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Full-length Article

Selective dentate gyrus disruption causes memory impairment at the early stage of experimental multiple sclerosis

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ABSTRACT

Memory impairment is an early and disabling manifestation of multiple sclerosis whose anatomical and biological substrates are still poorly understood. We thus investigated whether memory impairment encountered at the early stage of the disease could be explained by a differential vulnerability of particular hippocampal subfields. By using experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, we identified that early memory impairment was associated with selective alteration of the dentate gyrus as pinpointed *in vivo* with diffusion-tensor-imaging (DTI). Neuromorphometric analyses and electrophysiological recordings confirmed dendritic degeneration, alteration in glutamatergic synaptic transmission and impaired long-term synaptic potentiation selectively in the dentate gyrus, but not in CA1, together with a more severe pattern of microglial activation in this subfield. Systemic injections of the microglial inhibitor minocycline prevented DTI, morphological, electrophysiological and behavioral impairments in EAE-mice. Furthermore, daily infusions of minocycline specifically within the dentate gyrus were sufficient to prevent memory impairment in EAE-mice while infusions of minocycline within CA1 were inefficient. We conclude that early memory impairment in EAE is due to a selective disruption of the dentate gyrus associated with microglia activation. These results open new pathophysiological, imaging, and therapeutic perspectives for memory impairment in multiple sclerosis.

1. Introduction

Multiple sclerosis is the most frequent inflammatory disorder of the central nervous system (Compston and Coles, 2008). Among symptoms, episodic memory impairment is frequent (Planche et al., 2016), greatly impacts quality of life (Ruet et al., 2013) and occurs early during the course of the disease (Feuillet et al., 2007). Evidence from clinical studies links episodic memory impairment with hippocampal alteration. Nevertheless, these human data are either post-mortem histological studies inherently biased toward the chronic stage of the disease (Papadopoulos et al., 2009; Dutta et al., 2011), or *in vivo* studies based on measurement of hippocampal atrophy on magnetic resonance imaging (MRI), which reflects again a late process and don't inform on the underlying cellular modifications (Sicotte et al., 2008; Koenig et al., 2014). Therefore, there is a need for a better understanding of the substrate of early memory deficit associated with multiple sclerosis.

The cellular mechanisms that could be potentially disrupted in the hippocampus during the early stage of multiple sclerosis are mainly those involved in long-term synaptic plasticity within the trisynaptic loop, which is one of the major substrates for learning and memory (Citri and Malenka, 2008). These mechanisms can only be explored by using experimental autoimmune encephalomyelitis (EAE), the most widely accepted animal model of multiple sclerosis ('t Hart et al., 2011). Early synaptic dysfunctions have

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been reported in EAE and associated with pro-inflammatory cytokines released by macrophages, microglia or astrocytes (Centonze et al., 2009; Nisticò et al., 2013; Di Filippo et al., 2016; Habbas et al., 2015), leading to the concept of "inflammatory synapthopathy" (Mandolesi et al., 2015). However, it is currently unknown whether some particular synaptic pathways could be differentially targeted by activated glial cells during the course of multiple sclerosis or EAE.

Indeed, the hippocampus is composed of different interconnected regions and layers (the Cornu Ammonis subfields, the dentate gyrus and the subiculum) which are very different in their morphological, molecular, electrophysiological and functional profiles. This implies that it is not enough to consider the hippocampus as a unitary and homogeneous entity. By analogy with other diseases such as Alzheimer or post-traumatic stress disorder that differentially target distinct subregions of the hippocampus circuit, we can envision that groups of hippocampal neurons are afflicted at the early stage of multiple sclerosis while neighboring ones are not (Small et al., 2004; Small, 2014). Human MRI data also suggest that some hippocampal regions could be more affected than others in multiple sclerosis because some authors have reported hippocampal volume loss localized to specific subfields (Sicotte et al., 2008; Gold et al., 2010; Longoni et al., 2015; Rocca et al., 2015). However, these studies provide controversial results which may come from measure of atrophy performed at different stages and with different methodology. Whether hippocampal regions/subfields could be differentially vulnerable to early pathophysiological mechanisms in multiple sclerosis (prior to atrophy) is therefore currently unclear and can only be addressed with the EAE model.

Consequently, in this study, we looked for the substrate of early memory impairment in multiple sclerosis by combining multi scale complementary approaches in the different hippocampal subfields of EAE-mice. Similarly to the human disease, we demonstrated that EAE-mice showed hippocampal-dependent memory impairment prior to hippocampal atrophy. Then, we used diffusion tensor imaging (DTI) as an *in vivo* "screening procedure" to pinpoint potential regional vulnerability and focus the other experiments. We identified the locus of early hippocampal disruption in the dentate gyrus with concordant DTI, morphological and electrophysiological experiments. This selective vulnerability was associated with early microglial activation and was prevented with systemic injections or local intra-dentate gyrus infusions of the microglial inhibitor minocycline. Hence, we identified the anatomical source of memory impairment in early multiple sclerosis which could be exploited to clarify pathogenesis and which could be translated to patients as it can be captured with *in vivo* imaging.

2. Materials and methods

2.1. Animals and experimental autoimmune encephalomyelitis (EAE)

Experiments were performed on 7–9-week-old females C57BL6/J (Janvier Labs). EAE was induced with a subcutaneous injection of 150 μg of Myelin Oligodendrocyte Glycoprotein peptide 35–55 (MOG35-55, Anaspec) emulsified in 150 μL of Complete Freund's Adjuvant (CFA, Difco) containing 6 mg/mL of desiccated *Mycobacterium tuberculosis* (H37Ra, Difco). Animals received intraperitoneal (IP) injections of Pertussis Toxin (Sigma) on the day of immunization and 2 days later (250 ng/injection). Control mice were injected with 150 μL of CFA emulsified in phosphate-buffered saline (PBS). All animals were weighted daily and scored for clinical symptoms using the standard grading scale: 0, unaffected; 1: flaccid tail; 2: hind limb weakness and/or ataxia; 3: hind limb paralysis; 4: paralysis of all four limbs and 5: moribund.

All experiments reported in this article were performed early in the course of the disease at 20 days post-injection (d.p.i), except electrophysiological experiments which were performed between 18 and 22 d.p.i. All animal care and experiments were conducted in accordance with the European directive (2010/63/EU) and after approval of the local ethical committee (approval number 02046.01).

2.2. Contextual fear conditioning

Memory functions were assessed with a particular contextual fear conditioning procedure (Fig. 1B) that does not require important motor skills unlike other tests, such as the Morris water maze. Acquisition of fear conditioning took place in a Plexiglas box (30 × 24 × 22 cm, Imetronic) in a brightness of 60 lx, given access to different visual-spatial cues in the experimental room. The floor of the chamber consisted of stainless-steel rods connected to a shock generator. The box was cleaned with 70% ethanol. The training procedure consisted on a pseudo-random distribution of tones and shocks as followed (tone-shock unpairing procedure): 100 s after being placed into the chamber, animals received a footshock (0.4 mA squared signal, 1 s), then, after 20 s, a tone (65 dB, 1 kHz, 15 s) was presented twice (30 s delay); finally, after 30 s, mice received a second shock and returned to the home cage 20 s after. One day after the acquisition, mice were re-exposed to the tone alone during 2 min in a safe and familiar chamber where they had been pre-exposed the day before conditioning (opaque PVC chamber with opaque floor, brightness of 15 lx, cleaned with 4% acetic acid). One hour later, animals were re-exposed to the conditioning context alone, without the tone. Animals were recorded on videotape for off-line manual scoring of freezing behavior. We previously repeatedly showed that such conditioning procedure normally leads to the identification of the conditioning context as the correct predictor of the threat (*i.e.* footshock) as animals display conditioned fear when re-exposed to the context but not to the discrete tone (Calandreau et al., 2006; Desmedt et al., 1999; Kaouane et al., 2012).

Conditioned fear to the context was assessed by measuring the percentage of total time spent freezing during the first 2 min period of context re-exposure. In order to assess conditioned fear to the tone, a freezing ratio was calculated as follow: [% freezing during the tone presentation − (% pre-tone period freezing + % post-tone period freezing)/2]/[% freezing during the tone presentation + (% pre-tone period freezing + % post-tone period freezing)/2]. Two different observers performed the measurements independently and blinded of experimental groups (EAE, n = 12 and CFA, n = 12).

Slowing of information processing speed is frequent in multiple sclerosis (Chiaravalloti and DeLuca, 2008) and can confound memory tests. Thus, we reasoned that EAE-mice could need more time than CFA-mice to process and memorize a new context independently of hippocampal dysfunction. To disentangle these phenomena, we designed a new experimental procedure where the conditioning period was longer: 130 s into the chamber, first shock, 40 s delay, first tone, 50 s delay; second tone, 50 s delay, second shock, 20 s and back to the home cage. Tone, shock, context parameters and testing conditions were the same as described above (EAE, n = 12 and CFA, n = 12).

2.3. Magnetic resonance imaging: acquisition and analyses

The afternoon following contextual fear conditioning, the same animals underwent MRI exploration. Imaging was performed on a 4.7T scanner (Biospec 47/20, Brucker) equipped with a high-performance gradient system (capable of 660 mT/m maximum strength and 110 μs rise time). A 86 mm-diameter volume coil