine replacement medications or other FDA approved medications and psychosocial treatments.

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Introduction

Aggressive educational and legislative efforts as well as improved tobacco dependence treatments resulting from basic and clinical research findings have helped reduce the prevalence of tobacco dependence in the United States; however, there is a need to continue to improve the treatment outcomes and better match individual patients to specific treatments. In addition to education about the health consequences of tobacco use, an important component of ongoing public education must be on the addictive potential of nicotine and tobacco use. A neurobiological understanding of nicotine addiction helps reduce stigma put on those who have difficulty quitting and provides increased understanding of the problem and possible solutions for individuals not responding to current treatments (Brewster et al., 2002). Implementation of health care parity requires having an underlying biological basis for the disorders that are to be reimbursed in health care plans. A clear neurobiological explanation of tobacco addiction helps these efforts. This paper will initially review some of the key clinical phenomena explaining nicotine addiction (early tolerance, nicotine withdrawal, and craving) and place these important issues in the context of the reward and sensitization-homeostasis theories of nicotine addiction and current information from imaging, genetic, and basic laboratory studies, including nicotine receptor subtypes. This information provides a foundation for the concluding section on potential therapeutic targets. A better understanding of the rapid clinical progression to tobacco dependence can inform neurobiological research questions which may lead to new advances in treatment.

Addiction Due to Early Tolerance and Nicotine Withdrawal

Until a few years ago, it was widely assumed that nicotine dependence did not begin until a person had smoked for several years (American Psychiatric Association, 2000). It was thought that nicotine withdrawal symptoms were experienced only by people who smoked more than five cigarettes every day, and that people who experienced nicotine withdrawal had to maintain the presence of nicotine in the blood throughout the day to suppress withdrawal symptoms. Thus, nicotine dependence was envisioned as developing very slowly in the face of long exposures to constant heavy doses of nicotine. Basic science researchers attempted to replicate prolonged heavy exposures in animal models of nicotine addiction. This assumption is proving to be inaccurate.

The past few years have brought the revelation that nicotine withdrawal symptoms are quite commonly experienced by novice smokers who smoke only a few cigarettes per week (DiFranza et al., 2007a; Gervais et al., 2006; Kandel et al., 2007). These smokers do not have to smoke at least five cigarettes daily, because their withdrawal symptoms can be suppressed for many days at a time by smoking a single cigarette.

The duration of relief from withdrawal that is provided by smoking a single cigarette shrinks with repeated exposure to nicotine. A youth who was able to keep withdrawal in check by smoking one cigarette every few days finds that, over time, he or she must smoke at more and more frequent intervals to keep withdrawal in check. Eventually, most smokers who have ready access to tobacco will find that they are smoking 10 to 20 cigarettes per day just to keep withdrawal under control. The interval between finishing a cigarette and the onset of withdrawal is termed the latency to withdrawal (DiFranza & Ursprung, 2008). It can vary from many days in length in novice smokers, to just a few minutes in smokers with advanced dependence. The shortening of the latency to withdrawal represents dependence-related tolerance. The challenge for neurobiologists is to determine not only what causes withdrawal, but why the latency to withdrawal shortens with repeated exposure to nicotine. The fact that the impact of one cigarette can last long after nicotine has been cleared from the blood suggests that perhaps other factors are responsible for propagating or sustaining nicotine's effects after it is gone. Nicotine stimulates the release of dopamine, but many other brain neurotransmitters, including g-aminobutyric acid (GABA), glutamate, neurepinephrine, serotonin, cannabanoid and beta-endorphin are also involved (Benowitz, 2008).

Another challenge for researchers is to determine how the processes that produce nicotine addiction can develop as quickly as they do. The most vulnerable youth experience symptoms of nicotine addiction after smoking only one or two cigarettes (Scragg et al., 2008). What could change in the brain with such rapidity? The least vulnerable youth can smoke more than 100 cigarettes before experiencing symptoms of nicotine addiction, and about 95 percent of individuals smoking 100 cigarettes in their lifetime will become addicted to tobacco (Scragg et al., 2008). These facts have tremendous public policy and public education impact. They also highlight the need to focus clinical and neurobiological research on these phenomena.

Are these differences in individual vulnerability genetically determined? The reaction to the first inhalation from a cigarette is the best predictor of which youth will become addicted. Those who experience a sense of relaxation are at triple the risk of addiction (DiFranza et al., 2007b). Do individuals differ in their responses to nicotine and their vulnerability to addiction because they have a different compliment of nicotinic receptors? Other neurobiological changes with mental illness or other addictions are associated with increased rates of nicotine addiction and more difficulty quitting (Ziedonis &Williams, 2003).

Novice smokers can keep withdrawal in check by smoking only a few cigarettes per week, but long-term smokers cannot do this, even after having quit smoking for many years (Wellman et al., 2006). The fact that relapsed smokers retain a short latency to withdrawal is an indication that the brain does not revert to its nicotine-

naïve state after smoking cessation. This has been confirmed in animal experiments (Slotkin et al., 2007).

The Sensitization-Homeostasis Theory of Nicotine Addiction

Based on the new discoveries about the clinical nature of nicotine addiction, members of our team have postulated the sensitization-homeostasis theory to explain how nicotine might act on the brain to cause addiction (DiFranza & Wellman, 2005). In a departure from traditional theories that postulate that drugs cause addiction by stimulating pleasure centers in the brain, the sensitization-homeostasis theory postulates that nicotine causes addiction by inhibiting brain circuits that are responsible for generating craving for rewarding experiences (DiFranza & Wellman, 2005). This is consistent with the observation that many individuals do not find nicotine to be pleasurable until after they are addicted. Throughout this paper we will expand the description of the sensitization-homeostasis theory as it relates to the particular neurobiological research being described. Also, although we have focused on this theory in the article, we acknowledge that the reward theory and other models continue to be important to understand the complex and multidimensional components of addiction. The sensitization-homeostasis theory provides a framework for clinicians and policy makers to better organize the findings being presented. Other important models include the reward model, social-ecological model, genetic theories, biological and conditioning exposure theories, and adaptation theories. We will not review these other approaches, although we recognize that they have been helpful in developing current tobacco control and psychosocial clinical interventions (Ziedonis, 2004).

Dopamine, the Reward Theory, and the Neuroanatomy of Dependence and Withdrawal

The idea that addiction may be initiated by the first dose of nicotine strongly supports a neurobiological basis of this addiction versus being based on habit or socialization. Consistent with this idea, laboratory studies have shown that reward, sensitization, withdrawal, tolerance, conditioned cues/memory, motivation, executive functioning, and impulse control are all components of addiction with a neurobiological basis (Difranza & Wellman, 2005).

The main action of nicotine's addictive effects is thought to be mediated through the mesocorticolimbic dopamine pathways. Dopamine neurons within these circuits originate in the ventral tegmental area (VTA) and project to the nucleus accumbens (NAc) and the prefrontal cortex (PFC, mesocortical). The amount of nicotine typically inhaled by smoking and found in smokers' blood is able to acutely increase the firing of dopaminergic neurons in the VTA, which causes increased dopamine release in the nucleus accumbens (Pidoplichko, DeBiasi, Williams, & Dani, 1997). Originally thought to directly mediate the rewarding effects of abused drugs, more

recent evidence suggests that dopamine release may be a predictor of reward (Schultz, 2004). Regardless of dopamine's exact role, nicotine-induced release of dopamine is critical for the onset and maintenance of dependence (Corrigall & Coen, 1991; Picciotto et al., 1998). Pharmacological blockade of dopamine receptors or destruction of dopamine neurons or the nucleus accumbens reduces self administration in rats (Corrigall & Coen, 1991; Corrigall, & Coen, 1992). These findings support the dopamine reward theory; however, the recognized importance of other neurotransmitters, such as GABA and glutamate, cannot be easily explained with only this theory. GABA (a major inhibitory neurotransmitter) inhibits dopamine release, and glutamate (a major excitatory neurotransmitter) provides excitatory input into the VTA coming from multiple regions of the brain (George, 2007).

In humans, nicotine withdrawal causes craving, irritability, insomnia, restlessness, and impatience (Hughes, 2007; Kenny & Markou, 2001). Some of these symptoms are mood-subjective feelings oriented (affective) and some are physical (somatic). Given the varied nature of these symptoms, the sensitization-homeostasis theory proposes that withdrawal symptoms result from homeostatic imbalance in multiple independent brain systems, so there may be several mechanisms underlying withdrawal (DiFranza & Wellman, 2005). Central affective withdrawal symptoms have been associated with a decrease in dopamine release in the nucleus accumbens and striatum (Fung, Schmid, Anderson, & Lau, 1996; Hildebrand, Nomikos, Hertel, Schilström, & Svensson, 1998). Somatic withdrawal has both central and peripheral nervous system components to explain the symptoms; these physical withdrawal symptoms can occur without changes in brain striatal dopamine levels (Carboni, Bortone, Giua, & DiChiara, 2000). The affective withdrawal behaviors and symptoms appear to be directly correlated with decreases in brain dopamine levels (Kenny & Markou, 2001). Local injection of the non-specific nicotine receptor antagonist, mecamylamine, into the VTA decreases dopamine release in the nucleus accumbens and precipitates both somatic and affective withdrawal symptoms in nicotine dependent, but not nicotine naïve, rats (Hildebrand, Panagis, Svensson, & Nomikos, 1999). Caution must be used in the interpretation of these animal models of withdrawal. For example, the blocker mecamylamine induces withdrawal in animals but not in human smokers (Rose et al., 2001). Interestingly it was the opioid antagonist naloxone that precipitated withdrawal in nicotine dependent rats and in human smokers (Malin, Lake, Carter, Cunnigham, & Wilson, 1993).

NICOTINIC RECEPTORS

Of particular importance to nicotine addiction and withdrawal is the fact that dopamine neurons, as well as GABA interneurons, within the VTA are rich in nicotine receptors (nAChRs) expressed both at the cell body, where they contribute to

neuronal excitability, and in presynaptic terminals, where they modulate the release of either excitatory neurotransmitters such as glutamate or inhibitory factors such as GABA. Thus, nicotine activates or inhibits dopaminergic VTA neurons through multiple mechanisms. VTA neurons express multiple nicotinic receptor (nAChR) subunits (including α4, α5, α6, α7, β2 and β3 to be discussed later in the article), and these different combinations of subunits imply that different receptors have different properties (Dani, 2001).

A major goal of nicotine addiction research is to identify specific nicotine receptor (nAChR) subtypes critical for nicotine dependence and withdrawal (Laviolette & van der Kooy, 2004). Once identified, these receptors should provide therapeutic targets for smoking cessation. Because nicotinic receptors serve as modulators of virtually every neurotransmitter system in the brain (Benowitz, 2008), the transition to a nicotine dependent state likely involves many brain regions outside of the traditional dopaminergic "reward pathway." Thus, another major goal is to understand how other brain regions and neurotransmitters are involved in nicotine dependence and withdrawal. Brain imaging is an invaluable tool in this effort.

Sensitization

The potential role of sensitization in human addiction has been the focus of basic science research for over seven decades (Post, 1980). Sensitization refers to a process whereby subsequent doses of a drug have a greater impact than the initial dose. Like other addictive drugs, nicotine induces sensitization in animals which is seen as increased stimulation of locomotion. Following its induction, sensitization persists as a latent state: activity is normal when nicotine is absent, but when nicotine is re-administered, sensitization is expressed as augmented locomotion (DiFranza & Wellman, 2007). The sensitization-homeostasis theory proposes that sensitization to nicotine causes an exaggerated response to subsequent doses of nicotine. Since the postulated effect of nicotine is to inhibit activity in a craving generation system, sensitization of the response to nicotine would cause a "super physiologic" inhibition of activity in this system. The sensitization-homeostasis theory contends that the process that results in nicotine addiction can be set in motion by the first cigarette (DiFranza & Wellman, 2005). This is consistent with data suggesting that the neurological adaptations underlying sensitization begin with the first dose. Sensitization can be observed when the second dose of nicotine is administered in rats (Bevins & Besheer, 2001; Hahn, Stolerman, & Shoaib, 2000).

Unpublished data show that rats express sensitization in the form of increased locomotion only when nicotine is given after a period of abstinence (King, Shields, DiFranza, & Rane, 2009). When doses are too closely spaced, a sensitized response cannot be evoked. For this reason, the sensitization-homeostasis theory postulates

that novice smokers may get more impact from smoking single cigarettes spaced days apart than inveterate smokers get from smoking constantly throughout the day. It may not seem that a single cigarette could have much impact, but it was recently demonstrated that the nicotine from a single cigarette is sufficient to occupy 88% of the nicotinic receptors (nAChRs) in the brain (Brody et al., 2006). Widely spaced doses of nicotine are sufficient to invoke sensitization in rats, and to trigger the development of nicotine withdrawal symptoms in novice adolescent human smokers (DiFranza et al., 2007a; Gervais et al., 2006; Kandel et al., 2007; DiFranza & Wellman, 2005). These are very important findings in the public policy and education on adolescent smoking risks. The challenge for neurobiologists is to determine if there is a common mechanism behind these phenomena.

Like addiction in general, sensitization cannot be attributed to a single neurological alteration, but rather entails changes in multiple systems (Pierce & Kalivas, 1997). Researchers (Kalivas, Sorg, & Hooks, 1993) have proposed that sensitization involves alterations in the neural circuitry that is subservient to how the brain translates motivationally relevant stimuli into adaptive motor responses. Sensitization has been induced and expressed by direct injection of nicotine into the VTA brain region (Kita, Okamoto, & Nakashima, 1992; Panagis, Nisell, Nomikos, Chergui, & Svensson, 1996); however, this effect is complex and is linked with dopamine neurons, GABA neurons, and the pre-frontal cortex (Kalivas et al., 1993; Pierce & Kalivas, 1997).

Utilization of Imaging Techniques to Study the Neurobiology of Nicotine Addiction

Imaging Sensitization in Preclinical Models

Functional magnetic imaging (fMRI) has been utilized to determine which brain circuits are involved with sensitization. In our own laboratory, we have utilized fMRI to show that when nicotine is administered to sensitized animals as compared to controls, brain activation is more prolonged in many key regions for nicotine addiction (i.e., the hippocampus, nucleus accumbens, prefrontal cortex, ventral pallidum, and ventral tegmentum), and there is more intense maximal activation in the hippocampus, prefrontal cortex, and ventral tegmentum area (Li, DiFranza, Wellman, Kulkarni, & King, 2008). In addition, sensitization was associated with a relative decrease in activation in the anterior cingulate gyrus (Li et al., 2008). Furthermore, despite the rich endowment of nicotinic receptors in the visual cortex, there were no changes in activation with sensitization, thus establishing the selectivity of the process (Li et al., 2008). The relative inhibition in activity seen in the anterior cingulate gyrus in sensitized animals is of interest because the sensitization-homeostasis theory holds that nicotine works through inhibition of key circuits.

The clinical and public policy take-home message from these imaging studies is that within four days of the initial dose of nicotine, the brain already demonstrates striking changes in how it responds to nicotine. This corresponds well with reports from youth that some of them are already feeling symptoms of addiction within days of their first cigarette.

Human Imaging Studies and Craving

Traditional addiction theories have commonly discussed a reward pathway, but not a craving generation system. With the ability to image brain activity in humans there has been a burst of interest in identifying brain structures tied to craving. Cue-induced craving for nicotine is associated with increased fMRI activity in the anterior cingulate gyrus, the orbitofrontal cortex, the temporal lobe, nucleus accumbens, the amygdala, and the ventral striatum (McBride, Barrett, Kelly, Aw, & Dagher, 2006; McClernon et al., 2007; Smolka et al., 2006). In a perfusion fMRI study, researchers (Franklin et al., 2007) demonstrated that craving intensity correlated positively with increases in neural activation in the dorsolateral prefrontal cortex and posterior cingulated cortex. Many similar experiments demonstrate brain activation with similar patterns in addicts in response to cues for many addictive substances and underlying mechanisms that may modulate activation of this drug craving network (Volkow et al., 2008). Together these studies provide strong support for the idea that there are circuits of neurons that are responsible for the generation of craving for addictive substances as proposed by the sensitization-homeostasis theory.

The sensitization-homeostasis theory postulates that nicotine's addictive potential comes from its ability to suppress activity in a postulated "craving generation system." With sensitization, nicotine would elicit super-physiological levels of inhibition in this circuit. Homeostatic adaptations would develop in the presence of nicotine to restore the activity of the craving generation system to normal. If these neuroadaptations could develop literally overnight, craving for another cigarette could develop very soon thereafter. Indeed, many smokers report that their first cigarette tasted terrible and provided no pleasure, and yet, within a day or two they had a strong craving for another.

Further support for this theory comes from a recent report that nicotine perfusion decreased craving and regional cerebral blood flow in a distributed network of brain regions including the cingulate cortex and amygdala (Zubieta et al., 2005). Much more work will be needed to establish that nicotine's primary addiction-related mode of action is through the inhibition of activity in the craving generation system, but the accumulated evidence encourages additional exploration.

Sensitization and Brain Epigenetic Changes on a Molecular Level

Consistent with the proposal that early exposures to nicotine trigger brain changes in the nucleus accumbens and prefrontal cortex, nicotine sensitization is associated with an increase of a non-specific indicator of neuronal metabolic activation called c-fos-like (Panagis et al., 1996; Shim et al., 2001), as well as marked alterations in the early response genes (Schochet , Kelley, & Landry, 2005). In early addiction, the drug can cause tolerance and reduce the individual's reward circuit by switching on a molecule (CREB - cyclic AMP response element-binding protein) which causes a cascade of events, including binding to specific genes and causing them to produce new proteins that these genes encode. Whether the drug causes tolerance or sensitization rapidly is due to the balance of the CREB versus fos-like molecule activity in the nucleus accumbens (Nestler, 2001). Usually there is an initial increase in CREB leading to tolerance and then a fall in levels. In contrast, fos-like substances will stay increased for an extended period of time after the substance use occurs, resulting in long-term sensitization (Nestler, 2001).

Which nAChR Subtypes are involved in Nicotine Dependence?

Identifying the role of specific nicotinic receptor (nAChR) subtypes is important to efforts to design new drug therapies. Nicotine exerts its physiological effects through activation of brain receptors that are receptive to the natural neurotransmitter, acetylcholine. Nicotinic receptors (nAChRs) straddle the nerve cell membrane and allow positively charged ions to pass through a central channel when they are activated. These receptors are comprised of five subunits, and the subunit composition of each channel determines its electrophysiological properties and agonist binding affinities.

There are 12 varieties of nicotinic receptor (nAChR) subunits thus far identified (α2 to α10 and β2 to b4) (Elgoyhen et al., 2001; Gotti et al., 2007; Lustig Peng, Hiel, Yamamoto, & Fuchs, 2001; Wonnacott, 1997). Each of these is part of the five units needed to assemble a receptor. Although all combinations have not been identified, there appears to be a great number of possible varieties of nicotinic receptors with each having different properties. The α2 to α6 subunits can assemble with either the β2, β3, or β4 subunits to form heteromeric nicotinic receptors (e.g., α4β2 or α3β4). In some cases, there are multiple a subunits (e.g., α4α5β2) or multiple β subunits (e.g., α6β2β3) (Gotti et al., 2007). In contrast, the α7, α8, and α9 subunits are able to form homomeric receptors (composed of 5 identical subunits) that are blocked by the chemical a-bungarotoxin.

The critical point is that the various mixtures of receptor subunits generate pharmacologically and functionally distinct nicotinic receptor subtypes. While the complete repertoire of native nAChR subtypes has not been fully elucidated, it is

clear that a staggering diversity of receptor subtypes may exist, with each subtype playing a specific physiological role. Nicotine receptors are involved in several fundamental physiological processes such as learning, memory and attention (Dani & Bertrand, 2007). The diversity of assembled nAChR types might provide neurons with an ability to engage in homeostatic regulation by switching the type of receptor that is displayed or the relative proportions of mixtures of receptor types.

Nicotine stimulates an increase in the number of nAChRs in the brain (Volk et al., 2007). When it was discovered that some youth experienced symptoms of nicotine addiction soon after smoking their first cigarette, there was an interest in determining how rapidly the brain could undertake a remodeling of nAChRs. Animal studies revealed that one dose of nicotine and one day was all that were required to demonstrate an increase in the number of nicotine receptors (Abreu-Villaca et al., 2003). This rapid increase in number of receptors (upregulation) shows the brain's ability and effort to change for homeostasis—even in response to one dose.

Much of what we know about the individual nicotinic receptor (nAChR) subtypes involved in nicotine dependence stems from mouse genetic studies where a given gene encoding a specific nAChR subunit is either interrupted in the animal such that the protein product is not expressed (i.e., "knock out", KO, mouse) or modified such that the subunit being studied forms hypersensitive nAChRs (i.e., "knock in" mice), allowing for selective activation by exquisitely small doses of nicotine that do not activate non-mutant, wild-type receptors (Champtiaux & Changeux, 2004). This strategy allows one to answer the question, "Is a nAChR subtype containing subunit X necessary and/or sufficient for nicotine dependence?"

Neuronal nAChRs containing the β2 subunit represent the most abundant high affinity nAChR in the central nervous system (CNS) (Picciotto et al., 1995). Mice that do not express these receptors (β2 KO mice) fail to maintain self-administration of nicotine and exhibit impaired tolerance to the drug (McCallum, Collins, Paylor, & Marks, 2006; Picciotto et al., 1998). In addition, an injection of nicotine fails to increase the concentration of dopamine in the nucleus accumbens in these animals (Picciotto et al., 1998). Furthermore, re-expression of β2 nAChRs in the VTA via viral-mediated gene delivery restores nicotine self-administration, drug-induced dopaminergic neuron activation, and DA elevation in nucleus accumbens (Maskos et al., 2005).

Neuronal nicotinic receptors containing the α4* nicotinic receptor subunit have also been strongly implicated in nicotine dependence (the * indicates that other unidentified, nicotinic subunits co-assemble with α4 subunits to form functional receptors). Knock in mice genetically engineered to express α4* nAChR with a single point mutation that render these receptors hypersensitive to agonists are fifty-fold more sensitive to nicotine tolerance, sensitization, and reward, indicating that

selective activation of α4* nAChRs are sufficient for these behaviors (Tapper et al., 2004). Supporting the importance of these molecules in nicotine dependence, α4 KO mice do not develop tolerance to nicotine nor do they exhibit increased dopamine release in accumbens when challenged with the drug (Marubio et al., 2003; Tapper, McKinney, Marks, & Lester, 2007). Not surprisingly, the majority of β2* nAChRs also contain the α4 subunit (Marks, Meinerz, Drago, & Collins, 2007; Marks et al., 1999). Thus, it is becoming increasingly clear that nicotine dependence is initiated through the activation of α4β2* nAChRs. Because most high affinity nAChRs are assembled from combinations of different subunit types, a vast array of receptor subtypes exist in the reward circuitry, several versions contain α4 and β2 subunits in addition to a third or fourth subunit including α6, α5, or β3 (Grady et al., 2007; Salminen et al., 2007; Salminen et al., 2004). Future studies will likely address the role of non-α4β2 subunits in nicotine addiction.

How Do Specific Nicotinic Receptor Subtypes Mediate Different Symptoms of Withdrawal?

In an effort to both better understand the neurobiology of nicotine withdrawal as well as develop new medication treatments to better manage withdrawal, animal studies have focused on specific nicotine receptor subtypes and compared them in regards to the physical (somatic) symptoms of withdrawal versus the affective (mood and anxiety) symptoms. In the mouse model, somatic withdrawal symptoms in mice are characterized by increased licking, grooming, shaking, and scratching (Damaj, Kao, & Martin, 2003; Kenny & Markou, 2001; Malin et al., 1994). Mouse affective symptoms include increased anxiety, hypo-locomotion, and conditioned place aversion when they receive nicotinic receptor antagonist chemicals/medications (Damaj et al., 2003; Jackson, Martin, Changeux, & Damaj, 2008; Suzuki, Ise, Tsuda, Maeda, & Misawa, 1996). In animals, but not people, withdrawal symptoms can be precipitated by administration of nicotinic receptor antagonist chemicals during a time of chronic nicotine exposure. To date, knockout mouse lines that do not express α7, β2 or β4 nAChR subunits have been utilized to analyze the role of these subunits in withdrawal. Our own summary review of the literature suggests a role for α7 and β4* nAChRs in somatic withdrawal symptoms, whereas high affinity β2* nAChR expression may be necessary for affective symptoms (Jackson et al., 2008; Salas, Main, Gangitano, & De Biasi, 2007). The results from these studies reinforce the idea that different withdrawal symptoms may be linked to activation of different nAChR subtypes. Identification of these subtypes may provide unique pharmacological targets for smoking cessation. The relevance of these withdrawal studies to humans is unclear as smokers do not display similar withdrawal symptoms (except anxiety) and nicotinic blockers do not trigger withdrawal in humans.

Neuronal Nicotine Receptors in Alcohol Dependence and Psychiatric Disorders

Many current smokers in the United States have a mental illness or another addiction (Ziedonis, 2004; Ziedonis & Williams, 2003), and this group appears to have particular difficulty with nicotine withdrawal. There is a need for treatment to be available for these individuals with multiple disorders. Also, all these disorders have their primary biological basis in the central nervous system and new discoveries in one area may help others. Clinicians and public policy makers need to target these populations, including encouraging research to examine possible common ground.

For example, most drugs of abuse increase dopamine release; however, alcohol (ethanol) is a non-specific drug that must interact with a myriad of receptors and ion channels to get the effect on dopamine release (Harris, 1999). Ethanol only modestly increases dopamine activity via direct action on the dopamine neuron, and its activation of nicotine receptors appears paramount for alcohol dependence (Lupica & Brodie, 2006). Interestingly, a nicotine receptor blocker, mecamylamine, can block the reinforcing aspects of alcohol in rats (Larsson et al., 2002), and humans given this type of medication report reduced pleasurable effects of alcoholic beverages (Chi & de Wit, 2003). Specific nicotine receptor subtype(s) may underlie ethanol reinforcement and addiction, and understanding other psychiatric disorders may be helped by better understanding nicotinic receptors (George, 2007).

Neuronal Nicotinic Acetylcholine Receptors: The Genes

Important to understanding the functions of the nicotinic receptors is the need to discover how and where the nAChR genes and proteins are expressed. These receptors are found throughout the body in such diverse sites as the intestinal epithelium, adipose tissue, skin, the thymus, and the oral epithelium where they are thought to be involved in inflammation, cell adhesion, and wound healing (Gahring & Rogers, 2006). The expression of nicotinic receptors in the respiratory system is thought to contribute directly to the etiology of lung cancer (Dasgupta & Chellappan, 2006; Minna, 2003). Thus, understanding the mechanisms underlying the complex expression patterns of the nAChR genes is likely to have a large impact on our understanding of a number of pathological conditions in which nicotinic signaling is disrupted.

A family of closely related genes encodes the subunits that assemble to form functional nicotinic receptors. Clinically, this raises the possibility that each subtype may represent a distinct drug target in treating nicotine dependence. Each of the receptor subunit genes exhibits distinct and common patterns of expression suggesting that they are both commonly and independently regulated (Drago, McColl, Horne, Finkelstein, & Ross, 2003; Wada et al., 1989; Zoli, Le Novere, Hill, & Changeux, 1995) and both transcriptional (RNA level) and post-transcriptional (post-RNA synthesis) processes are involved (Fornasari, Battaglioli, Terzano, &

Clementi, 1998). Genetic differences in the expression of receptor types might account for individual differences in reactions to the first dose of nicotine, variability in managing withdrawal, and other factors.

Potential Therapeutic Targets for Nicotine Addiction

Nicotine Receptor

Progress has been made over the past two decades in understanding the physiological basis of nicotine addiction, and this has resulted in the development of seven Food and Drug Administration (FDA) approved medications. These include nicotine replacement therapy (e.g., patch, gum, spray, inhaler, and lozenge), varenicline (Chantix®), and the monoamine uptake inhibitor, bupropion (Zyban®). The research that led to the development of these medications has focused on the suppression of craving and withdrawal and particularly the nicotine receptor (agonists, partial agonists). Other medications are in investigation, such as dianicline (SSR591813, Sanofi-Aventis) which recently completed Phase III clinical trials and is an α4b2 complete agonist.

In spite of these advances, there continues to be a need to develop better pharmacological treatments and to integrate these with psychosocial treatments. Patient matching may be an important strategy. Even with finding improved new medications, the success rate of smoking cessation continues to be about 20% to 25% at one year (Gonzales et al., 2006), and with longer clinical experience there has been an increased awareness of potential adverse side effects (e.g., Nides, 2008; Popkin, 2008). For example, varenicline, a partial agonist of α4b2 nAChRs, has demonstrated the best effectiveness of the FDA approved options, but has also had concerns about rare but serious side effects including vision problems, heart trouble, depression, suicide, and changes in mood and behavior (Moore, Cohen, & Furberg, 2008). These concerns prompted the FDA to issue two alerts regarding its use in 2008 and the Federal Aviation Administration banned varenicline use by pilots and air traffic controllers (Moore et al., 2008; Federal Aviation Authority, 2008; Food and Drug Administration, 2007, 2008). These concerns resulted in a drop in varenicline prescriptions of 50% in the first 5 months of 2008 (Hensley, 2008). For the clinician, the rare serious side effects of varenicline are weighed against the efficacy of the medication and the 50% mortality associated with continued smoking (Doll et al., 2004). These issues highlight the need to develop new medications.

Drugs targeting the α4β2 and α7 subtypes remain attractive targets for potential therapies and considerable effort is being expended to identify other, hopefully more potent and safe, drugs for smoking cessation (Arneric, Holladay, & Williams, 2007). A recent wave of research has raised the idea that the α3α5β4 subtype may also be a good target. The genes encoding the α3, α5, and β4 subunits are tightly clustered

in the genome suggesting that their expression may be co-regulated (Boulter et al., 1990). This is important in light of recent genome-wide association studies (GWAS) suggesting a critical role for the α3α5β4 subtype in nicotine dependence. Two groups have shown that variability (i.e., single nucleotide polymorphisms) within the α3/α5/β4 gene cluster is a critical determinant of tobacco use, age of onset of use, and even lung cancer (Berrettini et al., 2008; Schlaepfer et al., 2008), and the referenced articles will provide details of interest to genetics and pharmacology experts. The α6 and β3 subunits may prove to be effective drug targets for nicotine intervention given their role in dopamine release (Exley et al., 2007). Given the complexity of nicotine dependence, it is not surprising that a number of functionally distinct nicotine receptor subtypes appear important for drug discovery (George, 2007).

Other Medication Development Strategies

Nicotine addiction is a complex disorder, and as such requires that we look beyond a limited number of nicotinic receptor subtypes as drug targets. Other receptors and chemical messengers modulated by nicotine are also important, including the mu opioid receptor (Xue & Domino, 2008) and the chemical GABA. On another front, a novel approach to tackle nicotine dependence has been the development of anti-nicotine vaccines (Cerny & Cerny, 2008). The vaccines are composed of nicotine molecules coupled to a carrier protein and an adjuvant. These complexes are used to stimulate antibody production. An attractive aspect of vaccination is that the target is the drug rather than a critical component of the brain. Thus far, results in early clinical studies are encouraging, suggesting lower rates of smoking or increased cessation in vaccinated subjects (Maurer et al., 2005; Wagena, de Vos, Horwith, & van Schayck, 2008). Treatment matching based on genetic profiles may be a strategy in the future. Clusters of gene variants have been identified that are present more frequently in successful quitters, and the gene variants differed in subjects who were successful with buproprion treatment versus those who were successful with nicotine replacement therapy (Uhl et al., 2008). The identified genes encode proteins spanning a wide variety of biological functions such as cell adhesion, transcription, receptors, and enzymes. These results make sense if neuroplasticity is crucial to cessation—supporting the sensitization-homeostasis theory. The results provide molecular genetic evidence for the hypothesis that a person's ability to quit smoking has polygenic genetic components and suggest potential biological pathways for further research.

Conclusion

Tobacco addiction continues to be the most preventable cause of increased morbidity and mortality. Clinicians, researchers, and policy makers must work

together to reduce tobacco use and help individuals on a clinical and population level to quit smoking. This review reinforces the strong genetic and neurobiological underpinnings of this addiction. These hard facts must be known in public policy and education as they inform the parity discussion, stigma, the role of medications in treatment, the need for psychosocial treatments to help address cue-induced cravings, and the development of new medications. The article included a discussion of the reward and sensitization-homeostasis theories of nicotine addiction as models to organize animal and human laboratory findings. There is a need for more research to better understand the different subtypes of nicotinic acetylcholine receptors and important clinical phenomena such as rapid tolerance, withdrawal, sensitization, and craving. A better understanding of the biological basis of nicotine dependence may lead to parity in insurance coverage for medications and psychosocial treatments. In addition, information about the neurobiology of tobacco addiction can help inform public policy, fight stigma, and improve clinical treatment and research.

References

Abreu-Villaca, Y.A., Seidler, F.J., Qiao, D., Tate, C.A., Cousins, M.M., Thillai, I., & Slotkin, T.A.

2003 Short-term adolescent nicotine exposure has immediate and persistent effects on cholinergic systems: Critical periods, patterns of exposure, dose thresholds. *Neuropsychopharmacology, 28,*1935-49.

American Psychiatric Association

2000 *Diagnostic and statistical manual of mental disorders: DSM-IV-TR.* Fourth Edition, Text Revision.Washington, DC: American Psychiatric Association.

Arneric, S.P., Holladay, M., & Williams, M.

2007 Neuronal nicotinc receptors: A perspective on two decades of drug discovery research. *Biochemical Pharmacology, 74*, 1092-1101.

Benowitz, N.L.

2008 Neurobiology of nicotine addiction: Implications for smoking cessation treatment. *American Journal of Medicine, 121*, S3-S10.

Berrettini, W., Yuan, X., Tozzi, F. , Song, K., Francks, C., Chilcoat, H., Waterworth, D., Muglia, P., & Mooser, V.

2008 α-5/α-3 nicotinic receptor subunit alleles increase risk for heavy smoking. *Molecular Psychiatry, 13,* 368-373.

Bevins, R.A., & Besheer, J.

2001 Individual differences in rat locomotor activity are diminished by nicotine through stimulation of central nicotinic acetylcholine receptors. *Physiology & Behavior, 72,* 237-244.

Boulter, J., O'Shea-Greenfield, A., Duvoisin, R.M., Connolly, J.G., Wada, E., Jensen, A., Gardner, P.D., Ballivet, M., Deneris, E.S., McKinnon, D., & Heinemann, S., & Patrick, J.
1990 Alpha 3, alpha 5, and beta 4: Three members of the rat neuronal nicotinic acetylcholine receptor-related gene family form a gene cluster. *Journal of Biological Chemistry, 265*(8), 4472-4482.

Brewster, T., Farentinos, C., & Ziedonis, D.
2002. Response: The neurobiological model in community treatment programs. *Scientific Practices & Perspectives, 1*(1), 21.

Brody, A.L., Mandelkern, M.A., London, E.D., Olmstead, R.E., Farahi, J., Scheibal, D., Jou, J., Allen, V., Tiongson, E., Chefer, S.I., Koren, A.O., & Mukhin, A.G.
2006 Cigarette smoking saturates brain alpha 4 beta 2 nicotinic acetylcholine receptors. *Archives of General Psychiatry, 63*(8), 907-15.

Carboni, E., Bortone, L., Giua, C., & Di Chiara, G.
2000 Dissociation of physical abstinence signs from changes in extracellular dopamine in the nucleus accumbens and in the prefrontal cortex of nicotine dependent rats. *Drug and Alcohol Dependence, 58*(1-2), 93-102.

Cerny, E.H., & Cerny, T.
2008 Anti-nicotine abuse vaccines in the pipeline: An update. *Expert Opinion on Investigational Drugs, 17*(5), 691-696.

Champtiaux, N., & Changeux, J.P.
2004 Knockout and knockin mice to investigate the role of nicotinic receptors in the central nervous system. *Progress in Brain Research, 145*, 235-251.

Chi, H., & de Wit, H.
2003 Mecamylamine attenuates the subjective stimulant-like effects of alcohol in social drinkers. *Alcoholism-Clinical and Experimental Research, 27*(5), 780-786.

Corrigall, W.A., & Coen, K.M.
1991 Selective dopamine antagonists reduce nicotine self-administration. *Psychopharmacology, 107*, 171-176.

Corrigall, W.A., Franklin, K.B., Coen, K.M., & Clarke, P.B.
1992 The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacology, 107*, 285-289.

Damaj, M.I., Kao, W., & Martin, B.R.
2003 Characterization of spontaneous and precipitated nicotine withdrawal in the mouse. *Journal of Pharmacological and Experimental Therapeutics, 307*(2), 526-534.

Dani, J.A.
2001 Overview of nicotinic receptors and their roles in the central nervous system. *Biological Psychiatry, 49*(3), 166-74.
Dani, J.A., & Bertrand, D.
2007 Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annual Review of Pharmacology and Toxicology, 47*, 699-729.
Dasgupta, P., & Chellappan, S.P.
2006 Nicotine-mediated cell proliferation and angiogenesis. *Cell Cycle, 5*, 2324-2328.
DiFranza, J.R., Savageau, J.A., Fletcher, K., O'Loughlin, J., Pbert, L., Ockene, J.K., McNeill, A.D., Hazelton, J., Friedman, K., Dussault, G., Wood, C., & Wellman, R.J.
2007a Symptoms of tobacco dependence after brief intermittent use -The development and assessment of nicotine dependence in youth-2. *Archives of Pediatrics and Adolescent Medicine,161*(7), 704-10.
DiFranza, J.R., Savageau, J.A., Fletcher, K., Pbert, L., O'Loughlin, J., McNeill, A.D., Ockene, J.K., Friedman, K., Hazelton, J., Wood, C., Dussault, G., & Wellman, R.J.
2007b Susceptibility to nicotine dependence: the Development and Assessment of Nicotine Dependence in Youth-2. *Pediatrics, 120*, e974 - e83.
DiFranza, J. & Ursprung, W.
2008 The latency to the onset of nicotine withdrawal: A test of the Sensitization-Homeostasis Theory. *Addictive Behaviors, 33,*1148-53.
DiFranza, J. R., & Wellman, R.J.
2005 A sensitization-homeostasis model of nicotine craving, withdrawal, and tolerance: Integrating the clinical and basic science literature. *Nicotine & Tobacco Research, 7,* 9-26.
DiFranza, J. R., & Wellman, R.J.
2005 A sensitization-homeostasis model of nicotine craving, withdrawal, and tolerance: Integrating the clinical and basic science literature. *Nicotine & Tobacco Research, 7,* 9-26.
DiFranza, J.R., & Wellman, R.J.
2007 Sensitization to nicotine: How the animal literature might inform future human research. *Nicotine & Tobacco Research, 9,* 9-20.
Doll, R., Peto, R., Boreham, J., & Sutherland, I.
2004 Mortality in relation to smoking: 50 years' observations on male British doctors. *British Medical Journal, 328,* 1519.
Drago, J., McColl, C.D., Horne, M.K., Finkelstein, D.I., & Ross, S.A.
2003 Neuronal nicotinic receptors: Insights gained from gene knockout and knockin mutant mice. *Cellular and Molecular Life Sciences, 60,* 1267-1280.

Elgoyhen, A.B., Vetter, D.E., Katz, E., Rothlin, C.V., Heinemann, S.F., & Boulter, J.
2001 a10: A determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells. *Proceedings of the National Academy of Science, U.S.A., 98*(6), 3501-3506.

Exley, R., Clements, M.A., Hartung, H., McIntosh, J.M., & Cragg, S.J.
2007 Alpha-containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine neurotransmission in nucleus accumbens. *Neuropsychopharmacology, 33,* 2158-66.

Federal Aviation Authority
2008 *Advisory to pilots and air traffic controllers banning varenicline use.* May 21, 2008.

Food and Drug Administration.
2007 *Early communication about an ongoing safety review of varenicline.* November 20, 2007.

Food and Drug Administration
2008 *FDA issues public health advisory on Chantix®.* February 1, 2008.

Fornasari, D., Battaglioli, E., Terzano, S., & Clementi, F.
1998 Transcriptional regulation of neuronal nicotinic receptor subunit genes. In *Neuronal nicotinic receptors: Pharmacology and therapeutic opportunities*. Arneric, S.P. & Brioni, J.D. (Eds.), Wiley-Liss, Inc.

Franklin, T.R., Wang, Z., Wang, J., Sciortino, N., Harper, D., Li, Y., Ehrman, R., Kampman, K., O'Brien, C.P., Detre, J.A., & Childress, A.R.
2007 Limbic activation to cigarette smoking cues independent of nicotine withdrawal: A perfusion fMRI study. *Neuropsychopharmacology., 21*(11), 2301-2309.

Fung, Y.K., Schmid, M.J., Anderson ,T.M., & Lau, Y.S.
1996 Effects of nicotine withdrawal on central dopaminergic systems. *Pharmacology, Biochemistry and Behavior, 53(*3), 635-640.

Gahring, L.C., & Rogers, S.W.
2006 Neuronal nicotinic acetylcholine receptor expression and function on nonneuronal cells. *American Association of Pharmaceutical Scientists Journal, 7*, E885-E894.

George, T.P.
2007 Medication Treatments for Nicotine Dependence. Taylor & Francis: Boca Raton.

Gervais, A., O'Loughlin, J., Meshefedjian, G., Bancej, C., Tremblay, M.
2006 Milestones in the natural course of cigarette use onset in adolescents. *Canadian Medical Association Journal, 175*(3), 255-61.

Gonzales, D., Rennard, S.I., Nides, M., Oncken, C. , Azoulay S., & Billing, C.B.
2006 Varenicline, an a4b2 nicotinic acetylcholine receptor partial agonist, vs. sustained-release bupropion and placebo for smoking cessation. *Journal of the American Medical Association, 296*, 47-55.

Gotti, C., Moretti, M., Gaimarri, A., Zanardi, A., Clementi, F., & Zoli, M.
2007 Heterogeneity and complexity of native brain nicotinic receptors. *Biochemical Pharmacology, 74*, 1102-1111.

Grady, S.R., Salminen, O., Laverty, D.C., Whiteaker, P. , McIntosh, J.M., Collins, A.C., & Marks, M.J.
2007 The subtypes of nicotinic acetylcholine receptors on dopaminergic terminals of mouse striatum. *Biochemical Pharmacology, 74*(8), 1235-1246.

Hahn, B., Stolerman, I.P., & Shoaib, M.
2000 Kappa-opioid receptor modulation of nicotine-induce behavior. *Neuropharmacology, 39*, 2848-2855.

Harris, R.A.
1999 Ethanol actions on multiple ion channels: Which are important? *Alcoholism-Clinical and Experimental Research, 23*(10), 1563-1570.

Hensley, S.
2008 As bad news mounts, Chantix® prescriptions crumble. *Wall Street Journal* May 30, 2008.

Hildebrand, B.E., Nomikos, G.G., Hertel, P., Schilström, B., & Svensson, T.H.
1998 Reduced dopamine output in the nucleus accumbens but not in the medial prefrontal cortex in rats displaying a mecamylamine-precipitated nicotine withdrawal syndrome. *Brain Research,779*(1-2), 214-225.

Hildebrand, B.E., Panagis, G., Svensson, T.H., & Nomikos, G.G.
1999 Behavioral and biochemical manifestations of mecamylamine-precipitated nicotine withdrawal in the rat: Role of nicotinic receptors in the ventral tegmental area. *Neuropsychopharmacology, 21*(4), 560-574.

Hughes, J.R.
2007 Effects of abstinence from tobacco: Valid symptoms and time course. *Nicotine & Tobacco Research, 9*(3), 315-327.

Jackson, K.J., Martin, B.R., Changeux, J.P., & Damaj, M.I.
2008 Differential role of nicotinic acetylcholine receptor subunits in physical and affective nicotine withdrawal signs. *Journal of Pharmacological and Experimental Therapeutics, 325*(1), 302-312.

Kalivas, P.W., Sorg, B.A., & Hooks, M.S.
1993 The pharmacology and neural circuitry of sensitization to psychostimulants. *Behavioral Pharmacology, 4*(4), 315-334.

Kandel, D., Hu, M.C., Grieisler, P., & Schaffran, C.
2007 On the development of nicotine dependence in adolescence. *Drug & Alcohol Dependence, 91*, 26-39.
Kenny, P.J., & Markou, A.
2001 Neurobiology of the nicotine withdrawal syndrome. *Pharmacology, Biochemistry and Behavior, 70*(4), 531-549.
King, J., Shields, J., DiFranza, J., & Rane, P.
2009 Nicotine-induced behavioral sensitization in normal and ADHD rats. Unpublished personal communication.
Kita, T., Okamoto, M., & Nakashima, T.
1992 Nicotine-induced sensitization to ambulatory stimulant effect produced by daily administration into the ventral tegmental area and the nucleus accumbens in rats. *Life Sciences, 50*(8), 583-590.
Larsson, A., Svensson, L., Soderpalm, B., & Engel, J.A.
2002 Role of different nicotinic acetylcholine receptors in mediating behavioral and neurochemical effects of ethanol in mice. *Alcohol, 28,* 157-167.
Laviolette, S.R., & van der Kooy, D.
2004 The neurobiology of nicotine addiction: bridging the gap from molecules to behaviour. *Nature Reviews Neuroscience, 5*(1), 55-65.
Li, Z., DiFranza, J.R., Wellman, R.J., Kulkarni, P., & King, J.A.
2008 Imaging brain activation in nicotine-sensitized rats. *Brain Research, 11(*99), 91-99.
Lupica, C.R, & Brodie, M.S.
2006 Queer Currents, Steady Rhythms, and Drunken DA Neurons. Focus on Hyperpolarization-Activated Cation Current (Ih) Is an Ethanol Target in Midbrain Dopamine Neurons of Mice. *Journal of Neurophysiology, 95,* 585-586
Lustig, L.R., Peng, H., Hiel, H., Yamamoto, T., & Fuchs, P.A.
2001 Molecular cloning and mapping of the human nicotinic acetylcholine receptor alpha10 (CHRNA10). *Genomics, 73*(3), 272-283.
Malin, D.H., Lake, J.R., Carter, V.A., Cunningham, J.S., Hebert, K.M., Conrad, D.L., & Wilson, O.B.
1994 The nicotinic antagonist mecamylamine precipitates nicotine abstinence syndrome in the rat. *Psychopharmacology, 115*(1-2), 180-184.
Malin, D.H., Lake, J.R., Carter, V.A., Cunningham, J.S., & Wilson, O.B.
1993 Naloxone precipitates nicotine abstinence syndrome in the rat. *Psychopharmacology, 112*, 339-342.

Marks, M.J., Meinerz, N.M., Drago, J., & Collins, A.C.
2007 Gene targeting demonstrates that alpha4 nicotinic acetylcholine receptor subunits contribute to expression of diverse [3H] epibatidine binding sites and components of biphasic 86Rb+ efflux with high and low sensitivity to stimulation by acetylcholine. *Neuropharmacology, 53*(3), 390-405.

Marks, M.J., Whiteaker, P., Calcaterra, J., Stitzel, J.A., Bullock, A.E., Grady, S.R., Picciotto, M.R., Changeux, J.P., & Collins, A.C.
1999 Two pharmacologically distinct components of nicotinic receptor-mediated rubidium efflux in mouse brain require the beta2 subunit. *Journal of Pharmacological and Experimental Therapeutics, 289*(2), 1090-1103.

Marubio, L.M., Gardier, A.M., Durier, S., David, D., Klink, R., Arroyo-Jimenez, M.M., McIntosh, J.M., Rossi, F., Champtiaux, N., Zoli, M., & Changeux, J.P.
2003 Effects of nicotine in the dopaminergic system of mice lacking the alpha4 subunit of neuronal nicotinic acetylcholine receptors. *European Journal of Neuroscience, 17*(7), 1329-1337.

Maskos, U., Molles, B.E., Pons, S., Besson, M., Guiard, B.P. , Guilloux, J.P., Evrard, A., Cazala, P., Cormier, A., Mameli-Engvall, M., Dufour, N., Cloëz-Tayarani, I., Bemelmans, A.P., Mallet, J., Gardier, A.M., David, V., Faure, P., Granon, S., & Changeux, J.P.
2005 Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature, 436*(7047), 103-107.

Maurer, P., Jennings, G.T., Willers, J., Rohner, J., Lindman, Y., Roubicek, K., Renner, W.A., Müller, P., & Bachmann, M.F.
2005 A therapeutic vaccine for nicotine dependence: Preclinical efficacy, and Phase I safety and immunogenicity. *European Journal of Immunology, 35*(7), 2031-2040.

McBride, D., Barrett, S.P., Kelly, J.T., Aw, A., & Dagher, A.
2006 Effects of expectancy and abstinence on the neural response to smoking cues in cigarette smokers: An fMRI study. *Neuropsychopharmacology, 31*(12), 2728-2738.

McCallum, S.E., Collins, A.C., Paylor, R., & Marks, M.J.
2006 Deletion of the beta 2 nicotinic acetylcholine receptor subunit alters development of tolerance to nicotine and eliminates receptor upregulation. *Psychopharmacology, 184*(3-4), 314-327.

McClernon, F.J., Hiott, F.B., Liu, J., Salley, A.N., Behm, F.M., & Rose, J.E.
2007 Selectively reduced responses to smoking cues in amygdala following extinction-based smoking cessation: Results of a preliminary functional magnetic resonance imaging study. *Addiction Biology, 12*(3-4), 503-512.

Minna, J.D.
2003 Nicotine exposure and bronchial epithelial cell nicotinic acetylcholine receptor expression in the pathogenesis of lung cancer. *Journal of Clinical Investigation, 111*(1), 31-33.

Moore, T.J., Cohen, M.R., & Furberg, C.D.
2008 Strong safety signal seen for new varenicline risks. *The Institute for Safe Medication Practices QuarterWatch,* May 21, 2008,1-11.

Nestler, E.J.
2001 Molecular basis of long-term plasticity underlying addiction. *Nature Reviews Neuroscience, 2,* 119–28.

Nides, M.
2008 Update on pharmacologic options for smoking cessation treatment. *American Journal of Medicine, 121*, S20-S31.

Panagis, G., Nisell, M., Nomikos, G.G., Chergui, K., & Svensson, T.H.
1996 Nicotine injections into the ventral tegmental area increase locomotion and Fos-like immunoreactivity in the nucleus accumbens of the rat. *Brain Research, 730*(1-2),133-142.

Picciotto, M.R., Zoli, M., Lena, C., Bessis, A., Lallemand, Y., Le Novere, N., Vincent, P., Pich, E.M., Brulet, P., & Changeux, J.P.
1995 Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. *Nature, 374*(6517), 65-67.

Picciotto, M.R., Zoli, M., Rimondini, R., Lena, C., Marubio, L.M., Pich, E.M., Fuxe, K., & Changeux, J.P.
1998 Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature, 391*(6663),173-177.

Pidoplichko, V.I., DeBiasi, M., Williams, J.T., & Dani, J.A.
1997 Nicotine activates and desensitizes midbrain dopamine neurons. *Nature, 390*(6658), 401-404.

Pierce, R.C., & Kalivas, P.W.
1997 A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Research Reviews, 25*(2), 192-216.

Popkin, M.K.
2008 Exacerbation of recurrent depression as a result of treatment with varenicline. *American Journal of Psychiatry, 165*(6), 774.

Post, R.M.
1980 Intermittent versus continuous stimulation: Effect of time interval on the development of sensitization or tolerance. *Life Science, 26*(16), 1275-1282.

Rose. J., Behm. F., & Westman, E.
2001 Acute effects of nicotine and mecamylamine on tobacco withdrawal symptoms, cigarette reward and ad lib smoking. *Pharmacology Biochemistry and Behavior, 68,* 187-97.

Salas, R., Main, A., Gangitano, D., & De Biasi, M.
2007 Decreased withdrawal symptoms but normal tolerance to nicotine in mice null for the alpha7 nicotinic acetylcholine receptor subunit. *Neuropharmacology, 53*(7), 863-869.

Salminen, O., Drapeau, J.A., McIntosh, J.M., Collins, A.C., Marks, M.J., & Grady, S.R.
2007 Pharmacology of alpha-conotoxin MII-sensitive subtypes of nicotinic acetylcholine receptors isolated by breeding of null mutant mice. *Molecular Pharmacology, 71*(6), 1563-1571.

Salminen, O., Murphy, K.L., McIntosh, J.M., Drago, J., Marks, M.J., Collins, A.C., & Grady, S.R.
2004 Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Molecular Pharmacology, 65*(6), 1526-1535.

Schlaepfer, I.R., Hoft, N.R., Collins, A.C., Corley, R.P., Hewitt, J.K., Hopfer, C.J., Lessern, J.M., McQueen, M.B., Hyun Rhee, S., & Ehringer, M.A.
2008 The CHRNA5/A3/B4 gene cluster variability as an important determinant of early alcohol and tobacco initiation in young adults. *Biological Psychiatry, 63,* 1039-1046.

Schochet, T.L., Kelley, A.E., & Landry, C.F.
2005 Differential expression of arc mRNA and other plasticity-related genes induced by nicotine in adolescent rat forebrain. *Neuroscience, 135*(1), 285-297.

Schultz, W.
2004 Neural coding of basic reward terms of animal learning theory, game theory, microeconomics and behavioural ecology. *Current Opinion in Neurobiology, 14*(2), 139-147.

Scragg, R., Wellman, R.J., Laugesen, M., & DiFranza, J.R.
2008 Diminished autonomy over tobacco can appear after the first cigarette. *Addictive Behaviors, 33*, 689-98.

Shim, I., Avaid, J.I., Wirtshafter, J.D., Jang, S.Y., Shin, K.H., Lee, H.J., Chung, Y.C., & Chun, B.G.
2001 Nicotine-induced behavioral sensitization is associated with extracellular dopamine release and expression of c-Fos in the striatum and nucleus accumbens of the rat. *Behavioral Brain Research, 121*(1-2), 137-147.

Slotkin, T., MacKillop, E., Rudder, C., Ryde, I., Tate, C., & Seidler, F.
2007 Permanent, sex-selective effects of prenatal or adolescent nicotine exposure, separately or sequentially, in rat brain regions: Indices of cholinergic and serotonergic synaptic function, cell signaling, and neural cell number and size at six months of age. *Neuropsychopharmacology, 32,* 1082-97.

Smolka, M.N., Bűhler, M., Klein, S., Zimmermann, U., Mann, K., Heinz, A., & Braus, D.F.
2006 Severity of nicotine dependence modulates cue-induced brain activity in regions involved in motor preparation and imagery. *Psychopharmacology, 184*(3-4), 577-588.

Suzuki, T., Ise, Y., Tsuda, M., Maeda, J., & Misawa, M.
1996 Mecamylamine-precipitated nicotine-withdrawal aversion in rats. *European Journal of Pharmacology, 314*(3), 281-284.

Tapper, A.R., McKinney, S.L., Marks, M., & Lester, H.A.
2007 Nicotine responses in hypersensitive and knockout alpha 4 mice account for tolerance to both hypothermia and locomotor suppression in wild-type mice. *Physiological Genomics, 31(*3), 422-428.

Tapper, A.R., McKinney, S.L., Nashmi, R., Schwarz, J., Deshpande, P., Labarca, C., Whiteaker, P., Marks, M.J., Collins, A.C., & Lester , H.A.
2004 Nicotine activation of alpha4* receptors: Sufficient for reward, tolerance, and sensitization. *Science, 306*,1029-1032.

Uhl, G.R., Liu, Q.-R., Drgon, T., Johnson, C., Walther, D., Rose, J.E., David, S.P., Niaura, R., & Lerman, C.
2008 Molecular genetics of successful smoking cessation: Convergent genome-wide association study results. *Archives of General Psychiatry, 65*(6), 683-693.

Volk, H.E., Scherrer, J.F., Bucholz, K.K., Todorov, A., Hearth, A.C., Jacob, T., & True, W.R.
2007 Evidence for specificity of transmission of alcohol and nicotine dependence in an offspring of twins design. *Drug and Alcohol Dependence, 87*(2-3), 225-32.

Volkow, N.D., Wang, G.J., Fowler, J.S., & Telang, F.
2008 Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. Philosophical Transactions of the Royal Society of London *B-Biological Sciences, 363*(1507), 3191-200.

Wada, E., Wada, K., Boulter, J., Deneris, E., Heinemann, S., Patrick, J., & Swanson, L.W.
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. *Journal of Comparative Neurology, 284*(2), 314-335.

Wagena, E.J., de Vos, A., Horwith, G., & van Schayck, C.P.
2008 The immunogenicity and safety of a nicotine vaccine in smokers and nonsmokers: Results of a randomized, placebo-controlled phase 1/2 trial. *Nicotine & Tobacco Research, 10*(1), 213-218.

Wellman, R.J., DiFranza, J.R., Savageau, J.A., Godiwala, S., Savageau, N., Friedman, K., & Hazelton, J.
2006 The effect of abstinence on cigarette consumption upon the resumption of smoking. *Addictive Behaviors, 31*, 711-6.

Xue, Y., & Domino, E.F.
2008 Tobacco/nicotine and endogenous brain opioids. *Prog. Neuro-Progress in Neuro-psychopharmacology & Biological Psychiatry.* Elsevier, in press.

Ziedonis, D.M.
2004 Integrated treatment of co-occurring mental illness and addiction: clinical intervention, program, and system perspectives. *CNS Spectrum, 9*(12), 892-904.

Ziedonis, D.M. & Williams, J.
2003 Addressing Tobacco Use in Mental Health and Addiction Settings. *Psychiatric Annals 33*(7), 425-426.

Zoli, M., Le Novere, N., Hill, J.A., Jr., & Changeux, J.P.
1995 Developmental regulation of nicotinic ACh receptor subunit mRNAs in the rat central and peripheral nervous systems. *Journal of Neuroscience, 15*(3 Pt 1), 1912-1939.

Zubieta, J.K., Heitzeg, M.M., Xu, Y., Koeppe, R.A., Lisong, N., Guthrie, S., & Domino, E.F.
2005 Regional cerebral blood flow responses to smoking in tobacco smokers after overnight abstinence. *American Journal of Psychiatry, 162*(3), 567-577.