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Partial loss in septo-hippocampal cholinergic neurons alters memory-dependent measures of brain connectivity without overt memory deficits

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ABSTRACT

The functional relevance of septo-hippocampal cholinergic (SHC) degeneration to the degradation of hippocampus-dependent declarative memory (DM) in aging and Alzheimer's disease (AD) remains ill-defined. Specifically, selective SHC lesions often fail to induce overt memory impairments in animal models. In spite of apparent normal performance, however, neuronal activity within relevant brain structures might be altered by SHC disruption. We hypothesized that partial SHC degeneration may contribute to functional alterations within memory circuits occurring in aging *before* DM decline. In young adult mice, we studied the effects of behaviorally ineffective (saporin-induced) SHC lesions – similar in extent to that seen in aged animals – on activity patterns and functional connectivity between three main neural memory systems: the septo-hippocampal system, the striatum and the amygdala that sustain declarative, procedural and emotional memory, respectively. Animals were trained in a radial maze procedure dissociating the human equivalents of relational/DM and non-R/DM expressions in animals. Test-induced Fos activation pattern revealed that the partial SHC lesion significantly altered the brain's functional activities and connectivity (co-activation pattern) despite the absence of overt behavioral deficit. Specifically, hippocampal CA3 hyperactivity and abnormal septo-hippocampo-amygdalar inter-connectivity resemble those observed in aging and prodromal AD. Hence, SHC neurons critically coordinate hippocampal function in concert with extra-hippocampal structures in accordance with specific mnemonic demand. Although partial SHC degeneration is not sufficient to impact DM performance by itself, the connectivity change might predispose the emergence of subsequent DM loss when, due to additional age-related insults, the brain can no longer compensate the holistic imbalance caused by cholinergic loss.

Introduction

Cholinesterase inhibitors are commonly administered to Alzheimer's disease (AD) patients to compensate for the loss of cholinergic neurons in an attempt to rescue the loss of memory function (Bartus et al., 1985; Pepeu and Giovannini, 2009). However, the contribution of cholinergic degeneration to the characteristic deterioration of hippocampus-dependent declarative memory (DM) in aging and early AD is still poorly understood. Indeed, the reduction in cholinergic markers seen in aged patients and animals often correlates with the severity of their memory impairment (Gallagher and Rapp, 1997) but selective deafferentation of cholinergic input to the hippocampus by intraseptal injection of immunotoxin saporin in young animals does not reliably produce overt memory loss (Parent and Baxter, 2004).

To better understand the role of septo-hippocampal cholinergic (SHC) neurons in senescent memory decline, we examined here whether partial SHC degeneration similar in extent to that naturally occurring in aging – despite being inefficient to induce over memory impairment – could alter brain activity patterns. Indeed, senescence is associated with alterations in functional activity patterns and connectivity within memory-related brain circuits and some of these alterations occur before the appearance of overt DM degradation (Grady, 2008; Hedden and Gabrieli, 2005; Minati et al., 2007; Pihlajamaki et al., 2009; Sperling et al., 2010). We hypothesize that partial SHC degeneration may contribute to the functional alterations that precede irreversible DM decline. In particular, while hippocampal activation engaged by memory execution is typically reduced in cognitively impaired subjects, a paradoxical increase in hippocampal activity at prodromal stage of AD has been observed, which may be predictive of future pathological memory decline (Bassett et al., 2006; Dickerson

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and Sperling, 2008; Mondadori et al., 2006; Quiroz et al., 2010; Sperling, 2007). Moreover, indiscriminate hippocampal hyperactivation under DM as well as non-DM learning and striatal hyperactivation under DM learning have been previously observed in aged subjects, suggesting a senescence-related "de-differentiation" between DM and non-DM circuits (Dennis and Cabeza, 2011). Finally, aging-related reduction in hippocampo-amygdalar functional connectivity linked to memory retrieval has been reported in two studies (Murty et al., 2009; St Jacques et al., 2010).

Although the precise mechanisms leading to functional connectivity dysregulation remain to be delineated, the hypothesized contribution of SHC degeneration is based on current knowledge that SHC manipulations can alter hippocampal functional activity and the coordination among neural systems underlying different memory processes. First, computational models (Buzsaki, 1989; Hasselmo, 2006; Hasselmo and Bower, 1993) predict that the modulation of hippocampal function by acetylcholine modulates the balance between memory encoding and retrieval processes subserved by the hippocampus. Thus, SHC disruption may induce a shift in hippocampal computation, detrimental to memory encoding/promoting retrieval processes similar to that seen in aging (Gallagher et al., 2010; Ikonen et al., 2002; Toner et al., 2009; Yassa and Stark, 2011). Second, hippocampal cholinergic transmission seems to coordinate activity between neural systems sustaining DM and non-DM (Micheau and Marighetto, 2011) by balancing the relative contributions of the hippocampus and extra-hippocampal structures, such as the amygdala (Calandreau et al., 2006; McIntyre et al., 2002) and the striatum (Chang and Gold, 2003; McIntyre et al., 2003; Pych et al., 2005) involved in emotional and procedural memory, respectively (White and McDonald, 2002).

Here, we tested the hypothesis that senescence-related SHC degeneration could alter functional activity in the hippocampus and functional connectivity between the septo-hippocampal, striatal and amygdalar neural systems. To this end, memory-induced activation patterns in these brain areas were visualized by Fos imaging in young adult mice, and the impact of partial SHC neuronal loss (induced by intra-septal immunotoxin saporin) comparable in magnitude to that occurring naturally in aged animals examined. The partial lesions were expected to alter inter-region Fos activity patterns without affecting memory performance. A radial maze discrimination learning procedure was used to separately engage two forms of memory expression, thereby dissociating relational/DM and non-R/DM, through a change in the arrangement of discriminanda with any difference in informativeness (Mingaud et al., 2007). Fos activation patterns were examined in a two-way (lesions × memory engagement) factorial design contrasting lesioned and control mice having been subjected to either R/DM or non-R/DM retrieval in comparison with a treadmill non-learning control condition.

Methods and materials

Subjects

Male C57 BL/6J mice (Charles River, Lyon, France) were used.

First we evaluated the effect of aging on the density of SHC neurons by comparing the number of ChAT immunopositive neurons per mm^2 in the medial septum – the main source of the cholinergic innervations to the hippocampus (Mesulam et al., 1983) – in young (4–5 months, n = 9) and aged (23–24 months, n = 11) mice.

Having ascertained that aged mice suffered approximately a 20% reduction in the density of SHC cells, we went on to induce SHC loss of similar magnitude in young mice by intra-septal saporin infusion (saporin-injected mice, n = 27; vehicle-injected Sham controls, n = 29). Saporin and Sham mice were submitted to behavioral training

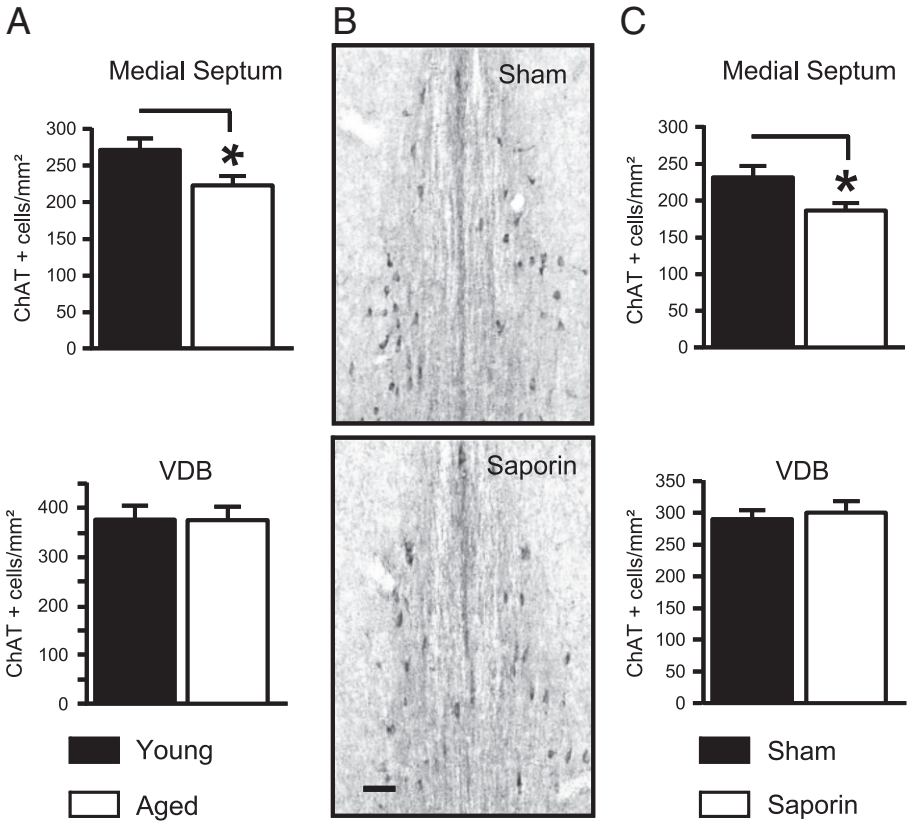

Fig. 1. *Impact of aging and intra-septal saporin infusion on the density of SHC neurons.* In the medial septum (M-Sept) but not in the vertical limb of the diagonal band (VDB), a ≈20% reduction in ChAT immunopositive cells' density is observed in 23–24 month old mice compared to 4-month old ones (A), and in saporin-treated mice compared to Sham mice of the same age (4–5 months, C). B: photomicrographs of the medial septum representative of the Sham and Saporin groups (scale bar: 50 μm).