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Altered cerebellar organization and function in monoamine oxidase A hypomorphic mice

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ABSTRACT

Monoamine oxidase A (MAO-A) is the key enzyme for the degradation of brain serotonin (5-hydroxytryptamine, 5-HT), norepinephrine (NE) and dopamine (DA). We recently generated and characterized a novel line of MAO-A hypormorphic mice (MAO-A^{Neo}), featuring elevated monoamine levels, social deficits and perseverative behaviors as well as morphological changes in the basolateral amygdala and orbitofrontal cortex. Here we showed that MAO-A^{Neo} mice displayed deficits in motor control, manifested as subtle disturbances in gait, motor coordination, and balance. Furthermore, magnetic resonance imaging of the cerebellum revealed morphological changes and a moderate reduction in the cerebellar size of MAO-A^{Neo} mice compared to wild type (WT) mice. Histological and immunohistochemical analyses using calbindin-D-28k (CB) expression of Purkinje cells revealed abnormal cerebellar foliation with vermal hypoplasia and decreased in Purkinje cell count and their dendritic density in MAO-A^{Neo} mice compared to WT. Our current findings suggest that congenitally low MAO-A activity leads to abnormal development of the cerebellum.

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1. Introduction

Monoamine neurotransmitters, including serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE), are known to exert a profound impact on early brain development through the regulation of neurogenesis, migration, differentiation, plasticity and other key morphogenetic processes (Azmitia, 2001; Gaspar et al., 2003; Schultz, 2007; Thompson and Stanwood, 2009).

Monoamine oxidase A (MAO-A) is the key enzyme that catalyzes the oxidative degradation of 5-HT and NE (Bortolato et al., 2008; Shih et al., 1999). Congenital deficiency of MAO-A results in high brain levels of 5-HT and NE (Cases et al., 1995), neurodevelopmental abnormalities of the cortex and other brain regions (Cases et al., 1996; Kim et al., 1997; Upton et al., 1999), as well as overt aggression and other emotional alterations (Cases et al., 1995).

We recently generated MAO-A^{Neo} mice, a line of MAO-A hypomorphic mutants. These transgenic animals harbor a neomycin-resistance cassette in intron-12 of the *Maoa* gene, which by alternative splicing of the *Maoa* mRNA, leads to a reduction in the levels of the functional MAO-A enzyme. MAO-A^{Neo} mice showed undetectable MAO-A enzymatic activity in the hippocampus and midbrain that was accompanied by high levels of 5-HT and NE, while low levels of MAO-A enzymatic activity in the prefrontal cortex and amygdala was accompanied by normal 5-HT levels and

Abbreviations: 5-HT, serotonin; ASD, Autism spectrum disorder; DA, Dopamine; CB, Calbindin; MAO-A, Monoamine Oxidase A; MAO-A^{Neo}, MAO-A hypomorphic mice; NE, Norepinephrine; PC, Purkinje cell; SSRI, selective serotonin reuptake inhibitor; WT, wild type.

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high NE levels. Notably, these animals exhibited a unique behavioral phenotype, different than their wild type (WT) and MAO-A knockout (KO) counterparts (Bortolato et al., 2011). In particular, we found that these mice exhibited social deficits and perseverative responses (Bortolato et al., 2011), two common traits observed in autism–spectrum disorder (ASD). The possibility that MAO-A hypomorphism may induce ASD-related alterations is in line with previous findings documenting monoaminergic dysregulations that have been implicated in these and other neurodevelopmental disorders (Simpson et al., 2011; Whitaker-Azmitia, 2005; Winter et al., 2008).

Neuropathological alterations of the cerebellum are among the most consistent morphological aberrances found in ASD subjects (Bailey et al., 1998; Bauman and Kemper, 1985; Kemper and Bauman, 1993; Ritvo et al., 1986; Whitney et al., 2008a). In particular, 72% of ASD cases reported in the literature exhibited a decreased number of Purkinje Cells (PCs) in the cerebellar cortex (Palmen et al., 2004). Furthermore, reports have also shown a decrease in the size of cerebellar vermis (Scott et al., 2009; Webb et al., 2009). Interestingly, anatomical and immunohistochemical studies have shown that the cerebellum is innervated by an extensive plexus of both 5-HT and NE fibers (Bishop and Ho, 1985; Fillenz, 1990; Hoffer et al., 1971; Takeuchi et al., 1982) and they modulate the firing rate of PC and cerebellar nuclei (Bickford-Wimer et al., 1991; Hoffer et al., 1973; Mitoma et al., 1994; Murano et al., 2011; Strahlendorf et al., 1991). Disruption of these monoamine systems during development could alter the microcircuitry within the cerebellum as well as their extra-cerebellar connections.

Based on these lines of information, the aim of the present study was to investigate the morphological and functional characteristics of the cerebellum in MAO-A^{Neo} mice. A portion of the data have been presented in an abstract form (Alzghoul et al., 2011).

2. Experimental procedure

2.1. Animals

All animals were treated in accordance with respective universities approved by the animal care and use committee and complied with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and National Institutes of Health (NIH) guidelines. Briefly, mice were generated from 129S6 mice (WT) by insertion of a floxed neomycin-resistance cassette in intron-12 of the *Maoa* gene, which by alternative splicing produces a truncated enzyme at the C-terminus. This leads to a reduction in the amount of functional transcript of the MAO-A enzyme and resulted in higher 5-HT and NE levels in many brain regions (Bortolato et al., 2011). A total of 47 male mice were used in this study (24 WT and 23 MAO-A^{Neo}). Among them, 24 mice (12 WT and 12 MAO-A^{Neo}) were used for behavioral studies, 10 mice (5 from each group) were used for the MR imaging study, and 7 WT and 6 MAO-A^{Neo} mice were used for histological and immunohistochemical analyses.

2.2. Behavioral testing

2.2.1. Footprint assay

Gait was assessed as described elsewhere (Carter et al., 1999). Briefly, we used a Plexiglas runway (1000 × 6 cm) lined with white paper. Mouse forepaws and hindpaws were dipped in blue and orange non-toxic tempera paint. Mice were placed on the runaway and allowed to traverse it. For each set of footprints, we measured the distance between paw axes (stride-width) for both forepaws and hindpaws. A minimum of six measurements were taken per mouse. Gait pattern was also analyzed by calculating the ratio between the hindpaw and forepaw stride-widths, as well as the overall stride length (forepaws and hindpaws) for each genotype.

2.2.2. Rotarod

Motor coordination was measured using a rotarod apparatus (Med-associates, St. Albans VT, USA). Mice were placed on a rotating rod (6 cm in length and 3 cm in diameter at 25 rpm) for 5 min. Mice that fell were quickly returned to the rotarod and the total number of falls was recorded.

2.2.3. Balance beam

To test for balance, we used a steel beam (40 cm in length × 1 cm in diameter) suspended 20 cm above a padded surface. Mice were placed on the beam and the latency to fall was recorded. A total of three trials were performed per mouse.

2.3. Behavioral data analysis

Normality and homoscedasticity of data distribution were verified by using Kolmogorov-Smirnov and Bartlett's tests. Parametric and non-parametric analyses were performed with one-way ANOVAs and Mann–Whitney tests, respectively. Significance threshold was set at 0.05.

2.4. Neuroimaging

Five adult WT and 5 adult MAO-A^{Neo} mice were transcardially perfused with 4% paraformaldehyde solution and T2-weighted magnetic resonance images (MRI) of the heads were acquired with a 21.1T/900 MHz/105 mm magnet, at 60um isotropic resolution, echo time (TE) = 10.5 ms, repetition time (TR) = 150 ms.

Brain MR images were aligned, with rigid transformations, to an average mouse brain MR scan (Ma et al., 2008), with the use of the freely available image registration software RView (http://www.colin-studholme.net/), and then analyzed using commercially available image analysis software (Amira, 4.1.1). Regions of Interest (ROI) included cerebral cortex, olfactory bulb, striatum, nucleus accumbens-olfactory tubercle, thalamus, hypothalamus, hippocampus, amygdala, midbrain, pons, medulla oblongata, pontine nuclei, inferior olive, cerebellum, and total brain. ROIs were manually highlighted from cross-sections at the coronal, horizontal, and sagittal planes and volumetric measurements were made. ROI volumes were compared between WT and MAO-A^{Neo} mice with independent *t*-tests, followed by the Benjamini–Hochberg correction for multiple comparisons (Thissen et al., 2002). Results from histo-anatomical analysis of the cerebellum prompted us to further analyze the cerebellar vermis. The thickness of the vermis was defined at 1400 μm along the midline of the cerebellum, and did not include any paravermal tissue in the rostral and caudal areas of the structure. Lobules I–III, IV–V, VI–VIII, and IX–X were segmented and their volumes were assessed using analysis of covariance (ANCOVA) with total brain volume as a covariate, followed by Bonferroni tests when appropriate.

2.5. Histological and immunohistochemical analysis

Adult mice (7 WT and 6 MAO-A^{Neo}) were perfused with saline followed by 3.5% buffered paraformaldehyde in 0.1 M phosphate buffer solution (PBS). The brains were postfixed in the same fixing solution for 24 h, followed by incubation overnight in 30% sucrose in 0.1 M PBS at 4 °C. Each brain was assigned a code number, and raters were unaware of the genotype in order to limit the risk of introducing bias by rater expectation. Later, 40 μm sagittal sections were made with a freezing microtome, and serial sections