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HIPPOCAMPAL FUNCTION IS COMPROMISED IN AN ANIMAL MODEL OF MULTIPLE SCLEROSIS

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Abstract—Multiple sclerosis (MS) is a progressive inflammatory autoimmune disease that is characterized by demyelination and axonal damage in the nervous system. One obvious consequence is a cumulative loss of muscle control. However, cognitive dysfunction affects roughly half of MS sufferers, sometimes already early in the disease course. Although long-term (remote) memory is typically unaffected, the ability to form new declarative memories becomes compromised. A major structure for the encoding of new declarative memories is the hippocampus. Encoding is believed to be mediated by synaptic plasticity in the form of long-term potentiation (LTP) and long-term depression (LTD) of synaptic strength. Here, in an animal model of MS we explored whether disease symptoms are accompanied by a loss of functional neuronal integrity, synaptic plasticity, or hippocampus-dependent learning ability. In mice that developed $MOG_{35–55}$-induced experimental autoimmune encephalomyelitis (EAE), passive properties of CA1 pyramidal neurons were unaffected, although the ability to fire action potentials became reduced in the late phase of EAE. LTP remained normal in the early phase of $MOG_{35–55}$-induced EAE. However, in the late phase, LTP was impaired and LTP-related spatial memory was impaired. In contrast, LTD and hippocampus-dependent object recognition memory were unaffected. These data suggest that in an animal model of MS hippocampal function becomes compromised as the disease progresses.

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Key words: LTP, LTD, EAE, object recognition, spatial memory, inflammation.

INTRODUCTION

Hippocampal function, both at the level of learning and synaptic plasticity, can be affected in neurodegenerative diseases, as well as in animal models of neurodegen erative diseases (Kim et al., 2001; Rowan et al., 2003; Costa et al., 2012; Balu et al., 2012; Denney et al., 2005; Usdin et al., 1999). Multiple sclerosis (MS) is a progressive inflammatory, demyelinating, and ultimately neurodegenerative disease of the central nervous system (CNS) that is characterized by the progressive accumulation of lesions in the brain and the spinal cord. Pathological features of MS comprise inflammation, demyelination, axonal loss and gliosis (Frohman et al., 2005). The most typical symptoms are visual, motor, sensory and autonomic dysfunctions, as well as a wide range of symptoms related to lesions located in the CNS (Compston and Coles, 2002). A hallmark of MS is also cognitive decline, which occurs even in the absence of physical impairments, and affects many aspects of a patient's daily life and social status (Chiaravalloti and DeLuca, 2008). Patients with MS often experience long-term memory impairments (Gaudino et al., 2001; Drake et al., 2006), attention deficiency (Beatty et al., 1996), reduced information processing speed (Litvan et al., 1988) and impairment in executive functions (Denney et al., 2005). Inhibition of transcallosal impulse conduction has been postulated to underlie some of these changes (Schmierer et al., 2000). However, the underlying mechanisms remain largely unknown.

In rodents, experimental autoimmune encephalomyelitis (EAE) is used to model disease progression in MS. EAE mirrors MS-like pathology and complexity (Gold et al., 2006; Constantinescu et al., 2011) and mimics most of the histopathological, clinical and immunological features of the disease (Fletcher et al., 2010). Moreover, the pathogenesis of EAE resembles that of MS (Baker and Jackson, 2007; Constantinescu et al., 2011). EAE that is induced by myelin oligodendrocyte glycoprotein 35–55 peptide ($MOG_{35–55}$-induced EAE) is characterized by gliosis, demyelination, axonal and neuronal loss (Herrero-Herranz et al., 2008; Peruga et al., 2011). Given that clinical studies have reported cognitive decline that correlates with hippocampal atrophy in MS patients (Sicotte et al., 2008; Roosendaal et al., 2009), the present study explored whether neuronal circuit

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Abbreviations: aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; CFA, complete Freund's adjuvant; EAE, experimental autoimmune encephalomyelitis; fEPSP, field excitatory postsynaptic potential; I/O, input–output; LTD, long-term depression; LTP, long-term potentiation; MS, multiple sclerosis; OVA, ovalbumin; PP, paired-pulse; PTX, pertussis toxin; s.e.m., standard error of the mean; TBS, theta burst stimulation.

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responsiveness and cognitive functions are affected following $MOG_{35–55}$-induced EAE.

Here, we examined synaptic plasticity in the CA1 stratum radiatum, hippocampus-dependent learning and memory, and the physiological properties of CA1 pyramidal neurons in the EAE model. We observed that impairments in hippocampal synaptic plasticity and hippocampus-dependent memory occur in EAE. Specifically, we observed impaired long-term potentiation (LTP) and spatial memory in the late, but not in the early phase of EAE. However, long-term depression (LTD) and hippocampus-dependent object recognition memory (Eichenbaum et al., 1999; Manns and Eichenbaum, 2009; Brown et al., 2012) were unaffected. Passive properties of CA1 pyramidal neurons were unaffected, although the ability to fire action potentials became reduced in the late phase of EAE, indicating that neuronal transmission becomes impaired. Taken together, our data suggest that as MS progresses hippocampal function becomes directly compromised. Given the presumed role of LTP in memory formation (Kemp and Manahan-Vaughan, 2007), the deficits that occur in hippocampal LTP may underlie memory-related cognitive deficits that occur in MS patients.

EXPERIMENTAL PROCEDURES

The present study was carried out in accordance with the European Communities Council Directive of September 22nd 2010 (2010/63/EEC) for care of laboratory animals and after approval of the local government ethics committee (Bezirksamt, Arnsberg). All efforts were made to minimize the number of animals used.

Active induction of EAE

EAE is a $CD4^+$ T cell-mediated autoimmune disease that generates a mouse model of MS (Miller et al., 2007), in which animals progressively exhibit motor disabilities and abnormalities that are similar to changes observed in MS patients. EAE is characterized by an early phase and a late phase (Fig. 1).

Following seven days of acclimatization to the animal housing facility, 8–10-week-old female C57BL/6 mice were anaesthetized with Ketamin/Xylazin and subcutaneously immunized with 100 µg of myelin oligodendrocyte glycoprotein ($MOG_{35–55}$) peptide (Institute for Medical Immunology, Charite, Berlin, Germany) that is a target antigen that leads to autoimmune-mediated demyelination (Berger et al., 2003). MOG was emulsified with 100 µg of complete Freund's adjuvant (CFA) containing *Mycobacterium tuberculosis* at a final concentration of 1 mg/ml. Additionally, mice received an intraperitoneal injection of 100 ng of pertussis toxin (PTX) (Sigma, St. Louis, MO, USA) on days 0 and 2 post immunization. CFA augments the effects of MOG and expedites the onset of symptoms (Freund, 1947; Gold et al., 2006). PTX is used to make the blood–brain barrier more permeable and thus increase the effectiveness of the immunization with MOG (Linthicum et al., 1982; Yong et al., 1993).

One control group of mice was immunized with ovalbumin (OVA)/CFA emulsion (100 µg) together with PTX (200 ng), whereas a second control group was immunized with OVA/CFA emulsion only. This was done so that we could exclude that any possible effects detected in hippocampal function were not caused by changes in the blood–brain barrier (elicited by PTX) and/or the presence of the adjuvant, CFA, together with an antigen. The antigen, OVA is not a myelin-associated protein, thus served as a control for MOG.

The mice were monitored for weight-loss and clinical symptoms of EAE on a daily basis. The immunization with $MOG_{35–55}$ peptide results in an ascending paralysis usually starting after 10 days and reaching its maximum within one week. According to the symptoms, the progression of the disease was assessed using a disability scale ranging from 0 to 10 (Hartung et al., 1988). This was scored in the following way: 0 = normal, 1 = less lively, 2 = impaired righting/limp tail, 3 = absent righting, 4 = ataxic gait, abnormal position, 5 = mild paraparesis, 6 = severe paraparesis, 7 = tetraparesis, 9 = moribund, 10 = death.

The early phase of EAE (between days 8 and 19 after immunization) is characterized by severe motor disabilities. Starting with the loss of tail tone, the first motor disabilities of EAE typically appear 8–12 days after immunization (Peruga et al., 2011; Zorzella-Pezavento et al., 2013). The peak of clinical signs appears between days 14 and 19 after immunization, i.e. when clinical symptoms are maximally severe. In the current experiments, we assessed early EAE effects in mice 14 and 19 days after immunization. The maximal disability score (Hartung et al., 1988) we observed was three or four.

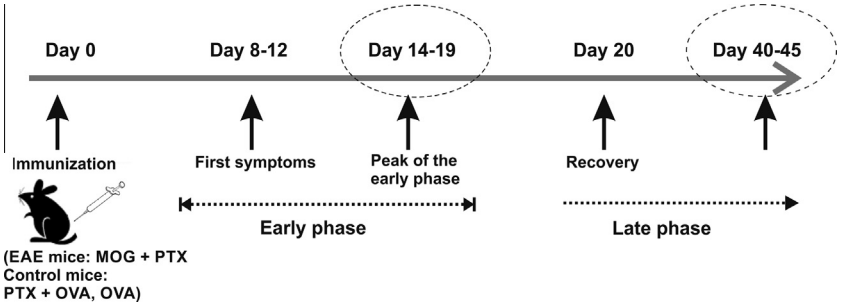

Fig. 1. Schematic illustration of the EAE progression. Two phases of the EAE course were distinguished – the early and the late phase. Mice that were in the early phase of EAE (between days 14 and 19 post immunization) as well as mice that were in the late phase of EAE (between days 40 and 45 post-immunization) were used for the experiments (indicated by dashed circle lines).