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REVIEW

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The cholinergic mesopontine tegmentum is a relatively neglected nicotinic master modulator of the dopaminergic system: relevance to drugs of abuse and pathology

U Maskos

Institut Pasteur, Neurobiologie intégrative des systèmes cholinergiques, Paris, France

The mammalian mesopontine tegmentum (MPT) contains two cholinergic nuclei, the pedunculopontine tegmental nucleus (PPTg) and the laterodorsal tegmental nucleus (LDTg). These provide the cholinergic innervation of, among other brain areas, the dopaminergic A9 and A10 cell groups. Their axons are thus the source of endogenous acetylcholine (ACh) acting on somato-dendritic acetylcholine receptors in the substantia nigra (SN) and ventral tegmental area (VTA). The anatomy, physiology, functional and pathological implications of these interactions with the nicotinic subtype of acetylcholine receptors (nAChRs) are discussed with a view of the important role of the MPT as a master regulator of nicotinic dopaminergic signalling in the brain, including for nicotine addiction.
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Abbreviations: ACh, acetylcholine; DA, dopaminergic; GABA, γ-aminobutyrate; MPT, mammalian mesopontine tegmentum; nAChRs, nicotinic subtype of acetylcholine receptors; PD, Parkinson's disease; PPTg, pedunculopontine tegmental nucleus; PFC, prefrontal cortex; SN, substantia nigra; VTA, ventral tegmental area

Introduction

The midbrain dopaminergic (DA) system is a key element in the control of cognition and locomotion, and it is also the primary target of drugs of abuse (Bjorklund and Dunnett, 2007). For 50 years it has been the subject of a substantial research endeavour, focusing to a large extent on downstream events in the DA signalling cascade (Carlsson, 1993; Roberts and Koob, 1997; Robbins *et al*., 1998; Robbins, 2000). However, the DA system is also the target of a range of other neuromodulators, including the brain cholinergic system (Schmitz *et al*., 2003; Maskos, 2007). Cholinergic systems use acetylcholine (ACh) as their neurotransmitter, and act through metabotropic muscarinic receptors (Bymaster *et al*., 2003; Wess, 2003; Wess *et al*., 2003; Yamada *et al*., 2003) and ionotropic nicotinic receptors (nicotinic subtype of acetylcholine receptors (nAChRs)) (Changeux and Edelstein, 2005). The midbrain DA system has its cell bodies largely in the A9 and A10 brain nuclei, and these project their axons to the caudate putamen, nucleus accumbens and prefrontal cortex (PFC). The cholinergic modulation of DA release in these target areas is rather well understood, mainly because of a large body of experimental and theoretical work from key laboratories focusing on striatal function (Graybiel, 1990, 2005; Graybiel *et al*., 1994; Gerfen and Wilson, 1996; Zhou *et al*., 2001, 2002, 2003; Cragg, 2003, 2006; Cragg and Rice, 2004; Rice and Cragg, 2004; Ungless and Cragg, 2006), see also Exley and Cragg, 2008. However, the cholinergic modulation of DA cell bodies through nicotinic receptors in the somato-dendritic compartment warrants much greater elucidation. Here, I will review the nicotinic–cholinergic modulation of DA cells in the A9 and A10 areas.

Correspondence: U Maskos, Neurobiologie intégrative des systèmes cholinergiques, Département de Neuroscience, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France.
E-mail: umaskos@pasteur.fr
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Anatomy of the mammalian mesopontine tegmentum with respect to cholinergic nuclei

The pedunculopontine tegmental nucleus (PPTg) and laterodorsal tegmental nucleus (LDTg) are identified as the cholinergic cell groups Ch5 and Ch6, respectively (Mesulam

et al., 1983). The anatomical literature dates mainly from before 1990, and several groups contributed to it substantially, as published more recently (Butcher, 1995; Blaha *et al.*, 1996; Butcher and Woolf, 2003). The generally accepted definition is that the PPTg extends from the posterior pole of the substantia nigra (SN, where ectopic cholinergic neurons from the PPTg are occasionally found; Martinez-Murillo *et al.*, 1989a) back to the lateral tip of the superior cerebellar peduncle. The LDTg is dorsal and caudal to the neighbouring PPTg, dorsally abutting the aqueduct, and at its caudal extreme positioned between the locus coeruleus and the fourth ventricle, as schematically indicated in Figure 1a.

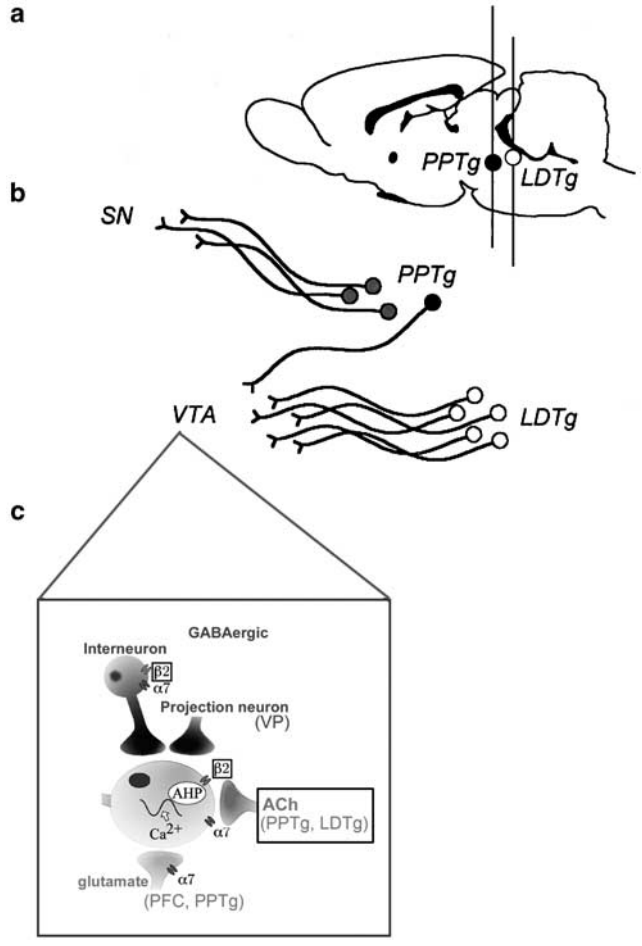

Figure 1 (**a**) A schematic of the mouse brain in sagittal section highlighting the two cholinergic nuclei of the mammalian mesopontine tegmentum (MPT), the pedunculopontine tegmental nucleus (PPTg), filled circle, and laterodorsal tegmental nucleus (LDTg), open circle. (**b**) The specificity of cholinergic axons from the PPTg, filled circles, is not 100% for the innervation of the substantia nigra (SN), grey-filled circles. The posterior portion of the PPTg, black-filled circle, innervates the ventral tegmental area (VTA). Cholinergic axons from the LDTg, open circles, project exclusively to the VTA. (**c**) In the blow up, the microcircuit of the VTA and its cholinergic modulation are indicated: dopaminergic (DA) neurons in the centre show rhythmic pacemaker activity that is calcium dependent and controlled through glutamatergic and GABAergic inputs (dark cells). These inputs derive, respectively, from the prefrontal cortex (PFC) or pedunculopontine tegmental nucleus (PPTg), and VTA GABAergic interneurons or projection neurons from the ventral pallidum (VP). The key signal gating the transition from tonic to phasic, burst firing, is the cholinergic input from the tegmentum, PPTg and LDTg (boxed). Note that the glutamatergic afferents contain nicotinic receptors of the $\alpha 7$ subtypes, and are thus also modulated through tegmental acetylcholine (ACh). Additionally, both the DA and γ-aminobutyrate (GABA)ergic neurons in the VTA contain both $\alpha 7$* and $\beta 2$* (boxed) nAChRs.

The composition of the two nuclei is heterogeneous, composed of mainly large cholinergic neurons and non-cholinergic cells (Honda and Semba, 1995; Takakusaki *et al.*, 1996; Steininger *et al.*, 1997), that are glutamatergic (Clements and Grant, 1990; Clements *et al.*, 1991; Grofova and Zhou, 1998) and γ-aminobutyrate (GABA)ergic (Ford *et al.*, 1995; Jia *et al.*, 2003). This distinct group of non-cholinergic cells of the PPTg is referred to as the midbrain extrapyramidal area, or also PPTg pars dissipata, by some authors (Rye *et al.*, 1987; Lee *et al.*, 1988; Steininger *et al.*, 1992). As discussed by Winn (2006), it is possible to see there a structural organization within the PPTg that resembles the way the SN is set up: there, a fairly homogeneous compact group of DA cells, the pars compacta, is connected to a distinct GABAergic cell group, the pars reticulata. The homogeneous ACh cell group of the PPTg innervates this homogeneous DA group of the pars compacta, thus interconnecting these anatomically defined parts of the two structures.

In several species, different neurotransmitters have been detected in the same PPTg neuron, especially with respect to cholinergic and glutamatergic markers (Clements and Grant, 1990; Clements *et al.*, 1991; Lavoie and Parent, 1994).

Cholinergic connectivity

The cholinergic efferents of the mammalian mesopontine tegmentum (MPT) received a lot of attention in the 1970s and 1980s, due to their implication in thalamic function: a majority of cholinergic cells in the MPT project to both the thalamus and the basal forebrain (Losier and Semba, 1993), the thalamus and the pontine reticular formation (Semba *et al.*, 1990), and the thalamus and DA mesencephalon (Oakman *et al.*, 1999). The role of these thalamic afferents has been reviewed in other places (Woolf and Butcher, 1986; McCormick, 1989; Steriade, 2004), and I will only discuss the MPT involvement in the basal ganglia.

Tract-tracing studies using double labels have established a certain topographic organization: the LDTg projects mainly to the ventral tegmental area (VTA) (Cornwall *et al.*, 1990), the PPTg innervates predominantly the SN, with a minor projection from its posterior part also going to the VTA (Semba and Fibiger, 1992; Steininger *et al.*, 1992; Oakman *et al.*, 1995, 1999), see also Figure 1b. These assignments are not only anatomical but also based on function: Blaha and colleagues have established the underlying pharmacology of this topographic innervation as discussed below (Blaha and Winn, 1993; Blaha *et al.*, 1996; Forster and Blaha, 2003). The laboratory of Tony Grace has pioneered a different functional approach based on activating different parts of the MPT, and according to these recent data, cholinergic afferents from the PPTg to the VTA are solely responsible for the release of ACh there, with no LDTg involvement, whereas the LDTg acts as a kind of 'switch' to activate the PPTg connection (Floresco *et al.*, 2003; Goto and Grace,

2005, 2007; Grace, 2006; Lodge and Grace, 2006; Grace *et al.*, 2007), as discussed further below.

The cholinergic projection to the mesopontine DA cells at the ultrastructural level

Double-label tract-tracing studies have also been used to elucidate the ultrastructural features of this cholinergic innervation using specific markers, typically the enzyme synthesizing ACh, choline acetyl transferase (Bolam *et al.*, 1991), and the vesicular transporter of ACh (Schäfer *et al.*, 1998; Garzon *et al.*, 1999). These studies established the synaptic connectivity between cholinergic afferents and DA cell dendrites, and to a lesser extent, the soma. In these papers, clearly delineated asymmetric, that is, excitatory, cholinergic synapses onto the DA cell, typically at the level of its dendrite, were identified (Beninato and Spencer, 1988; Martinez-Murillo *et al.*, 1989b; Bolam *et al.*, 1991; Garzon *et al.*, 1999). Further synapses were clearly established between cholinergic synaptic boutons and non-DA, most likely GABAergic, neurons.

The presence of nicotinic receptors in DA cell bodies had first been described by nicotine autoradiography in 1985 (Clarke and Pert, 1985; Clarke *et al.*, 1985), but it is only 10 years later that postsynaptic nAChRs were clearly identified in the postsynaptic membrane (Sorenson *et al.*, 1998; Arroyo-Jim nez *et al.*, 1999). These studies employing antibodies to the α4 subunit of the nAChR were carried out in the rat. Caution has to be exercised, however, as a recent publication strongly discourages the use of these antibodies, certainly in the mouse, where cross-reactivity was also observed in the mouse knockout for the corresponding subunit (Moser *et al.*, 2007).

Functional consequences of the nicotinic–cholinergic innervation

The nicotinic modulation of DA cell activity was first studied by Lichtersteiger and Hefti 30 years ago (Lichtensteiger *et al.*, 1976, 1982). Following the groundbreaking work of Grace, it was then seen that an important feature of DA function is the capacity of the DA cell to fire in bursts, that is, rapid spiking, separated one from the next by less than 80 ms (Grace and Bunney, 1983a–c, 1984a, b). This has to be distinguished from the rhythmic pacemaker-like activity of DA cells obtained in slice electrophysiology where afferents from connected brain regions, such as the PFC, subthalamic nucleus or the PPTg, have been cut. The consequences of this lack of afferent modulation have been discussed in detail by Kitai and colleagues (Kitai, 1998; Kitai *et al.*, 1999). They ascribe the rhythmic activity observed in slices to the absence of cholinergic innervation in *in vitro* preparations. This cholinergic action on the postsynaptic DA cell inhibits a slow outward K^+ current that, when uninhibited, prevents bursting. The main finding in support of this explanation is that application in slices of apamin, a specific blocker of this Ca^{2+}-activated slow outward K^+ current, does lead to burst firing. In this study, the involvement of the nAChRs in mediating burst firing could not be established.

As reviewed in detail recently, the physiological and behavioural consequences between these two firing modes are strikingly different (Grace *et al.*, 2007): whereas only small amounts of DA are released in the striatum as a consequence of rhythmic activity, very high concentrations of DA in the synaptic cleft are reached through burst firing (Suaud-Chagny *et al.*, 1992, 1995; Chergui *et al.*, 1994; Garris and Wightman, 1994).

Recent *in vivo* work has now established the physiological basis for these findings, and implicates nicotinic–cholinergic mechanisms stemming from the MPT (Lodge and Grace, 2006; Mameli-Engvall *et al.*, 2006; Ungless and Cragg, 2006): *in vivo* electrophysiology carried out in the VTA of the anaesthetized mouse confirmed, in the mouse after mainly rat studies, the typical alternation in firing behaviour between phasic and tonic episodes using single extracellular recordings. However, in the mouse knockout for the high-affinity nicotinic–cholinergic receptor subunit β2 (Picciotto *et al.*, 1995, 1998; Maskos *et al.*, 2005; Maskos, 2007), burst firing is almost completely absent (Mameli-Engvall *et al.*, 2006), but could be restored by the specific lentivirus-mediated re-expression of the β2 subunit in the cell bodies of the VTA, thus restoring locally the high-affinity receptor (Maskos, 2007). Following up on the discussion of the work of Kitai and colleagues, it is therefore ACh released in the VTA, acting on the β2*-nAChR, that is responsible for the switch between phasic and tonic activity. This cholinergic input thus serves as a 'gate' that enables DA neurons to respond to a glutamatergic input deriving most likely from the PFC, or also the glutamatergic cells of the MPT (Geisler and Zahm, 2005). This glutamate release is itself regulated also by ACh, or nicotine, acting on α7* nicotinic receptors (Svensson *et al.*, 1998; Schilstrom *et al.*, 1998a, b, 2000, 2003; Nomikos *et al.*, 2000). The microanatomy of this VTA circuit is highlighted in Figure 1c: the glutamatergic input is offset by GABAergic afferents from the ventral pallidum and by GABAergic interneurons that are themselves modulated by ACh acting through both the α7* and β2* nAChRs, rendering the VTA microcircuit truly complex with respect to cholinergic-nicotinic modulation, requiring a lot of further work to elucidate the mechanisms fully.

Pharmacological consequences

Further functional evidence for the specificity of the cholinergic innervation to the VTA and the SN comes from studies of Blaha and Winn (Blaha and Winn, 1993; Blaha *et al.*, 1996). They injected nicotinic agonists or the acetylcholinesterase inhibitor neostigmine into the A9 or A10 DA area and compared the DA efflux, measured by *in vivo* chronoamperometry and microdialysis, in the respective projection areas, nucleus accumbens and caudate putamen, as a function of the lesioning of either PPTg or LDTg. A potentiation of DA efflux after neostigmine injection following a lesion was seen as a functional correlate of cholinergic innervation of this area. They could thus establish that, functionally, the innervation to the VTA originates in the LDTg, and the innervation to the SN in the PPTg.

Using electrical stimulation of the PPTg, the Blaha lab could show that DA efflux in the striatum is mediated through nicotinic and glutamatergic receptors in the SN, with also a contribution of muscarinic M5 receptors responsible for a prolonged activation of the DA system (Forster and Blaha, 2003).

Role in behaviour

The behavioural role of the cholinergic tegmentum has mainly been assessed by a complete lesion of the PPTg or LDTg, to assess their impact on, for example, nicotine self-administration (Laviolette *et al*., 2002). Only very recently has a toxin been developed that will allow a fine tuned dissection of the cholinergic involvement (Clark *et al*., 2007). When discussing classical lesion studies caution needs to be exercised, therefore.

Further pharmacological manipulations of the PPTg have also been carried out recently (Lança *et al*., 2000a, b; Corrigall *et al*., 2001, 2002): Both, lesions that reduce the number of PPTg cholinergic neurons and microinfusion of dihydro-β-erythroidine, a high-affinity antagonist selective for non-α7* nAChRs, reduce nicotine self-administration in the rat. A more sophisticated lesion paradigm was carried out more recently, showing that the posterior PPTg is responsible for the control of i.v. nicotine self-administration in the rat (Alderson *et al*., 2006). This shows that also these cholinergic nuclei themselves are under the control of cholinergic mechanisms, and that their efferents to the midbrain DA system play a crucial role also in nicotine reinforcement. After nicotine administration, Fos-positive nuclei are observed in the cholinergic tegmentum, *but* almost exclusively in the GABAergic and glutamatergic neurons (Lança *et al*., 2000b). Moreover, of several agents micro-infused into the PPTg, GABA antagonists reduce nicotine self-administration selectively. These studies thus implicate non-cholinergic parts of the Ch5 and Ch6 nuclei, and will not be discussed further here. For an excellent recent discussion see (Laviolette and van der Kooy, 2004).

The LDTg is also critically involved in the locomotor response to nicotine injections in the rat (Alderson *et al*., 2005): after repeated injections of nicotine, the locomotor response that is elevated in normal animals was significantly lower in LDTg-lesioned rats compared to controls. The authors infer VTA dysfunction arising from this loss of LDTg regulation.

Role in pathology

There has been increasing interest for some time in pathologies associated with the cholinergic MPT, especially schizophrenia and Parkinson's disease (PD). Garcia-Rill *et al*. (1995) reported an intriguing study on a significantly increased number of cholinergic neurons in the MPT. They postulated that this potential increase in cholinergic drive on DA neurons could account for some of the symptoms in schizophrenic patients, a study challenged by subsequent work of other groups (German *et al*., 1999).

Loss of neurons in the PPTg is apparent in a number of pathologies, such as Alzheimer-type dementia, where tangles also appear in the cholinergic cells (Mufson *et al*., 1988), multiple system atrophy and progressive supranuclear palsy (Zweig *et al*., 1987). However, it is the involvement of the PPTg in PD that has been studied most (Pahapill and Lozano, 2000; Winn, 2006): in idiopathic PD neuronal loss in the PPTg is very apparent (Hirsch *et al*., 1987; Jellinger, 1988), including in hydrocarbon-induced PD (Pezzoli *et al*., 1996), while cholinergic innervation of the SN *increases* (Anglade *et al*., 1993, 1995a, b). Thus, in early PD, PPTg cholinergic neurons are intact and working to keep DA neurons functional, *or*, they are intact and driving DA neurons excessively leading to pathology. Induced loss of neurons from the pontine tegmentum does lead to SN cell death (McGeer and McGeer, 1984), but PPTg lesions can also protect against DA nigrostriatal cell death (Takada *et al*., 2000). This clearly represents another rewarding avenue for future research. These first studies raise intriguing questions as to a potential cholinergic contribution to PD, and also a potential route to pharmacological intervention addressing the nAChRs, and thus only indirectly the DA system, unlike conventional PD drugs (Schapira *et al*., 2006).

A related issue is the clear neuroprotection against PD afforded in smokers, well established in meta-analyses (Allam *et al*., 2004). Many recent studies are trying to approach this potentially important finding in animal studies, reviewed in Quik *et al*. (2007). Unfortunately, so far, discrepancies in mouse models after 1-methyl 4-phenyl 1,2,36-tetrahydropyridine or 6-OH dopamine lesions limit the mechanistic understanding of nicotinic signalling in neuroprotection *in vivo*, as a large variability in the protective effect of nicotine is observed in the mouse. In the rat, however, there are several studies of nicotine protection, or perhaps delayed death, in 6-hydroxy dopamine models (Quik, 2004; Visanji *et al*., 2006).

Summary

As we have seen, many interesting issues with respect to the cholinergic MPT and its functional role have only partially been addressed in the past. This may be due to a relative neglect of the cholinergic system that since the 1980s has lost ground compared with the DA or serotonin systems (Bjorklund and Dunnett, 2007). However, as ACh, and by extension, nicotine, are in a position to affect the release of every other neurotransmitter in the brain through pre-synaptic mechanisms (Wonnacott, 1997), and neuroscientists are realizing this increasingly, interest has been rekindled recently. The cholinergic MPT seems an obvious candidate for further in-depth studies as it directly controls the DA system through somato-dendritic events, and possibly indirectly through presynaptic mechanisms in the VTA.

Outlook

As we have seen in the previous sections, many unanswered questions remain regarding the precise role of cholinergic

neurons of the Ch5 and Ch6 cell groups. Progress will certainly come from a couple of sources: classical lesioning studies in which the whole nucleus is destroyed need to be replaced by more specific intervention, for example, through cholinergic-specific ablation as will be possible shortly (Clark *et al*., 2007). An alternative is clearly the use of genetic paradigms where the enzymes responsible for the synthesis or release of ACh can be targeted. Enzymes such as choline acetyl transferase, the choline transporter or the vesicular ACh transporter can be inactivated locally through strategies of RNA interference, or perhaps more efficiently through the local expression of Cre recombinase on a mouse background containing floxed alleles of the gene of interest in the genome.

An alternative lies in the interference with the cholinergic receptor in the target area: mice knockout for the β2 subunit show reduced exploratory behaviour in the open-field paradigm, a phenotype that can be completely reversed by the targeted expression of the β2 subunit in the VTA (Maskos *et al*., 2005; Maskos, 2007). There, clearly, endogenous ACh released from afferents of the cholinergic tegmentum can act solely through the high-affinity nicotinic receptor in the VTA. Further studies are needed, and are under way, to more clearly establish the role of endogenous ACh acting on the midbrain DA system (Avale *et al*., 2006, 2007).

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Conflict of interest

The author states no conflict of interest.

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