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Altered cognitive performance and synaptic function in the hippocampus of mice lacking C3

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ABSTRACT

Previous work implicated the complement system in adult neurogenesis as well as elimination of synapses in the developing and injured CNS. In the present study, we used mice lacking the third complement component (C3) to elucidate the role the complement system plays in hippocampus-dependent learning and synaptic function. We found that the constitutive absence of C3 is associated with enhanced place and reversal learning in adult mice. Our findings of lower release probability at CA3–CA1 glutamatergic synapses in combination with unaltered overall efficacy of these synapses in C3 deficient mice implicate C3 as a negative regulator of the number of functional glutamatergic synapses in the hippocampus. The C3 deficient mice showed no signs of spontaneous epileptiform activity in the hippocampus. We conclude that C3 plays a role in the regulation of the number and function of glutamatergic synapses in the hippocampus and exerts negative effects on hippocampus-dependent cognitive performance.

Introduction

Complement is part of the innate immune system and plays a major role in the elimination of pathogens and inflammatory response to injury. There is, however, an accumulating body of evidence in support of a range of non-immunological functions of complement, in particular in the CNS. The C3-derived peptide C3a induces intracellular [Ca_{2+}] elevation and neutrophin expression in cultured microglia (Heese et al., 1998; Möller et al., 1997), and regulates migration and neuronal differentiation of neuronal progenitor cells as well as neurite outgrowth in vitro (Shinjyo et al., 2009). In vivo studies demonstrated the involvement of C3a in neuronal differentiation during the development of rat cerebellum (Bénard et al., 2008) and signaling through C3a receptor stimulates neurogenesis in the adult hippocampus (Rahpeymai et al., 2006). In the immature brain, C3a is protective against hypoxic-ischemic injury and C3a treatment ameliorates hypoxia–ischemia-induced cognitive impairment (Järlestedt et al., 2013). The complement system has also been implicated in developmental elimination of synapses in the thalamus (Stevens et al., 2007) and the sensorimotor cortex (Chu et al., 2010). Microglia play an important role in this process, since inhibition of microglial motility resulted in delayed synapse elimination in the hippocampus (Paolicelli et al., 2011) and disrupting microglia-specific complement receptor 3 (CR3)/C3 signaling led to sustained deficits in synaptic connectivity in the retinogeniculate system (Schafer et al., 2012).

In the adult brain, the hippocampus plays a crucial role in the formation of certain types of memory, such as episodic memory and spatial memory (Kesner et al., 2000; Squire, 1992), and hippocampal lesions

Abbreviations: C3, the third complement component; C1q, the first complement component subunit.

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are associated with impaired spatial learning and spatial pattern separation (Gallagher and Holland, 1992; Gilbert et al., 1998; Morris et al., 1982); in particular when the environment complexity is high (Moses et al., 2007). Both synaptic plasticity and generation of new neurons have been implicated in hippocampal function (Deng et al., 2010; Dragoi et al., 2003; Morris et al., 1986; Nakazawa et al., 2003). As C3 seems to regulate both the number of synapses and hippocampal neurogenesis, in the present study, we sought to determine the effects of constitutive genetic ablation of C3 on the functions of an unchallenged adult hippocampus by assessing place and reversal learning ability and synaptic function in C3 deficient (C3 KO) mice. Our results show that C3 KO mice have an enhanced hippocampus dependent learning and provide evidence that supports the involvement of the complement system in excitatory synapse elimination in the hippocampus. Further, we report that the C3 deficiency does not result in spontaneous epileptiform activity in the hippocampus as the increased number of functional synapses is compensated by reduced presynaptic glutamate release probability.

Methods

Animals

C3 KO mice (Pekna et al., 1998) were backcrossed onto the C57BL/6 background for 13 generations. Homozygous C3 KO and wild type (WT) control mice were housed in standard cages with a 12-hour light/dark cycle and free access to food and water. All experiments were performed in accordance with the guidelines of the local ethical committee for animal research at the University of Gothenburg or the Malmö-Lund Ethical Committee for the use of laboratory animals and were conducted in accordance with European Union directives on animal rights.

Behavioral testing

Animals

Male C3 KO mice and WT controls were 2.5–3 months old (first experiment, Fig. 1A) and 2 months old (second experiment, Fig. 1B–D), respectively when behavioral testing began. Before weaning, all mice were anesthetized with isoflurane (Abbott Laboratories, North Chicago, IL, USA) and implanted subcutaneously with microtransponders (DATAMARS, PetLink, Youngstown, OH, USA) to allow individual animal identification in the IntelliCages. After weaning, the mice were, kept in groups of up to 10 and separated by genotype.

Testing of place and reversal learning using IntelliCage®

The IntelliCage platform (New Behavior, Zurich, Switzerland) for unbiased monitoring of mouse behavior in a home cage setting has been described elsewhere (Barlind et al., 2010; Galsworthy et al., 2005; Knapska et al., 2006; Onishchenko et al., 2007). The animals were

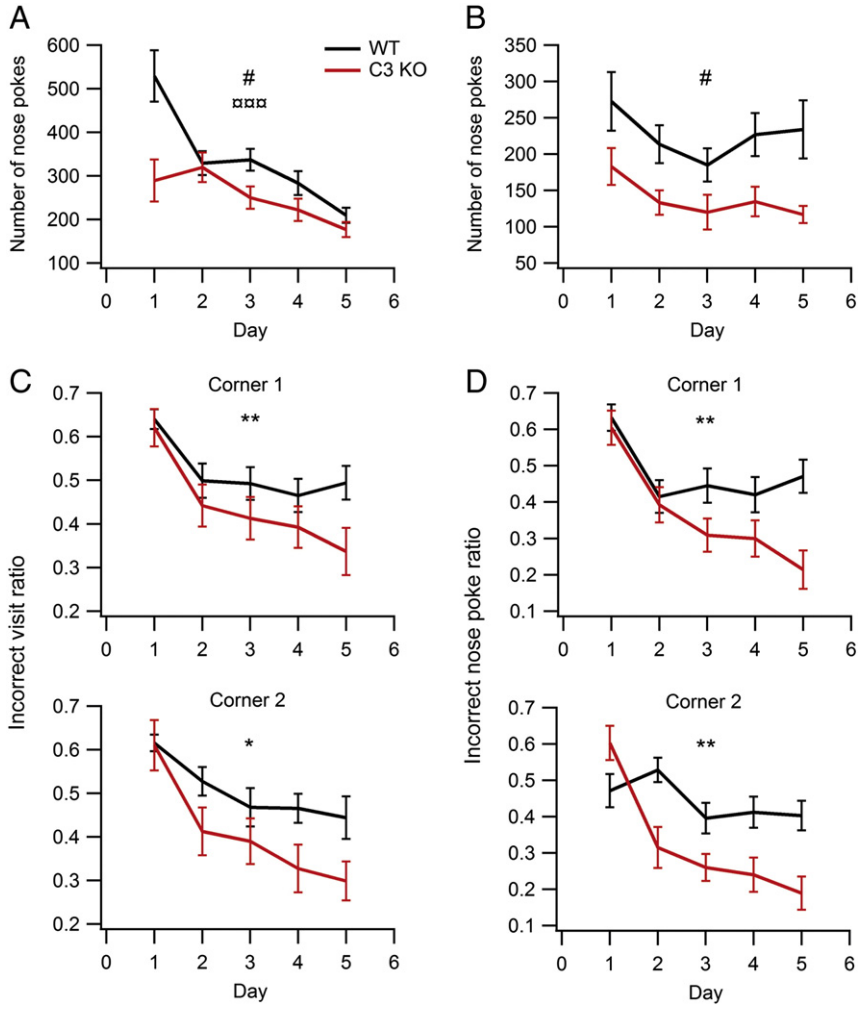

Fig. 1. C3 KO mice show enhanced place and reversal learning compared to WT mice. (A) Exploratory behavior, expressed as number of nose pokes, in the 5 first days declined over time in both groups of mice but the C3 KO mice (n = 10) were less active compared to WT mice (n = 15) (B) In a separate experiment, the exploratory activity of C3 KO mice (n = 9) was lower compared to WT mice (n = 10) during the place learning period (corner 1), and place learning (corner 1) and reversal learning (corner 2) showed significantly lower incorrect visit ratio in C3 KO compared to WT mice (C). (D) The C3 KO mice made less incorrect nose pokes over the 5 days of both learning periods (corner 1 and 2). * = time × genotype, ¤ = time, # = genotype, $P < 0.05^{*, \#}$, $P < 0.01^{**}$, $P < 0.001^{¤¤¤}$.