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► **To cite this version:**

Phil D. Livingstone, Susan Wonnacott. Nicotinic acetylcholine receptors and the ascending dopamine pathways. *Biochemical Pharmacology*, 2009, 78 (7), pp.744. 10.1016/j.bcp.2009.06.004. hal-00509505

**HAL Id: hal-00509505**

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Submitted on 13 Aug 2010

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## Accepted Manuscript

Title: Nicotinic acetylcholine receptors and the ascending dopamine pathways

Authors: Phil D. Livingstone, Susan Wonnacott

PII: S0006-2952(09)00451-1  
DOI: doi:10.1016/j.bcp.2009.06.004  
Reference: BCP 10212

To appear in: *BCP*

Received date: 7-4-2009  
Revised date: 4-6-2009  
Accepted date: 4-6-2009

Please cite this article as: Livingstone PD, Wonnacott S, Nicotinic acetylcholine receptors and the ascending dopamine pathways, *Biochemical Pharmacology* (2008), doi:10.1016/j.bcp.2009.06.004

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Receiving Editors: Daniel Bertrand, Murali Gopalakrishnan, Diana Donnelly-Roberts

# **Nicotinic acetylcholine receptors and the ascending dopamine pathways**

Phil D. Livingstone and Susan Wonnacott

Department of Biology & Biochemistry, University of Bath BA2 7AY, UK

**Corresponding author:** Professor Susan Wonnacott,  
Department of Biology & Biochemistry,  
University of Bath,  
Bath BA2 7AY,  
UK  
Email: [bsssw@bath.ac.uk](mailto:bsssw@bath.ac.uk)  
Tel: 44 (0)1225 386391  
FAX: 44 (0)1225 386779

**Total Number of:** Pages, 38  
Figures, 4  
Appendix, 1 (separate files, includes figure)  
Tables, 0  
Equations, 0  
Words, Whole Manuscript ; 10832  
Abstract ; 79  
Introduction; 444  
References, 159

**Running title:** nAChRs and dopamine pathways

*Abstract*

Nicotinic acetylcholine receptors (nAChRs) are widely expressed in midbrain dopamine neurons that project to dorsal striatum, nucleus accumbens and prefrontal cortex. Thus nAChRs can influence the functions of these three pathways; notably motor control, 'reward' and executive function, respectively. Diverse subtypes of nAChRs have been identified on dopamine cell bodies and terminals as well as on neighbouring afferents and interneurons. Here we review the molecular and cellular mechanisms through which nAChRs exert their influence on these pathways in rodents.

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*Abbreviations*

$\alpha$ Bgt	alpha-bungarotoxin
CPu	caudate putamen
CREB	cyclicAMP response element binding protein
DARPP-32	dopamine and cyclicAMP regulated phosphoprotein of 32KDa
DH $\beta$ E	dihydrobetaerythroidine
ELK	Ets-like transcription factor
EPSC	excitatory postsynaptic current
ERK	extracellular signal-regulated kinase
FSCV	fast-scan cyclic voltammetry
GABA	gamma-aminobutyric acid
iGluR	ionotropic glutamate receptor
IPSC	inhibitory postsynaptic current
LDT	laterodorsal tegmental nucleus
LTP	long-term potentiation
MLA	methyllycaconitine
NAc	nucleus accumbens
nAChR	nicotinic acetylcholine receptor
NMDA	N-methyl-D-aspartate
PFC	prefrontal cortex
PKA	protein kinase A
PPn	pedunculopontine tegmental nucleus
RRF	retrosubthalamic field
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
VTA	ventral tegmental area

## 1. Introduction

The association of nicotine with tobacco addiction has stimulated particular interest in how nicotine interacts with the 'reward' pathways of the brain. Most attention has been given to the ascending dopaminergic pathways that arise in the midbrain and project to aspects of the basal ganglia and prefrontal cortex (PFC; Fig. 1). In addition to mediating the reinforcing properties of addictive substances, dopamine is also central to the fine control of movement governed by the basal ganglia, whereas its actions in the PFC are critically important to 'executive' function [1,2]. This includes working memory, behavioural flexibility and decision making, and the PFC is also involved with the anticipation of reward [3, 4].

FIGURE ONE NEAR HERE

Nicotine interacts with the dopamine systems via nicotinic acetylcholine receptors (nAChRs), a family of ligand-gated pentameric cation channels constituted from at least nine  $\alpha$  and  $\beta$  subunits ( $\alpha$ 2-7 and  $\beta$ 2-4) expressed in the mammalian brain [5]. nAChR subtypes can differ in their sensitivities to nicotine or acetylcholine (ACh), their channel characteristics (including their propensity to desensitise) and their cellular distribution. A high relative permeability to  $\text{Ca}^{2+}$ , a notable characteristic of the  $\alpha$ 7 nAChR subtype, enables nAChRs to interface with a variety of intracellular  $\text{Ca}^{2+}$ -dependent mechanisms [6]. The localisation of nAChRs on dopamine cell bodies in the ventral tegmental area (VTA) or substantia nigra pars compacta (SNc) allows nicotine to directly modulate dopaminergic cell firing. Additionally, nAChRs on dopamine terminals can influence transmitter release. nAChRs present on afferents projecting to the VTA and SNc or to the terminal fields can also influence the activity of dopaminergic neurons. The nicotinic modulation of dopamine release has generated interest in nAChRs as therapeutic targets for conditions involving dopaminergic dysfunction, including schizophrenia, attention deficit hyperactivity disorder, Parkinson's disease, Alzheimer's disease and age-related cognitive impairment [7-10].

In this review we focus on the mechanisms through which nAChRs modulate the activity of dopamine neurones of the principal ascending pathways (Fig. 1). We will consider

nicotinic influences on somatodendritic and terminal fields, with the aim of integrating the diverse studies on nAChR actions. As the majority of this work has been undertaken in rodents, we will focus on these species. Although much of this research has been driven by the desire to understand nicotine addiction or the potential of nAChRs as therapeutic targets, the underlying physiological roles of nAChRs and the influence of their endogenous agonist ACh is also considered. Because nAChRs alter the firing pattern of dopaminergic neurons and dopamine release, this is often the endpoint of research studies. However, dopamine levels determine several critical downstream signals. Therefore the molecular and cellular consequences of dopamine release in response to nicotinic modulation will also be briefly explored.

## 2. *Nicotinic regulation at the level of the dopaminergic cell bodies*

Of the three anatomically defined groups of mesencephalic dopamine neurons (see Appendix), those of the VTA and SNc have been extensively studied with respect to the roles of nicotine and nAChRs. These neurons are tonically active, firing single action potentials at low frequency ('irregular single spike firing' [11]). In addition to increases in single spike firing rate, these neurons can switch to a burst firing mode in which rapid bursts of action potentials serve to "boost the gain of neural signalling" of important or novel events [12,13]. A switch from tonic to burst firing is observed during presentation of a rewarding stimulus [14] and this switch occurs in response to nicotine [15,16]. ACh, glutamate, GABA and dopamine itself determine the excitability of dopaminergic cell bodies, and orchestrate changes in firing rate. nAChRs on dopamine cell bodies, local interneurons and afferent terminals play a significant role in this process by responding to endogenous transmitter or exogenous nicotine (Fig. 2A).

### FIGURE 2 NEAR HERE

In the rodent VTA/SNc, in-situ hybridisation and single-cell RT-PCR have revealed the expression in dopaminergic cell bodies of  $\alpha 3$ - $\alpha 7$ ,  $\beta 2$  and  $\beta 3$  nAChR subunits (note the relative lack of  $\alpha 2$  and  $\beta 4$  only).  $\alpha 4$ ,  $\beta 2$ ,  $\alpha 7$  and  $\beta 4$  subunits were found in GABAergic interneurons or in other non-dopaminergic cells [17,18] (Fig. 2B). 6-Hydroxydopamine lesions to mimic dopaminergic cell loss in Parkinson's disease preferentially decrease the



expression of  $\alpha 5$ ,  $\alpha 6$  and  $\beta 3$  nAChR subunits in the rat SNc [19]. The VTA expresses the same complement of nAChR subunit mRNAs as SNc but in a slightly lower percentage of neurons [18]. These findings suggest that the dopaminergic neurons are likely to be well endowed with a great diversity of nAChR subtypes, but mRNA expression data are not informative about nAChR subunit composition or subcellular distribution.

There are functional nAChRs on dopaminergic cell bodies in both SNc [20] and VTA [21,22]; autoradiographical evidence for the labelling of these neurons with [ $^3$ H]nicotine supports the presence of high affinity ( $\beta 2$ -containing) nAChRs [23] and colocalisation of  $\alpha$ bungarotoxin ( $\alpha$ Bgt) with tyrosine hydroxylase corroborates the presence of  $\alpha 7$  nAChRs [24]. More detailed pharmacological analysis, in conjunction with single cell PCR and comparison of tissue from null mutant mice lacking a particular subunit, has provided evidence for both  $\alpha$ -conotoxinMII-sensitive and -insensitive nicotine- and ACh-evoked currents (tentatively ascribed the subunit composition  $\alpha 4\alpha 6\beta 3(\beta 2)_2$  and  $(\alpha 4)_2\alpha 5(\beta 2)_2$ , respectively), as well as  $\alpha 7$  nAChR-mediated responses [17].

The main influence of nAChRs expressed on cell bodies of dopamine neurons is to modulate the rate of action potential firing and consequent level of dopamine released at synapses [25]. Cholinergic inputs to the SNc arise in the pedunclopontine tegmental nucleus (PPn) and those to the VTA are from both the PPn and the laterdorsal tegmental nucleus (LDT) [26,27]. Burst firing in dopaminergic neurons is dependent on inputs from the LDT [28]. Evidence for the importance of endogenous nicotinic signalling comes from knockouts of  $\beta 2$  or  $\alpha 7$  nAChR subunits in which spontaneous firing in VTA dopaminergic neurons is altered, either by attenuation of high frequencies and high bursts ( $\beta 2$  -/-) or reduction of other, more subtle firing modes ( $\alpha 7$  -/-) [25] (Fig. 2C). Selective re-expression of the  $\beta 2$  subunit in the VTA of  $\beta 2$  -/- mice restored normal frequency and bursting patterns of action potential firing to these neurons. Thus both  $\alpha 7$  and  $\beta 2^*$  nAChRs (where \* denotes additional unspecified subunits) are implicated, with the latter having the more prominent role.

Focus on the VTA reflects the importance given to this region with respect to reward-related events, including responding to nicotine [29-31]. Lack of nicotine self-administration in  $\beta 2$  knockout mice has led to the conclusion that  $\beta 2$  subunit-containing nAChRs are critical for nicotine addiction [32] and a necessary and sufficient role for both  $\alpha 4\beta 2^*$  and  $\alpha 6\beta 2^*$  nAChRs in the VTA in nicotine self-administration has been demonstrated [33]. The importance of  $\alpha 6^*$  nAChRs is highlighted in a study of mice in which these receptors harbour a gain of function mutation. This results in increased firing rates in VTA and SNc dopaminergic neurons, compared to wild type controls, in response to nicotine [34]. It would be interesting to know how this mutation affects nicotine self-administration, with respect to rate of acquisition and rate of responding.

How does the predominance given to  $\beta 2^*$  nAChRs in the above studies relate to the view that burst firing is largely influenced by NMDA receptors on dopamine cell bodies, activated by glutamate released from afferents from the PFC [13,35]? In this model nicotine increases burst firing via stimulation of presynaptic  $\alpha 7$  nAChRs on the glutamate afferents. There is immunocytochemical evidence for  $\alpha 7$  nAChRs on glutamate terminals in the VTA [24] (Fig. 2D), supporting functional evidence that nicotine can sustain long-lasting presynaptic facilitation and NMDA receptor-dependent long term potentiation [30,36]. In vivo single cell recordings from rat VTA indicated that  $\beta 2^*$  nAChRs contribute to increased firing rate but not bursting [37]. However, systemic nicotine was unable to alter the firing mode of dopaminergic neurons in the VTA in  $\beta 2^{-/-}$  mice, an effect reversed by re-expression of the  $\beta 2$  subunit in this region [25]. Thus in  $\beta 2^{-/-}$  mice, activation of  $\alpha 7$  nAChRs is insufficient to elicit burst firing. Conversely, the observation that  $\alpha 7^{-/-}$  mice can switch to burst firing following systemic nicotine also emphasises the importance of  $\beta 2^*$  nAChRs in this phenomenon. A caveat to this interpretation is that excitability in the VTA may be altered in  $\alpha 7$  knockout animals, for example,  $\alpha 7$  nAChRs may also be expressed by VTA GABAergic neurons [17,24] and / or regulation of the excitability of glutamatergic afferents may be compromised by the absence of  $\alpha 7$ -mediated inhibition of pyramidal outputs from the PFC [38,39].

A concerted hypothesis for the nicotinic control of burst firing [40] posits that nicotine rapidly and preferentially desensitises  $\alpha 4\beta 2$  nAChRs on GABAergic interneurons [41], relieving inhibition of midbrain dopamine neurons while maintaining a sustained activation of  $\alpha 7$  nAChRs on dopaminergic cell bodies as well as glutamate terminals [30].  $\beta 2^*$  nAChRs on dopamine cell bodies provide a coincident depolarisation that enhances excitability, and this is necessary for LTP and /or to drive changes in firing pattern. Thus multiple nAChR subtypes play essential and complex roles in modulating the repertoire of firing patterns in midbrain dopamine neurons and a balance of activation and desensitisation of nAChRs is critical [42]. However our understanding of their roles is incomplete: for example, the contribution of  $\alpha 7$  nAChRs on GABAergic neurons is unclear. The regulation of burst firing is further complicated by the possibility that somatodendritic dopamine release can promote glutamate release in the VTA, presumably by acting at D1 receptors on glutamatergic afferents [43,44]. In addition to the well characterised nicotinic modulation of dopamine release in the terminal regions, nicotine also promotes somatodendritic dopamine release in the VTA/SNc [45]. Moreover, endogenous nicotinic signalling must be disentangled from responses to acute versus chronic nicotine, made more challenging by the complex pharmacodynamics and pharmacokinetics of modelling human nicotine consumption through cigarette smoking (see [46] ). Extrapolation between in vivo and in vitro studies is also problematic as inherent firing patterns may not be preserved in the latter. A recent study [16] overcame this problem by recording firing patterns from midbrain dopamine neurons of freely moving rats in vivo and subsequently reproducing the same firing rates in striatal slices in vitro, allowing for a measurement of dopamine release probability across the striatum during firing modes representative of nicotine administration in the midbrain.

### 3. *The nicotinic modulation of dopamine release in the dorsal striatum and NAc*

The firing rates of midbrain dopaminergic neurons are the major determinants of dopamine release in the terminal fields. Basal firing patterns maintain a low concentration of extracellular dopamine whereas burst firing efficiently increases dopamine concentration in a spatially and temporally circumscribed manner [47]. As discussed

above, nAChRs in the SNc and VTA influence the firing rates of these neurons and the VTA is the locus of action of systemically administered nicotine with respect to increased dopamine release in the NAc [29-31,48]. However, local application of nicotine to the NAc or dorsal striatum in vivo increases dopamine overflow [49,50], and striatal dopamine terminals express multiple nAChR subtypes that promote the release of radiolabelled dopamine from in vitro preparations (synaptosomes or prisms; Fig. 3A). The presynaptic regulation of dopamine release has been extensively studied [51,52].

#### FIGURE 3 NEAR HERE

Selective pharmacological tools, immunoprecipitation, knockout mice and lesion studies have contributed to defining the subunit composition of presynaptic nAChRs on dopamine terminals [17,53-55].  $\beta 2$  subunits are ubiquitous in these nAChRs;  $\alpha 4\beta 2^*$  and  $\alpha 6\beta 2^*$  subtypes can be distinguished on the basis of their differential sensitivity to  $\alpha$ -conotoxinMIII (Fig. 3C). These have been further sub-divided into five  $\beta 2$ -containing nAChRs:  $\alpha 4\beta 2$ ,  $\alpha 4\alpha 5\beta 2$ ,  $\alpha 6\beta 2$ ,  $\alpha 6\beta 2\beta 3$  and  $\alpha 4\alpha 6\beta 2\beta 3$  [52], with the  $\alpha 4\alpha 6\beta 2\beta 3$  subtype deduced to have the highest sensitivity to nicotine [56]. These presynaptic receptor subtypes are consistent with mRNA expression in the dopaminergic cell bodies of the VTA/SNc [17,18]; Fig 2B). Segregation of  $\alpha 6$ -containing and  $\alpha 4$ (non- $\alpha 6$ )-containing nAChRs to distinct populations of dopaminergic fibres with different firing patterns has been proposed [57]. nAChR heterogeneity is further enhanced by the variable stoichiometry of the  $\alpha 4\beta 2$  subtype, with the  $(\alpha 4)_2(\beta 2)_3$  configuration conferring higher sensitivity to ACh than  $(\alpha 4)_3(\beta 2)_2$  [58,59]. Evidently in a nAChR pentamer the fifth 'accessory' subunit that does not contribute directly to the agonist binding sites nevertheless influences the sensitivity of the nAChR to agonist. In heterologous expression systems the switch from low to high sensitivity stoichiometry is promoted by exposure to chronic nicotine [58]. Similar stoichiometric variation can be anticipated for the  $\alpha 6\beta 2$  nAChR subtype; multiple stoichiometries may contribute to the biphasic nature of ACh-stimulated  $^{86}\text{Rb}^+$  efflux (a neurochemical assay of ion flux through open nAChR channels) from mouse striatal synaptosomes [60].

Despite the expression of  $\alpha 7$  nAChR subunit mRNA in a proportion of midbrain dopaminergic neurons (Fig. 2B), there is little evidence for the presence of  $\alpha 7$  nAChRs on dopamine terminals, as judged by their inability to influence [ $^3$ H]dopamine release from isolated nerve terminals [61-63]. Therefore this subtype may be restricted to somatodendritic regions of dopamine neurons. However, presynaptic nAChRs capable of modulating dopamine release in the striatum are not exclusively expressed on striatal dopaminergic terminals and in vitro approaches using more intact tissue preparations (slices, or prisms) can reveal neighbouring influences arising from the nicotinic modulation of other neurotransmitters, and a role for  $\alpha 7$  nAChRs is seen in this context.

The presence of  $\alpha 7$  nAChRs on glutamatergic afferents was initially inferred from the ability of a variety of antagonists selective for  $\alpha 7$  nAChRs or ionotropic glutamate receptors (iGluR) to partially inhibit [ $^3$ H]dopamine release evoked by the nicotinic agonist anatoxin-a in rat striatal prisms [64]. This arrangement is consistent with the release of [ $^3$ H]aspartate in response to activation of  $\alpha 7$  nAChRs and the release of [ $^3$ H]dopamine following stimulation of iGluRs observed in striatal synaptosome preparations [65-67]. Subsequently, the availability of novel  $\alpha 7$  nAChR-selective agonists, antagonists, and positive allosteric modulators has confirmed that  $\alpha 7$  nAChRs induce [ $^3$ H]dopamine release in striatal preparations [68,69] (Fig. 3D), and this response is absent in  $\alpha 7$  knockout mice [70] (Fig 3C). The modulation of striatal dopamine release by two local populations of nAChRs ( $\alpha 7$  and non- $\alpha 7$ ) is also observed in vivo. Administration of drugs directly into the striatum by reverse dialysis in freely-moving rats resulted in a partial blockade of nicotine- or anatoxin-A-induced dopamine overflow by methyllycaconitine (MLA), an antagonist that is relatively selective for  $\alpha 7$  nAChRs, or  $\alpha$ -Bgt [71,72].

Neuronal architecture of the striatum is well defined (Fig. 3A): dopamine terminals form typical symmetric synapses onto the dendritic spines and shafts of medium spiny GABAergic neurons of the striatum. These neurons are principally influenced by cortico-striatal glutamatergic afferents that make asymmetric synapses onto the heads of dendritic spines [73,74]. Thus glutamatergic terminals are in close proximity to

dopaminergic boutons to facilitate transmitter crosstalk. In addition to GABAergic projection neurons, the striatum also contains of a small population of GABAergic interneurons [75]. The nicotinic facilitation of [<sup>3</sup>H]GABA release from mouse striatal synaptosomes has implicated  $\alpha 4\beta 2^*$  and  $\alpha 4\alpha 5\beta 2^*$  nAChRs on GABAergic terminals, from comparison of nAChR subunit null mutant mice [76]. It is unclear if these receptors indirectly modulate dopamine release, although the influence of dopamine on local GABA release is well documented [77,78]. There is no evidence that striatal cholinergic interneurons, the source of endogenous agonist, are modulated by nAChRs [79] although  $\alpha 7$  and  $\beta 2$  subunit mRNA has been detected in these cells [80].

Given the diversity of presynaptic nAChR subtypes in the striatum, and their propensity to facilitate the release of dopamine and other transmitters, one has to consider what their physiological function might be. This is particularly intriguing in light of the dominant role of midbrain nAChRs in driving the firing patterns of dopamine neurons and the coupling between rate of action potential firing and dopamine release. At rest, tonic regular firing occurs at a rate of 2 – 5 Hz, this can increase to bursts of action potentials having a frequency of 15 – 100 Hz during ‘reward presentation’ [14] but firing can also ‘pause’, and this phenomenon is observed during reward omission [81]. Insight into the contributions of presynaptic nAChRs to dopamine release from active neurons has come from the application of fast-scan cyclic voltammetry (FSCV) to monitor endogenous dopamine release from coronal striatal slices with subsecond temporal resolution [16,82,83]. Typical firing rates can be simulated by electrical stimulation of striatal slices in vitro.

These experiments have led to the proposition that presynaptic nAChRs act as a ‘filter’ to enhance the contrast between different firing patterns with respect to dopamine release [16,84]. Thus during low frequency action potential firing in dopaminergic afferents, presynaptic  $\beta 2^*$  nAChRs are activated by ACh released from tonically active cholinergic interneurons, and increase dopamine release, ensuring that it is sustained throughout this mode of firing [85]. Desensitisation of nAChRs is avoided by the rapid turnover of ACh due to acetylcholinesterase activity. When dopaminergic firing pauses,

so do the cholinergic interneurons and dopamine release is reduced. Burst firing in dopaminergic axons is also accompanied by a pause in cholinergic activity; sustained dopamine release is observed without the intervention of presynaptic nAChRs [83] (Fig. 3B). The lack of nicotinic potentiation of dopamine release during burst firing is proposed to avoid depletion of the readily releasable pool of neurotransmitter. It is noteworthy that neither GABA nor glutamate promotes dopamine release measured using FCSV, despite the evidence for  $\beta 2^*$  and  $\alpha 7$  nAChRs on striatal GABAergic terminals and glutamatergic afferents respectively [86,87].

In this model, the effects of nicotine on dopamine release are opposite to those of endogenous ACh [82,83]. Thus responses to tonic low frequency stimulation are reduced in magnitude whereas phasic bursts of action potentials result in increased levels of dopamine. This paradoxical action reflects the propensity of nicotine to desensitise  $\beta 2^*$  nAChRs. nAChR antagonists produce similar effects (Fig. 3B), which could explain, in part, the failure of DH $\beta$ E to have any effect on nicotine self administration when locally infused into the NAc of rats [29]. Thus nicotine, by its contrasting effects in response to tonic and phasic firing patterns, could enhance the salience of rewarding information and this might be germane to its addiction liability. Prior chronic nicotine treatment in vivo resulted in increased dopamine release (monitored by FSCV) in response to burst firing in striatal slices, compared with tissue from untreated controls. However, this response was reduced (rather than enhanced as in Fig. 3B) by the  $\alpha 6\beta 2^*$ -selective nAChR antagonist  $\alpha$ -conotoxinMII [88]. It is possible that this ex vivo analysis may reflect the initial withdrawn state.

The use of  $\alpha$ -conotoxinMII highlights the importance of  $\alpha 6\beta 2^*$  nAChRs in regulating striatal dopamine release [88]. The striatum can be subdivided anatomically and functionally (see Appendix) and there is evidence for  $\alpha 6\beta 2^*$  having a greater role in the NAc than in the dorsal striatum [57,83]. A similar conclusion was reached by Drenan et al [34] using 'knock-in' mice with gain-of function  $\alpha 6\beta 2^*$  nAChRs that are hypersensitive to agonist. The mice are hyperactive and acute administration of a low dose of nicotine

produces locomotor activation. In contrast wildtype mice are insensitive to low nicotine doses and exhibit locomotor depression in response to higher doses. The behavioural phenotype of the mutant mice is consistent with enhanced dopamine release and may reflect enhanced responding to novelty (exploratory behaviour) governed by the mesolimbic pathway [34]. A striking difference between the shell of the NAc and dorsal striatum with respect to dopamine release probability was shown using FSCV [16]. The extent to which  $\alpha 6\beta 2^*$  nAChRs contribute to this difference is not known. It would be illuminating to know the effect of gain-of function  $\alpha 6\beta 2^*$  nAChRs on endogenous dopamine release evoked by tonic vs phasic stimulation of striatal slices. One can speculate that the mutated receptors would be less prone to desensitisation and hence they could initially enhance release during bursts but this would be unsustainable due to pool depletion and synaptic depression [84]. Further studies are required to establish the contribution of presynaptic nAChRs to dopamine release in vivo.

#### 4. *Nicotinic receptor-mediated facilitation of dopamine release in the PFC.*

It is now well-established that rodents have a functional prefrontal cortex able to perform many of the same rudimentary processes seen in primates and humans [89], including aspects of cognition, motor and reward anticipation [90,91] (see Appendix). Changes in dopamine, glutamate and GABA transmission in this region can lead to cognitive or attentional impairment, resulting in conditions such as schizophrenia and attention deficit hyperactivity disorder [92-95] and an imbalance in prefrontal dopamine versus glutamate systems underpins many hypotheses about the development of schizophrenia [96]. The potential of nAChRs in the PFC to modulate these neurotransmitter systems has prompted characterisation of their properties (Fig 4A).

#### FIGURE 4 NEAR HERE

Local administration of nicotine into the rat PFC improves attentional performance in the five-choice serial reaction time task [97], consistent with the presence of functional nAChRs in this brain region. However, the PFC has been relatively neglected with respect to the characterisation of nAChR subtypes present. Many earlier studies into nAChR distribution either failed to examine the anterior frontal cortex or did not



distinguish it from the rest of the frontal cortex. Comparison of the autoradiographical distributions of [ $^3\text{H}$ ]nicotine and [ $^{125}\text{I}$ ] $\alpha\text{Bgt}$  binding, specific labels for  $\beta 2^*$  and  $\alpha 7$  nAChR subtypes, provided evidence for the presence of both classes of nAChR, with complimentary distributions, in the PFC in sagittal rat brain sections [98].

Immunoprecipitation experiments to define the nAChR subunits present in rat PFC tissue identified  $\alpha 4$ ,  $\alpha 5$  and  $\beta 2$  but levels of  $\alpha 6$  or  $\beta 3$  subunits did not reach significance [69] (Fig 4B). Moreover, although nicotine induces [ $^3\text{H}$ ]dopamine release from PFC prisms and this is blocked by DH $\beta$ E, indicative of  $\beta 2^*$  nAChRs, it is insensitive to  $\alpha$ -conotoxinMII [69,99]. Therefore, in contrast to striatum,  $\alpha 6^*$  nAChRs are absent from PFC. Because  $\alpha$ -conotoxinMII blocks both  $\alpha 6\beta 2^*$  and  $\alpha 3\beta 2^*$  nAChRs, this result also excludes the latter subtype so a more limited portfolio of nAChR subtypes (comprised of  $\alpha 4$ ,  $\alpha 5$  and  $\beta 2$  subunits only) exists to modulate dopamine release. Thus mesocortical projections have arisen from a population of dopamine neurons in the VTA that is distinct from those giving rise to the mesolimbic pathway [17].

The glutamatergic pyramidal cells in layer V of the PFC do not express functional nAChRs [39] but nicotine increases spontaneous excitatory postsynaptic currents (EPSCs) in these cells, attributed to its ability to increase glutamate release from neighbouring afferents [39,100,101]. Thalamocortical glutamatergic afferents projecting to layer V of the PFC are endowed with functional  $\beta 2^*$  nAChRs [100,101] (Fig 4C). Indeed, stimulation of PFC synaptosomes with a  $\beta 2$ -selective agonist increases the release of [ $^3\text{H}$ ]D-aspartate, consistent with the location of  $\beta 2^*$  nAChRs on glutamatergic terminals [102]. In the rat PFC  $\alpha 7$  nAChRs have also been localised to glutamatergic terminals, as well as GABAergic terminals and cell bodies [103].  $\alpha 7$  nAChR agonists also induce [ $^3\text{H}$ ]D-aspartate release from PFC synaptosomes, but via a distinct cellular mechanism compared with  $\beta 2^*$  nAChRs [102]. It is not known if  $\alpha 7$  and  $\beta 2^*$  nAChRs co-exist on the same glutamate terminals.

Nicotinic modulation of GABAergic transmission also contributes to PFC activity. A diverse array of GABAergic interneuron types exist in rat PFC [104,105]. These can be crudely separated into two classes; fast spiking and non-fast spiking. Single cell mRNA analysis indicates that in the mouse PFC non-fast spiking interneurons express  $\alpha 4$ ,  $\beta 2$  and  $\alpha 7$  nAChR subunits, whereas fast spiking cells do not express any of these nAChR subunits but can be influenced by nicotinic potentiation of excitatory inputs [39]. This study focused on the ability of nicotine to increase inhibitory postsynaptic currents (IPSCs) (Fig. 4D) and reduce LTP in layer V pyramidal cells, due to augmented release of GABA from interneurons. As a result, the threshold for spike-time dependant plasticity in layer V pyramidal cells of the medial PFC is increased. Potentially, this modulation of the PFC neuronal network by nicotine can change the rules for synaptic plasticity [39]. Such a mechanism could enhance the accuracy of rodents when performing cognitive tasks but in both rats and humans this depends on the baseline cognitive state [106,107].

Dopamine and glutamate systems can act in synergy with GABA to control pyramidal cell excitability [108-110] and nicotine has been instrumental in revealing the nicotinic modulation of these interactions, but how is this achieved by the endogenous transmitters ACh and choline? Cholinergic projections to the PFC from the nucleus basalis of Meynert will release ACh in response to activation of  $\alpha 4\beta 2^*$  nAChRs residing on prefrontal dopaminergic and glutamatergic afferents [111,112]. The known changes in tonic to phasic release of ACh from these neurons during increased performance in a cue detection task may permit alterations in GABAergic interneuron firing, hence affecting PFC network function [113]. It is noteworthy that muscarinic receptors in the PFC can also increase GABA release and depress pyramidal cell excitability [114].

Dopamine is a prominent modulator of executive function [115] and nicotine delivered systemically [116] or locally [50] elicits dopamine release in the medial PFC in conscious, freely moving rats. To address the subtypes of nAChR responsible within the PFC, subtype-selective agonists have been employed. Local delivery of either  $\beta 2^*$ - or  $\alpha 7$ -selective agonist into the rat medial PFC increased dopamine overflow measured by

microdialysis, implying a positive role for both  $\beta_2^*$  and  $\alpha_7$  nAChRs on dopamine release, in agreement with in vitro data for release from PFC prisms [69]. The in vitro experiments indicate that the action of  $\alpha_7$  nAChRs is indirect, requiring the intervention of glutamate release to activate ionotropic glutamate receptors (Fig 4D), as previously described for striatum. The novel  $\alpha_7$  nAChR-selective positive allosteric modulator PNU-120596 enhanced responses to  $\alpha_7$  nAChR agonist in vitro and in vivo. This modulator can be used to detect the presence of endogenous agonist in vivo, as it is ineffective at activating  $\alpha_7$  nAChRs on its own [117]. Interestingly, PNU-120596 elicited dopamine overflow in the medial PFC when administered systemically, and this response was attenuated by systemic MLA, but it was ineffective when delivered locally into the PFC [69] (Fig. 4E). Tonic cholinergic activity, possibly arising from the pontine nuclei projecting to the VTA (Fig. 2A), may account for the extracortical stimulation. However, during cognitive performance (not encompassed in Livingstone et al. [69]) ACh would be released in the PFC [113] allowing locally administered PNU-120596 to become effective in releasing dopamine under these conditions. Hence positive allosteric modulators of  $\alpha_7$  nAChR have therapeutic potential for restoring cognitive deficits in conditions involving PFC dysfunction [10]. Indeed, a role for  $\alpha_7$  nAChRs in PFC function is implicit in the deficits in attention and working memory performance in knockout mice lacking this nAChR subunit [118,119], although it is unclear in these studies if the critical  $\alpha_7$  nAChRs are located in the PFC or elsewhere in the brain.

##### 5. *Downstream consequences of dopamine release.*

The previous sections have established that dopamine release in the terminal fields of the ascending dopamine pathways is influenced by a variety of nAChR subtypes on dopaminergic cell bodies, terminals and neighbouring neurons. Postsynaptic dopamine receptor activation results in numerous downstream responses, including phosphorylation of strategic proteins and changes in gene expression, and results in altered target cell excitability. Here we briefly consider the impact of nAChR activation on these downstream effectors.

In the striatum, dopamine can increase the excitability of medium spiny neurons of the striatonigral direct pathway via dopamine D1 receptors and can suppress those neurons of the striatopallidal indirect pathway, mainly via dopamine D2 receptor activation [120,121]. The key effector protein, downstream of D1 receptors and inhibited by D2 receptor activation, is protein kinase A (PKA) [108,121,122]. Activation of PKA phosphorylates dopamine- and cyclic AMP-regulated phosphoprotein of 32kDa (DARPP-32) at Thr34, making it a potent inhibitor of protein phosphatase 1. This protein is abundant in all medium spiny neurons and operates as a 'molecular switch' [123,124]. The status of DARPP-32 can ultimately result in changes in the excitability of medium spiny neurons. Longer term changes may reflect the function of DARPP-32 as a critical modulator of ERK, ELK and CREB signalling [123, 125].

Nicotine has been reported to alter DARPP-32 activity, in vitro and in vivo. In mouse striatal slices in vitro, nicotine produced differential, concentration-dependent changes in DARPP-32 activity, reflecting the activation of D2 and D1 receptors by low and high dopamine concentrations, respectively [126]. D2 activation was attributed to low nicotine concentrations activating  $\alpha 4\beta 2$  nAChRs on dopaminergic terminals whereas higher nicotine concentrations were inferred to also activate  $\alpha 7$  nAChRs on neighbouring glutamate afferents, resulting in greater dopamine release (see Fig. 3A). In vivo, nicotine, like other psychostimulants, increases the phosphorylation of ERK in the NAc and prefrontal cortex in a D1-dependant manner [127]. ERK phosphorylation has an important impact on synaptic plasticity by increasing gene transcription in the post synaptic neuron and this occurs in the NAc as a direct consequence of DARPP-32 phosphorylation [125]. Increased phosphorylation of CREB accompanies DARPP-32 phosphorylation in the NAc in response to psychostimulant administration [128]. CREB acts as a transcription factor to alter gene expression of immediate early genes such as c-Fos. Nicotine administration (systemic, or locally into the VTA) increases c-Fos transcription in the NAc [129,130]. Such changes allow for a reduced excitability threshold in the medium spiny neurons of the direct pathway and behavioural adaptations to administered drugs such as cocaine [131].

Chronic nicotine produces a complex set of changes in phosphorylation [132] and expression of immediate early genes [133-136], perhaps most notably in NAc. Persistent increases in FosB expression were observed in NAc of rats following periadolescent nicotine exposure [136]. Accumulation of a long-lived truncated form of FosB, deltaFosB, following drug administration (including nicotine) [135] has been proposed as a sustained molecular change that contributes to the persistence of drug dependence and the propensity to relapse [137]. The loci of action of nicotine in these in vivo treatments is likely to be complex but the overall contribution of mesolimbic nAChR activation to downstream effectors in medium spiny neurons of the striatum will depend on the opposing actions of dopamine upon both D1 and D2 receptors of the direct and indirect pathways [126,138] as well as D2 autoreceptors on dopaminergic nerve terminals [139].

In the PFC the role of DARPP-32 is less clear than in striatum. Neither changes in ERK phosphorylation [125] nor increases in excitability of fast spiking GABAergic interneurons [140] depend on the phosphorylation of DARPP-32. It is likely that pathways involving DARPP-32 and ERK in the PFC are less integrated than in the NAc. The ability of dopamine in vitro to increase ERK phosphorylation in the PFC is sufficient to induce LTP in pyramidal cells, when NMDA receptors are also activated [141]. However, there is some evidence for negative regulation through DARPP-32: D2 receptors on pyramidal neurons are coupled to DARPP-32, resulting in reduced excitability [142]. How nAChRs in the PFC interface with these signalling pathways remains to be determined.

## **6. Concluding remarks**

From this review of the literature it is striking that nAChRs can influence dopamine pathways in a variety of ways, reflecting their subcellular localisation: somatodendritic, presynaptic or on neighbouring neurons. Their influence ranges from key roles in determining firing rates to modulating transmitter release and enhancing the contrast between different firing modes, achieved through a delicate balance between activated and desensitised states of nAChR subtypes. The consequences of these actions include short and long term changes in downstream effector molecules. The different modes of

nicotine administration (acute, repeated or sustained) impacts on all these parameters. We have highlighted, where there is evidence, the contrasting roles of nicotine and the endogenous agonist, ACh. Although a wealth of details have been accrued in recent years there remain many gaps in our knowledge, particularly of how cellular mechanisms elucidated in vitro are integrated in the functioning of the brain in vivo. nAChRs in additional neuronal circuits will also influence the behaviour of the dopamine system, for example in addiction. Further studies are needed to enhance our understanding of the physiologically relevant nAChR-mediated mechanisms, their contributions to disease and therapy, and how these mechanisms are hijacked by nicotine, leading to addiction.

### Acknowledgements

Due to space constraints we have been unable to cite all original papers and we apologise to authors whose work in this area has been omitted. Work in our laboratory is supported by grants from the Biological and Biotechnological Sciences Research Council (BBSRC; BBS/B/15600) and The Parkinson's Disease Society (G-0613) to SW and a postgraduate studentship from the BBSRC to PDL.

Accepted Manuscript

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*Figure legends*

**Figure 1.** Major ascending dopamine pathways of the rodent brain. Projections arise from cell bodies of the mesencephalon from one of two regions, the ventral tegmental area (VTA, orange) or substantia nigra pars compacta (SNc, including the retrorubral field, light orange). The caudate-putamen (CPu) or dorsal striatum (light blue) is innervated by dopaminergic neurons from the SNc forming the nigrostriatal pathway and, to a lesser extent, from the VTA (dashed line). Two major dopamine pathways originate in the VTA; the mesolimbic pathway that terminates in the nucleus accumbens (NAc, dark blue) and the mesocortical pathway in the prefrontal cortex (green). For more anatomical detail see Appendix.

**Figure 2.** Contribution of nAChRs to dopamine cell firing in the VTA / SNc. **(A)** Model indicating the key locations of  $\alpha 4\beta 2^*$  and  $\alpha 7$  nAChR subtypes. ACh is released from terminals of projections from pedunculo pontine and laterodorsal tegmental nuclei (PPn and LDTn). GABAergic interneurons bear  $\alpha 4\beta 2^*$  nAChRs and glutamate afferents from the prefrontal cortex (PFC) and elsewhere express  $\alpha 7$  nAChRs ( $\alpha 7$  nAChRs might also be present on GABAergic interneurons and afferents, not shown). NMDA receptors on dopamine cell bodies determine firing patterns. **(B)** Expression of nAChR subunit mRNA in tyrosine hydroxylase positive (dopaminergic) and negative (non-dopaminergic) neurons using *in situ* hybridisation. Many dopaminergic neurons in the SN / VTA express nAChR subunits (left side). The relative expression of subunits in non-dopaminergic cells (right side) shows significant expression of  $\alpha 4$ ,  $\alpha 7$  and  $\beta 2$  in SN and  $\beta 4$  in SN/VTA. Horizontal black bar denotes sensitivity limit for tyrosine hydroxylase-negative cells (Modified from [18]). **(C)** VTA dopamine cell firing modes and changes observed in nAChR subunit knockout mice. Single cell *in vivo* electrophysiology recordings from a dopaminergic neuron show tonic and burst firing patterns (top left). Four firing modes in wild-type (WT) mice (top right) consist of high or low frequencies (HF or LF) with high or low bursting activity (HB or LB). Frequency of spikes plotted against percentage of spikes in a burst (bottom) shows the distribution of the four firing modes in WT VTA (lower left panel). The HFHB mode is absent in  $\beta 2$  subunit knockout mice (red; middle panel) whereas in VTA

from  $\alpha 7$  knockout mice the HFHB mode is preserved. (blue; right panel; Reproduced from [25], with permission from Elsevier). **(D)** Localisation of  $\alpha 7$  nAChRs using  $\alpha$ -bungarotoxin ( $\alpha$ Bgt) in rat VTA. Top left shows  $\alpha 7$  nAChRs (alexa fluor 488-conjugated  $\alpha$ Bgt, green arrow), vesicular glutamate transporter 2 (VGluT2), a marker of glutamate terminals, (red) and tyrosine hydroxylase (blue) in fixed sections. Yellow arrows indicate examples of colocalisation of  $\alpha 7$  nAChRs with VGluT2. Bottom left panel, subcellular co-localisation of biotinylated  $\alpha$ Bgt (small black particles, green arrow) and VGluT1/2 (large black particles, red arrow), using anti-biotin 1nm gold and secondary antibodies to vGluT1/2 conjugated to large gold particles, respectively. An axon terminal is decorated with particles of both sizes, indicative of co-localisation. Right panel, in an aldehyde-fixed section of VTA  $\alpha 7$  nAChRs labelled with biotinylated  $\alpha$ Bgt and anti-biotin gold, followed by silver enhancement (black particles, some indicated by arrows), are found in a vGluT1/2-immunopositive axon terminal (AT; revealed by diaminobenzidine reaction product). (I.W. Jones and S. Wonnacott, unpublished).

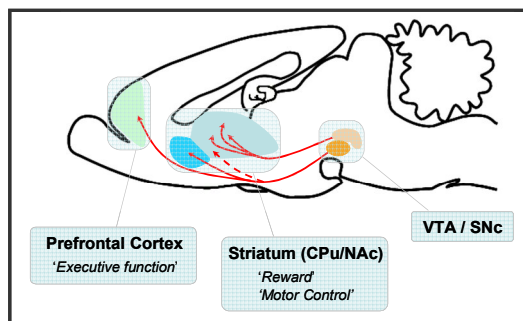
**Figure 3.** Heterogeneity of presynaptic nicotinic receptors modulating striatal dopamine release. **(A)** Model of nAChRs on dopamine and glutamate afferents. Dopamine terminal from ventral tegmental area (VTA) or substantia nigra pars compacta (SNc) forms a synapse with a GABAergic striatal medium spiny neuron. nAChRs of the  $\alpha 4\beta 2^*$  and  $\alpha 6\beta 2^*$  subtypes, including  $\alpha 4\alpha 6\beta 2\beta 3^*$ , are situated on dopamine terminals (they are not necessarily colocalised on the same terminal) and  $\alpha 7$  nAChRs reside on glutamate terminals. Tonically active cholinergic interneuron firing is coupled with dopamine cell firing. GABA interneurons are omitted for clarity. **(B)** Effect of electrical pulse stimulation of nucleus accumbens coronal slices on extracellular dopamine concentration measured using fast-scan cyclic voltammetry. nAChR antagonists (DH $\beta$ E and  $\alpha$ -conotoxinMII) enhance the difference between responses to one and four pulses (100 Hz; left side). Peak height (right side) highlights the increased contrast in dopamine release produced by blockade of  $\beta 2^*$  (DH $\beta$ E) or  $\alpha 6^*$  ( $\alpha$ -conotoxinMII) nAChRs, an effect mimicked by nicotine (not shown). (Reproduced from [84], with permission from Nature Publishing Group) **(C)** Evidence for  $\beta 2^*$  and  $\alpha 7$  nAChRs facilitating [ $^3$ H]dopamine release in mouse

striatal prisms. Inhibition of responses to 1  $\mu$ M nicotine by  $\alpha$ -conotoxinMIII (light grey bars) or DH $\beta$ E (open bars) or in wild type mice implicates both  $\alpha$ 6 and non- $\alpha$ 6 nAChR subtypes. Their contribution is unchanged in  $\alpha$ 7 knockout (K/O) mice. Choline-evoked responses in wild type mice are absent in  $\alpha$ 7 knockout animals. (Reproduced from [70], with permission from Springer Publishing). **(D)** Contribution of  $\alpha$ 7 nAChR to rat striatal [ $^3$ H]dopamine release in tissue prisms. Choline (3 mM), with or without the allosteric modulator PNU-120596 (10  $\mu$ M blue bars) increases release of [ $^3$ H]dopamine in an  $\alpha$ Bgt-sensitive manner. The AMPA<sub>R</sub>/Kainate<sub>R</sub> antagonist DNQX (200  $\mu$ M) and the NMDA<sub>R</sub> antagonist MK801 (5  $\mu$ M) partially inhibit choline plus PNU-120596-mediated release and fully attenuate the effect when combined. [ $^3$ H]dopamine release evoked by nicotine (1  $\mu$ M) is insensitive (yellow bars) to DNQX or MK801. (Modified from [69]).

**Figure 4.** nAChRs in the PFC. **(A)** Converging afferents and interneurons bearing nAChRs influence PFC glutamatergic pyramidal cells in PFC layer V. Two categories of GABAergic interneurons, fast spiking and non-fast spiking, are distinguished; only non-fast spiking interneurons express nAChRs ( $\beta$ 2\* and  $\alpha$ 7) (these interneurons also project to pyramidal cell apical dendrite in layers II/III). Presynaptic  $\alpha$ 7 or  $\alpha$ 4 $\beta$ 2\* nAChRs may be segregated to distinct populations of glutamatergic afferents (dashed line). Dopamine terminals express  $\alpha$ 4 $\beta$ 2\* nAChRs and are in proximity to glutamatergic afferents bearing  $\alpha$ 7 nAChRs. Cholinergic innervation is from the nucleus basalis of Meynert (nBM). Reciprocal crosstalk between glutamate, dopamine and GABA neurons affects activity and synaptic plasticity in the projection neurons (pyramidal cells). **(B)** Immunoprecipitation of nAChR subunits from rat PFC using subtype-selective antibodies. Modified from [69]. **(C)** Nicotine and ACh increase excitatory postsynaptic currents (EPSCs) in single pyramidal cells. These responses are sensitive to thalamic lesions (left, insert: section showing extent of lesion, black) and are absent in  $\beta$ 2 nAChR subunit knockout (K/O) mice (right), compared to wild type (WT). (Reproduced from [101], with permission from Nature Publishing Group). **(D)** Nicotine also increases inhibitory postsynaptic currents (IPSCs) in PFC layer V pyramidal neurons. Both high (10  $\mu$ M, left) and low (300 nM, right) nicotine concentrations augment spontaneous IPSC frequency.

(Reproduced from [39], with permission from Elsevier). **(E)**  $\alpha 7$  nAChRs increase dopamine overflow in medial PFC in freely moving rats. Local perfusion (bar) of a subthreshold concentration of the  $\alpha 7$  nAChR selective agonist Compound A plus the positive allosteric modulator PNU-120596 increases dopamine overflow whereas PNU-120596 alone had no effect (left). Systemic administration of PNU-120596 does increase dopamine overflow and this is attenuated by systemic methyllycaconitine (MLA, right). Modified from [69].

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Nicotinic acetylcholine receptors (nAChRs) affect dopamine cell firing and release probability in three major ascending pathways; nigrostriatal, mesolimbic and mesocortical. nAChRS are therefore poised to modulate multiple brain functions.

Figure 1

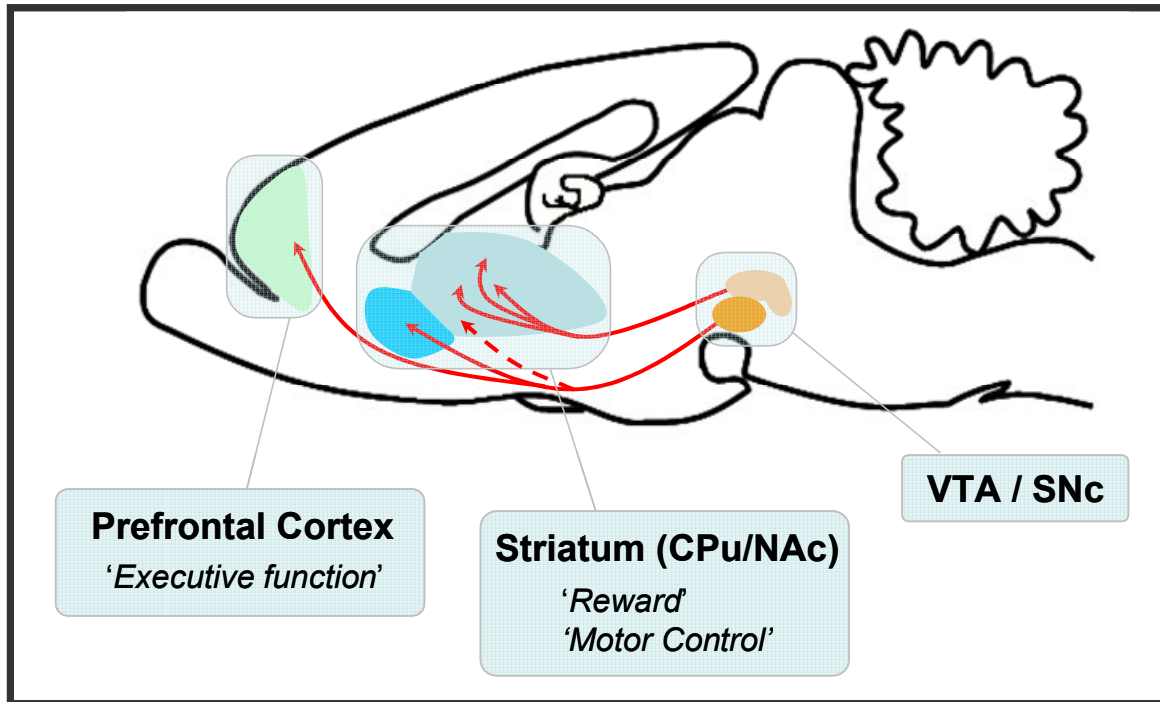


Figure 2

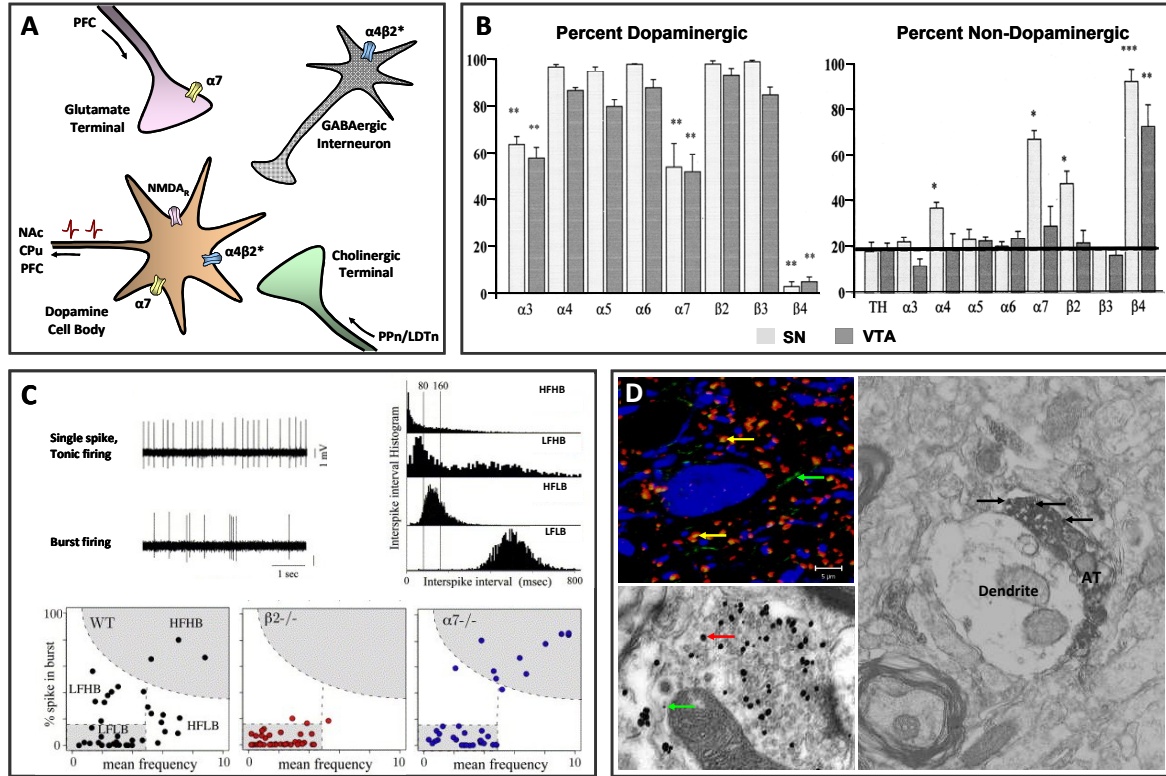




Figure 3

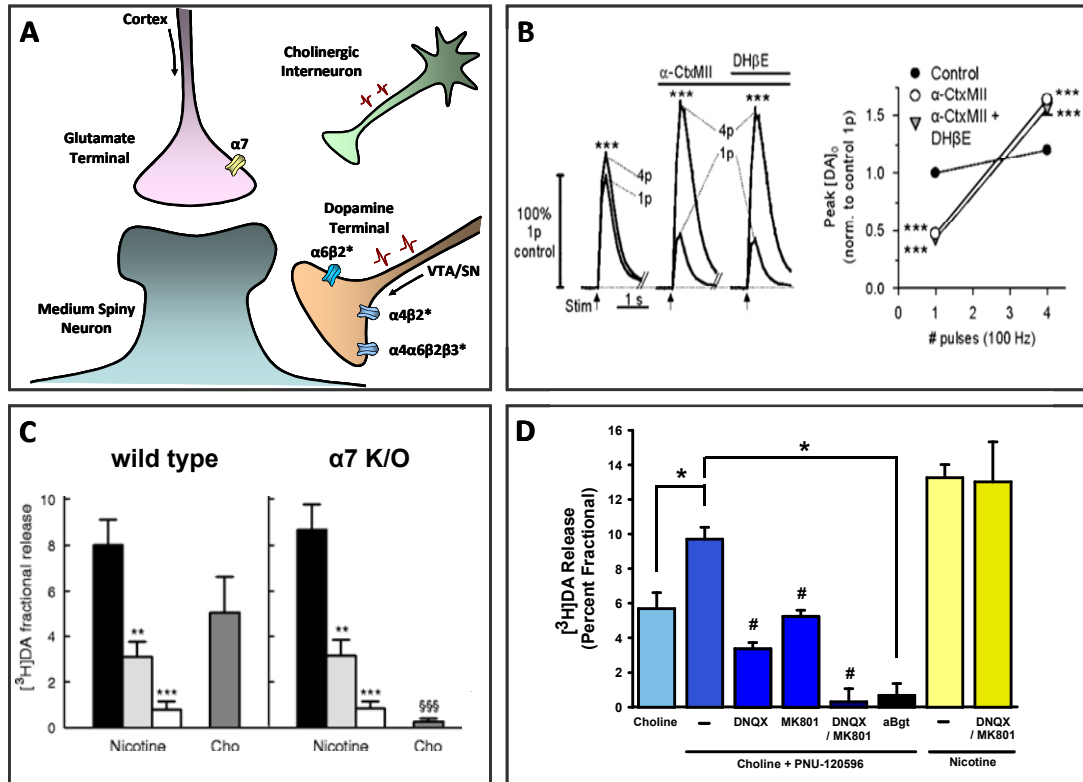


Figure 4

