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Loss of T cells influences sex differences in behavior and brain structure

Kelly C. Rilett [a], Miriam Friedel [c], Jacob Ellegood [c], Robyn N. MacKenzie [b], Jason P. Lerch [c,d], Jane A. Foster [b,e,*]

[a] Neurosci. Grad Program, McMaster Univ., Hamilton, ON, Canada
[b] Psychiatry & Behavioural Neurosciences, McMaster Univ., Hamilton, ON, Canada
[c] Mouse Imaging Ctr., Hosp. for Sick Children, Toronto, ON, Canada
[d] Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada
[e] Brain-Body Institute, St. Joseph's Healthcare, Hamilton, ON, Canada

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ABSTRACT

Clinical and animal studies demonstrate that immune–brain communication influences behavior and brain function. Mice lacking T cell receptor β and δ chains were tested in the elevated plus maze, open field, and light–dark test and showed reduced anxiety-like behavior compared to wild type. Interestingly sex differences were observed in the behavioural phenotype of *TCR*$\beta-/-\delta-$ mice. Specifically, female *TCR*$\beta-/-\delta-$ mice spent more time in the light chamber compared to wild type females, whereas male *TCR*$\beta-/-\delta-$ spent more time in the center of the open field compared to wild type males. In addition, *TCR*$\beta-/-\delta-$ mice did not show sex differences in activity-related behaviors observed in WT mice. *Ex vivo* brain imaging (7 Tesla MRI) revealed volume changes in hippocampus, hypothalamus, amygdala, periaqueductal gray, and dorsal raphe and other brain regions between wild type and T cell receptor knockout mice. There was also a loss of sexual dimorphism in brain volume in the bed nucleus of the stria terminalis, normally the most sexually dimorphic region in the brain, in immune compromised mice. These data demonstrate the presence of T cells is important in the development of sex differences in CNS circuitry and behavior.

1. Introduction

The importance of sex differences to clinical and behavioural neuroscience has moved to the forefront. An emerging body of literature reveals striking sex differences in prevalence, vulnerability to disease, age of onset, and course of psychiatric disorders (Harkness et al., 2010; Keers and Aitchison, 2010; McLean and Anderson, 2009; Rutter et al., 2003; Vesga-Lopez et al., 2008). For example, the lifetime risk for major depressive disorder (MDD) is twice as high for females compared to males and the risk for MDD increases dramatically after puberty, particularly in females (Essau et al., 2010; Hankin et al., 1998). In addition, distinct clinical features are displayed by men and women across mood and anxiety-disorders related to incidence, age of onset, and clinical presentation (Earls, 1987; Seeman, 1997; Steiner et al., 2005; Vesga-Lopez et al., 2008). Despite recent attention to sex differences in both psychiatric and neuroscience research, there is limited information about the biological factors that influence sex differences in neurodevelopment. In the present study, we examined sex differences in behavior and in brain structure in immune compromised mice.

Researchers in psychiatry and behavioural neuroscience are increasingly recognizing the importance of the adaptive immune system in behavior. For example, lymphocytes have been shown to influence anxiety-like behaviors in mice (Cushman et al., 2003; Oliveira-Dos-Santos et al., 2000). Despite the evidence of sexually dimorphic immune functioning (Da Silva, 1999; De

Abbreviations: 6Cb, lobule 6 of the cerebellar vermis; 10Cb, lobule 10 of the cerebellar vermis; Pr, prepositus nucleus; 4V, 4th ventricle; PM, paramedian lobule; V2, secondary visual cortex; Aq, cerebral aqueduct; PAG, periaqueductal gray; PDR, posterodorsal raphe nucleus; CEnt, caudomedial entorhinal cortex; PnO, pontine reticular nucleus oral part; DG, dentate gyrus; DLG, dorsal lateral geniculate nucleus; LHbL, lateral habenular nucleus, lateral; Re, reunions thalamic nucleus; 3V, third ventricle; ic, internal capsule; VM, ventromedial thalamic nucleus; PHD, posterior hypothalamic area, dorsal; VMH, ventromedial hypothalamic nucleus; MePD, medial amygdaloid, posterodorsal part; S1, primary somatosensory cortex; M1, primary motor cortex; A24, cingulate cortex, area 24; CPu, caudate putament (striatum); BNST, bed nucleus of the stria terminalis; LV, lateral ventricle; CxA, cortex-amygdala transition zone.

* Corresponding author at: Department of Psychiatry and Behavioural Neurosciences, McMaster University at St. Joseph's Healthcare, 50 Charlton Ave. E, T3308, Hamilton, ON L8N 4A6, Canada. Tel.: +1 905 522 1155x35993; fax: +1 905 540 6593.

E-mail address: jfoster@mcmaster.ca (J.A. Foster).

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Leon-Nava et al., 2009; Weinstein et al., 1984), little attention has been given to a possible role for immune–brain crosstalk in sex differences in behavior. Recently, we observed loss of sexual dimorphism in activity, exploratory behaviors in mice lacking both β-2microglobulin and transporter associated with antigen processing genes (*β2M−/−TAP−/−*) (Sankar et al., 2012). Loss of both β2M and TAP proteins leads to a functional loss of class I major histocompatability (MHC) molecules and depletion of cytotoxic CD8+ T cells (Ljunggren et al., 1995). The interpretation of these data is confounded by the findings that expression of class I MHC is expressed throughout the developing brain and in key regions of the adult brain including regions that remain plastic throughout life such as the hippocampus and olfactory bulb (Corriveau et al., 1998; Huh et al., 2000; Linda et al., 1999; Oliveira et al., 2004a) and has been linked to normal neuronal development and activity-dependent neuronal plasticity (Glynn et al., 2011; Huh et al., 2000; McConnell et al., 2009; Needleman et al., 2010; Oliveira et al., 2004b). We therefore sought to test the hypothesis that T lymphocytes influences sex differences in behavior in mice lacking all functional T cells through knockout of the T cell receptor β and δ chains (*TCRβ−/−δ−/−*).

In parallel we used magnetic resonance imaging to determine the relationship between loss of functional T cells and neuroanatomical structure. High resolution imaging in mouse models has become increasingly useful in examining subtle differences or changes in the neuroanatomy at the mesoscopic scale. Studies have shown that with MRI one can detect highly significant differences in the neuroanatomy after only 5 days of training in the Morris Water Maze (Lerch et al., 2011b) as well as over the course of the estrous cycle (Qiu et al., 2013). Further, mice with known behavioural phenotypes are often found to have a corroborating neuroanatomical phenotype (Nieman et al., 2007). Several disease models have also been examined including Huntington's disease (Lerch et al., 2008; Zhang et al., 2010), Alzheimer's disease (Cramer et al., 2012; Lau et al., 2008), Parkinson's disease (Goldberg et al., 2005), and autism (Ellegood et al., 2011, 2012, 2013). While several groups have examined behavior in immune compromised mice (Clarke et al., 2013; Cushman et al., 2003; Heijtz et al., 2011; Kipnis et al., 2004; Neufeld et al., 2011a,b), to the best of our knowledge no studies have examined how loss of adaptive immune function influences brain structure or circuitry. Here we examined volumetric changes in T cell deficient mice (*TCRβ−/−δ−/−*) compared to C57Bl/6 wild type mice using MRI.

2. Methods

2.1. Animals

T cell deficient mice on C57Bl/6 background, *TCRβ−/−δ−/−* mice, characterized by a loss of T cell receptor function by double knock-out of the β and δ chains (Mombaerts et al., 1994), were obtained from Dr. Andrew McPherson at McMaster University and breeding colonies maintained at the central animal facility at McMaster University. Adult B cell deficient mice on C57Bl/6 background, *Ighm−/−* mice, lacking the immunoglobulin heavy constant μ gene, were purchased from Jackson Labs (Bar Harbor, Maine). WT (C57Bl/6) mice were purchased from either Taconic (T cell match, Mississauga, ON) or Jackson Labs (B cell match, Bar Harbor, Maine). Mice were housed in ventilated racks Four independent experiments were carried out on separate cohorts of mice (Table 1). Experiment 1 – Immune phenotyping: Adult male and female *TCRβ−/−δ−/−* mice and age (8–10 weeks) and sex-matched wild type (WT; C57Bl/6) mice ($n = 8$ per sex per genotype) were included. Experiment 2a – Behavioral studies: Adult male ($n = 26$ per genotype) and female ($n = 21$ per genotype) *TCRβ−/−δ−/−* mice and age (14–16 weeks) and sex-matched WT (C57Bl/6) mice were included in the analysis. Experiment 2b – Behavioral studies: Adult (10–12 weeks) male and female ($n = 12$ per sex per genotype) B cell deficient mice, *Ighm−/−* mice, lacking the immunoglobulin heavy constant μ gene, and age and sex-matched WT mice were included in the analysis. Experiment 3 – Imaging study: Adult male ($n = 9$ per genotype) and female ($n = 8$–9 per genotype) *TCRβ−/−δ−/−* mice and age (P60) and sex-matched wild type (WT; C57Bl/6) mice were included in the analysis. All experimental procedures were approved by the Animal Research Ethics Board, McMaster University in accordance with the guidelines of the Canadian Council on Animal Care.

Table 1
Overview of experiments.

Experiment	Mice	Number of mice			
		WT ♂	*TCR/Ighm* ♂	WT ♀	*TCR/Ighm* ♀
1. Immunophenotype – FACS	WT vs *TCRβ−/−δ−/−*	8	8	8	8
2a. Behaviour – EPM, L/D, OF	WT vs *TCRβ−/−δ−/−*	21	21	26	26
2b. Behaviour – EPM, L/D, OF	WT vs *Ighm−/−*	11	12	12	12
3. Imaging – MRI	WT vs *TCRβ−/−δ−/−*	9	9	8	9

WT – C57Bl/6; *TCRβ−/−δ−/−* – T cell deficient; *Ighm−/−* – B cell deficient; EPM – elevated plus maze; L/D – light/dark test; OF – open field.

2.2. Flow cytometric analysis (FACS) of spleens and bone marrow

Spleens harvested at endpoint from experimental animals were processed via flow cytometric analysis to define their immune cell profiles. For each spleen, the tissue was pulverized and suspended in 1 ml FACS Buffer (FB; 1% Fetal Bovine Serum in 1% PBS). Bone marrow was collected from the femurs with a syringe containing 1% PBS and then transferred to FACS Buffer. Samples containing 10^6 cells per tube run in duplicate were prepared for each spleen and stained with 100 µl antibody solution for 30 min in the dark. T cells were stained for using CD3 antibody tagged with PE-A fluorophore (1:300 dilution; eBioscience, San Diego, CA). B cells were stained for using CD19 antibody tagged with PerCP-Cy5.5 fluorophore (1:300 dilution; eBioscience, San Diego, CA). Macrophages were stained for using F4/80 antibody tagged with APC-A fluorophore (1:200 dilution; eBioscience, San Diego, CA). In addition to the samples, compensation beads stained with 1 antibody (or no antibody) were included for FACS compensation. Finally, cells were resuspended in 200 µl FB and stored in the dark at 4 °C up to 2 days before flow cytometry was performed. Samples were run using the BD FACSCanto flow cytometer (San Jose, CA) connected to a computer on which data was analyzed using FlowJo (Ashland, OR).

2.3. Open field

Behaviour in the open field was measured at 14–16 weeks of age using the Kinder Scientific Smart Cage Rack System consisting of a 24 cm wide × 45 cm long × 24 cm high cage rack system, with 22 infrared beams (7 *X* & 15 *Y*) and a rearing option (22 additional beams). Beginning 1 week prior to behavioural testing and throughout the behavioural testing mice were handled 2 min each 3 times per week. Mice were tested in a non-colony room and were habituated to the room for 1 h prior to open field testing. Testing occurred in low light (100 lux). Behaviours were recorded for 60 min (6 mice in parallel units), using Kinder Scientific Motor Monitor software (Poway, CA).