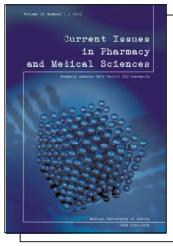

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# The role of acetylcholine in drug addiction

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**ABSTRACT**

Acetylcholine ACh, is involved in many CNS functions like sensory and motor processing, sleep, nociception, memory, mood, stress response, attention, arousal, motivation and reward. Due to the interaction with the dopaminergic reward system mainly in the ventral tegmental area (VTA), nucleus accumbens (NAc) and prefrontal cortex (PFC), it is believed that ACh plays a significant role on the initiation and maintenance of the drug addiction. Substances affecting the cholinergic system by increasing the levels of ACh, like cholinesterase and butyrylcholinesterase inhibitors (donepezil, rivastigmine, physostigmine, tacrine, galantamine), reduced the symptoms of addiction in a drug-induced CPP test as well as locomotor activity caused by morphine, cocaine and heroin. Unfortunately, donepezil failed to inhibit methamphetamine-CPP as well as the locomotor sensitization caused by this drug. For comparison, it has been indicated that mice lacking M5 receptor showed a reduction in cocaine self-administration and morphine-induced CPP. The stimulation of M4 receptor decreased DA release in NAc and the administration of non-selective antagonist of mAChRs, scopolamine decreased cocaine-induced locomotor activity in rats. Moreover, mecamylamine, a noncompetitve antagonist of nAChRs, prevented the increase in cocaine consumption as well as blocked cocaine and amphetamine-induced behavioral sensitization. Thus, drugs affecting cholinergic transduction could be approached as potential therapeutic agents for patients who abuse different psychoactive substances. These issues, however, require further studies.

**Keywords:** acetylcholine, acetylcholinesterase inhibitors, nucleus accumbens, addiction, conditioned place preference test, reward system

## INTRODUCTION

Acetylcholine (ACh) was the first discovered neurotransmitter that has been found to be involved in many central and peripheral nervous system functions. It is synthesized from acetyl-CoA and choline by choline acetyltransferase (ChAT) and in synaptic cleft it is hydrolyzed by enzymes from cholinesterase group such as: acetyl- (AChE) and butyrylcholinesterase (BuChE). In peripheral nervous system (PNS) ACh is responsible for muscle contraction and functioning of the autonomic nervous system [29].

In central nervous system (CNS) ACh is involved in many functions like sensory and motor processing, sleep, nociception, memory, mood, stress response, attention, arousal, motivation and reward [34]. There are two types of acetylcholine receptors (AChR) that bind ACh and transmit its signal: muscarinic AChRs and nicotinic AChRs, which are named after the agonists muscarine and nicotine, respectively. These receptors are functionally different, the muscarinic type being G-protein coupled receptors (GPCRs) that mediate a slow metabolic response via second messenger cascades, while the nicotinic type are ligand-gated ion channels, consisting of pentametric combinations of 12 subunits ($\alpha_2$-$\alpha_{10}$ and $\beta_2$-$\beta_4$), that mediate a fast synaptic transmission of the neurotransmitter [29].

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## ACETYLCHOLINE, DOPAMINE AND DRUG ADDICTION

A lot of publications also emphasize the significant role of ACh on the initiation and maintenance of the drug addiction [18,19] due to the interaction of the cholinergic system with the dopaminergic reward system mainly in the *ventral tegmental area* (VTA), *nucleus accumbens* (NAc) and *prefrontal cortex* (PFC).

Many years of research have demonstrated an important role of dopamine (DA) in the reward system and in the acute reinforcing effects of the drugs [22]. DA is the principal neurotransmitter in three major neural systems in the midbrain : 1) the ***nigrostriatal pathway*** which originates from dopamine-synthesizing neurons of the midbrain substantia nigra complex and innervates the dorsal striatum (caudate-putamen), and whose degeneration leads to Parkinson's disease); 2) the ***mesolimbic system*** which originates in the midbrain VTA and innervates the ventral striatum (NAc and olfactory tubercle) and part of the limbic system – this system influences motivated behavior, including activity related to reward; 3) the VTA also gives rise to the smaller ***mesocortical pathway***, which innervates part of the frontal cortex (FC) and may be involved in certain aspects of learning and memorization.

Psychostimulants like cocaine elevate extracellular DA level in NAc and this action is responsible for the rewarding effect of drug. It was observed that intravenous cocaine administration increased DA level in NAc. Moreover, DA fiber destruction in the mesocorticolimbic system caused inhibition of cocaine self-administration, as well as DA fiber damage limited to NAc. For comparison, during withdrawal from ethanol, there was a dramatic reduction in the level of DA associated with a significant reduction in spontaneous firing rates [22].

As mentioned above, ACh is also involved in the rewarding effects of addictive substances. There are two main cholinergic projections that innervate parts of the reward system. The first projection from the forebrain leads to nucleus basalis magnocellularis (NMB), which in turn innervates cortical and subcortical structures of the HPP and Amy [1]. This pathway is mainly associated with Parkinson's and Alzheimer's diseases, but can also be involved with learning and memory associated with the process of addiction. The second projection leads from the mesopontine cell group (Ch 5,6 = pedinculopontine tegmental and laterodorsal tegmental nuclei LDT) directly to the VTA, which in turn provides projections to the NAc, which regulates drug reinforcement [1].

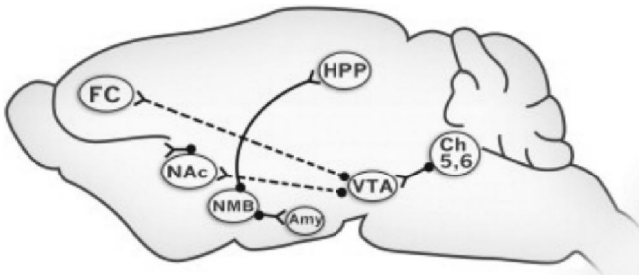


Fig.1. Regions of the brain with cholinergic influence on drug reward and withdrawal [1]. Broken lines represent DA projection, while solid lines represent ACh projections. HPP- hippocampus; NAc- nucleus accumbens; FC- frontal cortex; Amy- amygdala; VTA- ventral tegmental area; Ch 5,6 -pedinculopontine tegmental and laterodorsal tegmental nuclei; NMB- nucleus basalis magnocellularis.

The activity of neurons in the NAc is modulated not only by the DA neurons, but also by ACh interneurons, which represent less than 1% of all neurons in this region [1, 13]. Moreover, cholinergic interneurons within NAc have large dendritic arbors and they can modulate DA cell firing and mesolimbic DA release [17]. DA neurons and axons terminals in NAc are rich in mAChRs and nAChRs, whose stimulation results in an increased release of DA and reinforced behavior [38]. Similarly in the VTA, stimulation of $\beta_2$-containing nAChR leads to an increased release of DA in brain regions like the NAc and PFC [29]. The activation of neuronal $\alpha_7$-containing nAChR on the terminals of the PFC increases levels of glutamate, which facilitates the release of DA in the NAc. This mechanism is critical for the rewarding action of nicotine [29].

The VTA-NAc projection and the cholinergic neurons in the PFC may play a role in learning and memory, which are involved in effects of addictive substances such as the desire to eat substances (craving), searching for a substance (drug-seeking) or return to the consumption of substances of abuse (relapse). The neuroimaging studies revealed decreased activity of PFC in drug abusers, which manifested poor sustained attention, response inhibition, and decision-making [29].

The addictive substances like amphetamine and cocaine increase ACh release in PFC [29]. ACh release caused by amphetamine is accompanied by the development of psychomotor sensitization, which may suggest that PFC is involved in the development of neuronal adaptive changes. The correlation between ACh release and the DA system has been proven by studies conducted on rats. It has been shown that administration of dopamine $D_1$ receptor agonist, increases ACh release in the NAc, and the administration of $D_2$ receptor agonist reduces the level of ACh [5]. The difference in activity is because $D_1$-dopaminergic receptor is excitatory, while $D_2$-dopaminergic is inhibitory [29].

## MACHR AND NACHR AS POTENTIAL TARGETS IN TREATING ADDICTION

In the synaptic cleft, ACh interacts with two types of receptors: mAChRs and nAChRs. mAChRs, that are GPCRs are divided in two main groups based on their G-coupling mechanisms: $M_1$ ($M_1$+$M_3$+$M_5$) and $M_2$ ($M_2$+$M_4$). The $M_1$ mAChRs are expressed mainly in the cerebral cortex, hippocampus and striatum and are involved in learning and memorization process. These receptors can be potential therapeutic target in treating Alzheimer disease [29]. The $M_3$ mAChRs are not well characterized. The $M_5$ mAChRs are widely expressed in dopaminergic neurons in the substantia nigra and VTA and agonists binding to these receptors cause the release of DA in the NAc. Electrical stimulation of the laterodorsal tegmental nucleus (LDT) in the mice causes accumbal DA efflux in three consecutive phases. The last strongest efflux DA in a long third phase is absent in $M_5$ knockout mice [11]. Moreover, in mice lacking $M_5$ receptor in VTA, there was observed a reduction in self-administration of low-to-intermediate doses of cocaine [31], reduction of morphine-induced conditioned place preference (CPP) [3] and a decrease of morphine-induced locomotion by approximately 40-50% [28].

The $M_2$ and $M_4$ receptors also regulate the neurotransmitter release. Blockade of $M_2$ receptor increases cholinergic activity, while the stimulation of $M_4$ receptor decreases DA release in NAc. Interestingly, the use of microdialysis showed an increase of DA level in $M_4$ knockout mice. Indeed, in some brain regions $M_4$ receptors act as auto receptors to exert a negative feedback of control over ACh release. Inhibition of these receptors leads to increase in the impact of excitatory cholinergic input to dopaminergic neurons, which leads to DA release in NAc [32].

mAChRs probably play a role in reinforcement, since the administration of nonselective antagonist of these receptors, scopolamine, to the medial prefrontal cortex (MPC) enhanced cocaine self-administration in rats. Moreover, intra-MPC injections of scopolamine increased locomotor activity, but decreased cocaine-induced locomotor activity [21]. In the same experiment, it was showed that intra-NAc microinjections of scopolamine did not affect the number of lever presses made and infusions delivered, what can suggest, that blockade of mAChRs in the NAc does not affect cocaine self-administration [21].

As it was mentioned above nAChRs are ligand-gated ion channels, consisting of pentametric combinations of 12 subunits ($\alpha_2$-$\alpha_{10}$ and $\beta_2$-$\beta_4$), mostly located presynaptically in the CNS, where they modulate the release of ACh and DA. They are permeable to sodium, potassium, calcium ions, and most of them contain $\alpha_4$, $\beta_2$ or $\alpha_7$ subunits. Those nAChRs localized postsynaptically on the DA neurons in VTA, can be critical for addictive properties of substances. It was observed that animals treated by nicotine showed a significant increase in the number of cocaine infusions, compared to saline group. Moreover, during extinction phase when cocaine was unavailable, it was observed that injections of nicotine caused reinstatement of the lever-press behavior, what indicates that nicotine during activation of nAChR, can facilitate cocaine reinforcement [4].

Several studies have also demonstrated a significant role of nAChR antagonists in reducing drug-related behaviors. The mecylamine (MEC), a noncompetitive antagonist all nAChR subtypes, added to cocaine solution prevented the increase in cocaine consumption, but did not eliminate cocaine self-administration [17]. The cocaine and amphetamine-induced behavioral sensitization was blocked by MEC as well [27]. For comparison, Hiranita et al. revealed that nicotine and donepezil, which is AChE inhibitors (IA ChE), can attenuate the reinstatement of methamphetamine-seeking behavior. This donepezil's effect on methamphetamine-seeking behavior was blocked by a nAChR antagonist MEC, but not by mAChR antagonist- scopolamine [20].

The effect of MEC was also studied in cocaine-dependent rhesus monkeys. Monkeys were trained to self-administer cocaine or food by responding on appropriate lever. Chronic adminstration of varenicline, which is selective nAChR agonist, reinforced cocaine self-administration, while MEC did not have any influence on cocaine self-administration at doses that maintained food-reinforced responding. Varenicline did not have any abuse potency and did not substitute for cocaine in discriminative schedule but administered with MEC or nicotine increased discriminative effects of cocaine. In this model, varenickline failed to be effective in treating cocaine addiction [13].

## CHOLINESTERASE AND ITS INHIBITORS

Substances affecting the cholinergic system by increasing the levels of ACh can be used as a potential treatment target of addiction. One group of such drugs is cholinesterase inhibitors (IChE) widely used for example in the treatment of Alzheimer's disease and other dementia diseases. The level of ACh in the synapses of the CNS cholinergic neurons is mainly responsible for AChE, an enzyme that degrades the neurotransmitter [12].

AChE is an enzyme specific for the hydrolysis of ACh. There are two types, namely: synaptic AChE localized in the postsynaptic membrane, which directly hydrolyzes ACh after nerve impulse conduction, and deprived of the possibility of a soluble ACh binding to the membrane receptor. Its function is nonsynaptic hydrolysis of ACh, before it reaches the postsynaptic membrane. BuChE (pseudocholinesterase) is a nonspecific enzyme, hydrolyzing not only ACh but also all compounds having in their structure ester bonds. BuChE is located on neurons, glial cells, endothelium, and a number of areas supplied by cholinergic neurons. The enzyme decomposes the poisons, especially plant alkaloids, pesticides, insecticides. It plays an important role in the metabolism of certain drugs such as aspirin or diazepam [16].

AChE inhibitors (IAChE) are divided into irreversible and reversible. Reversible AChE inhibitors are clinically useful in the treatment of Alzheimer's disease. This disease is characterized by a substantial loss of *cholinergic innervation* in the cerebral cortex and hippocampus, which leads to the loss of neurons in the basal forebrain. The loss of cholinergic neurons and the consequent decrease in cholinergic neurotransmission is responsible for the weakening of memory processes and dementia [34]. At a later stage of the disease when many cholinergic neurons rich in AChE die, BuChE are beginning to play a significant role in the regulation of cholinergic system [34]. Studies have shown that selective blocking of BuChE in the brain cortex of old rats resulted in increased concentration of ACh in the region and improvement of cognitive function, despite an unchanged activity of AChE [16].

The first known reversible IAChE- physostigmine and tacrine showed a slight improvement in cognitive function in humans. Clinical studies have shown that they are characterized by low activity after oral administration, poor brain penetration, low pharmacokinetic parameters, and high hepatotoxicity. Because of that, tacrine is no longer available in the market. A more specific and having fewer side effects (diarrhea, nausea etc.) are the newer cholinesterase inhibitors, like donepezil, galantamine or rivastigmine [34].

## CONDITIONED PLACE PREFERENCE- ANIMAL MODEL OF DRUG REWARD AND RELAPSE

To assess the rewarding actions of addictive substances and relapse (even after long periods of abstinence), there are used several animal models e.g. intra-cranial electric stimulation, self-administration models and conditioned place preference (CPP), which can assess conditioned responses related to reward. The majority of animal models of relapse are based on reinstatement models, which are a recovery of a learned response to having a place where animals are exposed to specific stimuli after extinction of such response,

e.g. CPP paradigm. This method is based on classical conditioning in which the originally neutral environment characteristics (texture, smell, color) associated with the subjective effects of administration of drugs of abuse, acquire rewarding properties (improved mood, euphoria). Addictive substances cause strong preference of animals to a place associated with their administration [33]. Being in this room evokes feelings comparable to the effects of the reward [2].

The CPP method consists of two phases: acquisition and expression. The first seems to be related with reward and learning, while the second one with motivation and memory [25].

## IACHE AS A POTENTIAL TARGET FOR THE TREATMENT OF DRUG ADDICTION

The literature shows that in experimental animals IAChE weaken some of the symptoms of addiction to psychoactive substances. Hikida at al. [18, 30] showed that donepezil injected before cocaine or morphine, inhibited the CPP caused by these drugs in wild-type mice. For comparison, cholinergic cell-eliminated transgenic mice did not respond to donepezil and they showed morphine CPP comparable to saline-paired mice. Hikida at al. [18] also studied the role of accumbens ACh in the development of cocaine-induced sensitization. The studies showed that in mice, in which cholinergic interneurons were destroyed by immunotoxin-mediated cell targeting techniques, administration of cocaine resulted in a progressive increasing of locomotor activity much higher compared to the wild-type littermates [18, 19]. It was also reported that administration of IAChE donepezil, prior to cocaine, abolished the induction of locomotor activity caused by cocaine in mice. In the same experiment, it was also examined that the other IChE, galantamine, also inhibited cocaine-induced hyperlocomotion [18]. Galantamine also inhibited nicotine-withdrawal symptoms in the form of memory impairment in mice [35] and inhibited the dextroamphetamine-induced unrest, arousal and stereotypy in cebus monkeys [9]. There was only one study on humans, where galantamine was observed to reduce smoking compared to placebo in 114 alcohol-dependent volunteers [10].

Another group of researchers led by Takamatsu [30] also examined the impact of donepezil on behavioral changes in mice caused by administration of cocaine and methamphetamine. It was found that pretreatment with donepezil, abolished signs of cocaine-induced CPP, as well as locomotor sensitization, but not the development of locomotor sensitization to cocaine, which confirmed the results of Hikida et al. [18]. Unfortunately, in the same experiment, they found that pretreatment with the same dose of donepezil failed to inhibit methamphetamine-CPP as well as the locomotor sensitization caused by this drug. The different effect of donepezil on cocaine and metamphetamine action is probably due to a mechanism of action on various neurotransmitters and membrane transporters. However, these require further study [30].

In a different experiment, IAChE-tacrine inhibited cocaine self-administration in rats [14]. In one study, 17 human volunteers abusing cocaine were treated with donepezil. The drug was well tolerated, but did not change cocaine-use behavior. Unfortunately, these results should be confirmed by further studies, because a group of volunteers was too small for statistical evaluation, and used only one dose of donepezil [37].

For comparison, in another study rivastigmine, IAChE as well as butyrylcholinesterase inhibiotor (IBuChE), did not decrease methamphetamine self-administration in humans addicted to this substance, but might reduce positive effects [8]. Grasing et al. confirmed the difference between donepezil and rivastigmine on cocaine self-administration in rats. It was observed that both compounds decreased cocaine self-administration, but in a different potency. Rivastigmine decreased cocaine self-administration in greater potency compared to donepezil. Rivastigmine produces a greater increase in *ACh level than donepezil,* because it inhibits both AChE and BuChE while donepezil does only IChE [9].

Physostigmine, another IAChE, given to the hippocampal CA1 field, decreased morphine-induced CPP in rats [26]. In another study, cholinergic enhancement caused by this drug, decreased cocaine self-administration in rhesus monkeys [9].

The role of cholinergic transmission was conducted on rats trained to nose-poke for intra-venous (i.v.) heroin administration. It was observed that physostigmine pretreatment reduced the acquisition of heroin-administration, and this effect can be reversed by pretreatment of scopolamine but not MEC [38]. After 14 days of extinction, the animals were subjected to quenching action items originally associated with reward (like house light or the sound of infusion pump), but without an addictive substance, to initiate cue-dependent reinstatement. It was observed that physostigmine reduced cue-induced reinstatement, and only the dose of 0.5mg of the drug was effective in reducing nose-poke responding on the extinction test [38].

In the same experiment, physostigmine was microinjected to NAc or VTA to evaluate the regions mediating the effects of systemic drug treatment on reinstatement [38]. It was observed that microinjection of the drug to NAc inhibited cue-induced heroin-seeking, without an influence on extinction phase. The microinjection of the drug to VTA caused inhibition of cue-induced heroin-seeking as well as extinction responding. Moreover, inactivation either NAc or VTA by tetrodotoxin, inhibited both extinction responding and cue-induced reinstatement, what confirmed that ACh is involved in heroin-seeking behavior [38].

## CONCLUSIONS

Many studies have revealed that ACh plays a significant role in the processes underlying drug addiction [6]. Using IAChE such as donepezil and rivastygmine, Hikida et al. [18] indicated an inhibition of the development and persistence of behavioral effects associated with administration of cocaine and morphine [18]. In addition, donepezil and the other IAChE, galantamine inhibited locomotor activity in mice caused by cocaine [18]. Furthermore, there are indica-

tions that other IAChE inhibitor, tacrine attenuated cocaine self-administration in rats [13], galantamine inhibited nicotine withdrawal-symptoms in mice [35], and physostigmine decreased morphine-induced CPP [26] and cue-induced heroin-seeking in rats [38].

On the other hand, it has been indicated that mice lacking $M_5$ receptor indicated a reduction in cocaine self-administration [31] and reduction of morphine-induced CPP [3]. The stimulation of $M_4$ receptor decreased DA release in NAc [32] but the administration of non-selective antagonist of mAChRs, scopolamine, to the MPC enhanced cocaine self-administration in rats, but decreased cocaine-induced locomotor activity [21]. On the other hand, MEC, a noncompetitve antagonist of nAChRs, added to cocaine solution, prevented the increase in cocaine consumption, and blocked cocaine and amphetamine-induced behavioral sensitization [17]. MEC also blocked donepezil effect on metamphetamine-seeking behaviors, while scopolamine failed to block it [20].

In summary, IAChE and substances affecting different ACh receptors are not only useful for a better understanding of mechanisms underlying drug addiction but also provide *potential* therapeutic *targets* for the *treatment of addiction*.

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