*Neuroscience* 345 (2017) 130–141

# REVIEW

# CHOLINERGIC CIRCUITS IN COGNITIVE FLEXIBILITY

**VANIA F. PRADO,** [a,b,c,d]* **HELENA JANICKOVA,** [a] **MOHAMMED A. AL-ONAIZI** [a,b] **AND MARCO A. M. PRADO** [a,b,c,d]

[a] *Robarts Research Institute, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario N6A5K8, Canada*

[b] *Department of Anatomy and Cell Biology, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario N6A5K8, Canada*

[c] *Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario N6A5K8, Canada*

[d] *Graduate Program in Neuroscience, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario N6A5K8, Canada*

**Abstract—Cognitive flexibility, the ability to adjust behavior in response to new and unexpected conditions in the environment, is essential for adaptation to new challenges and survival. The cholinergic system is an important modulator of this complex behavior however, the exact cholinergic circuits involved in this modulation and the precise influence of acetylcholine (ACh) in the process is still not fully understood. Here we review the role of different cholinergic circuits in cognitive flexibility. Strong evidence indicates that cholinergic interneurons (CINs) from the dorsomedial striatum are essential for facilitating the establishment of a new selected strategy; an effect that seems to depend mainly on activation of muscarinic receptors. Cholinergic neurons from the nucleus basalis magnocellularis (nBM), which project to the prefrontal cortex, seem to modulate the initial inhibition of a previously learned strategy, however, this concept is still controversial. Additionally, some studies suggest that basal forebrain cholinergic neurons projecting to the hippocampus, basolateral amygdala, and posterior parietal cortex may also participate on the modulation of cognitive flexibility. We highlight the fact that when investigating effects of ACh on behavioral flexibility, or any other behavior, one has to keep in mind two important particularities of the cholinergic system: (1) Many cholinergic neurons in the brain co-release glutamate or GABA with ACh. Methodologies that rely on neuronal silencing or ablation lead to simultaneous elimination of both neurotransmitters, making interpretation of results complex. (2) The cholinergic gene locus has a unique organization, with the vesicular acetylcholine transporter (*VAChT*) gene present within the intron between the first and second exons of the choline acetyltransferase (*ChAT*) gene. Thus, behavioral studies using transgenic animals generated with *ChAT* bacterial artificial chromosome (BAC) clones should be considered carefully, taking into consideration that these mice may overexpress *VAChT* and therefore, present a hypercholinergic tone that can be a confounder in behavioral studies.**

**This article is part of a Special Issue entitled: Cognitive Flexibility.** 

**Key words: cholinergic, cognitive flexibility, striatum, frontal cortex, reversal learning, set-shifting.**

Contents



## INTRODUCTION

Cognitive flexibility, the ability to appropriately adjust one's behavior in response to new and unexpected conditions in the environment, is essential for adaptation and survival. This complex behavior depends on effective engagement of different brain processes to identify salient changes in the surroundings, direct attention to changed elements, determine that a previous strategy is no longer appropriate, inhibit previous responses and establish a new strategy (Dajani and Uddin, 2015; Nilsson et al., 2015). Deficits in cognitive flexibility have been associated with diverse pathologies such as autism, schizophrenia, Parkinson's disease, Alzheimer's disease and attention deficit hyperactivity disorder (Daban et al., 2006; Schoenbaum et al.,

*Correspondence to: V.F. Prado, Robarts Research Institute, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario N6A5K8, Canada.

*Abbreviations:* ACh, acetylcholine; BAC, bacterial artificial chromosome; *ChAT*, choline acetyltransferase; ChR2, channelrhodopsin-2; CINs, cholinergic interneurons; MWM, Morris Water Maze; nAChR, nicotinic acetylcholine receptors; nBM, nucleus basalis magnocellularis; nbM, nucleus basalis of Meynert; PPT, pedunculopontine tegmental nucleus; *VAChT*, vesicular acetylcholine transporter; VGLUT3, vesicular glutamate transporter 3.

http://dx.doi.org/10.1016/j.neuroscience.2016.09.013
0306-4522/

2006; Nakaaki et al., 2007; Ceravolo et al., 2012; D'Cruz et al., 2013; Karvat and Kimchi, 2014; El Haj et al., 2015; Hill et al., 2015; Miller et al., 2015).

It has long been recognized that the prefrontal cortex and the striatum, which are part of the cortico-basal-ganglia–thalamic circuit, work together to modulate cognitive flexibility and a wealth amount of data indicate that acetylcholine (ACh) transmission facilitates the process [for a review see (Ragozzino, 2003)]. For instance, systemic administration of cholinesterase inhibitors to boost cholinergic transmission improved reversal learning and attentional set-shifting deficits in old rats (Tait et al., 2013; Nikiforuk et al., 2015), in rat models of schizophrenia (Alexander et al., 2013), as well as in rats with immunolesion of basal forebrain cholinergic neurons (Cutuli et al., 2009). Conversely, mice with forebrain cholinergic signaling impairment, which includes the whole cortical mantle, hippocampus, amygdala and striatum showed consistent deficits in reversal learning assessed using the Morris Water Maze (MWM) (Martyn et al., 2012; Al-Onaizi et al., 2016), as well as a severe reversal learning impairment in a visual discrimination task (Kolisnyk et al., 2013a). Moreover, selective decrease of hippocampal cholinergic tone disturbs reversal learning in the MWM similarly to mice in which forebrain cholinergic tone is disrupted (Al-Onaizi et al., 2016). Systemic administration of scopolamine, a muscarinic receptor antagonist, impaired set shifting and reversal learning in rats while having no effect on discrimination performance (Soffie and Lamberty, 1987; Chen et al., 2004). In addition, systemic injections of a selective M1 muscarinic agonist (CDD-0102A) had a significant effect on enhancing strategy switching under changing environmental demands in rats (Ragozzino et al., 2012). Systemic administration of nicotinic agonists (Allison and Shoaib, 2013; Terry et al., 2016), as well as of α7-nAChR-positive allosteric modulators (Nikiforuk et al., 2015) also showed significant effect on enhancing strategy switching under changing environmental demands.

Because cholinergic innervation is widespread throughout the brain, including the entire cortical mantle and the striatum, and ACh can signal through a large variety of ionic and G-protein-coupled receptors that are present pre- and post-synaptically on distinct neuronal systems, it has been hard to determine the exact role of specific cholinergic circuits on the modulation of cognitive flexibility. However, in the last decade, cholinergic circuits and brain mechanisms underlying this behavior have been extensively studied. Here we will briefly describe important components of the cholinergic system as well as the location of main cholinergic nuclei and their projection to different areas of the brain. We will shortly describe how analyses of reversal learning in discrimination tasks and/or attentional set-shifting tasks are used to investigate behavioral flexibility. We will review biochemical, pharmacological and behavioral studies implicating cholinergic interneurons (CINs) from the striatum as well as basal forebrain cholinergic projections to the cortical mantle, amygdala and hippocampus in the modulation of cognitive flexibility. We will discuss evidence that CINs from the striatum facilitate exploration of new strategies when conditions change. We will also discuss a possible role for basal forebrain cholinergic projections on determining whether a previous strategy is no longer appropriate.

## THE CHOLINERGIC SYSTEM IN THE BRAIN

ACh synthesis at the nerve terminal depends on the uptake of choline by the high-affinity choline transporter (CHT1) (Ribeiro et al., 2006) and on the enzyme choline acetyltransferase (*ChAT*); which catalyzes the acetylation of choline with acetyl-CoA (Fig. 1A); [see review on (Prado et al., 2002, 2013)]. The neurotransmitter is then loaded into synaptic vesicles by the vesicular acetylcholine transporter (VAChT) [see review on (Parsons, 2000; Prado et al., 2002, 2013)], a process dependent on the electrochemical gradient generated by a V-type proton ATPase (Parsons, 2000). Arrival of the nerve impulse to the terminal leads to membrane depolarization and consequent $Ca^{2+}$ influx, which triggers vesicle fusion and release of ACh into the synaptic cleft. Released ACh binds to both pre- and postsynaptic muscarinic (mAChR) and nicotinic (nAChR) receptors. Half-life of ACh at the synaptic cleft is short as it is hydrolyzed within milliseconds by the enzyme acetylcholinesterase or butyrylcholinesterase (Prado et al., 2002; Zimmerman and Soreq, 2006). Binding of ACh to pre- and postsynaptic receptors present on distinct neuronal systems can alter neuronal excitability, influence synaptic transmission, induce synaptic plasticity, and coordinate firing of groups of neurons [for excellent reviews see (Picciotto et al., 2012; Dineley et al., 2015; Soreq, 2015)] and as a result produce diverse consequences for brain activity. Cholinergic tone can also regulate long-term changes in target cells by modulating microRNA and RNA metabolism with consequences for gene expression and alternative splicing (Berson et al., 2012; Kolisnyk et al., 2013a, 2016; Soreq, 2015), which has important consequences for cognitive function, neuronal resilience and Alzheimer's disease pathology.

The brain is highly innervated by cholinergic neurons and ACh modulates numerous brain functions. Most cholinergic innervations originate from projection neurons that target distal areas. The basal forebrain (BF) complex for instance (Fig. 1C, D), which includes the nucleus basalis magnocellularis (nBM), the medial septum (MS), the nuclei of the diagonal band (nDB) and substantia innominata (SI), provides the major input to the whole cortical mantle, hippocampus, thalamus and amygdala (Mesulam et al., 1983). The pedunculopontine–laterodorsal tegmental complex (PPTg–LDT) innervates midbrain regions (Fig. 1E, F), the thalamus, the striatum and pontine targets (Hallanger and Wainer, 1988). The medial habenula (MHb) nuclei projects to the interpeduncular nucleus in the midbrain (Ren et al., 2011). In the striatum (Fig. 1B), most cholinergic innervations come from interneurons (Woolf and Butcher, 1981; Bolam et al., 1984) with some projections coming from PPT cholinergic neurons (Dautan et al., 2014). Striatal CINs (also named tonically active neurons or TANS) are