

**Review Article**

**THE CONTRIBUTION OF CHOLINERGIC NEUROCIRCUITS OVER THE MODULATION OF DOPAMINE ACTIVITY IN THE STRIATUM**

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**ABSTRACT**

The dopaminergic neurons and cholinergic interneurons of the striatum play key roles in motivational and sensorimotor processing by the basal ganglia. The striatal dopaminergic transmission in the forebrain is modulated by the excitatory cholinergic projections from the pedunculopontine and the laterodorsal tegmental nuclei of the hind brain by activating nicotinic and muscarinic acetylcholine receptors within the substantia nigra and ventral tegmental area. Furthermore, acetylcholine-containing interneurons in the striatum also constitute an important neural substrate for modulating dopamine activity. The striatal muscarinic acetylcholine receptors, by inhibiting acetylcholine release from cholinergic interneurons and thus modifying nicotinic acetylcholine receptor activity, offer variable control of dopamine release probability. In addition, it appears that the striatal influence of dopamine and acetylcholine cannot be fully appreciated without an understanding of their reciprocal interactions. Impairments or abnormality in these reciprocating interactions is often manifested in the form of various neuropsychiatric disorders and require extensive research to establish the optimized drug therapy. Hence the present review will serve as an aid to study the anatomical and physiological aspects of dopamine modulation as influenced by acetylcholine in striatum.

**Keywords:** Dopaminergic system, Mesopontine cholinergic system, Striatum, Cholinergic interneurons.

**INTRODUCTION**

Cholinergic cell bodies are located in a loosely contiguous axis running from the cranial nerve nuclei of the brain stem to the medullary tegmentum and pontomesencephalic tegmentum, continuing rostrally through the diencephalon to the telencephalon [1]. There are three major cholinergic subsystems above the brain stem that innervate nearly every neural area. One cholinergic system arises from neurons mainly in the pedunculopontine tegmentum (PPTg) and the laterodorsal pontine tegmentum (LDTg), providing widespread innervation to the thalamus and midbrain dopaminergic areas and also descending innervation to the caudal pons and brain stem. The second major cholinergic system arises from various basal forebrain nuclei that make broad projections throughout the cortex and hippocampus. The third major cholinergic subsystem arises from a collection of cholinergic interneurons located in the striatum.

The cholinergic neurons of the LDTg mainly innervate the Ventral tegmental (VTA) neurons, influence their burst firing and the consequent release of dopamine in the nucleus accumbens and thereby influence goal-directed behaviors. In contrast, the PPTg neurons innervate both Substantia nigra pars compacta (SNc) and VTA neurons. The PPTg neuronal activity alters dopamine release in the dorsal as well as ventral striatum and thereby modifying whole basal ganglia activity through its effects on striatal outputs and ultimately behavior.

Thus the cholinergic and dopaminergic systems have a potent reciprocal relationship within the striatum. It has long been recognized that Acetylcholine (ACh) and Dopamine (DA) have dense overlapping axonal arborizations. ACh regulates striatal DA neurotransmission via actions at both Muscarinic acetylcholine receptors (mAChRs) and nicotinic acetylcholine receptors (nAChRs) in a manner which differ according to the striatal sub region and DA neuron activity. In addition, it is widely accepted that the mesopontine cholinergic system modulates the midbrain dopaminergic system, which is the focus of the present review.

**BASIC ANATOMY OF THE STRIATUM**

The basal ganglia is a group of nuclei of varied origin situated at the base of the forebrain and are strongly connected with the cerebral

cortex, thalamus and other brain areas. The main components of the basal ganglia are the striatum (the largest component), pallidum, substantia nigra and subthalamic nucleus. The striatum is the major input station of the basal ganglia system, extremely rich in acetylcholine (ACh) and its associated enzymes Acetyl cholinesterase (AChE; the ACh degrading enzyme), Choline acetyl transferase (ChAT; the ACh synthesizing enzyme), and cholinergic receptors (muscarinic and nicotinic; mAChRs and nAChRs, respectively). As the input nucleus of the basal ganglia, the striatum receives dopaminergic inputs from the midbrain alongside excitatory inputs from both cortex and thalamus. Once the cortical information is integrated at the striatal level, it is conveyed to basal ganglia output nuclei (e. g., the globus pallidus) via the striatal medium spiny neurons (MSNs). The integration is strongly modulated by striatal ACh interacting with dopamine (DA) [2].

The principal neurons of the striatum are the Medium spiny neurons (MSNs), which make up about 90-95% of the total striatal neuronal population and form the striatal output. These neurons are inhibitory, using  $\gamma$ -aminobutyric acid (GABA) as their primary neurotransmitter. These cells have dendrites densely covered with dendritic spines; hence their name. The remaining minority of neurons is a diverse collection of inter neurons that can be classified into three groups. There are the rapidly firing GABAergic interneurons that express the calcium binding protein parvalbumin, and the somatostatin and nitric oxide expressing interneurons that mainly fire in bursts. The much larger cholinergic interneurons make up the third group [3]. Each of these three groups of interneurons contributes about 1-3% of the striatal neurons.

Based on connectivity and function the rodent striatum can be divided into dorsal and ventral portions. The dorsal striatum (or neostriatum) consists of the caudate-putamen, and is mainly sensorimotor related. It receives DA innervations primarily from the SNc and to a lesser degree from the VTA. The dorsal striatum receives glutamatergic inputs from sensorimotor related cortical areas [4]. The medium spiny GABAergic projection neurons in the dorsal striatum project to the substantia nigra reticulata (SNr) and the internal segment of the globus pallidus (GPI), forming the so-called direct, monosynaptic pathway. Other striatal medium spiny

neurons project to the external segment of the globus pallidus (GPe) multisynaptically, via intermediate connections forming the indirect pathway. However, these two pathways are not strictly separated, as some MSNs project to the internal segment of the globus pallidus and also send axon collaterals to the external segment of the globus pallidus [5, 6]. The thalamus is another major input region of the neostriatum, with glutamatergic thalamostriatal neurons [2].

The ventral striatum which is composed of the NAc (nucleus accumbens) and portions of the olfactory tubercle is mainly limbic related. It receives DA innervations primarily from the VTA and to a lesser degree from the SNc. The NAc sends inhibitory projections to the VTA and surrounding DA areas, and receives its major glutamatergic input from the prefrontal cortex, the hippocampus, and the amygdala [4, 7, 8]. Figure 1 explains the connectivity to and from the striatum.

The NAc affects cortical arousal, attention, and cognitive function through its projections to the basal forebrain cholinergic neurons [9]. The NAc participates in reward-based learning and addiction by serving as limbic-motor or motivation-action interface [4, 10].

A single dopamine neuron projecting to the striatum forms highly branched arbors, which constitutes 5.7% of the volume of the striatum, with a high density of axonal varicosities approximately 1 million and form dopamine synapses at an incidence estimated at 1 in every 10-20  $\mu\text{m}^3$  in the rat [11,12]. Although dopamine fibers make synaptic connections, DA receptors and DA uptake transporters exist extra synaptically [13-15]. Spillover of dopamine from the synapse into the extracellular space mediates extrasynaptic/volume transmission [16, 17, 18, 19], whereas ACh acts mainly via non-synaptic and diffuse transmission, released by the varicosities in addition to synaptic transmission [20].

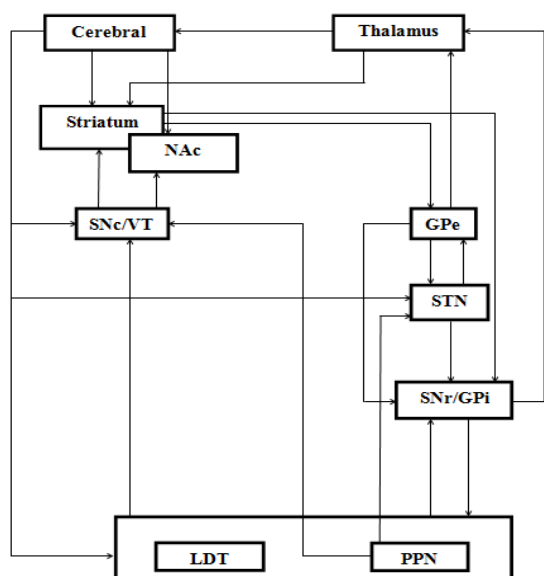


Fig. 1: Correlation of various parts of brain within the striatum

### Cholinergic interneurons (ChIs) in the striatum

The striatum is extremely rich in cholinergic interneurons [21]. The cholinergic interneurons in the striatum were identified as giant interneurons by Kolliker, through his classic studies of a combination of Golgi staining [22, 23] and intracellular labeling with immunochemistry for choline acetyl transferase [24]. In the striatum the cholinergic input arises solely from cholinergic striatal interneurons [1, 25, 26]. The ratio of medium sized neurons (mostly spiny projection neurons) to large cells (mostly cholinergic interneurons) is about 100 to 1 or 2 [27]. It is estimated that a total of 2.8 million neostriatum neurons are found on one side of a rat brain [28], and the number of cholinergic interneurons in a rat is approximately 40,000 per side. When compared to other cells in the neostriatum, the cholinergic interneurons are the one with large and

extremely dense axonal arbors. The cholinergic arbors within the dorsal and ventral striatum are more reminiscent of dopaminergic arbours. As per quantitative electron microscopic studies there are approximately  $2 \times 10^8$  ACh varicosities/ $\text{mm}^3$  in the striatum and each striatal cholinergic interneuron has 500,000 axon varicosities. A 10  $\mu\text{m}$  radius sphere of striatal neutrophil contains about 400 dopaminergic as well as cholinergic axon terminals [25, 29]. These cholinergic interneurons are spontaneously active in the striatum [30-32].

Not only dopaminergic neurons, but also the cholinergic interneurons are critically involved in signaling unexpected primary rewards as well as signaling learning associated with events of high salience [26-37]. The cholinergic interneurons signal these events with a pause in firing, in a burst-pause-burst pattern [38, 39]. Like dopaminergic neurons striatal cholinergic interneurons form synapses primarily onto distal dendrites and dendritic spine necks in the striatum. In addition to the direct action at synaptic sites, ACh like DA may also influence striatal function via volume transmission [20, 40]. The highest expression of ACh, DA, tyrosine hydroxylase, choline acetyl transferase and acetylcholine esterase due to the extensive axonal arborization ensures that the cholinergic and dopaminergic system are positioned to interact within the striatum [31].

### Dopamine receptors in the striatum

Dopamine receptors are a class of metabotropic G protein coupled receptors. There are at least 5 subtypes of dopamine receptors as identified by molecular cloning studies [41]. They are classified into  $D_1$  like family receptors ( $D_1$  &  $D_5$ ) which are coupled to G protein  $G_s$  that stimulates adenylyl cyclase and  $D_2$  like receptors ( $D_2, D_3, D_4$ ) which are coupled to  $G_i$  and inhibit adenylyl cyclase. The relative abundance of  $D_1$  like and  $D_2$  like receptors in the brain is in the order of  $D_1 > D_2 > D_3 > D_5 > D_4$  [41, 42, 43, 44]. The striatum has the highest expression of dopamine receptors in the brain. DA inputs to the striatum arise predominantly from the "A9-A10" dopamine neurons in the midbrain. The  $D_1$  and  $D_2$  receptors (and to a lesser extent  $D_3, D_4, D_5$ ) on the striatal spiny projection neurons can be classified into sub populations based on their expression levels [45, 46, 47, 48, 49]. The activity of striatal spiny neurons can be altered by dopamine by modulating voltage dependent ion channels as well as excitatory and inhibitory synaptic inputs [49]. Based on some previous studies it is found that membrane excitability of striatal neurons is increased by the activation of  $D_1$  like receptors and decreased by the activation of  $D_2$  like receptors [18, 50, 51]. The state of the neurons also influences the effects caused by DA. The cholinergic interneurons in the striatum contain  $D_2$  receptors in both the short and long forms and to a lesser extent contain  $D_5$  receptors mainly in the samotodendritic areas [46, 52]. These  $D_5$  receptors may regulate the excitability and ACh release from cholinergic interneurons. Activation of  $D_1$  like (probably  $D_5$ ) can depolarize cholinergic interneurons and enhance ACh release [53, 54, 55, 56, 57], whereas activation of  $D_2$  like receptors inhibits striatal ACh efflux [55], through suppression of N type  $\text{Ca}^{2+}$  channels that directly initiate ACh release [52].

### Muscarinic and nicotinic receptors in striatum

Autoradiography studies using tritiated agonist identified that cholinergic receptors expressed in the striatum are of both metabotropic muscarinic (mAChR) and ionotropic nicotinic (nAChR) families. Thus the striatum is richly endowed with both classes of cholinergic receptors. Nicotinic ACh receptors are composed of five subunits, which can be categorized into three classes based on their evolutionary relationship, their pharmacology and their physiology. These include muscle nAChRs and two classes of neuronal nAChR one class is formed from  $\alpha$ - $\beta$  subunit combinations ( $\alpha 2$ - $\alpha 6, \alpha 10$  and  $\beta 2$ - $\beta 4$ ), the other class from subunits which can form homo oligomeric neuronal nAChRs ( $\alpha 7$ - $\alpha 9$ ) and in the third class only the  $\alpha 7$  subunit is widely distributed in the mammalian CNS [58]. Among the most commonly expressed nAChR subunits, the  $\alpha 4$  and  $\beta 2$  subunit is the first and is widely expressed throughout the mammalian brain including the midbrain DA areas and the striatum [59, 60] but the  $\alpha 7$  containing nAChRs which are the next most common [61], are not highly expressed in the striatum. In the

mesostriatal DA system of the mouse, the  $\beta_2$  nAChRs are the most widely expressed, followed by  $\alpha_4$  and  $\alpha_6$  subunits. In the VTA and SN  $\beta_2$  nAChR are expressed on all DA neurons and most of the GABA neurons [62, 63, 64], whose activation increases excitability, action potential firing, and  $Ca^{2+}$  influx [62, 64, 65, 66]. DA axon terminals in the striatum express nAChR as indicated by histochemical and ion flux measurements with synaptosomes [60, 67, 68, 69]. Electrophysiological data, support the fact that striatal nAChRs are present only on the GABAergic interneurons, but not on the medium spiny neurons or cholinergic interneurons [58]. Molecular cloning has identified five different types of mAChRs,  $M_1$ - $M_5$  present on a variety of neurons and axon terminals throughout the striatum. They are divided into  $M_1$  like ( $M_1, M_3, M_5$ ) and  $M_2$  like ( $M_2$  and  $M_4$ ) in a similar manner as that of the DA receptors  $M_1$  like receptors are coupled to the  $G_s$  class of G2 proteins, whereas  $M_2$  like receptors are coupled to the  $G_i$  protein.  $M_1$ ,  $M_2$  and  $M_4$  are the dominant striatal muscarinic subtypes [70, 32]. MSNs express primarily  $M_1$  and  $M_4$ , while  $M_2$ ,  $M_3$  and  $M_5$  subtypes are present in very low or undetectable levels [71, 72, 73, 74, 75, 76]. In contrast to MSNs, the striatal cholinergic interneurons have dominant expression of  $M_2$  and  $M_4$  mAChRs [77, 78, 79, 80].

### DOPAMINERGIC PATHWAYS IN CNS

Nigrostriatal and mesocorticolimbic dopaminergic pathways are the two major dopaminergic (DA) systems in the CNS. The nigrostriatal DA system innervates the striatum and mediates excitatory and inhibitory influence, via dopamine  $D_1$  like ( $D_1/D_5$ ) and  $D_2$  like ( $D_2, D_3, D_4$ ) receptors and acetylcholine (ACh) muscarinic  $M_1$  like ( $M_1/M_3/M_5$ ) and  $M_2$  like ( $M_2/M_4$ ) receptors, of direct and indirect GABAergic striatal output pathways. Striatal neurons in the direct pathway utilize dopamine  $D_1$  receptors, whereas those in the indirect pathway utilize  $D_2$  receptors.  $D_1$  receptors activation stimulates adenylate cyclase there by activating the GABAergic substance P containing medium spiny output neurons, whereas  $D_2$  receptors activation inhibits adenylate cyclase, there by inhibiting GABAergic enkephalin containing output neurons [81]. Thus the direct (via  $D_1$ ) and indirect (Via  $D_2$ ) pathways have opposing actions, but the net outcome activating motor regions of the cortex is the same. In some, the striatal dopamine released from the nigrostriatal pathway finally facilitates voluntary movements through increasing thalamocortical activity either by direct or indirect reduction of GPi/SNr activity [82].

The Mesocorticolimbic DA system consists of dopaminergic neurons originating in the VTA projecting via the medial forebrain bundle (MFB) to forebrain limbic and cortical areas [83]. Based on the localization of dopamine containing cell bodies within the VTA and their projection targets, it is further divided into two subsystems. The mesolimbic DA pathway is the one in which the dopaminergic cells of the paranigral VTA subdivision project to the NAc, amygdala, and hippocampus and (the central part of the reward system) the mesocortical DA pathway is the one in which the DA neurons of the parabrachial VTA subdivision project to the cortical structure [84]. However when compared to the other VTA projection target nuclei, the NAc is more established in the maintenance of goal directed behavior related to obtaining natural and artificial rewards as well as stimulus reward associations [85, 86].

According to traditional views, nigrostriatal system with dopamine containing cell bodies in the substantia nigra pars compacta (SNc) of the midbrain and projecting to the dorsal striatum, play a role in the expression of motor acts, while the mesocorticolimbic system with dopamine containing cell bodies originating in the VTA of the midbrain and projecting largely to the nucleus accumbens and other ventral striatal regions such as hippocampus, amygdala and medial prefrontal cortex play a role in reinforcement/incentive motivational processes [83, 84, 87, 88]. However, it is not just mesocorticolimbic dopamine that is important for reward function; nigrostriatal dopamine is similarly important.

The nigrostriatal and mesolimbic dopamine "systems" are not simply differentiated anatomically, and significant functional interactions between the two systems have been recently suggested [4, 89]. While it is the mesocorticolimbic dopamine system that is most frequently associated with brain stimulation reward, the

dopaminergic fibers from the SN and the VTA each enter and project along the MFB [90], and reward sites are found in both SN and VTA [91].

Despite several kinds of heterogeneity both between SN and VTA dopamine neurons [92,93,94,95] and also within SN [96] and VTA [97,98,99] dopamine groupings, subsets of both SN and VTA dopamine neurons show the characteristic responses to reward and reward-predictive stimuli and to reward omission [100]. Indeed, the responses of SN and VTA neurons to rewards and reward-predictors are so indistinguishable that the electrophysiological data from cells in the two regions are traditionally pooled [100]. SN and VTA dopamine neurons share the same qualifying characteristics for whatever role dopamine neurons play in reward function.

### Afferents modulating the activity of dopaminergic neurons in the VTA

Accumbal DA release is modulated by various afferents to the VTA such as the glutamatergic afferents from the lateral hypothalamus [101], bed nucleus of stria terminalis [102] and the superior colliculus [103], the GABAergic input, e. g. from the NAcc shell and medial part of ventral pallidum and the noradrenergic afferents from the locus coeruleus [104]. Activation of the GABAergic interneurons in the VTA inhibits the release of accumbal dopamine [105]. 5-HT afferents from the dorsal and medial raphe and the hypothalamic opioidic provide input via the ventral tegmental GABAergic interneurons [106, 107], thereby modulating the activity of the DA neurons in VTA. Moreover, the orexin containing projections from lateral hypothalamus to VTA also regulates the activity of ventral tegmental DA neurons [108,109,110]. Cholinergic neurons in additionally, the mesopontine areas i. e. the pedunculopontine tegmental area (PPTg) and laterodorsal tegmental area (LDTg) provide GABAergic input and thereby have an important modulatory role [108, 111, 112, 113].

### Distinct action potential firing patterns of the dopaminergic neurons in the VTA

Based on previous microelectrode studies two cell types are identified in the striatum which differ in the action potential firing patterns [114]. The cells which are often silent for seconds, but fire in brief episodes are commonly called phasically active neurons (PANS), which include medium spiny projection neurons of the striatum [115]. On the other hand cells which fire tonically at a rate that might vary, but which doesn't exhibit long periods of silence are called tonically active neurons (TANS), cholinergic interneurons are of this type [116]. The mesostriatal DA neurons exhibit two broad and well defined firing modes *in vivo*; single spike firing in regular or irregular patterns (10Hz) and burst firing of 3-5 spikes at a frequency of 15-100Hz [117,118,119] and in between DA neurons are in a hyper polarized, quiescent state. A change from single to burst firing enhances and prolongs the signal strength, which in turn increases the DA levels in NAcc [120]. Initially presentation of a novel food reward [121], as well as conditioned stimulus associated with it leads to phasic burst firing of the DA neurons [122]. Later on as the reward becomes expected due to training the DA neurons loses their phasic bursting.

### CHOLINERGIC REGULATION OF THE VENTRAL TEGMENTAL AREA

Cholinergic neurons are widely distributed throughout the brain [123, 124] and have cognitive functions such as learning [125], memory [126] and attention [127]. Interestingly, cholinergic neurons have been identified in the mesopontine area i. e. the pedunculopontine tegmental area (PPTg) and (LDTg). These neurons project to various brain regions such as the thalamus, hypothalamus, basal forebrain, substantia nigra and medial limbic cortex. Additionally, the cholinergic neurons in the mesopontine area provide the only known cholinergic projections to the VTA [123]. Specifically, the cholinergic neurons of the LDTg projects to the VTA, whereas the PPTg mainly projects to substantia nigra [113-131]. The cholinergic neurons originating in the LDTg regulate the activity of ventral striatum while that of the PPTg regulates the activity of dorsal striatum [132-134]. However, it is unlikely that these relationships are wholly exclusive, as the VTA receives bilateral innervations not only from the LDT but also the PPN

(pedunculopontine tegmental nucleus) [130-137]. As seen with PPN, the LDT cholinergic neurons also project ipsilaterally to SNc dopaminergic neurons, but the projection is not as dense as that arising in the PPN [1,130,138]. Thus the PPN, rostral part of the brainstem cholinergic system projects ipsilaterally to the SNc, most caudal part of the midbrain dopaminergic system, whereas the medial and caudal parts of the brainstem cholinergic system (PPN caudal and LDT) project bilaterally to the VTA, the most rostral part of the midbrain dopaminergic system. The cholinergic projection from the LDTg to the VTA involves regulation of the dopaminergic, rather than the GABAergic mesoaccumbal neurons. And also it is important to note that the PPN cholinergic neurons are intermingled with non-cholinergic neurons such as GABAergic and glutaminergic [139]. The cholinergic projection from the LDTg, together with the mesolimbic DA system constitutes the cholinergic dopaminergic reward link. This link is composed of the cholinergic projection from the LDTg to the VTA and the dopaminergic projection from the VTA and the dopaminergic projection from the VTA to the NAc. Activation of LDTg causes a release of ACh in the VTA which by interacting either with thenAChR and/or muscarinic mAChR stimulates the mesolimbic DA system causing a release of DA in NAc [140]. The cholinergic projections via mAChRs exert a tonic excitatory and the nAChRs exert phasic influence on the mesolimbic DA system respectively. The projections from the rostral part of the brain stem cholinergic system (PPN rostral) reach the most caudal part of the midbrain dopaminergic system (SNc).

#### Mesopontine cholinergic modulation of the nigrostriatal dopaminergic system

The mesopontine cholinergic system arises from neurons located in the LDT and PPT of the hindbrain [82]. Based on *in vivo* electrochemical studies activation of mAChRs located in the PPT inhibits striatal dopamine release by hyper polarization of mesopontine cholinergic cells [141,142] and a net decrease in excitation to SNc DA cells [134]. The  $M_2$  like mAChRs function as cholinergic autoreceptors as they are localized presynaptically on PPT cholinergic neurons and regulate the information received by the PPT [82]. Intra PPT infusions of scopolamine, a non-selective mAChR antagonist enhances striatal DA release as opposed to cholinergic agonist carbachol [143,144].

Activation of PPT cholinergic neurons could evoke striatal dopamine release through innervations of dopaminergic cells in the substantia nigra pars compacta directly by cholinergic and glutamatergic neurons in PPT and indirectly via glutamatergic neurons in the subthalamic nucleus (STN)[82]. *In vivo* fixed potential amperometry studies concluded that STN mAChRs, particularly of the  $M_3$  subtype, may be involved in the indirect activation of SNc DA cells via PPT-STN-SNC pathways [145].

#### Mesopontine cholinergic modulation of the mesocorticolimbic da system

The cholinergic input from the LDT is required for burst firing of DA cells in the VTA [146], which conveys motivational information to NAc, which is involved in reward related processes [147]. LDT mAChRs, most likely  $M_2$  auto receptor stimulation, hyperpolarizes LDT cholinergic neurons [141]. Nicotinic receptors containing  $\alpha 7, \beta 2$  and non  $\alpha 7$  subunits mediate nicotinic actions on LDT cholinergic neurons [148].

Electrical stimulation of the LDT as well as chemical stimulation of both mAChRs and nAChRs in the VTA excite mesolimbic DA neurons [149,150,151], and further facilitate dopamine release in the NAc [131,152,153]. The excitatory effect of ACh acting on VTA muscarinic and nicotinic AChRs influences incentive related behavior driven by DA activity. In rats, blockade of mAChRs in the VTA disrupts responding for food reward [154].

#### INTERACTION OF THE CHOLINERGIC AND DOPAMINERGIC SYSTEM

The dopaminergic and cholinergic systems are the primary and secondary largest neuromodulatory systems in the striatum [155]. Interactions between them are important and have been suggested to be mediated via correlated changes in neuron activities, reciprocal presynaptic regulation of neurotransmitter release, and

through postsynaptic interactions [26, 35, 38, 58, 156]. Initial studies suggested that activation of the dopaminergic system generally inhibits the release of ACh. Indeed, later studies showed that  $D_2$  activation reduces whereas activation of the  $D_1$  receptor using specific agonists facilitates ACh release.

In addition to the dopaminergic system modulating the cholinergic system, the cholinergic system also affects activity of the striatal dopaminergic system. However, the data are contradictory, and the mechanisms unresolved. Poor ligand selectivity has made it difficult to define the mAChR type(s) involved [157]. Some studies report that mAChRs enhance DA release [158-163], whereas some report suppression [160-165]. Recent data revealed that based on the activity of DA neurons, mAChRs regulate DA bidirectionally [166]. Activation of striatal mAChRs suppressed DA release by single pulses or low, tonic-like frequencies of presynaptic activity, but enhanced the sensitivity of DA release to frequency, thereby increasing DA released by higher frequencies [166]. Thus the sensitivity of DA release to frequency is enhanced either by activation of mAChRs or inhibition of nAChRs [167,168]. To investigate cholinergic influence over dopamine release, carbon-fiber microelectrodes were placed into mice striatal brain slices, and fast cyclic voltammetry was used to monitor the concentration of action-potential-dependent dopamine release in real time. They found that cholinergic interneurons acting via nAChRs containing the  $\beta 2$  subunit potentially regulate dopamine release.

However, unlike for striatal  $\beta 2^*$ -nAChRs, there is no anatomical evidence that striatal mAChRs are present on Dopaminergic axon terminals to influence DA release directly [32, 63, 164]. In contrast, many other striatal neurons express and are regulated by mAChRs, including striatal cholinergic interneurons [77, 78, 169, 170], GABAergic interneurons [40], GABAergic projection neurons [26-74], and glutamatergic afferents [26]. In contrast, the dynamic regulation of endogenous striatal DA release by mAChRs shown here requires ACh tone at  $\beta 2^*$ -nAChRs on DA axons: blockade of ACh input from cholinergic interneurons (ChIs) to  $\beta 2$ -nAChRs precluded effects of mAChR agonists and vice versa. The somato dendritic and axonal mAChRs which are expressed on striatal cholinergic interneurons [78,169,171], are autoreceptors as seen with inhibition/desensitization of nicotinic receptors. When these auto receptors are activated cholinergic interneurons become silenced and ACh release is inhibited [26,32,77,80,159,171,172,173], which further deactivates nAChRs on dopaminergic axons and in turn, increases the sensitivity of DA release to presynaptic depolarization frequency. These data offer support generally for the hypothesis that the outcome of changes in DA neuron activity will depend critically on any accompanying changes in ChIs [38].

Dopaminergic neurons may possess  $M_5$ -mAChRs in midbrain for modulation by mesopontine afferents [71,174,175], but there is currently no anatomical evidence that mAChRs are located on Dopaminergic axons. Striatal ChIs in contrast, which release ACh and regulate DA release via  $\beta 2$ -subunit-containing nicotinic AChRs ( $\beta 2^*$ -nAChRs) on dopaminergic axons, express  $M_2$ - and/or  $M_4$ -mAChRs [77, 78, 79, 80, 173]. These  $M_2/M_4$ -mAChRs regulate cholinergic interneuron's activity and ACh [77,159,160] and might therefore gate  $\beta 2^*$ -nAChR function by controlling ACh availability. Since  $\beta 2^*$ -nAChRs either inhibits or facilitates DA release depending on the frequency of presynaptic activity in DA axons [156,176,177], mAChRs on ChIs might also regulate DA release probability in a bidirectional manner.

mAChR antagonist shimbacine and tropicamide, at concentrations selective for  $M_2/M_4$ -mAChRs, competed with the effects of mAChR agonists, implicating  $M_2/M_4$ -type mAChRs throughout striatum. This finding is consistent with the expression of  $M_2/M_4$ -mAChRs by ChIs [77, 78, 79, 80, 173]. However, several pieces of data suggested differing  $M_2/M_4$  function between striatal territories. Furthermore, whereas knock-out of the  $M_4$ R prevented mAChR effects throughout striatum, knock-out of  $M_2$ R prevented mAChR control of DA release only in caudate-putamen (CPu). These data suggest that  $M_4$ Rs are necessary for mAChR regulation of DA (and ACh) release throughout the striatum but that  $M_2$ Rs are necessary only in CPu, where both  $M_4$ Rs and  $M_2$ Rs are required [166].

Thus, distinct muscarinic mechanisms/receptors in subpopulations of ChIs may explain the differing mAChR control of DA release in CPU and NAc. These regional differences in M<sub>2</sub>/M<sub>4</sub> function could ultimately be exploited for discrete modulation of DA/ACh. For example, ACh/DA function might be modified selectively in NAc by activation of M<sub>4</sub>Rs, and in CPU by M<sub>2</sub>R inhibition [166]. DA release probability within the striatum is relatively high following a single action potential, whereas subsequent action potentials within a burst of action potentials are limited by short term depression [178,179]. However, ACh and presynaptic nAChRs control the DA release probability based on the activity of DA neurons and the multiple nAChR subunits [31,166,176,177,180,181]. Administration of nAChR antagonist or desensitization of nAChRs using nicotine reduces ACh action at nAChRs thereby reducing initial DA release probability and subsequently relieves short term depression [176,177]. Activation of nAChRs by ACh regulates DA axons release in response to single or low frequency action potentials as well as high frequency action potential, e. g. in a burst. Thus, nAChRs limits striatal DA release in response to frequency of activity of DA neurons in midbrain by acting as a low frequency pass filter and when they are turned off they act as a "high frequency pass" filter as the striatal DA release becomes highly sensitive to the frequency of activity of a neuron [156,176]. Thus reduced nAChR activity can either increase DA release during bursts of DA neuron activity (>20Hz) such as that seen after presentation of an unexpected reward or conditioned salient stimuli [119,182] and reduce DA release during low DA neuron activity such as that seen after omission of an expected reward [183]. By acting at the source of DA (in the midbrain) and at the target of DA fibers (in the striatum), nicotinic mechanisms exert multiple regulatory influences over DA signalling. Via those normal nicotinic mechanisms, the addictive drug, nicotine, exerts modulatory influences over the mesostriatal, mesocortical, and mesolimbic DA systems. Previous studies using the  $\alpha_6$  selective nAChR antagonist  $\alpha$ -CTXMII has revealed that  $\alpha_6$  is the dominant nAChR subunit responsible for DA transmission in ventral striatum [180], whereas  $\alpha_6$ ,  $\alpha_4$ ,  $\beta_2$  nAChRs may dominate  $\beta_2$ nAChR function in NAc [184].

## CONCLUSION

The cholinergic neurons in the pons region of the Hind brain, the PPT and LDT, provide the only known cholinergic projections to the SNc and VTA respectively. The activation of mesopontine cholinergic projection innervating the VTA causes a release of ACh which by interacting with nAChR/mAChR produces excitation of dopamine neurons and facilitates dopamine release in the NAcc. Thus dopamine's incentive-related behaviors are influenced by the excitatory effect of ACh in the VTA.

Furthermore, the mAChRs are known to regulate dopamine bidirectionally depending upon the activity of DA neurons. They do not simply suppress or enhance DA release but can do both depending on the frequency of depolarization and this variable mAChR control of DA release is not via variable striatal neuron types but is via the control of ACh release from striatal ChIs. While the cholinergic projections of mAChRs exert a tonic excitatory influence, nAChRs exert a phasic influence on the mesolimbic DA system.

As many neuropsychiatric disorders are manifestation of fluctuations in ACh and DA levels, their interaction should be given appropriate consideration.

Previous studies indicated that dopamine is necessary to elicit neural activity in the accumbens that drives the behavioral responses to cues. However the actual contribution of ACh towards this behavioral response is not known. It will be of great value to determine whether ACh apart from dopamine is also a powerful modulator of goal-directed behavioral responses.

Further research detailing the nature of the interactions between dopamine and ACh is necessary to precisely define their contributions towards these goal-directed behavioral responses.

## CONFLICT OF INTEREST

We declare no conflict of interest.

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