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Research Report

Differential development of central dopaminergic and serotonergic systems in BALB/c and C57BL/6J mice

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ABSTRACT

Although the etiology of autism is unclear, disruptions of the dopaminergic and serotonergic systems have been associated with the disorder. Based on behavioral differences observed in the BALB/c strain of mice in comparison to other strains, notably, C57BL/6J mice, it has been suggested that the BALB/c strain may serve as an animal model of autism. However, to date, most work investigating neural and behavioral abnormalities in this strain has been performed in adult animals. Therefore, the present study was conducted to examine the development of the central dopaminergic and serotonergic systems of BALB/c mice as compared to C57BL/6J mice. Levels of dopamine, serotonin, and their metabolites in several different brain regions and at three ages during development were measured. Alterations in both monoaminergic systems associated with age and strain were detected across brain regions indicating that there are neurochemical differences between these strains early in life. However, despite these differences in the development of brain monoaminergic systems, it remains difficult to declare this strain as a valid model of autism.

1. Introduction

Impairments in social behaviors are the major diagnostic feature of autism and include an inability to establish peer relationships, demonstrate empathy, or interpret body language (APA, 2000). These are the most challenging autistic symptoms to treat either pharmacologically or behaviorally (Gerlai and Gerlai, 2004; McDougle et al., 2005). Additional behavioral symptoms of autism include increases in anxiety, aggression, and self-injurious behavior, and it is these secondary symptoms that are often alleviated following pharmacological intervention. Unfortunately, the neuropathology of the social deficits underlying autism is not well understood.

Individuals with autism appear to have disruptions in dopaminergic and serotonergic activity, and these disruptions could play a part in mediating some of the behavioral symptoms of the disorder. With respect to the dopaminergic system, dopamine antagonists alleviate the hyperactivity, stereotypies, aggression, and self-injurious behavior while, in contrast, drugs that increase dopamine activity exacerbate those symptoms (Volkmar, 2001). Collectively, these pharma-

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Abbreviations: HPLC, high-performance liquid chromatography; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, Serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; EDTA, ethylenediamine tetraacetic acid

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cological observations may indicate that autistic individuals have dopaminergic overactivity (Lam et al., 2006). In addition to the pharmacological evidence in support of dopamine's role in autism, mothers of autistic children are twice as likely to have two missing alleles for dopamine-beta-hydroxylase enzyme, which results in chronic, high maternal levels of dopamine (Robinson et al., 2001).

With respect to the serotonergic system, about 30% of the individuals with autism have platelet hyperserotonemia (Schain and Freedman, 1961); more than 25 studies have confirmed that this platelet hyperserotonemia appears in roughly one third of all individuals diagnosed with autism (Lam et al., 2006). In autistic individuals who have hyperserotonemia, the platelet serotonin is approximately 50% higher than in control subjects (McBride et al., 1998). In addition, several autism susceptibility genes that have been identified code for proteins that are known to be involved in the regulation of serotonin transport (SLC6A4 and 5-HTTLPR) or serotonin receptors (HTR2A) (Guhathakurta et al., 2009). Finally, some individuals with autism have been shown to have altered serotonin synthesis capacity as compared to nonautistic children (Chugani et al., 1999).

Despite the fact that one third of the individuals with autism have platelet hyperserotonemia, there has been no evidence to suggest that there are increases in brain levels of serotonin. In fact, several pieces of evidence suggest that individuals with autism may actually have decreased central serotonergic activity. McDougle et al. (1996) have shown that depleting brain levels of tryptophan leads to an increase in autistic symptoms. In addition, the most commonly prescribed treatment for autistic symptoms are the serotonin selective reuptake inhibitors which have been shown to reduce repetitive thoughts and actions, aggressive behavior, and improve some elements of social behavior (Brodkin et al., 1997; West et al., 2009).

Through the use of animal models, some insight into the biological basis of the behavioral deficits associated with autism may be ascertained. The use of the BALB/c mouse strain could be valuable, as it has been asserted that, relative to other strains, these mice exhibit behavioral characteristics similar to those seen in autism. For example, using a social conditioned place preference paradigm, Panksepp and Lahvis (2007) demonstrated that BALB/c mice are less responsive to social contact than several other inbred mouse strains, including C57BL/6J mice. Similarly, Southwick and Clark (1968) found that, of 14 inbred mouse strains, BALB/c mice showed the least amount of social grooming. Differences in sexual behavior, as well as maternal behavior, may also be indicative of decreased social interactions in this strain. Shoji and Kato (2006) showed that BALB/c mothers had reduced nursing posture, pup licking, and slower time to retrieve pups than CBA/CA inbred mice. In addition, in a review of mouse social behavior by Crawley et al. (1997), BALB/c mice exhibited the lowest amount of male copulatory behaviors with estrus females. Finally, BALB/c mice also exhibit other features that resemble autism including high levels of aggression and anxiety, large brain size, underdevelopment of the corpus callosum, and low adult levels of brain serotonin (Brodkin, 2007).

Because BALB/c mice exhibit behavioral deficits seen in autism, and monoamine disruption may have a role in mediating those deficits, an understanding of the neurochemistry of BALB/c mice may shed light on the neuropathology of this disorder. Therefore, the objective of the present study was to determine if there were differences between BALB/c and C57BL/6J mice in the development of brain serotonergic and dopaminergic systems. More specifically, evidence of dopaminergic overactivity and/or serotonergic hypoactivity was expected in the BALB/c pups as compared to the C57BL/6J pups.

2. Results

2.1. *Cerebellum*

Neurochemical analysis of the cerebellum revealed a significant effect of age as well as a strain × age interaction on dopamine levels [$F(2,105)=21.487$, $p<.0001$, and $F(2,105)=5.926$, $p=.0036$, respectively] (Fig. 1 and Table 1A). With respect to age, *post hoc* analysis revealed that cerebellar concentrations of dopamine in both strains decreased as the animals matured. Specifically, in BALB/c mice, dopamine concentrations declined significantly on P10 and P30 as compared to the concentrations observed on P3. Likewise, in C57BL/6J mice, dopamine concentrations declined on P30 as compared to P3. With respect to strain, *post hoc* analysis revealed that BALB/c mice had significantly more cerebellar dopamine on P3 as compared to C57BL/6J mice. Neurochemical analysis of DOPAC levels revealed no changes over age or strain. However, HVA analysis revealed significant effects of age and strain [$F(2,105)=6.612$, $p=.002$, and $F(1,105)=5.926$, $p=.0.0443$, respectively]. *Post hoc* tests indicated that in C57BL/6J mice, HVA concentrations decreased significantly on P30 as compared to both P3 and P10. In BALB/c mice, HVA levels increased significantly on P10 from concentrations on P3 and decreased significantly on P30 as compared to P10. With respect to strain, *post hoc* analysis revealed that BALB/c mice had significantly less HVA on P3 than C57BL/6J mice. As for turnover rates, cerebellar DOPAC/DA turnover showed a significant effect of age [$F(2,105)=7.644$, $p=.0008$], as turnover increased significantly on P30 for both strains, as compared to both P3 and P10. Neurochemical analysis of HVA/DA turnover, on the other hand, did not show an effect of age, but did reveal an effect of strain [$F(1,105)=4.468$, $p=.0369$]. *Post hoc* analysis showed that BALB/c mice had a significantly lower HVA/DA turnover than C57BL/6J mice on P3.

Neurochemical analysis of the cerebellum also revealed significant effects of age and strain on serotonin levels [$F(2,105)=15.20$, $p<.0001$ and $F(1,105)=5.16$, $p=.02$, respectively] (Fig. 2 and Table 1B). With respect to age, animals of both strains had significantly less serotonin at both P10 and P30 compared to P3. With respect to strain, *post hoc* analysis revealed that on P10, BALB/c pups had significantly less serotonin than C57BL/6J pups. However, by P30, BALB/c and C57BL/6J pups no longer had statistically different levels of serotonin. There was also a significant age effect for 5-HIAA, with 5-HIAA levels decreasing with age. BALB/c mice showed a statistically significantly decrease in 5-HIAA at each age, while 5-HIAA levels in C57BL/6J mice only showed a significant decrease between P10 and P30. Finally, there were significant strain and age effects for serotonin turnover [$F(1,105)=8.32$, $p=.004$ and $F(2,105)=25.53$, $p<.0001$, respectively]. *Post hoc* analysis revealed that BALB/c