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Altered cognitive-emotional behavior in early experimental autoimmune encephalitis – Cytokine and hormonal correlates

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ABSTRACT

Multiple sclerosis (MS) is often associated with co-morbid behavioural and cognitive impairments; however the presence of these symptoms does not necessarily correlate with neurological damage. This suggests that an alternate mechanism may subserve these impairments relative to motor deficits. We investigated whether these abnormalities could be studied in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. In myelin oligodendrocyte glycoprotein peptide (MOG_{35-55})-induced EAE mice, no motor deficits were observed until d9 after immunization. This enabled us to carry out a series of neurobehavioral tests during the presymptomatic stage, between d6 and d8 post-immunization. EAE mice spent more time in the outer zone in an open field test and in the closed arms of an elevated plus maze and, showed decreased latency for immobility in the tail suspension and forced swim tests and reduced social interaction compared with controls. These results are indicative of anxiety- and depression- like behavior. In addition, EAE mice appeared to exhibit memory impairment compared to controls based on their reduced time spent in the target quadrant in the Morris water maze and their faster memory extinction in the fear conditioning test. No demyelination, microglial activation or astrogliosis was observed in the brain at this early stage. Transcript analysis by RT-PCR from d6 to d8 brain revealed elevated interleukin (IL)-1β and TNF-α in the hypothalamus but not in the amygdala or hippocampus of EAE mice. Lastly, plasma corticosterone levels increased in EAE mice compared to controls. In conclusion, emotional and cognitive deficits are observed in EAE prior to demyelination and are associated with elevated IL-1β and TNF-α in the hypothalamus and changes in the hypothalamic–pituitary–adrenal axis.

1. Introduction

Multiple sclerosis (MS) is a debilitating neurological disease involving motor paralysis that affects thousands of individuals. It is characterized by inflammatory demyelination and axonal loss resulting in multifocal sclerotic plaques in the CNS (Compston and Coles, 2002; Lassmann et al., 2007). In addition to the physical impairments, MS is also associated with co-morbid behavioral, neuropsychiatric and cognitive impairment (Miller, 2012). For example, 50% of MS patients suffer from depression (Paparrigopoulos et al., 2010) and cognitive deficits (Jongen et al., 2012); anxiety affects more than 35% and chronic fatigue affects 95% (Amato and Portaccio, 2012) of MS patients. Anti-depressants are used to treat these symptoms, but they have limited effectiveness and compliance (Janardhan and Bakshi, 2002; Lobentanz et al., 2004). Although symptoms of fatigue, anxiety, depression and memory loss are very common, they are poorly understood, often under-diagnosed and frequently under-treated. Interestingly, these behavioral co-morbidities often do not correlate with physical impairments (Janardhan and Bakshi, 2002; Janssens et al., 2003; Lobentanz et al., 2004), raising an interesting possibility that a mechanism independent of demyelination and axonal damage may be at play.

The mechanism(s) underlying the development of MS are not clearly understood. However, inflammation is a cardinal feature of the disease (Martino et al., 2002, 2000) and both adaptive and innate immune systems appear to play an important role. Peripheral inflammation can result in a secondary, cytokine-mediated mirror response in the CNS, even in the absence of overt CNS damage (Nadeau and Rivest, 2001; Rivest, 2001). In particular, IL-1β, IL-6 or TNF-α in the brain are thought to be responsible for many of the sickness behaviors (Cartmell et al., 2001; Kent et al., 1992; Luheshi et al., 1996) associated with peripheral inflammation through their interactions with neurotransmitters, receptors and biosynthetic enzymes (Dunn, 2006; O'Connor et al., 2009; Schafers and Sorkin, 2008). They may also predispose or facilitate degenerative (Mangano and Hayley, 2009) and depressive disorders (Anisman et al., 2005; Dantzer et al., 2008). There are parallel anti-inflammatory mechanisms activated in both the brain and

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periphery to protect the CNS from uncontrolled cytokine signaling (Lidow et al., 2001; Tatro, 2000). Among several of these molecules, glucocorticoids are released following HPA activation and act both peripherally and centrally to reduce inflammation (Coelho et al., 1995; Linthorst et al., 1999; Morrow et al., 1996). However, excessive exposure to glucocorticoids itself is known to produce dramatic effects on cognitive and emotional behavior, often resembling what is seen in the depressed state (Bodnoff et al., 1995; Fernandes et al., 1997; Hill et al., 2003).

Given the impact and high incidence of neuropsychiatric impairments in MS, and lack of proper therapeutics, we have investigated these behavioral co-morbidities in an inflammatory animal model of MS, experimental autoimmune encephalomyelitis (EAE) (Constantinescu et al., 2011; Lassmann, 2007). EAE recapitulates several cardinal features of MS, including motor paralysis, weight loss, demyelination, and inflammation in the CNS. Even though this model has been well characterized in terms of immune modulation, little is known about possible behavior co-morbidities.

We hypothesized that inflammation, or the consequential increase in circulating glucocorticoids associated with EAE can lead to behavioral co-morbidities in MS, independent of demyelination in CNS. Therefore, we carried out behavioral tests in EAE mice prior to onset of motor dysfunction and determined the relationship of behavioral alterations to the inflammatory state in the periphery and in the hippocampus, amygdala and hypothalamus, structures involved in emotional and cognitive behaviors. Our data suggest an important role of immune and glucocorticoid changes in developing depression and memory deficits in the MOG_{35-55} EAE model, as they are altered in tandem with emotional-cognitive behaviors, but prior to the onset of motor symptoms. The finding that the EAE model of MS recapitulates the behavioral changes seen in the human condition suggest that this model could be used to explore the development of novel therapies for these co-morbidities.

2. Materials and methods

2.1. EAE induction in mice

All animal protocols were approved by the University of Calgary Ethics Committee. EAE was induced in 8–10 week old C57BL/6 female mice by subcutaneous immunization with 100 μg myelin oligodendrocyte glycoprotein (MOG_{35-33}) in emulsion mixed in a 1:1 volume with complete Freund's adjuvant (CFA, containing 4 mg/ml of heat killed *Mycobacterium tuberculosis*, Difco Laboratories). The mice were also injected intraperitoneally with 800 ng *Bordetella pertussis* toxin (PTX, List Biological Laboratories Inc.) in 1X Phosphate Buffered Saline (PBS) at the time of, and 2 days following immunization (Ousman et al., 2007). Mice injected with CFA and PTX were used as controls (referred to as CFA from here on). Animals were scored daily for assessment of motor deficits associated with EAE after disease induction based on the following scoring: 0-no disease, 1-limp tail, 2-hindlimb weakness, 3-complete hindlimb paralysis, 4-hindlimb + forelimb paralysis, 5-moribund or dead.

2.2. Behavior tests

All the behavior tests were performed between d6 and d8; motor performance was determined at this time using the open field and rotarod (Smith et al., 2007) test. In general, animals were tested in groups of 5, repeated twice or thrice (to account for cohort effect), to give an '*n*' of at least 10 each. Where tests were less 'stressful', for example open field test, the less stressful test occurred on day 7 and a more stressful test (e.g. tail suspension test) on day 8. Since we tested on pre-symptomatic mice, we wanted to make sure that the EAE mice displayed paralytic behaviour (as a validation for the EAE model). The mice that did not get paralysis were eliminated from the data set *post hoc*. Thus in some cases, *n* fell below 10, as indicated in Table 1. This table also shows which cohorts were used for each behavioral experiment.

Table 1
Distribution of cohorts for different behavioural experiments.

Cohort	Test performed	Order of testing	Number of animals used in the test	
			CFA	EAE
Cohort 1 and 2	Open field test	Day 7 p.i.	10	7
	Tail suspension test	Day 8 p.i.		
Cohort 3 and 4	Elevated plus maze test	Day 7 p.i.	10	10
	Forced swim test	Day 8 p.i.	10	10
Cohort 5 and 6	Sociability test day	7 p.i.	10	10
Cohort 7 and 8	Water maze test day	6 p.i.	10	10
	Fear conditioning test	Day 7–8 p.i.	10	10
Cohort 9	Water maze test	Day 6 p.i.	8	6

2.2.1. Open field test

Mice were placed in the center of a 1 m wide × 1 m long × 30 cm high white box. The perimeter of the box was divided into three zones: inside (centre), middle and outer. The activity of mice in the box was digitally monitored and analyzed using SMART2.5 video tracking system (Panlab, Harvard Quebec, Canada).

2.2.2. Elevated plus maze test

The elevated plus maze apparatus consisted of a central platform, two opposed open arms and two opposed closed arms of same size, but with opaque walls of 15 cm height. The apparatus was elevated 50 cm above the surface. Each mouse was placed in the central platform and its activity was video recorded. The video recordings were then analyzed for numbers of entries and time spent in open and closed arm.

2.2.3. Forced swim test

A 2 L acrylic glass beaker was filled with 1.5 L water at 30 ± 1 °C. A mouse was placed in the water for a 6-min period and its activity in the cylinder was video-recorded. A mouse was judged to be immobile when it remained floating in the water, making only those movements necessary to keep its head above water.

2.2.4. Tail suspension test

A mouse was suspended from an elevated surface by an adhesive tape placed approximately 1 cm from the tip of the tail for 6 min and its activity was video-recorded. The mouse was considered immobile when it ceased to struggle and remained without any body movement.

2.2.5. Sociability test

A sociability test was carried out in a 40 cm wide × 20 length × 20 cm wide cm three-chamber system. The right and left chambers (each 10 cm wide) were separated from the middle chamber (20 cm wide) by a 1 cm plexiglass wall which has an opening for the mouse to enter or leave either chamber. The test mouse was habituated by putting it in the middle chamber for 5 min. For the actual test, a control mouse was placed in the right or left of the chamber inside a wire mesh and an empty wire mesh was put in the opposite side. The test mouse was then placed in the middle chamber and its activity was monitored for 5 min by video recording. The number of entries and the time spent in the chamber with the control mouse was measured.