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Mini-Review

Rodent models of nicotine reward: What do they tell us about tobacco abuse in humans?

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ABSTRACT

Tobacco products are widely abused in humans, and it is assumed that nicotine is the key substrate in these products that produces addiction. Based on this assumption, several pre-clinical studies have utilized animal models to measure various aspects of nicotine addiction. Most of this work has focused on behavioral measures of nicotine and how other variables contribute to these effects. Here we discuss the most commonly used animal models including, self-administration (SA), place conditioning (PC), and the intracranial self-stimulation (ICSS) paradigms in rodents. The strengths, limitations and procedural variables of these models are reviewed, followed by a discussion of how the animal models have been used to study factors such as age, sex, stress, and the effects of tobacco products other than nicotine. These factors are discussed in light of their influences on human tobacco abuse. The rodent models are evaluated in the context of face, predictive, and construct validity, and we propose that inclusion of factors such as age, sex, stress and other constituents of tobacco aside from nicotine can increase the utility of these animal models by more closely mimicking human tobacco abuse.

Contents

1. Introduction

Tobacco use is a major health and economic concern. Although over 4800 chemical compounds have been identified in tobacco, the addictive nature of tobacco products is largely due to one compound,

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nicotine, a major alkaloid component (Stolerman and Jarvis, 1995). The seven main features of nicotine dependence have been formally described in the Diagnostic and Statistical Manual (DSM-VI). These include: tolerance, withdrawal, increasing use over a longer period than intended, unsuccessful efforts to discontinue use, large amounts of time spent obtaining drug, loss of social and occupational functioning, and continued use despite realization of harmful consequences. The DSM-VI requires a person to meet at least 3 of these criteria to be considered dependent and neither tolerance nor withdrawal alone is sufficient for a diagnosis of nicotine dependence.

Much pre-clinical work has focused on studying the neural mechanisms that mediate the rewarding effects of nicotine. Although, it is not possible to mimic all aspects of nicotine dependence as indicated by the DSM-VI criteria, animal models have attempted to mimic aspects of dependence including, tolerance, withdrawal, and possibly continued use and inability to discontinue use. The three most commonly used animal models to study the rewarding effects of nicotine include the self-administration (SA), place conditioning (PC) and intracranial self-stimulation (ICSS) paradigms.

The goal of this review is to provide a discussion of the most commonly used rodent models of nicotine addiction and to provide an evaluation of the validity of these models in measuring different aspects of tobacco abuse in humans. Our discussion includes a description of the methodology, parameters, and findings from studies using the SA, PC, and ICSS rodent models. We also include a discussion of how these models have been used to assess nicotine dependence and withdrawal since these are contributing factors to tobacco abuse in humans. An important aspect of our review is a consideration of these animal models with respect to the degree to which they assess face, predictive, and construct validity. Finally, we discuss how the inclusion of variables such as age, sex, environmental stressors, and additional tobacco ingredients in these rodent models can potentially increase the utility of these models by providing a better understanding of the mechanisms that mediate tobacco abuse in humans.

2. Animal models assessing the rewarding effects of nicotine

2.1. Nicotine SA

The SA paradigm is based on reinforcement principles involving strengthening of a behavioral response by presentation of nicotine after the operant response is performed. The operant behavior typically involves lever pressing, but often includes nose poke behavior in mouse preparations. Although oral nicotine SA has been established, this review will focus on the intravenous (IV) route of administration given that this route is more commonly used in animal studies and more closely mimics the rapid drug distribution of nicotine to the brain via inhalation.

Nicotine IVSA was first demonstrated in non-human primates (Goldberg et al., 1981), and subsequent reports using rodents focused on optimizing the parameters of nicotine IVSA. Using limited access schedules, manipulations such as a fast infusion delivery (approximately 1 s), and a pH of the drug at physiological levels have emerged as important variables that facilitate reliable nicotine IVSA in rats (Corrigall and Coen, 1989). Several laboratories have also reported that nicotine IVSA using limited access conditions (e.g. 1–2 h access) is facilitated by pre-training with food reinforcement and by maintaining animals on a food-restricted diet (approximately 80% of free feeding weight). The critical role of secondary reinforcers (i.e., cues predictive of nicotine) in maintaining nicotine IVSA in rats has also been examined. Indeed, when rats are permitted to make responses for nicotine in the absence of such cues, the operant response then extinguishes (Caggiula et al., 2002). Thus, it appears that a number of experimental manipulations (food pre-training, food-restriction, and the presence of secondary reinforcers) are important for rats to acquire and maintain nicotine IVSA. It is noteworthy, that such manipulations are not needed, or at least not needed to a great extent, for IVSA of other drugs of abuse such as cocaine. Thus, the need for such manipulations in order for rats to self-administer nicotine may call into question the validity of the IVSA paradigm in rats (at least under limit-access conditions) and whether nicotine really serves as a positive reinforcer on its own.

More recent studies have avoided food restriction procedures by giving the animals extended access to nicotine IVSA. For the extended procedures, animals are given up to 23 h of access to nicotine IVSA in a chamber where they are also able to respond for food and water delivery. Most studies using extended access procedures have employed the use of secondary reinforcers, indicating that the use of stimulus lights may also be necessary for the maintenance of nicotine intake in extended access procedures. However, to our knowledge no one has determined whether cues are as critical in extended access procedures as they have been shown to be in limited access procedures. The extended access paradigm is believed to model continuous availability of tobacco in humans. These studies have shown that rats display increased nicotine IVSA during the active/dark phase of the light cycle, and that the average daily nicotine intake is 0.18–1.5 mg/kg/day which approximates the levels of nicotine intake observed in human smokers (LeSage et al., 2003). Lastly, it has been demonstrated that rats given extended access to nicotine display physical signs of withdrawal and an increase in nicotine intake following abstinence from nicotine IVSA (O'Dell and Koob, 2007). This "nicotine deprivation effect" is believed to reflect the increase in tobacco use that is seen during relapse in abstinent smokers.

Although nicotine IVSA has been observed in mice, there are fewer studies in mice as compared to rats. In the initial studies, the tail vein was used as the IV portal due to the small size of the jugular vein in mice (Martellotta et al., 1995). Since the tail of the mouse is secured during the entire session to avoid disruption of drug delivery, it has been suggested that these studies may be limited with regard to modeling nicotine use in humans. Nicotine IVSA in mice has also been examined in animals that were trained initially to press for cocaine (Picciotto et al., 1998). This procedure may model how tobacco is commonly used in combination with other drugs of abuse. Also, there is a report of extended (12 h) access to nicotine in mice whereby animals SA nicotine during the active phase of their light cycle, which more closely mimics extended use of nicotine in humans during their active wake period (Stolerman et al., 1999).

2.2. Nicotine-induced PC

The PC paradigm assesses the motivational properties of a drug by means of Pavlovian conditioning. The drug is administered in a distinct environment and after several pairings the environment (conditioned stimulus=CS) becomes associated with the effects of the drug (unconditioned stimulus=UCS), thereby acquiring incentive-motivational properties. The environment contains cues that elicit either approach (i.e., conditioned place preference; CPP) or avoidance (i.e., conditioned place aversion; CPA) depending on whether rewarding or aversive properties of the drug were associated with the cues during conditioning.

There are some methodological issues to be considered when conducting PC studies with nicotine. Perhaps, the most important factor in regards to measuring nicotine reward is whether a *biased* or *unbiased* PC design is used. In a *biased* PC procedure, the animal receives repeated drug administration in their initially non-preferred environment (if examining the rewarding effects of a drug), or the preferred side (if examining the aversive effects of a drug). In an *unbiased* PC design, the animals are randomly assigned without regard to initial bias for either side of the conditioning apparatus.

In rats, nicotine has been reported to produce CPP, CPA, or no effect depending on the dose of nicotine that is used. In general, previous studies using a dose of nicotine within a 0.2–0.6 mg/kg dose range report CPP, whereas studies using a dose within a 0.8–1.2 mg/kg dose range report

Table 1
Validity of animal models used to study nicotine addiction

	Face validity	Predictive validity	Construct validity
	"Are the overt behavioral qualities seen in the human condition measured in the animal model?"	"Does the behavioral outcome in a particular test predict performance in the condition being modeled?" "Are pharmacotherapies used in clinical settings effective in the animal model?"	"Is the theoretical premise underlying human nicotine addiction similar to that in the animal model?"
Self administration (SA)	*High:* • Animals have control over nicotine delivery. • Provides a measure of compulsive nicotine-taking behavior. • The intravenous route is often employed, providing a route that is characterized by rapid absorption.	*Moderate–high:* • Animals self-administer nicotine. • Nicotine replacement therapies, bupropion, and mecamylamine decrease nicotine IVSA in rodents.	*Moderate–high:* • Provides a good measure of reinforcement since primary reinforcers (such as food and water) are also self-administered. • Studies have examined the importance of central cholinergic, dopaminergic, glutaminergic, gabaergic and serotonergic systems in mediating nicotine intake.
Place conditioning (PC)	*Low–moderate:* • Unlike human nicotine consumption, nicotine delivery is passive with this animal model. *However:* • Different routes of administration have been used. • Animals receive repeated injections of nicotine over many days. • On the test day, animals "seek" nicotine similar to humans. • The environment paired with nicotine produces a conditioned response (similar to humans).	*Moderate:* • Nicotine can produce PC, *although*, nicotine PC can be difficult to establish (see text). • Effectiveness of pharmacotherapies has not been assessed with PC.	*Moderate–high:* • PC paradigm provides a valid measure of both nicotine-induced reward and aversion (at higher doses). • PC models incentive motivational properties of drug-cue associations. • Only dopamine and cannabinoid systems in acquisition of nicotine PC have been examined.
Intracranial self-stimulation (ICSS)	*Low:* • ICSS does not overtly appear to mimic any component of tobacco use in humans. *However:* • Nicotine can facilitate the rewarding effects of alcohol as reflected by a decrease in ICSS threshold.	*Moderate:* • Nicotine lowers the threshold for ICSS similar to other reinforcing drugs. • Rewarding value of nicotine is predictive of relapse to smoking in humans. • Bupropion has been shown to reverse the increases in brain reward threshold observed during withdrawal.	*Moderate:* • Changes in threshold values appear to be a valid measure of nicotine reinforcement. • Dopaminergic systems appear to play a role in nicotine-induced changes in ICSS. • This model mimics malaise and other negative aspects associated with nicotine abstinence.

CPA (for a review see Le Foll and Goldberg, 2005). The majority of studies reporting nicotine-induced CPP in rats have used the biased procedure. The interpretation of CPP data using a biased procedure can be problematic because pairing the US with the initially non-preferred compartment can result in a preference shift, that may be due to a reduction of aversion as opposed to the rewarding effects of the drug per se (Torrella et al., 2004). However, a recent study using a biased design concluded that nicotine-induced CPP is due in part to a preference shift rather than a reduction in aversive effects per se (Brielmaier et al., 2008). Also, reliable nicotine-CPP using the IV route of administration has been shown using an unbiased procedure that is sensitive to the number of drug-pairings and nicotine doses (Wilkinson and Bevins, 2008).

Nicotine has also been demonstrated to produce a CPP, CPA, or have no effect in mice. In contrast to rats, an unbiased procedure has typically been used in mouse studies that reported nicotine CPP. There appears to considerable overlap between doses that have no effect, produce CPP, or produce CPA even within individual mouse strains. For example, in the outbred Swiss Webster mice, 0.18–2.0 mg/kg nicotine produce CPP, 2.0 mg/kg nicotine produces CPA, and 0.25–1.0 mg/kg nicotine have been shown to have no effect on PC (Martin and Itzhak, 2000; Risinger and Oakes, 1995; Sahraei et al., 2004). In the inbred C57BL/6 mice, CPP has been reported with 0.1–0.5 mg/kg nicotine, CPA has been reported with 0.7 mg/kg, and no effect has been reported with 0.25–1.0 mg/kg of nicotine (Grabus et al., 2006; Walters et al., 2006, 2005). In general, these rodent studies have shown that low doses of nicotine produce CPP, whereas high doses of nicotine produce CPA.

2.3. Nicotine-induced changes in ICSS behavior

In ICSS studies, rats are first implanted with an electrode that is typically placed in the medial forebrain bundle that consists of efferent projections to several reward-related structures of the mesolimbic dopamine pathway. The animals are allowed to SA small amounts of electrical current to the brain, which can engender high levels of operant responding for ICSS. Typically, the experimenters vary the levels of electrical stimulation to determine a baseline threshold level at which behavioral responses are reliably maintained. Once a threshold level of electrical stimulation is established, the rewarding effects of drugs of abuse are assessed by examining whether administration of a drug reduces ICSS thresholds. A lowering of the ICSS threshold is believed to reflect an increase in brain reward function due to the reinforcer, nicotine.

Several reports have demonstrated that nicotine lowers the current intensity threshold for ICSS similar to other drugs of abuse (Huston-Lyons et al., 1993; Kenny and Markou, 2006). A recent report demonstrated that nicotine IVSA increased the sensitivity of brain reward systems and that this effect persisted for at least 36 days (Kenny and Markou, 2006). This finding suggests that the effects of nicotine IVSA on brain reward function are long lasting and that nicotine intake may reset the sensitivity of reward systems to a new elevated level. Two recent reports have verified that the effect of nicotine on ICSS thresholds is similar in mouse preparations (Johnson et al., 2008; Stoker et al., 2008).

3. Validity of animal models

The extent to which an animal model can be used to understand the underlying nature of addiction in humans depends largely on the validity of the animal model and which aspect of the addiction process is being modeled (Willner, 1991). Traditionally, the validity of animal models have been evaluated using three main criteria: face, predictive, and construct validity (Willner, 1991). The degree to which an animal model serves as a valid index of a human condition is open to debate. Here we provide an assessment of the validity of each animal model as a means for investigating nicotine addiction in humans (see Table 1).

3.1. Face validity

Face validity refers to whether the overt behavioral qualities of the human condition are similar to what is observed in the animal model. This may be the easiest type of validity to assess because it involves a phenotypic comparison of the animal's behavior with what is overtly seen in human addicts. For example, a major criterion for drug dependence as emphasized by DSM-VI is that the drug has control over behavior. In animal studies, this is seen as an increase in motivation to acquire drug, extinction of behavior once the drug is removed, and relapse to drug-seeking once the drug is re-introduced. Thus, the SA model typically emerges as the model with the highest degree of face validity because it mimics voluntary consumption of tobacco in humans. It may be argued that the cost-benefit aspect of drug addiction is not fully addressed by the SA paradigm. However, studies employing progressive ratio (PR) schedules, where rats complete an increasing schedule of reinforcement to obtain drug, appear to address this issue. Thus, the PR schedule might assess the strength of the rewarding effects of a drug by determining how willing an animal is to work for a drug injection. Nicotine has been shown to maintain high levels of responding on a PR schedule in rats (Donny et al., 1999). Also, animals that reliably SA nicotine display a compensatory decrease in operant responding when nicotine is replaced with vehicle (i.e., extinction of IVSA behavior). Previous work has shown that extinction of nicotine IVSA is more robustly reinstated by presentation of drug-associated cues versus presentation of the drug itself (LeSage et al., 2004; O'Dell et al., 2007a). This is believed to reflect the importance of cues in maintaining and reinstating tobacco use in abstinent smokers.

The PC paradigm is generally thought to possess a low-moderate degree of face validity based on the fact that the drug is delivered passively and not dependent upon motivation for the drug. However, the PC paradigm has some degree of face validity given that environmental cues are repeatedly paired with the drug and eventually become a CS. Thus, when the animal is given access to both compartments, and the resulting effect is a CPP, the environment is said to have acquired secondary reinforcing properties much like tobacco cues elicit conditioned responses and craving in humans (McClernon and Gilbert, 2004). Relapse to nicotine seeking in humans has also been modeled using the PC paradigm. Following extinction of CPP, a nicotine prime can reinstate this effect presumably due to the persistence of nicotine-cue associations that persist after conditioning (Biala and Budzynska, 2006).

The ICSS model is generally seen as having weak face validity. This is based on the fact that this model does not overtly mimic drug taking in human tobacco users. However, the face validity of this model may apply to the ability of nicotine to influence the rewarding effects of other stimuli (in this case ICSS), since nicotine is most commonly used in combination with other rewarding substances such as alcohol (Hertling et al., 2005). Although still highly speculative, changes in ICSS behavior may model how the rewarding effects of nicotine are used to facilitate the pleasurable effects of other substances or environmental stimuli.

3.2. Predictive validity

Predictive validity refers to whether the behavioral outcomes in the animal model predict performance in the human condition being modeled (Willner, 1991). For example, animal models of drug reward with a high degree of predictive validity are able to evaluate whether a novel drug possesses abuse liability in humans. Each of the animal models has been useful with regard to predicting abuse liability and potential treatments for nicotine abuse.

The SA paradigm appears to have a high degree of predictive validity since nicotine SA behavior is reliably produced in rodents, and thus nicotine appears to be the major ingredient that motivates tobacco abuse in humans. The predictive validity associated with the SA model and the effectiveness of pharmacotherapies that are used for tobacco abuse in humans have been summarized in a recent review (Lerman et al., 2007). For example, systemic injections of the nicotinic antagonists dihydro-ß-erythroidine (DHßE) and mecamylamine decrease nicotine IVSA in limited IVSA procedures (Lerman et al., 2007). Bupropion is another pharmacotherapy that has been examined in nicotine IVSA studies using the limited access procedure. Our review of reports examining the effects of bupropion on nicotine IVSA yielded somewhat mixed results, but this may be due to a complex non-linear dose-effect function for bupropion that results in opposing effects in different portions of the dose range. Thus, the overall pattern of results may best be explained by the results of a thorough dose-response study demonstrating that low doses of bupropion increase nicotine IVSA whereas high doses of this drug reduce nicotine intake (Rauhut et al., 2003). The effects of high doses of bupropion may be due to non-specific decreases in responding, since the doses of bupropion that reduced nicotine IVSA also reduced responding for sucrose and amphetamine (Rauhut et al., 2003). These results with bupropion on nicotine IVSA in rats may make it difficult to conclude the degree to which the nicotine IVSA paradigm possesses predictive validity in terms of preclinical drug development. However, it should be noted that even in human clinical studies bupropion is not effective in all studies. Although, bupropion is thought to facilitate quit rates for smoking by reducing withdrawal and craving (Mooney and Sofuoglu, 2006), this may also depend on the motivational factors of the individual attempting to quit. For example, bupropion does not decrease but in fact increases smoking behavior following abstinence in smokers not attempting to quit (Cousins et al., 2001). In closing, there are many stages in the addiction cycle that have been modeled by the IVSA paradigm (initiation, maintenance, cessation, abstinence, relapse), and it remains to be seen whether all of these medications have different effects on various stages of addiction as measured in this and other animal models.

The PC paradigm appears to have a moderate degree of predictive validity as a measure of the rewarding effects of nicotine. One advantage of the PC paradigm with regard to predictive validity is the ability of this model to assess both rewarding (CPP) and aversive (CPA) effects depending on the dose that is delivered. This is similar to the subjective effects of nicotine in humans. The PC model also appears to model the weak reinforcing effects of nicotine as compared to other psychostimulants, based on the fact that it has been more difficult to establish nicotine-induced CPP compared to other drugs of abuse. In regards to establishing effectiveness of pharmacotherapies, studies have shown that mecamylamine and the cannabinoid antagonist Rimonibant can decrease nicotine-induced CPP (Lerman et al., 2007) indicative that this model can be used as a screen to predict effectiveness of novel pharmacotherapies. In rats bupropion attenuates physical signs of withdrawal and conditioned place aversion produced by nicotine withdrawal (Malin et al., 2006). Thus, it is possible that the ability of bupropion to attenuate the physical and affective properties of nicotine withdrawal adds veracity to the predictive validity of place conditioning procedures to examine putative treatments for nicotine withdrawal.

The ICSS paradigm appears to have a moderate degree of predictive validity with regard to its ability to detect the rewarding effects of nicotine. In this regard, nicotine produces an increase in brain reward function that is comparable to the increases produced by other abused drugs. Also, bupropion, which has shown some utility in treating clinical withdrawal signs in humans, reverses the reward deficits produced by nicotine withdrawal in ICSS procedures (Cryan et al., 2003).

It should be noted, however, that a limitation for all three models in regards to predictive validity is that all studies have examined the effects of nicotine in the absence of other chemicals found in tobacco smoke. As a result, the utility of these models for predicting abuse liability of tobacco in humans is largely unknown.

3.3. Construct validity

The final and most difficult aspect of validity to ascertain is construct validity which refers to whether there is a sound theoretical

rationale linking the human condition and the animal model (Willner, 1991). Construct validity is the highest standard but most difficult to achieve because the underlying etiology of addiction is not well understood. The underlying substrates mediating the addiction process are complex and involve both behavioral and physiological constructs.

In humans, substances such as nicotine become abused because they produce positive reinforcing subjective effects that elicit drug-seeking behavior. Similarly, a drug is said to maintain SA behavior in animals because it acts as a positive reinforcer. Also, there are a constellation of other aspects that influence nicotine IVSA including drug-associated cues, stress, age, and sex differences in a similar manner in rodents as well as humans. With the PC paradigm variables such as sex, age, and drug-associated cues have also been shown to mediate nicotine CPP. Indeed, drug-cue associations have been shown to elicit relapse during abstinence evident as a reinstatement of nicotine-CPP. Lastly, the construct validity of the ICSS paradigm must be considered from the theoretical premise that lowering brain ICSS thresholds is due to the rewarding effects of the drug. The ICSS model also appears to mimic certain negative aspects associated with nicotine withdrawal in humans. For example, recent reports have demonstrated that blocking brain stress systems with corticotropin-releasing factor (CRF) antagonists reverses changes in ICSS behavior associated with nicotine withdrawal (Bruijnzeel and Gold, 2005). Thus, this model is able to assess constructs such as stress that may play an important role in mediating tobacco abuse.

There have been numerous reports examining the underlying physiological substrates that mediate tobacco abuse. The mesolimbic dopamine system has emerged as playing a key role in mediating the rewarding effects of nicotine and animal studies involving IVSA, CPP, and ICSS are sensitive to manipulations of dopaminergic systems (Corrigall, 1991; Huston-Lyons et al., 1993; Spina et al., 2006). It is recognized that dopamine is not the sole system responsible for mediating tobacco abuse. Indeed, preclinical studies have begun to explore the importance of central cholinergic, glutaminergic, gabaergic, serotonergic, and cannabinoid systems in mediating the rewarding effects of nicotine (Liechti and Markou, 2008; Merritt et al., 2008; Paterson and Markou, 2007). Future studies are likely to provide a more comprehensive understanding of how these various neurotransmitter systems interact to mediate tobacco abuse.

4. Measuring nicotine withdrawal in animal models

Cessation from smoking in humans results in a number of withdrawal signs including emotional, cognitive, and physical changes that can vary in time of onset, magnitude, and duration (Ward et al., 2001). Nicotine withdrawal has been widely studied in rats following a period of chronic nicotine administration via subcutaneous osmotic mini-pumps. Several studies have demonstrated that the nicotine withdrawal syndrome is comprised of both physical and affective components. The physical signs of nicotine withdrawal in rats include abdominal constrictions, facial fasciculation, writhes, gasps, eye blinks, and ptosis (Malin, 2001; O'Dell et al., 2004). Although physical and affective properties of withdrawal are elicited in nicotine-dependent mice, higher doses of nicotine are needed over a longer period of time to produce these effects in mice as compared to rats (40 mg/kg for 28 days in mice versus 9 mg/kg for at least 7 days in rats; (Stoker et al., 2008).

The affective properties of nicotine withdrawal have been assessed using ICSS procedures. Withdrawal from chronic nicotine produces an increase in brain reward threshold that is thought to reflect a decrease in brain reward function (Epping-Jordan et al., 1998; Panagis et al., 2000). Recent reports have illustrated that nicotine-dependent mice and rats display an increase in current intensity thresholds during withdrawal (Johnson et al., 2008; Stoker et al., 2008). The need for higher current levels is believed to reflect a decrease in brain reward function during withdrawal.

The PC paradigm has also been used to measure aversive effects induced by nicotine withdrawal. In these studies, animals typically receive chronic nicotine via osmotic pumps for 5–7 days. During conditioning, the animal receives a nicotinic receptor antagonist (such as mecamylamine) to precipitate withdrawal and is confined to one side of the apparatus. On alternating days they receive saline in the other compartment. Following conditioning, nicotine-dependent adult rats reliably display a CPA for the compartment where they experienced withdrawal (O'Dell et al., 2007b; Suzuki et al., 1996). The ability of mecamylamine to produce CPA has also been shown in nicotine-dependent mice (Jackson et al., 2008).

5. Other variables influencing tobacco abuse that have been included in animal models

There are a myriad of factors that contribute to tobacco abuse in humans including age, sex, stress, and other non-nicotinic constituents of tobacco. Animal studies have begun to reveal the importance of these variables as pertaining to tobacco abuse in humans as outlined below.

5.1. Age-dependent differences

Developmental studies in rodents generally consider adolescence as a period of enhanced vulnerability to nicotine (for a review see (O'Dell, 2009). For example, both male and female adolescent rats acquire nicotine IVSA more readily and display higher levels of nicotine intake compared to their adult counterparts under both limited and extended access conditions (Chen et al., 2007; Levin et al., 2007; Levin et al., 2003). CPP studies have also demonstrated that across various doses of nicotine, adolescent male rats display enhanced CPP relative to adults (Torres et al., 2008). These findings are consistent with those from other laboratories demonstrating that adolescents display CPP at doses of nicotine that do not produce CPP in adult rats (Shram et al., 2006; Vastola et al., 2002). In addition, a single injection of nicotine has been shown to produce CPP in early adolescent (PND 28) but not late adolescent (PND 38) or adult (PND 90) rats, even when additional conditioning trials were given to the adults (Belluzzi et al., 2004).

Recent studies have also examined developmental differences to nicotine withdrawal. For example, adolescent rats display fewer somatic signs of nicotine withdrawal and lower CPA produced by nicotine withdrawal compared to adult rats (O'Dell et al., 2004, 2007b). Consistent with this, male adolescent mice display lowered CPA produced by nicotine withdrawal when compared to adult mice (Kota et al., 2007). Based on these findings, it has been suggested that tobacco abuse during adolescence is driven in large part by strong rewarding effects of nicotine that are unequally balanced against the aversive effects of nicotine withdrawal (for a discussion see O'Dell, 2009). Thus, these animal studies mirror the enhanced vulnerability to the rewarding effects of nicotine during adolescence in humans, and they suggest that animal models examining nicotine reward should carefully consider the age of the animals.

5.2. Sex-dependent differences

Clinical studies have shown that females are particularly vulnerable to tobacco use. For example, females consume more tobacco products, have more difficulty quitting, and experience more severe withdrawal symptoms compared to males (Schnoll et al., 2007).

Animal studies also suggest that females are generally more vulnerable to nicotine use than are males. For example, female adult rats display faster acquisition of nicotine IVSA at lower doses

compared to male adults (Donny et al., 2000). The latter study also demonstrated that female adults display higher motivation for nicotine intake, as they reach a higher break point for nicotine infusions on a progressive ratio schedule of reinforcement relative to males. Consistent with this, recent unpublished observations in our laboratory have shown that female rats display enhanced nicotine-induced CPP relative to males and that the magnitude of this effect does not differ across the 4 day estrous cycle in adult females. Collectively, these studies suggest that the rewarding effects of nicotine are enhanced in female rodents as in clinical studies. Thus, sex differences are important to consider when assessing the rewarding effects of nicotine in animals.

5.3. Stress-related effects

The complex interplay between tobacco abuse and stress has been well established in humans and animal models. Smoking behavior in humans is enhanced by stress, and can be alleviated by tobacco use (Parrott, 1995). Nicotine can induce the release of stress-related hormones such as corticosterone (CORT), adrenocorticotropic hormone (ACTH), and prolactin (Lutfy et al., 2006; Sharp and Beyer, 1986). Nicotine IVSA increases levels of CORT following the first day of nicotine intake; however, tolerance rapidly develops to this effect (Chen et al., 2008). Furthermore, animals that SA nicotine displayed a chronic increase in their stress-responses to a novel stressor compared to naïve animals. Collectively these data are consistent with human clinical studies showing that nicotine exposure generally increases stress responses in people with a history of nicotine use (Chen et al., 2008).

Exposure to stressors has also been associated with a greater likelihood of relapse to tobacco use in humans (Bruijnzeel and Gold, 2005). In animals, exposure to foot-shock reinstates nicotine-seeking behavior following extinction from extended nicotine access (Buczek et al., 1999; Martin-Garcia et al., 2008). The neurobiological substrates that mediate stress-induced reinstatement of nicotine-seeking behavior have not been thoroughly investigated. However, a recent study reported that the corticotropin-releasing factor (CRF) antagonist D-Phe CRF(12e41) and the α2-adrenergic receptor agonist, clonidine, significantly attenuate stress-induced reinstatement of nicotine-seeking behavior in rats, suggesting that a heightened CRF and noradrenergic response may mediate stress-induced reinstatement of nicotine-seeking (Zislis et al., 2007). The ICSS procedure has been used to assess the effects of the CRF system on nicotine withdrawal in rats. Specifically, blockade of CRF receptors via D-Phe CRF(12–41) administration prevents the elevations in brain reward thresholds produced by precipitated, but not spontaneous nicotine withdrawal (Bruijnzeel et al., 2007). These concordant findings suggest that stress can be an important mediator of the rewarding effects of nicotine as well as the relapse to nicotine seeking.

5.4. Tobacco ingredients other than nicotine

Although nicotine has been shown to be a major component of tobacco that leads to addiction, it is possible that other components of tobacco augment the rewarding effects of nicotine. Thus, inclusion of these compounds should be incorporated in these animal models to more clearly model tobacco dependence in humans given that this is how the industry designs its products to be maximally addictive.

Non-nicotine smoke components such as ammonia-forming ingredients are added as flavorants and processing agents. These chemicals may influence the reinforcing effects of tobacco products in several ways: (1) by potentially increasing the amount of nicotine that enters the circulatory system; (2) by directly affecting nicotinic receptors; and (3) by enhancing the sensory cues of cigarettes and thus making the tobacco more palatable (Henningfield et al., 2004).

Additional compounds that block the breakdown of monoamines such as monoamine oxidase (MAO) inhibitors have been identified in tobacco smoke (Berlin and Anthenelli, 2001; Fowler et al., 2003). The inhibition of MAO leads to decreased metabolism of dopamine and other monoamines. A decrease in dopamine metabolism causes an increase in dopamine transmission, and this may contribute to tobacco abuse. It is likely that inhibiting the actions of MAO can synergize with nicotine to enhance smoking behavior by prolonging the activity of dopamine. A recent study reported that tranylcypromine, an irreversible MAO inhibitor, can enhance the acquisition of nicotine IVSA in rats (Villegier et al., 2007). Also, it has been shown that knockout mice lacking the MAO_A subtype do not exhibit nicotine-induced CPP relative to their wildtype controls (Agatsuma et al., 2006). Acetaldehyde, which is one of the most abundant constituents in tobacco smoke (Seeman et al., 2002) has been shown to have reinforcing properties on its own using both the SA and PC models (Talhout et al., 2007). Acetaldehyde also can enhance the acquisition of nicotine IVSA in adolescent but not adult rats (Belluzzi et al., 2005). Finally, metabolites of nicotine can also contribute to the addictive nature of tobacco products. For example, one important metabolite that has received much attention is nornicotine. Although only about 8% of nicotine is metabolized to nornicotine in the periphery, nornicotine has a much longer half-life (8 h) compared to nicotine in humans and nornicotine may accumulate in the brain following repeated tobacco use (Green et al., 2001). In animal models, nornicotine is self-administered and also stimulates DA release in the nucleus accumbens via stimulation of local nicotinic receptors (Green et al., 2001). Collectively, theses studies suggest that there may be several other products in tobacco that contribute to its rewarding value in clinical populations. Thus, future studies should take into consideration these additional components of tobacco to more accurately study tobacco abuse in humans.

6. Concluding remarks

Nicotine plays a critical role in maintenance of tobacco abuse in humans. The IVSA, PC, and ICSS paradigms have been used to assess the rewarding properties of nicotine in rodents. The rewarding effects of nicotine have however, been more difficult to establish than those of other abused drugs possibly due to factors such as the weak reinforcing effects of nicotine, age and/or sex differences, environmental stress, and the absence of other tobacco products in animal studies using nicotine alone. We acknowledge that all aspects of tobacco abuse in humans cannot be modeled in animals—especially psychological human constructs that are difficult to assess in animals. Although this may call into question the usefulness of these models, we suggest that the inclusion of relevant factors, such as sex, age, stress, and other tobacco components increases the utility of these animal models by providing converging lines of evidence leading to a comprehensive understanding of tobacco abuse in humans. Also, these models provide a valuable approach for testing the effectiveness of potential treatment medications, and a way in which the basic biological mechanisms that mediate the rewarding effects of nicotine can be examined. In this way, studies using these models can provide their unique contribution towards a holistic understanding of tobacco addiction in humans.

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