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A Threshold Model for Opposing Actions of Acetylcholine on Reward Behavior: Molecular Mechanisms and Implications for Treatment of Substance Abuse Disorders

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Abstract

The cholinergic system plays important roles in both learning and addiction. Medications that modify cholinergic tone can have pronounced effects on behaviors reinforced by natural and drug reinforcers. Importantly, enhancing the action of acetylcholine (ACh) in the nucleus accumbens and ventral tegmental area (VTA) dopamine system can either augment or diminish these behaviors. A threshold model is presented that can explain these seemingly contradictory results. Relatively low levels of ACh rise above a lower threshold, facilitating behaviors supported by drugs or natural reinforcers. Further increases in cholinergic tone that rise above a second upper threshold oppose the same behaviors. Accordingly, cholinesterase inhibitors, or agonists for nicotinic or muscarinic receptors, each have the potential to produce biphasic effects on reward behaviors. Pretreatment with either nicotinic or muscarinic antagonists can block drug- or food-reinforced behavior by maintaining cholinergic tone below its lower threshold. Potential threshold mediators include desensitization of nicotinic receptors and biphasic effects of ACh on the firing of medium spiny neurons. Nicotinic receptors with high- and low-affinity appear to play greater roles in reward enhancement and inhibition, respectively. Cholinergic inhibition of natural and drug rewards may serve as mediators of previously described opponent processes. Future studies should evaluate cholinergic agents across a broader range of doses, and include a variety of reinforced behaviors.

Keywords

acetylcholine; acetylcholinesterase inhibitors; cocaine; donepezil; galantamine; nicotinic receptor; muscarinic receptor; self-administration; reinforcement (Psychology); rivastigmine

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Introduction

ACh is widely distributed in the central nervous system, where it functions as a signal for local circuits and projection neurons. Both types of cholinergic neuron are involved in brain learning and reward functions. Synaptic levels of ACh are regulated by choline acetyltransferase, the rate-limiting enzyme for formation of ACh, and cholinesterases that inactivate it. ACh activates two categories of receptor: nicotinic and muscarinic. Neuronal nicotinic ACh receptors (nAChRs) are a family of ligand-gated ion channels that are made of combinations of type 2 through 9 alpha subunits, and type 2 through 4 beta subunits, arranged to form a pentameric pattern. Different subunit combinations give rise to various types of nAChRs, which differ in sensitivity to nicotine, calcium conductance, and propensity to desensitize [1], discussed in greater detail below. In contrast, muscarinic receptors are members of the superfamily of G protein-coupled receptors. Five muscarinic subtypes have been cloned which function through either activation of phospholipase (types 1, 3, and 5) or inhibition of adenylate cyclase to decrease the concentration of intracellular cAMP (types 2 and 4) [2]. Dopamine neurons express multiple types of muscarinic and nicotinic ACh receptors, and a dense mingling of dopaminergic and cholinergic neurons in limbic areas of the brain allows coordinated functioning of these neurotransmitter systems [3,4].

The cholinergic system is well known for its role in learning, memory, and attention. In general, cholinergic activation modifies these functions with an inverted-U dose-effect relationship [5,6]. Accordingly, nicotinic or muscarinic cholinergic antagonists can disrupt learning and memory in human or animal experiments, with this effect reversed by restoring ACh function [7,8]. Either cholinesterase inhibitors or cholinergic agonists with nicotinic or muscarinic selectivity can enhance learning under conditions in which cholinergic function is diminished, but disrupt the same behaviors when administered at higher doses [9,10], which can be associated with signs of yawning, tremor, involuntary jaw movements, and diarrhea in animals [11]. Overall, these findings are consistent with an optimal level of central cholinergic activity for learning and memory, with deviations in either direction capable of impairing learning and memory. Parallel to this, interaction of the ACh and dopamine systems to modulate drug-reinforced and drug-seeking behaviors can also be interpreted using an inverted-U dose-effect relationship.

Behavioral Significance of Striatal Acetylcholine Elevations

Augmented release of ACh in the striatum and nucleus accumbens has been observed under a number of qualitatively different conditions [12]. Locomotor activity in rats is correlated with dialysate levels of ACh in the striatum, hippocampus, frontal cortex [13,14]. Handling of rats increases extracellular ACh in both the nucleus accumbens core and shell, with repeated exposure to an open field further increasing values in the shell but not the core region [15]. Importantly, disruption of an established contingency that requires learning of a new pattern of responding appears to increase extracellular ACh. In the dorsal striatum, reversal of maze requirements for food reward caused pronounced increases in ACh which resolve as rats learn to maximize correct responding [16].

Activation of cholinergic neurons has also been implicated in the rewarding effects of both natural and drug reinforcers [17]. Repeated exposure to different classes of abused substances can produce persistent increases in the activity of cholinergic neurons in the nucleus accumbens [18]. Psychostimulant-reinforced behavior can cause long-lasting decreases in levels of choline acetyltransferase in the nucleus accumbens [19]. During cocaine self-administration, greater increases in ACh occur in dialysate from the nucleus accumbens shell [20] or VTA [21], relative to neurotransmitter increases that occur in animals that receive drug noncontingently. This early-session accentuation also occurs in cocaine-trained animals evaluated during extinction (substitution of inert injections) [21]. As rats acquire reinforcement in a runway model, psychostimulant, opiate, or food induced elevations in ACh in the nucleus accumbens core increase over consecutive trials, while levels of dopamine do not change [22,23]. In these experiments, drug-induced increases in ACh also did not vary in magnitude for rats that received noncontingent injections.

ACh elevations have also been linked to satiety caused by feeding and aversive states [24]. In deprived rats, both ACh and dopamine in the nucleus accumbens increased in response to food or water [25]. For freely feeding rats, extracellular ACh in the nucleus accumbens increases and reaches a maximum as satiety occurs. In drug-dependent animals, withdrawal produced by blockade of opiate, nicotinic, or benzodiazepine receptors increases ACh concentration in the nucleus accumbens [26]. Exposure to a flavor that has been paired with lithium-induced illness increases ACh concentration in dialysate from the nucleus accumbens, and infusion of a cholinesterase inhibitor into the nucleus accumbens can produce conditioned taste aversions [27]. Aversive hypothalamic stimulation (AHS) releases ACh in the nucleus accumbens, and rats that lever press to terminate AHS decrease their concentration of ACh in accumbal dialysate [28]. Apparently, elevated levels of ACh in the nucleus accumbens can serve as a neural indicator of aversiveness, or as a signal that inhibits appetitive behaviors. In many instances, accumbal levels of dopamine change in an opposite direction to that of ACh in response to an aversive stimulus. For example, exposure to an aversively-conditioned flavor [27] or precipitated withdrawal [26] can cause decreases in dopamine that accompany increases in accumbal ACh. Taken together, these findings show that elevations of ACh occur in a variety of settings, with the common element being ecological significance.

A Threshold Model for Cholinergic Effects on Reinforced Behavior

Cholinergic influences can be broadly divided into treatments that either enhance or attenuate reinforced behavior. A two-phase model has been developed to explain seeming discrepancies between these competing actions. It is based on the following two opposing systems, described in Table 1 and shown schematically in Figure 1. Both thresholds are part of normal physiology, functioning in the absence of drug treatments. Short-term increases in VTA-accumbal cholinergic transmission that exceed a low-level threshold increase the probability of reward, but further increases above an upper threshold decrease its probability (reward enhancement and inhibition, respectively). Probability is the likelihood that an organism will choose a given behavior in the future. VTA-accumbal cholinergic transmission is defined as agonist activity at nicotinic and muscarinic receptors within these two brain regions.

The model predicts that behavior which influences cholinergic activation through non-pharmacologic mechanisms that are either psychological or physical can also modify the probability of reward through either threshold. Therefore, reward enhancement and inhibition encompass both psychological and pharmacological constructs. For example, behavior causing increases in endogenous cholinergic tone in the nucleus accumbens and VTA that are intermediate in magnitude, in the absence of drug treatments, are supported (positively reinforced). In contrast, alternative behavior causing larger and sustained increases of endogenous ACh in the same brain regions without drug exposure would be inhibited. Both scenarios can be mimicked by drug treatments leading to intermediate or large increases in cholinergic activation, respectively. As described by Solomon and Corbit [29,30], reward inhibition can be viewed as an opposing process that is a relatively delayed in onset and long-lasting. Reward enhancement and inhibition can occur through activation of nicotinic receptors, muscarinic receptors, or both classes of receptor, as is seen with administration of cholinesterase inhibitors. Desensitization of nicotinic receptors is one potential mediator of differential responding by cholinergic systems: phasic activation for brief periods increases the probability of reward, with an opposite effect after a period of prolonged receptor activation. This property allows the same molecule to produce different effects after dissimilar patterns of cholinergic activity.

In addition to ACh, dopamine and various other neurotransmitter systems work in concert as mediators of motivated behaviors. Because relatively few studies have attempted to decipher the influences of multiple neurotransmitters on reward behavior, the model focuses on the role of ACh. Positive and negative effects of dopaminergic tone may act additively with actions of ACh. More precise characterization of this interaction should be an important goal of future work.

In this review, relevant findings are interpreted in terms of the model and its potential mediators. For more comprehensive accounts of cholinergic influences on reward, the reader is referred to previous reviews by Williams and Adinoff [31], Sofuoglu and Mooney [32], and Mark et al. [33]. Key evidence for the threshold model is based on systemic treatments with cholinergic agonists and antagonists, which may involve more than one functional circuit. Therefore, the review is organized according to type of receptor (nicotinic or muscarinic) and or agent (agonist or antagonist).

Thresholds Mediated by Nicotinic Receptors

Background—As reviewed by Tuesta et al. [34], different subtypes of the nicotinic receptor underlie the reinforcing effects of nicotine, psychostimulants, and other drugs of abuse. Nicotine stimulates release of dopamine by direct activation of high-affinity nAChRs on both the cell bodies [35] and terminal fields [36,37] of midbrain dopaminergic neurons that project to the ventral striatum. VTA dopaminergic neurons express several types of nicotinic receptor subunit, including $\alpha 3$ to $\alpha 7$ and $\beta 2$ to $\beta 4$ [38-41]. The $\alpha 4\beta 2$ subtype is the most abundant nicotinic receptor in mammalian brain [42], and variants of the CHRNA4 gene encoding the $\alpha 4$ subunit are associated with altered nicotine dependence in humans [43]. In the striatum, high affinity $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs are expressed on dopaminergic

and GABAergic neurons, where activation leads to calcium influx and greater excitability. This facilitates action potential firing and modulates dopamine release [44,45].

Homomeric $\alpha 7$ nAChRs are activated by both ACh and its ubiquitous breakdown product, choline [46]. In the VTA, receptors containing the $\alpha 7$ subunit are localized on glutamatergic axons that are distinct from cholinergic terminals, suggesting activation through diffusion of ACh or choline; a portion of these receptors are localized to cholinergic terminals in the nucleus accumbens [47]. Homomeric $\alpha 7$ nAChRs are typically activated only after relatively high agonist concentrations, in micromolar amounts [48]. In comparison, activity of $\alpha 4\beta 2^*$ receptors is most often observed with only nanomolar concentrations of ACh or nicotine [49]. Although the $\alpha 7$ -subunit may not form exclusively homomeric receptors in vivo, low agonist sensitivity also appears to be a property of native heteromeric receptors that include this subunit [50,51].

Nicotinic receptors exhibit a characteristic biphasic response to stimulation which is important for behavioral effects: brief, low levels of phasic stimulation can augment ion flux; but prolonged agonist exposure results in decreased responsiveness, termed desensitization [49]. Phasic events are those that are relatively brief, and resolve quickly. There is great variability in the degree to which the nicotinic receptors associated with individual neurons desensitize in response to agonist exposure. After more prolonged exposure to higher agonist concentration, recovery from desensitization typically requires longer periods. Certain subtypes of the nicotinic receptor are activated by phasic release of ACh but become desensitized after sustained ACh binding over seconds to minutes [52]. For VTA dopaminergic neurons, application of either micromolar concentrations of nicotine or rapid bursts of millimolar ACh desensitizes inward nicotinic currents [53]. At high firing rates under physiologic conditions, choline produced by ACh hydrolysis appears to be present at sufficient concentrations to cause homomeric $\alpha 7$ nAChRs to desensitize [54].

The firing of dopaminergic neurons in the VTA is also modulated by GABAergic and glutamatergic inputs that desensitize over different intervals after treatment with nicotine [55]. Because of enhanced glutamatergic transmission combined with inhibition of GABA release, there is a net facilitation of dopamine release that is thought to occur at micromolar concentrations of nicotine [56].

Nicotinic Agonists—Depending on the dose administered, systemic pretreatment with nicotine can have opposite effects on intravenous self-administration of cocaine in a rat model under a simple fixed-ratio-1 (FR-1) schedule in which each lever press resulted in a cocaine injection [57]. In this study, drug taking was enhanced by low-dose nicotine, but diminished after a higher dose. This biphasic effect of systemic nicotine on cocaine-reinforced behavior can be interpreted in as reward enhancement and inhibition mediated by the two thresholds shown in Figure 1. Low levels of nicotinic activation by ACh or other nicotinic agonists favor State A being advanced to State B as a lower threshold is exceeded, increasing the probability of reward. If nicotinic activation continues to increase, and reaches higher levels that exceed an upper threshold, the probability of reward is diminished, corresponding to State C.

Varenicline is a medication approved for use in smoking cessation that acts as a partial agonist at nicotinic $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ receptors, and a full agonist at $\alpha 7$ nAChRs [58]. In rhesus monkeys, varenicline can enhance cognitive function across a wide range of doses, 0.001 to 0.3 mg/kg [59]. In some instances, it has been associated with biphasic effects on cocaine-reinforced behavior which are consistent with reward enhancement and inhibition by low- and high-doses, respectively. For example, pretreatment with 0.01 to 0.56 orally in rhesus monkeys potentiated the reinforcing effects of cocaine [60], while 1.0 and 2.0 mg/kg administered subcutaneously to rats attenuated cocaine self-administration [61]. However, low (0.1 and 0.3 mg/kg) varenicline doses also attenuated cue- and cocaine- induced reinstatement of non-reinforced responding in the latter study. Low varenicline doses (0.004 and 0.04 mg/kg-hour) were reported to be ineffective in modifying cocaine reinforcement [62]. But, this protocol was not designed to detect increases in drug-reinforced responding, as monkeys self-administered at their limit of maximal available cocaine injections. Varenicline doses that attenuate cocaine-reinforced behavior in rats are well above those approved for clinical use. Consistent with this, varenicline treatment was found to be ineffective in facilitating abstinence from use of cocaine or opiates in treatment-seeking patients, but did decrease smoking in this population [63].

Knockouts and Antagonists—Nicotinic acetylcholine-receptor systems are important mediators of cognitive function. Pretreatment with the nonselective, noncompetitive nicotinic antagonist mecamylamine can impair learning and memory with threshold systemic doses of 1.0 to 5.0 mg/kg in rats [64]. For rats performing below criterion (less than 80% correct responses at baseline) on a food-reinforced, five-choice serial reaction time task [65], pretreatment with nicotine or an $\alpha 4\beta 2^*$ agonist increased correct responding, and an $\alpha 7$ -selective agonist was without effect. This may correspond to augmentation of nicotinic tone from a relatively low level to exceed a lower cholinergic threshold, causing reward enhancement. In rats that initially performed above criterion, mecamylamine pretreatment decreased accuracy. In addition to negative effects on learning and memory, nicotinic blockade may also decrease the value of food reinforcers, because cholinergic transmission falls short of its lower threshold. Systemic pretreatment with antagonists with $\alpha 4\beta 2^*$ or $\alpha 7$ selectivity in this study did not alter responding [65], which may reflect involvement of other neurotransmitters.

After knock-out of the CHRN2 gene which codes for the $\beta 2$ nicotinic subunit, elderly mice exhibit impaired fear conditioning, diminished spatial learning, and elevations of basal plasma corticosterone [66,67]; implicating a role for endogenous ACh in learning and stress reactivity during aging. CHRN2 deletion also disrupts nicotine-induced dopamine release by the ventral striatum [68], as well as nicotine self-administration [69]. In addition, intravenous self-administration of nicotine is prevented by deletion of either the CHRNA4 or CHRNA6 genes, which code for the $\alpha 4$ and $\alpha 6$ subunits respectively [70]. This same study showed that drug-reinforced behavior can be restored by re-expression of CHRNA4, CHRNA6, or CHRN2 genes in the VTA. An additional experiment that used self-administration procedures based on chronic rather than acute drug taking found similar rates of nicotine-reinforced responding in CHRNA4 knockout and wild-type strains [71]. Diminished reward after genetic knockout of subunits that make up high-affinity nAChRs

may be mediated by low levels of cholinergic transmission that fail to exceed a lower cholinergic threshold, preventing reward enhancement.

Acquisition of nicotine- or cocaine- induced conditioned-place preference is attenuated by a β_2^* antagonist delivered either intracerebroventricularly or to the nucleus accumbens, but not the septum [72]. Place preference conditioned by nicotine in this study was also disrupted by deletion of either the *CHRNA4* or *CHRNA6* gene. Although an earlier study found that that nicotine-induced conditioned-place preference was supported after *CHRNA4* knockout, it used a different mouse strain, route of nicotine delivery, and conditioning procedure [71]. Effects of *CHRNA6* deletion cited above could be overcome by high-dose nicotine, indicating a shift in its dose-response to the right [72]. In contrast, *CHRNA4* knockout prevented place preferences for each of the nicotine doses evaluated. Place preference after *CHRNA6* knockout produced by high-dose nicotine was blocked by pretreatment with an antagonist for β_2^* nAChRs [72]. Cocaine-induced conditioned-place preference was disrupted after knockout of the *CHRNA6* but not the *CHRNA4* genes in this study. Because the magnitude of nicotine-induced place preference can be increased by *CHRNA6* knockout, a role for reward inhibition by $\alpha_6\beta_2^*$ receptors is implicated after nicotine but not cocaine treatment. The broad role of the β_2^* subtype in preferences occurring under different conditions suggests an important contribution to reward enhancement (but see further discussion below, regarding reward inhibition after prolonged nicotine treatment).

The magnitude of conditioned-place preference for low-dose nicotine is increased after knockout of the *CHRNA7* gene, which codes for the α_7 nicotinic subunit; but decreased by knock-in of the same gene [73]. This is opposite to genetic effects of β_2^* expression, and implicates a role for low-affinity α_7 homomeric nAChRs in reward inhibition after acute treatment with nicotine. Consistent with this, there is a graded increase in the strength of nicotine-induced place preference across genetically different mouse strains, which correlates with lower levels of α_7 subunit expression in the nucleus accumbens [73]. Genetic variation in α_7 subunit expression may explain why earlier studies failed to identify an effect of *CHRNA7* knockouts on nicotine-induced conditioned-place preference or intravenous self-administration [70,74]. Pretreatment with a nicotinic agonist selective for α_7 nAChRs prevented conditioning of place preference by nicotine, with this effect blocked by an antagonist for α_7 receptors [73]. Cocaine-induced conditioned-place preference was unaffected by α_7 -selective agents in these studies, which could be explained by activation of other nicotinic subtypes or muscarinic receptors.

When each dose of cocaine self-administered under FR-3 in rats was combined with 70 μ g of mecamylamine, escalation of drug taking with exposure to daily extended (6 hour) access was prevented, with no effect on self-administration during one-hour sessions [75]. Systemic pretreatment with 1.0, 2.0, or 4.0 mg/kg of mecamylamine also decreased self-administration of cocaine under FR-1 in rats, with only the highest dose decreasing food reinforcement [76]. This study is unusual in that mecamylamine pretreatment was administered daily over a seven-day period. Neither effects of single mecamylamine doses nor trends over daily sessions were reported, and it is unclear whether chronic exposure was used as a strategy to diminish variance or was required to enhance drug action. The

requirement for a higher mecamylamine dose to decrease responding for food pellets was interpreted as a demonstration of the selective involvement of ACh for cocaine-reinforced behavior. However, food reinforcement supported a three-fold higher level of responding than cocaine in this study [76]. Palatable rewards can surpass cocaine as a motivator [77]. Use of a palatable non-drug reinforcer associated with greater responding may have caused drug reward to be more susceptible to blockade of nAChRs, with food reinforcement to appearing to be resistant. According to the threshold model, behavior motivated by either reinforcer is attenuated because cholinergic transmission is prevented from reaching its initial lower threshold for reward that is achieved in vehicle-treated animals, blocking reward enhancement (Figure 1, compare conditions A and B). Consistent with this, laboratory measures of cue-induced craving in human cocaine addicts can be enhanced or attenuated by acute pretreatment with nicotine [78] or mecamylamine [79], respectively.

Nicotinic Mechanisms—Activation of midbrain dopaminergic neurons in the VTA by either local drug treatments or optogenetic stimulation can enhance reward behavior (see review by Ikemoto et al. [80]). The threshold model predicts that delivery of agonists with selectivity for either nicotinic or muscarinic receptors to the VTA can exceed the lower cholinergic threshold. Delivery of 50 nM or more of nicotine after smoking appears to activate dopaminergic neurons [81], corresponding to in vitro estimates of its EC₅₀ (50% effective concentration) [82]. By preventing breakdown of ACh, cholinesterase inhibitors can function in a similar manner. For example, local infusion of either the nonselective agonist carbachol or the cholinesterase inhibitor neostigmine into the VTA can produce conditioned place preferences, which are blocked by muscarinic or nicotinic antagonists [83]. Cocaine-seeking behavior can also be blocked by delivery of antagonists with either muscarinic or nicotinic selectivity to the VTA [84]. These results implicate midbrain dopaminergic neurons in the VTA as mediators of reward enhancement.

Desensitization of nAChRs can be viewed as a ‘nuisance’ that occurs only after drug treatments under experimental conditions [49]. Alternatively, it may contribute to physiological processing in the normal and diseased brain [85]. Consistent with this, desensitization could limit or reverse nicotinic effects after sustained increases in endogenous or drug-induced ACh, by acting as an upper, inhibitory cholinergic threshold. Although classically viewed as involving rapid oscillation between active and inactive conformational states across milliseconds, more detailed models encompass multiple states that include long-term loss of nicotinic receptor function that can persist over 24 hours, and resolve at least partially through synthesis of new receptors [86]. In vitro studies of muscle-type nAChRs have shown multiple desensitization states, with increased duration of agonist exposure associated with reductions in function over successively longer periods, described as ‘deeper’ desensitization states [87]. Deeper levels of desensitization may underlie reward inhibition that follows extended nAChR activation by drug treatments or sustained increases in endogenous ACh.

Delivery of mecamylamine to the VTA blocks increases in dopamine following systemic cocaine [33]. Cocaine-induced conditioned-place preference can be disrupted by either mecamylamine pretreatment, knockout of nAChRs containing the CHRN β 2 subunit, or chronic intermittent nicotine treatment [88]. Disruption of cholinergic transmission by

antagonist pretreatment or gene knockout is likely to block reward enhancement, as cholinergic transmission is prevented from rising above its lower threshold. For mice with a genetically deleted *CHRNA2* nicotinic subunit, changes in dopamine metabolism and immediate-early gene-product expression were also deficient, consistent with a role of this receptor subtype in dopaminergic neurotransmission underlying reward processes [88]. Low, ineffective doses of cocaine and nicotine did cause a place preference if administered together, suggesting that greater nicotinic activation by combined treatment did allow the lower cholinergic threshold to be exceeded.

Disruption of cocaine-induced conditioned-place preference by chronic intermittent nicotine treatment [88] can be explained by reward inhibition, as prolonged exposure to agonist causes nicotinic receptors to become refractory. The pattern of intermittent nicotine dosing used in this study was aimed at causing tolerance to nicotine's physiologic effects, including reductions in nicotine-induced dopamine release [89,90], perhaps through nAChR desensitization. Similarly, systemic dopamine blockade and microinjection of nicotine to the VTA can produce a place preference in vehicle-treated rats, but are aversive after chronic infusion of nicotine by osmotic pump [91]. This dichotomy may reflect acute β_2^* reward enhancement by activation beyond the lower threshold in nicotine-naïve animals which potentiates drug-motivated behavior, and reward inhibition after prolonged nicotine levels that raise cholinergic transmission above its upper threshold.

Through low sensitivity to agonist, α_7 -containing nAChRs may act as a mediator of the upper cholinergic threshold, favoring reward inhibition after acute exposure to relatively high agonist levels. As noted above, activation of the α_7 nAChR decreases acute nicotine reward [73], with an opposite effect of high-affinity β_2^* receptors [70]. Knockout of the corresponding *CHRNA7* gene in mice prolongs nicotine-induced increases in extracellular dopamine in the nucleus accumbens [92]. This suggests that α_7 receptors inhibit dopamine release, which is again opposite to the effect of β_2^* receptors [68]. After relatively high levels of ACh, choline, or nicotinic agonist; α_7 -mediated reductions in dopamine release may underlie reward inhibition, serving an auto inhibitory role [93]. This may occur through phosphorylation of β_2^* receptors, associated with attenuated nicotine-induced increases in the firing of VTA dopaminergic neurons [94].

After nicotine dependence is established, systemic treatment with an antagonist for β_2^* nAChRs elevates anxiety-like behavior [95], and infusion of a β_2^* antagonist to the VTA increases the amount of current required to initiate intracranial self-stimulation [96]. Requirement for greater amounts of current to elicit intracranial self-stimulation to the medial forebrain bundle in the lateral hypothalamus is an animal model of human anhedonia and depressed mood [97]. For either spontaneous or precipitated withdrawal, knockout of the *CHRNA2* gene prevents increases in anxiety measures [98] and elevations in reward threshold [99]. In contrast, an antagonist for α_7 nAChRs can decrease locomotor activity and cause tremor and other somatic signs of nicotine withdrawal in dependent animals [100]. After genetic deletion of the *CHRNA7* gene, withdrawal-induced increases in nociception are lost; with anxiety, reward-threshold, and somatic measures remaining intact [98]. In nicotine-dependent mice, genetic disruption of either the α_7 or β_4 nicotinic subunit delayed the onset of spontaneous withdrawal [101]. In the same study, genetic disruption of the α_7

subunit caused biphasic effects on antagonist precipitated increases in reward threshold, which were potentiated and diminished by low- and high doses of mecamylamine, respectively.

Anxiety, elevated reward threshold, and somatic changes associated with withdrawal form an unconditioned contingency with changes in nicotinic activity. Prompt relief from the negative effects of withdrawal occurs after nicotine treatment [95], and supports further drug taking through negative reinforcement. A similar effect would also occur after actions that re-elevate endogenous ACh; reinforcing associated behavior. The differential sensitivity of low- and high- affinity nAChRs suggests a hierarchy of effects on reward that modify behavior through delayed consequences. Relatively modest, but sustained increases in either ACh or exogenous agonist that activate high-affinity receptors containing the $\beta 2$ subunit are associated with delayed increases in anxiety [98] with an elevation of reward threshold [99]; larger agonist doses or increases in either endogenous ACh or choline which activate low-affinity homomeric receptors also produce heightened nociception, tremor, and other somatic effects [98,100]. In either case, behavior associated with nicotinic activation can either be punished by these effects, or allow negative reinforcement after relief of unpleasant consequences associated with reward inhibition. The key factor supporting motivation is short-term positive effects on mood, lowered reward threshold, and diminished somatic symptoms (reward enhancement); and delayed effects with an opposite (aversive) valence.

Thresholds Mediated by Muscarinic Receptors

Background, Muscarinic Systems—GABAergic medium spiny neurons make up more than 90% of neurons in the striatum, and serve as its major output [102]. They are named for their far-reaching dendritic trees that contain numerous small spines, allowing them to receive extensive synaptic input. Glutamatergic input from various cortical and thalamic structures provides the major excitatory input to medium spiny neurons, presumably allowing temporal and spatial direction [103]. Though activation of type 1 and 2 dopamine receptors, activities of medium spiny neurons are modulated in a biphasic manner, in part by facilitation or suppression of glutamatergic transmission [104,105]. Medium spiny neurons also express both M_1 and M_4 muscarinic receptors [106]. In the nucleus accumbens, glutamatergic input is generally potentiated or inhibited by nicotinic or muscarinic (M_1 or M_4) receptors, respectively [107].

Muscarinic M_5 receptors on dopamine neuron terminals enhance striatal dopamine release, whereas M_2 and M_4 autoreceptors on cholinergic interneurons inhibit ACh release and subsequent nicotinic-receptor-dependent dopamine release [108,109]. Within the VTA and substantia nigra, RNA for the M_5 receptor is the predominant muscarinic subtype expressed [110]. In these brain regions, most neurons express message for both the M_5 muscarinic receptor and the dopamine D_2 receptor [111].

ACh in the striatum and nucleus accumbens is released by cholinergic interneurons that express choline acetyltransferase. Striatal cholinergic interneurons are presumed to be the tonically active neurons of electrophysiologic studies, which serve as mediators of stimulus-reward associations [112]. They spontaneously produce wide action potentials at a frequency of 2 to 10 Hz in vivo, and comprise less than 2% of neurons in the nucleus accumbens [113].

Even so, cholinergic interneurons are the major source of ACh in the nucleus accumbens. The density of their local projections implies an important role of ACh in modulating medium spiny neuron function, which is supported by electrophysiologic and optogenetic studies. With exposure to behaviorally relevant stimuli or reward, most cholinergic interneurons respond with a brief pause in firing of approximately 200 to 300 milliseconds duration, which may be preceded or followed by increased firing rates [114]. Excitatory cortical and thalamic inputs appear to play a role in initiating pause-excitation events that follow reward-predicting stimuli [115]. Pauses are associated with increases in firing by midbrain dopaminergic neurons [116]. In addition, about 25% of GABA-releasing neurons in the VTA project to the nucleus accumbens [117]. Stimulation of GABA projection neurons in the VTA can produce inhibitory effects that facilitate pauses by accumbal cholinergic interneurons, which are associated with enhanced discrimination of motivationally important stimuli [118].

Optogenetic stimulation of burst firing by VTA dopaminergic neurons is followed by increased firing and a subsequent pause of cholinergic interneurons in the nucleus accumbens shell [119]. In contrast, optogenetic stimulation of cholinergic interneurons in the striatum augments dopamine release through β_2^* nAChRs [120,121]. In the nucleus accumbens, release by this mechanism relies at least partially on glutamatergic AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, appears to be opposed by muscarinic activation, and is independent of midbrain dopaminergic cell bodies [122]. Direct optogenetic stimulation of cholinergic interneurons in the nucleus accumbens suppresses most adjacent medium spiny neurons (81%), while exciting a minority of cells (19%); with an opposite effect of optogenetic inhibition [123]. In this study, systemic treatment with cocaine markedly increased the firing rate on cholinergic interneurons. Optogenetic suppression or facilitation of cholinergic interneuron firing has no obvious effect on spontaneous behavior, but suppression did attenuate cocaine-induced conditioned-place preference. Reward-associated pause-excitation events in cholinergic interneurons appear to inhibit neighboring medium spiny neurons by nicotinic excitation of GABAergic neurons [124]. Because of their specific cortical, hippocampal, and amygdalar afferents, individual medium spiny neurons in the nucleus accumbens are thought to encode different stimulus-action associations [125]. This allows selection of a certain action through inhibition of its corresponding medium spiny neurons.

Muscarinic Agonists—The threshold model predicts biphasic effects on reward behaviors, which are enhanced at low doses of muscarinic agonists, but suppressed by higher doses of the same agents. A recent study examined effects of the muscarinic agonist xanomeline, which is selective for both type M_1 and M_4 receptors, on cocaine- and food-reinforced behaviors [126]. Rats were allowed to choose reinforcement with either liquid food or different doses of cocaine. Daily treatment with xanomeline produced complex effects, generally shifting cocaine dose-effect curves to the right (suppressing responding for low doses of cocaine doses, while increasing drug taking for higher doses). For some cocaine-xanomeline dose combinations, behavior was reallocated from cocaine to food reinforcement. The net effect was that overall cocaine intake was augmented by lower xanomeline doses (1.8 and 3.2 mg/kg-day), with a trend for reduced intake in animals

receiving a higher dose of xanomeline (5.6 mg/kg-day). This pattern likely reflects reward enhancement and inhibition by low- and high- xanomeline doses, respectively. It resembles biphasic effects of nicotine, which can also potentiate or diminish cocaine-reinforced behavior when administered at intermediate or high doses, respectively [57].

The study by Thomsen et al. described in the preceding paragraph [126] is unusual because of the broad range of muscarinic agonist doses evaluated during chronic dosing, with cocaine also tested across a broad range of doses that supported self-administration behavior. Furthermore, drug and food were both available under fixed-ratio-5 (FR-5), in which reinforcement was provided after 5 or more lever presses. This design is highly relevant for evaluation of potential human treatments for substance abuse disorders. Most other studies have evaluated a narrower dose range cholinergic agents, which may correspond to one limb of a biphasic dose-response. For example, pretreatment with single 1.0 mg/kg doses of xanomeline or two other muscarinic agonists attenuated self-administration under FR-1 across a broad range of cocaine doses in mice [127], implicating reward inhibition. Responding for the maximally reinforcing cocaine dose was attenuated by about two-thirds. Although food-reinforced behavior was not modified by pretreatment with M₁-selective agonists, food pellets supported a higher response requirement than cocaine injections in this study as well as the earlier report by Thomsen et al. [126], again raising question as to whether this effect was selective for drug reward. Systemic pretreatment with nonselective muscarinic agonists or a partial agonist attenuated cocaine self-administration behavior in mice [128], again corresponding to reward inhibition. Lower agonist or cocaine doses which may have produced conditions leading to enhanced drug reward were not evaluated in this experiment.

Muscarinic Antagonists—If muscarinic receptors contribute to reward enhancement by augmenting cholinergic transmission above the lower threshold shown in Figure 1, preventing this action should disrupt behavior supported by natural or drug reinforcers. Pretreatment of rhesus monkeys during a single session with the muscarinic antagonist atropine increased self-administration of cocaine, with no effect observed after pretreatment with methylatropine which has limited brain penetration [129]. Enhanced responding under these conditions may correspond to a short-term increase in drug-seeking behavior that would have extinguished if it were evaluated over additional sessions or under a schedule with a greater response requirement. Consistent with this interpretation, cocaine combined with the nonselective muscarinic antagonist scopolamine, maintained lower rates of drug-reinforced responding in Rhesus monkeys evaluated under either a FR-25 or progressive-ratio schedule [130]. Systemic treatment with a relatively high 5.0 mg/kg dose of scopolamine attenuated cocaine-induced reinstatement by approximately one-half, with 0.5 mg/kg being ineffective [131]. Although not statistically significant, the 5.0 mg/kg dose of scopolamine also decreased responding during a sucrose-induced reinstatement procedure by more than one-third. It should be noted that a wide variety of memory-related tasks are impaired by scopolamine [132]. Scopolamine doses that exceed 0.1 mg/kg can nonselectively diminish performance by modifying attention, stimulus discrimination, and motor activity [133]. Anxiety, associated with peripheral effects of decreased salivation and pupillary dilatation, has been implicated.

In rats performing the runway task described above, microinjection of atropine or mecamylamine into the nucleus accumbens core blocked opiate reward (consistent with blockade of reward enhancement), without affecting performance maintained by sweetened, condensed milk [22,23]. Although not statistically significant, run times were more than 100% prolonged in atropine-treated food-reinforced rats in the study by Crespo et al. [23], relative to vehicle- or mecamylamine- treated animals. These longer values may reflect a role for accumbal muscarinic receptors in supporting food-reinforced behavior. Greater preferences for sucrose over cocaine reward in rodents [77] is an additional consideration that again makes it difficult to evaluate the relative selectivity for drug and food rewards. There is a need for further research that focuses on evaluating a broader range of alternative, non-drug reinforcers, which support different levels of responding.

Microinjection of scopolamine into the nucleus accumbens core attenuated non-reinforced responding for either cocaine or sucrose (again consistent with blockade of reward enhancement); but injection into the nucleus accumbens shell unambiguously attenuated responding for only cocaine [131]. An additional study reported that blockade of muscarinic but not nicotinic receptors in either the nucleus accumbens core or shell decreased sucrose-reinforced behavior [134]. Importantly, delivery of a muscarinic antagonist dose to the nucleus accumbens core that attenuated liquid food intake also produced flavor- and place-aversions [135]. Comparable effects were not observed after blockade of NMDA, dopamine D₁, or opiate receptors. Rather than cognitive impairment, these results are more consistent with muscarinic blockade decreasing the reinforcing attributes of food or causing a “general hedonic suppression”.

Infusion of a muscarinic antagonist into the VTA of rats attenuated cocaine-induced increases in VTA dopamine by more than 50% [21]. With access to self-administration under FR-1, rats increased their rate of lever pressing and contingent injections, allowing them to maintain VTA elevations of dopamine that were comparable to animals not treated with an antagonist; use of a nicotinic antagonist was ineffective. After cocaine self-administration was completed, dopamine levels dropped more rapidly in rats pretreated with the muscarinic antagonist. The authors concluded that blocking muscarinic input to the VTA increased cocaine intake; this increase offset the reduction in cholinergic input, resulting in the same VTA dopamine levels as were seen in the absence of the ACh antagonists. Put another way, rats worked to maintain dopaminergic output from the VTA at an intermediate level by increasing lever pressing in the absence of muscarinic activation to this brain region that would normally enhance dopamine release [136] and drug reward. This appears similar to increases in cocaine-reinforced behavior under FR-1 after atropine treatment in monkeys [129]. An important message is that acute interventions under simple response requirements that decrease central mediators of reward via changes in cholinergic tone may actually increase drug motivated behavior. Presumably, evaluation under chronic conditions using a higher response requirement, such as multiple lever presses to obtain a drug injection, would lead to decreases in cocaine self-administration after cholinergic antagonist treatment that prevents reward enhancement.

Antagonists with selectivity for the M₁ muscarinic receptor may exhibit greater selectivity for disrupting memory without modifying attention or food motivation [137]. In rats,

infusion of a muscarinic agonist into the nucleus accumbens shell decreases cocaine self-administration behavior, with this effect blocked by pretreatment with an antagonist for the M₁ muscarinic subtype [138]. This effect may be mediated by cholinergic transmission that exceeds an upper cholinergic threshold, causing locally-mediated reward inhibition in this brain region. Alternately, muscarinic agonists may also facilitate reward if administered at low enough doses, as cholinergic transmission rises above a lower cholinergic threshold.

Pretreatment with the M₁ selective muscarinic antagonist biperiden alone does not cause either preference or aversion [139], but can attenuate the expression of cocaine-induced conditioned-place preference [140], consistent with blockade of reward enhancement. Conditioning of place preferences with cocaine is also diminished if biperiden is administered 5 minutes after exposure to cocaine pairings, implicating interference with the process of memory consolidation [141]. Both effects relied on a higher biperiden dose (10 mg/kg) associated with decreased response rate and diminished short-term memory, but without negative effects on attention or food-reinforced responding [137]. Lower doses of biperiden were ineffective in modifying expression of place preference [140]. In humans with cocaine-use disorder, biperiden administered over eight weeks (2 mg three-times-daily) improved treatment compliance and decreased craving intensity when combined with group-based counseling [142]. However, single 2 mg doses of biperiden cause broad declines on a subset of cognitive measures in humans, which include episodic memory, immediate recall, motor learning, and visual-spatial performance [143].

Morphine- and cocaine- induced conditioned-place preference are attenuated by either genetic disruption of the M₁ muscarinic receptor or combining drug treatments with an antagonist for the M₁ receptor [144]. In these studies, knock-out or antagonist treated animals correspond to State A in Figure 1, with wild-type and vehicle treated animals corresponding to State B in which activation of the M₁ receptor allows ACh to facilitate food- or drug- reinforced behaviors.

Knock-out of the M₅-muscarinic receptor decreases drug-induced place preference across a broad range of morphine doses, and also attenuates morphine-induced dopamine increases in the nucleus accumbens [145,146]. After knock-out of the M₅-muscarinic receptor, mice self-administered some but not all cocaine doses at approximately one-half the rate of wild-type animals [147]. During conditioned- place preference testing, cocaine-induced increases in preference were also approximately one-half the magnitude of wild-type animals. A subsequent evaluation using more highly inbred M₅-deficient mice observed lower rates of acquisition for cocaine self-administered under FR-1, but no differences in cocaine-experienced mice [148]. The same study found that lower break points were achieved by M₅- deficient mice under a progressive-ratio schedule for 0.03 or 0.32 mg/kg per injection of cocaine (reductions of approximately 50 and 25%, respectively). Although no differences in food-reinforced behavior were observed for either fixed- or progressive- ratio schedules, food reinforcement supported a two-fold higher rate of responding. Accordingly, activation of M₅ muscarinic receptors appears to preferentially contribute to reward enhancement, apparently through activation of VTA dopaminergic neurons. Nonetheless, M₅-deficient mice did not differ in cocaine self-administration under many of the conditions evaluated (i.e., a full range of cocaine doses evaluated using FR-1, and the higher two of four cocaine

doses tested under a progressive-ratio schedule) [148]. It may be that nicotinic signaling alone in the VTA can support drug-reinforced behavior in the absence of the M₅ muscarinic receptor in these instances.

Electrical stimulation of laterodorsal tegmental (LDT) nucleus cholinergic neurons increases dopamine release through an initial rapid phase (over approximately 2 minutes) which is dependent on VTA nAChRs, and a late phase (8 to 50 minutes post stimulation) which is dependent on M₅-muscarinic receptors [149]. Infusion of M₅-antisense mRNA causes a reversible rightward shift in the stimulation frequency required to maintain electrical brain stimulation [150]. Intra-VTA infusion of muscarinic or nicotinic antagonists causes a similar shift in the frequency required to maintain electrical brain stimulation [151]. Based on these effects, activation of M₅ muscarinic receptors on VTA dopaminergic neurons are believed to facilitate natural or drug reward, as cholinergic transmission exceeds its lower threshold. Because pharmacologic agents with M₅-selectivity are not available, it is unclear whether reward inhibition can be produced by agonists with selectivity for this subtype.

Muscarinic Mechanisms—As cited above, low doses of the muscarinic agonist xanomeline augmented cocaine intake by up to 40.1% [126]. In comparison, low doses of nicotine can enhance the amount of self-administered cocaine by approximately 120% [57]. Accordingly, nicotinic mechanisms appear to play a greater role in reward enhancement through the low-level threshold than that of the muscarinic system. This may be facilitated by more rapid nicotinic effects on ion flux, compared with slower muscarinic modulation of phospholipase and adenylate cyclase. Such a dichotomy echoes the time course of LDT activity cited above, with dopamine release stimulated over a several-minute rapid phase mediated by the nicotinic receptor, and a more prolonged phase produced by muscarinic receptors [149]. Nonetheless, both nicotinic and muscarinic agonists appeared to have the potential to produce either reward enhancement or inhibition in the studies cited above. Conclusions on the role of muscarinic receptors must again be qualified by the limitation that no direct agonists for the M₅ receptor are currently available, with this subtype implicated in muscarinic reward-enhancing actions [148].

In striatal slice experiments performed in mice lacking different muscarinic receptors, potassium-evoked release of dopamine is modulated in a biphasic manner. The authors infer that dopamine release is stimulated by activation of either type M₄ or M₅ receptors, inhibited by M₃ receptors, and unaffected by knock-out of M₁ or M₂ receptors [152,153]. Augmented release of dopamine is most consistent with reward enhancement, with reward inhibition expected after attenuation of neurotransmitter release. However, administration of an M₄ positive allosteric modulator strongly attenuates cocaine-induced increases in dopamine measured in the striatum by microdialysis, as well as cocaine self-administration [154]. Similarly, knockout of type M₄ muscarinic receptors appeared to facilitate dopamine efflux, augmenting cocaine- or amphetamine-induced locomotor activity [155]. Dissimilar actions of the muscarinic M₄ subtype on dopamine release may reflect experimental conditions that evaluate either reward enhancement or inhibition, mediated by different cholinergic thresholds.

Tonic activity by a subset of nucleus accumbens neurons appears to inhibit appetitive responding, with several-second pauses in firing associated with initiation of food-reinforced behavior [156]. The subset of cells in the nucleus accumbens shell that exhibit a pause in firing during feeding behavior have been described as type 1 neurons. Feeding is disrupted by electrical stimulation of type 1 neurons [157]. Declines in the activity of nucleus accumbens medium spiny neurons are also implicated in drug-reinforced behavior [158]. For example, the most frequent pattern of neural activity in the nucleus accumbens that follows self-administration of an intermediate dose of cocaine is a decline in firing rate that begins within 0.2 minutes of lever pressing, and persists over approximately one minute [159]. Overall, these findings implicate interventions that quiet or excite accumbal type 1 neurons as underlying reward enhancement or inhibition, respectively.

In striatal slices, either cholinesterase inhibition or low, micromolar concentrations of a nonselective cholinergic agonist suppressed electrically-evoked activity, with periods of burst firing observed after higher agonist concentrations [160]. After iontophoretic application of ACh in awake rats, the activity of most spontaneously active or glutamate-excited neurons in the nucleus accumbens is suppressed [161]. As noted above, optogenetic stimulation of cholinergic interneurons plays a role in cocaine reward, and suppresses the activity of most adjacent medium spiny neurons [123]. A role for ACh-induced electrical pauses in reward enhancement is consistent with a literature linking decreases in the rate of tonically active cholinergic interneurons in the striatum with exposure to behaviorally relevant stimuli in non-human primates [162].

In brain slices from the nucleus accumbens shell, treatment with a low concentration of a nonselective cholinergic agonist (1 μ M carbachol) suppresses repetitive firing in medium spiny neurons (consistent with reward enhancement), with this effect blocked by an M₁ selective antagonist [163]. Carbachol applied at greater concentrations (50 μ M) can enhance repetitive firing in medium spiny neurons (implicating reward inhibition) [107]. Short-term decreases in excitatory glutamatergic inputs mediated by muscarinic receptors [107,164] can also inhibit the activity of medium spiny neurons, possibly causing reward enhancement. In striatal brain slices, cholinergic agonists can depolarize GABAergic neurons through activation of nAChRs [165]. Inhibitory effects of enhanced GABA release may also increase the probability of pauses in firing by cholinergic interneurons and medium spiny neurons.

Biphasic effects of ACh on the firing rate of medium spiny neurons may underlie thresholds that modify reward behavior. Whether through ACh released by cholinergic interneurons, muscarinic agonists, or cholinesterase inhibition (see the following section), low-level binding at accumbal-VTA muscarinic receptors likely quiets the activity of type 1 accumbal medium spiny neurons [161,163]. This exceeds the lower cholinergic threshold, increasing the probability of reward behavior (reward enhancement). Further activation of the same muscarinic receptors rises above the upper cholinergic threshold, facilitating rhythmic activity by medium spiny neurons [107,160] and decreasing the probability of reward (reward inhibition).

Background, Cholinesterase Inhibition—Mammalian brain contains two forms of cholinesterase, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The

physiologic role of BuChE is unclear, but it can metabolize cocaine and other exogenous compounds and contributes to degradation of ACh. Cholinesterase inhibitors, which are widely used for treatment of Alzheimer's disease, increase synaptic levels of ACh by preventing its inactivation by AChE or BuChE. In general, these agents attenuate declines in functional status associated with Alzheimer's disease and other forms of dementia.

Clinically relevant doses of cholinesterase inhibitors can augment learning and memory in Alzheimer's disease and other neuropsychiatric disorders [166,167]. Depending on the specific task being evaluated, performance in healthy individuals may be impaired, augmented, or unaffected [168,169]. Cholinesterase inhibition in some settings may disrupt performance by producing a hyper-vigilant state, enhancing sensitivity to irrelevant stimuli that serve as distractions [170]. In a subset of patients, cholinesterase inhibitors administered at relatively high doses cause symptoms of anorexia, nausea, diarrhea, and weight-loss [171].

Cholinesterase inhibition that attenuated morphine-induced conditioned-place preference in control animals was ineffective in after ablation of cholinergic neurons from the nucleus accumbens [172], implicating the involvement of this brain region in reward inhibition. Cholinergic lesions to the posterior nucleus accumbens and ventral pallidum caused a shift to the left in cocaine-reinforced behavior, with enhanced self-administration of relatively low doses of cocaine [173], again implicating removal of an inhibitory influence.

Thresholds and Cholinesterase Inhibition—By preventing degradation of ACh in the synaptic cleft, the threshold model predicts that cholinesterase inhibition will also produce biphasic effects on behavior that are mediated by a combination of the nicotinic and muscarinic mechanisms outlined in the preceding sections. When applied to striatal slices, different cholinesterase inhibitors share a common biphasic effect on dopamine efflux: low (nanomolar) concentrations enhance release that is either electrically evoked or spontaneous, with maximal effects of 12 to 24%, while higher (micromolar) concentrations strongly attenuate release [174]. These in vitro effects may correspond to in vivo enhancement and inhibition or reward, respectively.

An early study found that pretreatment with single doses of the cholinesterase inhibitor physostigmine (0.1 to 0.5 mg/kg) decreased cocaine self-administration in rhesus monkeys under FR-1 and caused vomiting and diarrhea [129], consistent with its administration at relatively high dose causing reward inhibition. When either cocaine or procaine were self-administered by rhesus monkeys under FR-10, 0.02 to 0.05 mg/kg doses of physostigmine increased responding in 3 of 10 sessions, with declines uniformly observed following greater physostigmine doses [175]. This inverted "U" dose-response relationship is again consistent with reward enhancement and inhibition, respectively. It is unclear why augmented reward behavior is not more consistently observed at lower cholinesterase inhibitor doses, but this may reflect differences in the time-action or dose-response functions for nicotinic and muscarinic receptors. Cocaine-reinforced behavior evaluated under FR-5 was also attenuated in rats pretreated with cholinesterase inhibitors delivered as single bolus doses [176] or by infusion [177,178]. Pretreatment with tacrine or other cholinesterase inhibitors can also produce a dose-related attenuation of food- or water-reinforced responding in rats [179,180].

Daily pretreatment with 0.1 mg/kg of physostigmine modestly impaired acquisition of heroin self-administration by rats, and also attenuated cue-induced reinstatement 14 days later, although no further physostigmine was administered during extinction and reinstatement session [181]. In the same study, single doses of 0.5 or 2.5 mg/kg of physostigmine also attenuated self-administration in heroin-experienced animals. Although lower physostigmine doses did not modify heroin reward, the number of daily self-administered heroin doses was limited by design, decreasing the potential to detect increases in self-administration. Reductions in self-administration after 0.5 mg/kg of physostigmine were prevented by scopolamine but not mecamylamine, implicating a role for muscarinic receptors underlying inhibition of opiate reward caused by treatment with cholinesterase inhibitors. Physostigmine doses of 0.1 or 0.5 mg/kg decreased drug-seeking behavior after cue exposure, with the higher dose also effective during extinction. Cue- and extinction-induced responding for heroin were increased after local infusion of physostigmine into the VTA; but cue-induced responding was decreased after delivery to the nucleus accumbens, with responses during extinction unaffected [181]. Under these conditions, increased synaptic ACh in the VTA appeared to exceed the lower cholinergic threshold, increasing the likelihood of behaviors associated with reward (enhancement). Reductions in heroin-seeking behavior (reward inhibition) after delivery of physostigmine to the nucleus accumbens [181] may be due to ACh levels that preferentially activate the upper cholinergic threshold.

Administration of cholinesterase inhibitors can prevent acquisition of cocaine- or morphine-induced conditioned-place preference in mice [172] or rats [182], which again reflects reward inhibition. For the latter study, cholinesterase inhibition also attenuated place preference expression. In contrast, methamphetamine-induced conditioned-place preference in mice was not attenuated by donepezil in one study [183]. Because it stimulates reverse transport by the dopamine transporter [184], amphetamines can augment dopamine release more potently than cocaine [185]. This difference may explain why donepezil modified cocaine- but not methamphetamine-induced place preference in the study by Takamatsu et al. [183].

Treatment of cocaine-dependent human volunteers with 5 mg daily of donepezil, the lowest dose approved for Alzheimer's disease, increased ratings of 'good' drug or 'any' drug for low-dose of cocaine, without modifying the subjective effects intermediate-dose cocaine [186]. When donepezil was increased to a final dose of 10 mg daily in treatment-seeking patients receiving cognitive behavioral therapy, cocaine-positive urines were non-significantly increased, corresponding to values of 32 and 168% for placebo and active treatments respectively [187]. Apparently, donepezil administered at doses up to 10 mg daily interacts with the lower cholinergic threshold, augmenting drug-seeking behaviors through reward enhancement. Although a 23 mg daily dose of donepezil was approved which may offer greater improvements in some cognitive measures in Alzheimer's disease [188], its effects on the course of cocaine-use disorder are unknown.

In another human laboratory study, an intermediate dose of the cholinesterase inhibitor rivastigmine attenuated methamphetamine-induced increases in blood pressure, anxiety, and "desire" to use drug, with a lower dose being ineffective [189]. Neither dose of rivastigmine modified the number of methamphetamine doses self-administered by dependent volunteers,

but intermediate-dose rivastigmine was associated with a trend for attenuation of the positive subjective effects of self-administered methamphetamine [190]. Rivastigmine's pharmacokinetics may favor reward inhibition, in parallel to actions of cholinesterase inhibitors in rodents [172,178,182].

Galantamine is an additional clinically-available, reversible cholinesterase inhibitor, which also acts to allosterically potentiate nicotinic function [191]. The latter mechanism appears to augment extracellular levels of dopamine in medial prefrontal cortex [192] and nicotine-induced norepinephrine release in hippocampus [193]. In recently detoxified alcoholics, treatment with galantamine treatment did not prolong abstinence, but decreased the number of self-reported drinks per alcohol-use episode [194]. Although it did not include an overt strategy for smoking cessation, this trial also reported decreased cigarette use after galantamine treatment [195]. For newly abstinent chronic cocaine users, daily treatment with galantamine improved reaction time and sustained attention [196]. In a relatively small preliminary trial (7 active and 7 placebo-treated cocaine-dependent patients receiving methadone maintenance), an escalating schedule of galantamine treatment (initiated at 8 mg daily over 4 weeks, subsequently advanced to 16 mg daily for an additional 4 weeks) was associated with trends for decreased drug use determined by either self-report or urine drug screen, relative to placebo treatment [197]. Four days of galantamine treatment in abstinent smokers decreased cigarette craving, improved response control and attention measures, and attenuated some subjective and physiologic effects of intravenous nicotine [198]. Galantamine's unique dual mechanism of action of may allow it to preferentially cause reward inhibition by interacting with the upper inhibitory cholinergic threshold, without facilitating drug use through the lower threshold.

Alternative Interpretations—It has been proposed that reward processes are facilitated by VTA M₅ muscarinic receptors, but attenuated by activation of M₁ and M₂ subtypes [199]. As noted above, the absence of muscarinic agonists with M₅ selectivity prevents an evaluation of whether high agonist levels can prevent reward behavior. If the M₁ muscarinic subtype only functioned to diminish the probability of reward behaviors, pretreatment with antagonists or genetic disruption would be expected to augment drug- or food- motivated responding. As outlined above, both of these strategies instead have an opposite effect, attenuating the magnitude of drug reward in rodents [140,141,144] and decreasing craving intensity in humans [142].

In response to various seemingly contradictory results of cholinergic medications on drug reward, Zernig and colleagues [200] hypothesize the existence of different ensembles of accumbal cholinergic interneurons that are dedicated to specific reinforcement scenarios. These include psychostimulant self- administration, social interaction, eating routine food, and consuming highly palatable treats. Variable effects of treatments that augment cholinergic transmission across different species and behaviors occur because of the specific ensemble type being activated. They posit considerable intra- and inter- individual variation in the spatial distribution of different ensembles. In support of this hypothesis, neuronal activation measured by expression of the early growth response 1 transcriptional regulator is shows distinct patterns in the nucleus accumbens across different individual rats. These can be described as diffuse, patchy, or mixed [200], and may allow information processing in the

nucleus accumbens to provide specificity of responding based on individual differences or ecologically dissimilar circumstances. Even so, the existence a more generalized system which can facilitate or dampen reward through the thresholds described above allows a more parsimonious interpretation of existing data.

Dopaminergic systems in the nucleus accumbens also exert opposing influences on motivation, with the direct and indirect pathways facilitating and inhibiting behavior, respectively [201]. For example, stimulation of direct pathway medium spiny neurons expressing the D1 dopamine receptor can enhance cocaine-induced conditioned-place preference, with attenuation of place preference after activation of neurons expressing the dopamine D2 receptor that are part of the indirect pathway [202]. Self- administration of cocaine can also be inhibited by activation of indirect pathway D2 expressing medium spiny neurons in the nucleus accumbens core [203]. Interestingly, accumbal D1-expressing MSNs have an opposite effect on food-reinforced behavior, consistent with fundamental differences in the neural control of food- and drug- reinforcers [204]. As noted above, cholinergic interneurons can stimulate dopamine release through activation of nicotinic receptors on dopaminergic axons [120,121]. Therefore, biphasic effects of ACh on reward behaviors could be mediated through differential influences on dopaminergic direct and indirect pathways. Dopaminergic neurons projecting to the medial prefrontal cortex have also been implicated in aversive signaling [205].

A subset of neurons in the lateral habenula are activated by punishment and associated stimuli, but suppressed by rewarding events [206]. Intravenous cocaine can produce an initial inhibition of some neurons in the lateral habenula, followed by delayed excitation occurring 15 to 30 minutes later [207]. Local injection of an antagonist for $\alpha 3\beta 4$ receptors into the habenular complex can have opposing effects on self-administration of intravenous nicotine: drug taking is decreased by injection into the medial habenula, the basolateral amygdala, or the dorsolateral tegmentum; but increased after delivery to the interpeduncular nucleus [208]. For primary cultures of neurons derived from the medial habenula and interpeduncular nucleus, repetitive activation of nAChRs can facilitate glutamatergic release for sustained periods of up to two hours; while prolonged exposure to nicotine can deactivate this response, requiring up to 24 hours for full recovery [86]. An important question is how function of the habenular complex interacts with thresholds mediated by the VTA-accumbal cholinergic transmission.

Clinical Implications of Cognitive Effects—Successful prevention of relapse in drug use disorders involves development of new coping strategies to situations that would otherwise stimulate drug-seeking behavior, and establishing new social contacts who do not use illicit substances. Depending upon the treatment program, relapse prevention may rely on attendance at clinical appointments, taking a medication to facilitate abstinence, and attempts at being employed. Cognitive-behavioral approaches can improve outcomes in cocaine use disorders, perhaps through enhancement of decision making. During recovery from each of the major forms of drug abuse, previous studies have demonstrated impairment in learning, memory, and attention [209-211]. Two outpatient-based studies have shown that greater delay after use of cocaine is associated with more severe impairment in cognitive performance [212,213]. Accordingly, protracted withdrawal from cocaine use leads to more

pronounced cognitive deficits, which are less severe immediately following use of cocaine. If so, at least part of the motivation for relapse to drug use may be cocaine's ability to normalize mental function during recovery from cocaine dependence.

Because impaired learning, memory, and attention is one of the more well-validated actions of cholinergic antagonists, these agents have at least a relative contraindication for use in patients with substance abuse disorders due their potential for exacerbating cognitive deficits. Even so, recent findings of improved treatment compliance and decreased craving intensity after muscarinic blockade in treatment-seeking patients recovering from cocaine use [142] would support a lesser role of cognitive actions. It is also unclear whether use of cholinergic agonists or cholinesterase inhibition can ameliorate cognitive deficits in this setting. Treatment with galantamine appears to do so during either nicotine withdrawal [198] or recovery from cocaine dependence [196]. Further studies are needed which combine measures of cognitive function and drug-motivated behaviors.

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Highlights

Different classes of cholinergic agents produce biphasic effects on appetitive responding.

Phasic activation of cholinergic tone in the nucleus accumbens and VTA above a low-level threshold appears to increase the likelihood of rewarded behaviors.

Greater and more prolonged activation in the same brain regions above a second higher-level threshold is associated with a decreased likelihood of reward.

Cholinergic effects on the nicotinic receptor function, dopamine release, and the firing of medium spiny neurons are potential mediators of thresholds that shape behavior.

High-affinity $\beta 2^*$ nicotinic receptors appear to play a greater role in reward enhancement, with low-affinity homomeric $\alpha 7$ receptors underlying reward inhibition.

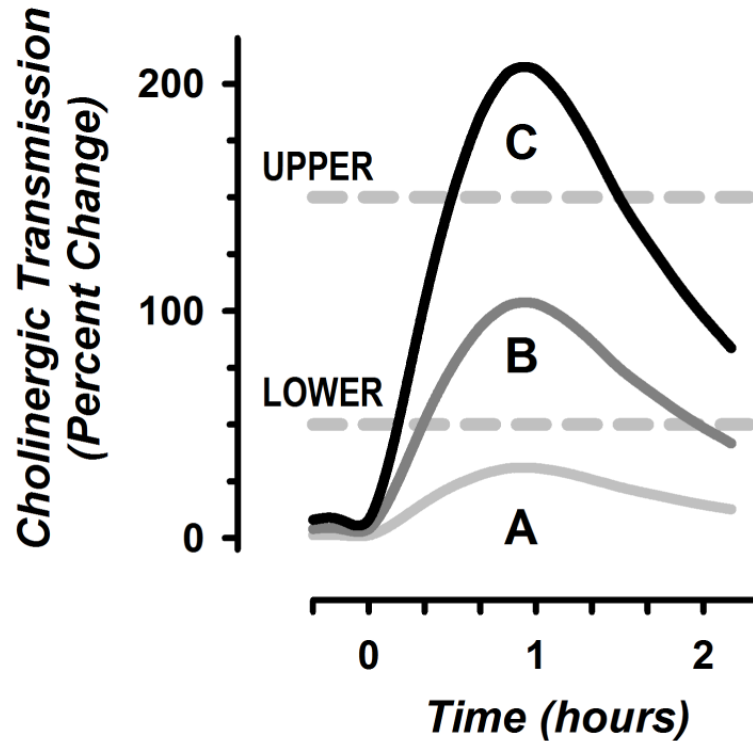


Figure 1. Schematic Outline of Biphasic Cholinergic Effects on Reinforcing Events

Hypothetical values for cholinergic transmission in the nucleus accumbens and VTA are shown on the vertical axis in arbitrary units, with time plotted horizontally. More precisely, the vertical axis corresponds to momentary agonist activity at cholinergic receptors. This can be provided by either endogenous ACh or medications with nicotinic or muscarinic agonist properties. Cholinergic transmission contributes to the rewarding properties of different events, if its level is increased to a magnitude between the lower and upper thresholds. Further increases above the upper threshold diminish the likelihood of reward. For example, pretreatment with the cholinesterase inhibitor donepezil prevents degradation of ACh in the synaptic cleft. Relatively low doses of donepezil typically used in humans can increase the subjective effects of low-dose cocaine as condition A is changed to condition B (reward enhancement) [186]. In contrast, if administered at relatively high doses in rodents, this agent can also change condition B to C, decreasing drug-reinforced behavior (reward inhibition) [172,178]. Because reward inhibition occurs at the upper limb of the dose-response for cholinergic agents, it is often associated with nonspecific disruption of behavior through adverse events.

Table 1

Properties of the cholinergic systems underlying reward enhancement and inhibition.

Threshold Level	Timing	Reward [and Affective] Effects	Description
Lower	Rapid, Short-Term	Enhancement [positive]	Under baseline, unstimulated conditions (Figure 1, State A), short-term, increases in VTA-accumbal cholinergic transmission that rise above a lower cholinergic threshold promote positive affective states, increasing the probability of reinforcement (Figure 1, State A is advanced to B).
Upper	Delayed, accumulates over Time	Inhibition [negative]	Larger and more prolonged increases in VTA-accumbal cholinergic transmission that exceed a second, higher value, have an opposite effect, facilitating negative affective states, and decreasing the likelihood of reinforcement (State B is advanced to C).

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