Behavioural Brain Research 311 (2016) 267–278

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Mice deficient for striatal Vesicular Acetylcholine Transporter (VAChT) display impaired short-term but normal long-term object recognition memory

Daniel Palmer[a], Samantha Creighton[a], Vania F. Prado[b,c,d], Marco A.M. Prado[b,c,d], Elena Choleris[a], Boyer D. Winters[a,*]

[a] Department of Psychology, University of Guelph, Guelph, Ontario, Canada
[b] Robarts Research Institute, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada
[c] Department of Physiology & Pharmacology, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada
[d] Department of Anatomy & Cell Biology, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada

HIGHLIGHTS

- Studied mice deficient for the Vesicular Acetylcholine Transporter in the striatum.
- Impairment seen in object recognition memory with short retention delays.
- No impairment with longer delays.
- No impairment on object location memory.
- Striatal acetylcholine is involved in short-term object memory.

ARTICLE INFO

Article history:
Received 11 February 2016
Received in revised form 20 May 2016
Accepted 23 May 2016
Available online 24 May 2016

Keywords:
VAChT
Acetylcholine
Striatum
Object recognition
Object location
Memory

ABSTRACT

Substantial evidence implicates Acetylcholine (ACh) in the acquisition of object memories. While most research has focused on the role of the cholinergic basal forebrain and its cortical targets, there are additional cholinergic networks that may contribute to object recognition. The striatum contains an independent cholinergic network comprised of interneurons. In the current study, we investigated the role of this cholinergic signalling in object recognition using mice deficient for Vesicular Acetylcholine Transporter (VAChT) within interneurons of the striatum. We tested whether these striatal VAChT$^{D2-Cre-flox/flox}$ mice would display normal short-term (5 or 15 min retention delay) and long-term (3 h retention delay) object recognition memory. In a home cage object recognition task, male and female VAChT$^{D2-Cre-flox/flox}$ mice were impaired selectively with a 15 min retention delay. When tested on an object location task, VAChT$^{D2-Cre-flox/flox}$ mice displayed intact spatial memory. Finally, when object recognition was tested in a Y-shaped apparatus, designed to minimize the influence of spatial and contextual cues, only females displayed impaired recognition with a 5 min retention delay, but when males were challenged with a 15 min retention delay, they were also impaired; neither males nor females were impaired with the 3 h delay. The pattern of results suggests that striatal cholinergic transmission plays a role in the short-term memory for object features, but not spatial location.

1. Introduction

Acetylcholine (ACh) has long been implicated in cognitive functions. Indeed, cognitive deficits in Alzheimer's disease, as well as those seen in other memory disorders such as mild cognitive impairment, are influenced by cholinergic cell death [1]. Previous research has implicated the cholinergic neurons of the basal forebrain in such cognitive impairment [2]. The cholinergic basal forebrain consists of a series of nuclei including the medial septum, diagonal band, and nucleus basalis, which project to most of the cortical mantle, hippocampus, and amygdala [3]. Excitotoxic or immunotoxic lesions of the cholinergic basal forebrain of rats can produce significant impairments in different cognitive domains,

* Corresponding author.
E-mail address: bwinters@uoguelph.ca (B.D. Winters).

http://dx.doi.org/10.1016/j.bbr.2016.05.050
0166-4328/

such as working and long-term memory [4–10], as well as attention [4,11–13].

A less well-characterized cholinergic network lies within the striatum, which contains large cholinergic interneurons that modulate the activity of striatal medium spiny neurons [14]. These cholinergic interneurons comprise a small percentage of the neurons within the striatum and have been previously theorized to have an inverse relationship with striatal dopamine [15]. Striatal ACh has also been shown to increase the excitability of cortico-striatal neurons through M1 receptor stimulation [16], and activation of striatal muscarinic receptors increases NMDA receptor-mediated currents [15]. Previous research has suggested that striatal cholinergic interneurons are involved in cognitive processes such as behavioural flexibility [17], memory for recent events [18], the acquisition of passive avoidance memory [19], fear memory [20], as well as reward based learning [21]. Additionally, striatal ACh release has been shown to increase as learning in a cross maze task is transitioning from a place- to a response-based strategy, consistent with a strong role for striatal ACh in stimulus-response and habit learning [22].

The role of ACh in cognitive functions is not a simple one, and cholinergic pathways have been implicated in various non-mnemonic functions [23], as well as, more recently, non-traditional roles in memory circuitry [24,25]. Indeed, it has been postulated that attentional deficits caused by loss of cholinergic function may underlie the memory deficits observed in other tasks [26]. It is likely that acetylcholine contributes to many different aspects of cognition and may be involved in memory acquisition through multiple mechanisms. Regardless of the specific processes involved, however, a large body of research indicates that ACh is required for the acquisition of declarative memory [27]. In rodents, declarative-like memory is often assessed by examining object recognition [28] or spatial memory [29]. Indeed, systemic injections of cholinergic antagonists can disrupt memory in spontaneous object recognition [30,31] and object location [32] tasks. Moreover, these effects are likely closely related to cholinergic basal forebrain transmission. Lesions of the cholinergic basal forebrain can impair object recognition [10,33,34] and object location [33] memory, but have also clearly been shown to spare several other forms of spatial memory [35–37]. Moreover, cholinergic lesions do not always produce behavioural impairments in recognition memory. For example, lesions to the basal forebrain do not impair performance in an episodic-like memory version of object recognition [38]. Indeed, the neurobiology behind recognition memory is complex, as lesions to various brain structures may only disrupt certain types of recognition while preserving recognition in other types [39–41]. While the impairing effects of cholinergic manipulations in many object memory tasks are most likely the result of effects on medial temporal lobe structures strongly implicated in object memory processing [42–44], the possibility remains that other cholinergic circuitry, such as striatal cholinergic interneurons, play a role in such cognitive functions [45].

The rodent literature regarding striatal involvement in object memory remains equivocal. For example, lesions to the caudate nucleus that caused motor response impairments in rats did not disrupt performance in a delayed non-match to sample object recognition task [46]; retention delays in this study, however, were very short (1–20 s), leaving open the possibility that striatal manipulations could affect object memory with delays of minutes to hours. Conversely, in mice, administration of the NMDA receptor antagonist AP-5 or the AMPA receptor antagonist DNQX into the nucleus accumbens prior to object exposure produced deficits in object recognition [47]. Similar effects were found on a spatial novelty task where positions of objects were manipulated, as opposed to object features [48]. The striatum has also previously been implicated in spatial learning; however, it is typically involved in procedural learning tasks with spatial information such as the Morris water maze [49,50]. There is some evidence, however, to suggest that the striatum may be involved in aspects of spatial and non-spatial novelty processing [51]. The exact role of the striatum in object recognition has not been assessed systematically to date, as there has been no study to investigate its involvement with different retention delays or for types of recognition tasks. Given the previous implication of the striatum in novelty detection and recognition tasks, the spontaneous recognition tasks employed in the current study appear well suited to an examination of the potential role of striatal ACh in cognition.

The current study investigated the role of striatal cholinergic activity in short- and long-term object recognition and location memory in mice. Although it is more commonly implicated in habit-like forms of learning and memory [22], the striatum – and diseases associated with striatal damage – has also been implicated in non-habit forms of cognition [52–55]; however, little is known about the potential involvement of striatal ACh in these functions. We used a recently created genetic mouse model, which has an elimination of the Vesicular Acetylcholine Transporter (VAChT) localized to the striatum ($VAChT^{D2-Cre-flox/flox}$)[56]. Mice deficient for the VAChT protein within the striatum show a hyper sensitivity to dopamine agonists, as well as an upregulation of D1 & D2 receptors. Elimination of VAChT within the striatum did not have any behaviour consequences on conventionally striatum-dependent tasks such as spontaneous locomotion and only modestly decreased the locomotion response to cocaine exposure [56]. Previous research with these mice also showed normal object recognition with a 1 h retention delay [56]. $VAChT^{D2-Cre-flox/flox}$ mice also have alterations to metabolic activity such as increases in insulin production and demonstrate increased wakefulness compared to controls [57].

Based on research suggesting that striatal ACh may be important for recent memory [18], we set out to assess systematically the involvement of striatal cholinergic activity in short term memory. To this end, we tested $VAChT^{D2-Cre-flox/flox}$ mice in the object recognition paradigm using multiple retention delays to determine if these mice have recognition deficits that are selective to shorter delays. We predicted that the $VAChT^{D2-Cre-flox/flox}$ mice would show a selective impairment with relatively short retention delays, while showing no impairment with a long retention delay. In addition to testing object recognition *per se*, we assessed $VAChT^{D2-Cre-flox/flox}$ mice using the object location task, in order to assess recognition memory for spatial location. It was expected that differences would exist between wildtype and $VAChT^{D2-Cre-flox/flox}$ mice in this task. In addition, we tested both male and female $VAChT^{D2-Cre-flox/flox}$ mice in order to determine if any sex differences are related to striatal acetylcholine. It was expected that sex differences would exist between male and female mice, as previously tested mice deficient globally for the VAChT protein were found to have differential memory deficits [58]. Male global VAChT knockdown mice displayed object recognition deficits with short term and long term retention delays, while female mice displayed only long term memory deficits [58]. Therefore it was expected that male mice would show greater deficits in these experiments compared to females.

2. Materials and methods

2.1. Subjects

$VAChT^{D2-Cre-flox/flox}$ mice were generated by crossing $VAChT^{flox/flox}$ [59] mice with D2-Cre mice (Tg(Drd2-cre)44Gsat) as described previously [56,57]. Mice used in this study have a mixed FVB/B6/129/Swiss background, although the C57BL/6J pre-