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Research Report

Neocortical molecular layer heterotopia in substrains of C57BL/6 and C57BL/10 mice

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ABSTRACT

Abnormal development of the neocortex is often associated with cognitive deficits and epilepsy. Rodent models are widely used to study normal and abnormal cortical development and have revealed the roles of many important genetic and environmental factors. Interestingly, several inbred mouse strains commonly used in behavioral, anatomical, and/or physiological studies display neocortical malformations including C57BL/6J mice, which are among the most widely utilized mice. In the present report we describe the prevalence and cytoarchitecture of molecular-layer heterotopia in C57BL/6J mice and related strains obtained from three commercial vendors as well as mice bred in academic vivaria from founders obtained commercially. In particular, we found that the prevalence of molecular-layer heterotopia varied according to the sex as well as the vendor-of-origin of the mouse. These data are relevant to the use of this strain as a mouse-model in the study of brain–behavior relationships.

1. Introduction

The laminated cytoarchitecture of the neocortex emerges from the orderly proliferation and migration of neurons during corticogenesis (reviewed in Gupta et al., 2002). Developmental disorders affecting the laminar positioning of cortical pyramidal neurons can have dramatic effects on cognitive function and are often associated with epilepsy (Leventer et al., 2008; Spalice et al., 2009). Cortical lamination disorders include those with fewer neuronal lamina, inverted lamina, band or periventricular heterotopia, or phenotypes lacking lamination altogether (reviewed in Gupta et al., 2002; Olson and Walsh, 2002). Neocortical molecular layer heterotopia (MLH), for example, consisting of clusters of misplaced neurons found in the molecular layer (layer I) were observed in the brains of dyslexic patients (Galaburda and Kemper, 1979; Galaburda et al., 1985; Humphreys et al., 1990), emphasizing a link between cortical lamination defects and cognitive dysfunction. The defining behavioral phenotypes of dyslexia such as learning impairment may therefore result from altered

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neuronal migration and subsequent aberrant organization of neuronal connections (reviewed in Galaburda, 2005; Galaburda et al., 2006).

We previously demonstrated that a number of different inbred mice often display MLH and that these malformations can be found in a variety of regions throughout sensory and motor cortices (Ramos et al., 2008; see also Sherman et al., 1985, 1987, 1990). The cytoarchitectonic features of MLH observed in mice are identical to those described in humans, suggesting a common developmental origin. The similarity of MLH found in humans and mice offers the possibility for the use of these mice as a model of cortical malformations found in humans with cognitive deficits such as dyslexia.

Inbred mice are important research tools in neuroscience. C57BL/6 mice are among the most popular strains of inbred mice used in behavioral, anatomical, and physiological studies. A number of different commercial vendors sell C57BL/6 substrains and recent studies indicate that there are substrain differences in behavioral performance as well as anatomical and biochemical differences among the different vendors (Bryant et al., 2008; Matsuo et al., 2010; Prasad and Richfield, 2008). C57BL/6J mice from Jackson Laboratories exhibit MLH with a prevalence of approximately 30% (Ramos et al., 2008; Sherman and Holmes, 1999) however the presence of MLH in C57BL/6 mice from other vendors is unknown. In the present report we examine the prevalence of MLH in C57BL/6 substrains and related strains purchased from different commercial vendors as well as animals bred in academic vivaria. These data are relevant to diverse studies using these mice in the study of cortical development as well as brain and behavior relationships.

2. Results

2.1. *Identification of MLH*

A number of different histological methods can be used to identify MLH most of which are simple, fast, and inexpensive. For example, very large MLH can be observed on the surface of the intact postmortem brain with a dissection microscope (Ramos et al., 2008) or even the naked eye. However, lack of visible MLH on the surface of the brain is not sufficient to rule out the presence of MLH which is only possible after sectioning and microscopic examination.

The method of tissue sectioning appears to have no effect on the ability to identify MLH as studies using vibratome- (Ramos et al., 2008), cryostat- (Ramos et al., 2008), and microtome- (Sherman et al., 1990) sections have revealed MLH in a diverse list of mice. In most cases, MLH are visible after inspection of free-floating vibratome sections under a dissection microscope and with oblique illumination such as from a fiber-optic illuminator. In unstained sections, MLH appear as areas with increased “whiteness” in the molecular layer due to the presence of abnormal arranged myelinated axons in MLH. Myelinated bundles of axons beneath MLH are also visible in layers 2–6 using this imaging technique. Representative examples of serial vibratome sections imaged free-floating from a brain containing a hetertopion are found in Fig. 1(A–B). When these same sections were then stained for myelin using gold chloride, intensely labeled bundles of axons were clearly visible consistent with observations made prior to staining (Fig. 1C–D; see also Supplementary Fig. 1). Moreover, when adjacent sections from this same brain were Nissl-stained to reveal somata, labeled cells in the molecular layer unequivocally confirmed the presence of a heterotopion (Fig. 1E–F). These data point to the utility that initial imaging of unstained sections may have on the selection of specific sections containing MLH for subsequent staining (ex. immunocytochemistry, in situ hybrid).

All data reported below are from tissue where MLH were positively identified following Nissl staining. In many cases, adjacent sections from brains found to have MLH using Nissl staining were stained to reveal myelin. The use of both Nissl and myelin allowed for identification of even very small MLH which were always accompanied by abnormal bundling of myelinated axons at the site of and below heterotopia. Bundling of myelinated axons is not seen elsewhere in the dorsal neocortex. A representative example of such a small MLH with associated bundling of myelinated axons is shown in Supplementary Fig. 2. These data point to our ability to identify even small MLH which is critical in comparing the prevalence of heterotopia between different strains.

2.2. *Prevalence of MLH in C57BL/6 and C57BL/10 substrains*

The prevalence of MLH in the different strains examined was determined by calculating the percentage of animals with MLH for each substrain according to the sex of the mice and whether the mice were bred in academic or commercial vivaria. This analysis revealed varying prevalence of MLH depending on the sex, substrain, and vendor origin of the brains examined. As shown in Table 1, all but one of the strains examined displayed MLH. Heterotopia were observed in both C57BL/6J and C57BL/10J mice from The Jackson Laboratory replicating our previous results (Ramos et al., 2008). Moreover, when C57BL/10J mice were bred in our vivaria, we observed MLH in brains examined from progeny born in our colony. These data suggest that MLH can be found in mice bred in diverse breeding, housing, and/or husbandry conditions such as those found in academic vivaria compared with those found at commercial mouse vendors. Both male and female C57BL/10J mice bred in our vivaria (founders obtained from The Jackson Laboratory) exhibited MLH, extending our observations in a previous report where only male C57BL/10J and C57BL/6J mice we examined (Ramos et al., 2008). In particular, MLH were observed in 50% of female compared to 60% of male C57BL/10J mice.

All C57BL/6 substrains currently sold by commercial vendors arose from C57BL mice originally derived by C.C. Little in 1921 (reviewed in Egan et al., 2007; Zurita et al., in press). With this in mind, we examined brains from several C57BL/6 substrains from Taconic Farms and Charles River Laboratories in order to determine if these strains also exhibit MLH. As shown in Table 1, we observed MLH in the C57BL/6NBomTac strain (53.33%, 8/15 brains) as well as in C57BL/10SgSnAiTac mice (25%, 4/16 brains). We observed a MLH in only 1 female mouse of the C57BL/6NTac strain (6.25%, 1/16 brains) but none in 16 male mice examined from this same strain. Interestingly, when we established colonies of C57BL/