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Age-related changes in nigrostriatal dopaminergic function in heterozygous mutant dopamine transporter knock-out mice

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ABSTRACT

In this report we compared three different parameters of nigrostriatal dopaminergic (NSDA) function – locomotor activity, striatal dopamine (DA) levels and 3,4-dihydroxyphenylacetic acid (DOPAC)/DA ratios between heterozygous mutant dopamine transporter mice (+/− DAT) and their wild type controls (+/+ DAT) at three different age range periods: 4–10, 11–17 and 18–24 months of age. Locomotor activity of the +/− DAT mice failed to differ over the three age periods sampled. In +/+ DAT mice a significant decrease in locomotor activity was obtained at the 18–24-month old period compared with scores at the two earlier age periods. In addition, locomotor scores of +/+ DAT mice at 18–24 months of age were significantly decreased as compared with scores of the +/− DAT mice at this age. Striatal DA concentrations of +/− DAT mice also failed to differ over the three age periods sampled, while that of +/+ DAT mice showed significant decreases in striatal DA at 11–17 and 18–24 months of age as compared to their 4–10-month old cohorts. Striatal DOPAC/DA ratios were significantly increased in both +/+ and +/− DAT mice at the 11–17 and 18–24 month age periods as compared with their respective 4–10-month old groups. Striatal DOPAC/DA ratios of +/− DAT mice were significantly greater than that of the +/+ DAT mice at 18–24 months of age. These findings reveal the significance of interactions between a mutation of the dopamine transporter and aging upon NSDA function and the importance of isolating such variables when using knock-out models.

As one means to study the role of genes on physiological functions, specific deletion models have been generated. Since work within our laboratory has been directed at understanding dopaminergic function, and, in specific, the role of the dopamine transporter (DAT), we have utilized DAT knock-out mice to evaluate DAT function. With these genetically altered mice there can exist a general perception that all changes obtained are attributable solely to the mutation. Such a perception has the potential of ignoring or diminishing the significance of other factors that may interact with this genetic alteration. For example, we have observed that a heterozygous mutation of the DAT (+/− DAT) does not exert similar effects within female and male mice. Specifically, striatal dopamine (DA) concentrations of +/− DAT female mice are significantly decreased as compared with their wild type controls (+/+ DAT) and +/− DAT males. In contrast, striatal DA of +/− DAT males does not differ from their wild type controls [9]. In addition, both protein and mRNA levels of DAT mice are significantly decreased in +/− DAT female, but not, male mice as compared with their respective wild type controls [8]. Two salient conclusions emerge from these findings. First, these mutations do not occur in a vacuum. That is, not all physiological differences or changes in these animals result simply from the presence of the specific mutation generated. Second, this heterozygous mutation does not necessarily produce the expected 50% reduction in function. It is typically reported that a linear decrease in function accompanies genetic deletions such that a heterozygous mutation results in an approximately 50% reduction in function, while null mutants result in a near complete elimination of function. The data related above reveal that such simple linear relationships may not be present in all circumstances.

In the present report we assess the influence of another ancillary variable that may interact with a DAT mutation, that of age. Specifically, we measured three different parameters of striatal dopaminergic function between +/+ and +/− DAT mice at three different age ranges. In this way, it was possible to establish whether an interaction may be present between the effects of a DAT genotype and the age of the mice. Since the nigrostriatal dopaminergic (NSDA) system is vulnerable to age-related deficits, in general [11,16] and, in specific, DAT densities decrease with age [3,4,15,16,18,19], we determined whether the variable of age

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would disproportionately affect and interact with the DAT genotype condition of the mice. Three different parameters of striatal dopaminergic function were measured in this report, locomotor activity, striatal DA concentrations and 3,4-dihydroxyphenylacetic acid (DOPAC)/DA ratios. Each of these three parameters was assessed at three different ranges of ages: 4–10, 11–17 and 18–24 months of age.

Male mice (C57/Bl6) identified as either wild type controls (+/+ DAT) or heterozygous DAT mutants (+/− DAT) were used in these experiments. Comparisons between +/−DAT and wild type mice were performed since a DAT deficit, as opposed to a complete DAT absence (−/− DAT), provides for a more realistic condition that would be applicable and relevant to the clinical setting as DAT numbers may decrease with age or pathology, but would generally not be completely absent [4,13]. In addition, interpretations of results derived from −/− DAT mice can sometimes be problematic due to the extreme nature of the deficit [17]. Due to the difficulty in achieving adequate sample sizes for this report it was necessary to pool animals into relatively wide age-range periods consisting of 4–10, 11–17 and 18–24 months. Breeding pairs of mice were provided through the generosity of Dr. Marc Caron at Duke University [7]. Mice were bred and raised in our colony rooms and were genotyped by polymerase chain reaction and 1% agarose gel of electrophoresis of tail DNA. They were housed under a 12 h light/dark cycle (lights on at 06:00 h) with food and water available *ad libitum*. Different groups of mice were used for the behavioral and neurochemical determinations. The Animal Care and Use Committee at NEOUCOM in accordance with NIH guidelines approved all experimental procedures. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Mice were placed in an animal activity monitor for a 60-min period to evaluate their spontaneous locomotor activity. The total distance traveled (cm) over this period was recorded from each mouse. Locomotor activity was measured between 09:00 and 12:00 h of the photoperiod.

Mice were euthanized by rapid decapitation and the striatum dissected. The striatum was weighed, placed in 500 μl cold 0.1N $HClO_4$, sonicated and an aliquot from the supernatant was removed for assay of DA and DOPAC. These were assayed using an ESA Inc. HPLC-EC system with a coulochem II electrochemical detector set at E1: 400 mV, R1: 2 μA, E2: −350 mV, R2: −100 nA. Biogenic amines were separated on a Supelco column (Discovery C-18, 10 cm × 3 mm, 5 μm). The DA and DOPAC standards were diluted in 0.1N $HClO_4$ to construct a standard curve and DA and DOPAC from striatal samples were determined by comparing peak heights and retention times with that of standards using the software program provided by ESA. Striatal DA and DOPAC levels were expressed as pg/mg of tissue.

Due to the variable and relatively small sample size for the locomotor activity scores, these data were subjected to analysis using non-parametric statistics. Separate Kruskal–Wallis ANOVAs followed by Mann–Whitney *U*-tests (for pairwise comparisons) were applied to the data from the +/+ and +/− DAT mice as a function of the three age periods sampled. For analyses of DA concentrations and DOPAC/DA ratios separate 2 (Genotype: +/+ versus +/− DAT) × 3 (age: 4–10 versus 11–17 versus 18–24 months of age) independent groups two-way ANOVA were used with these data. The Fisher's protected LSD test was used for pairwise post hoc comparisons. A $p < 0.05$ was required for results to be considered statistically significant.

A summary of the locomotor activity (Mean ± SEM in cm) is presented in Fig. 1. Locomotor activity scores of the +/+ DAT mice showed an overall statistically significant difference as a function of age ($H = 8.5$, $p = 0.014$). Pairwise post hoc comparisons revealed that locomotor activity of the 18–24 month old mice was significantly decreased as compared with the 4–10 month old ($U = 2$, $p = 0.0032$) and the 11–17 month old ($U = 9$, $p = 0.034$) mice. Locomotor activity of 18–24 month old +/+ DAT mice was significantly decreased as compared to that of 18–24 month old +/− DAT mice. No overall statistically significant differences in locomotor activity were obtained from the +/− DAT mice ($H = 0.14$, $p = 0.93$).

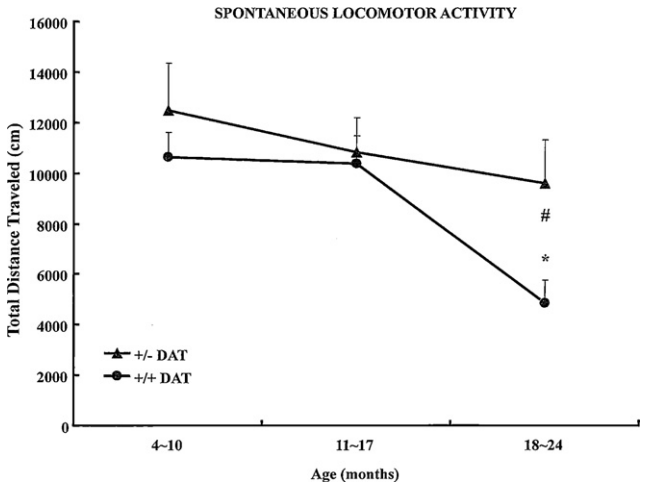

Fig. 1. Locomotor activity (Mean + SEM – total distance traveled in cm) over a 60-min period of heterozygous mutant dopamine transporter (+/− DAT) and wild type control (+/+ DAT) mice as sampled at three different age ranges (4–10, 11–17 and 18–24 months of age). Locomotor activity of +/+ DAT mice was significantly decreased at the 18–24-month old period ($N = 6$) as compared with that of +/+ DAT mice at the 4–10 ($N = 9$) and 11–17 ($N = 9$) month old age ranges as indicated by *. In addition, locomotor activity of +/+ DAT at 18–24 months of age was significantly decreased as compared with scores of the +/− DAT mice at 18–24 months of age as indicated by #. No overall statistically significant age-related differences in locomotor activity were obtained in +/− DAT mice. Sample sizes for the +/− DAT mice were: 4–10 month ($N = 5$), 11–17 month ($N = 8$) and 18–24 month ($N = 3$).

A summary of the striatal DA concentrations (Mean ± SEM in pg/mg) is presented in Fig. 2A. Analysis of striatal DA concentrations indicated a statistically significant effect for the age factor ($F_{2,41} = 8.40$, $p < 0.01$). Post hoc comparisons revealed that DA concentrations of the 4–10 month old +/+ DAT mice were significantly greater than that of the 11–17 and 18–24 month old mice. No statistically significant differences were obtained between these two latter age groups of the +/+ DAT mice. Nor were any overall statistically significant differences present among the three age groups for the +/− DAT mice.

A summary of the striatal DOPAC/DA ratios is presented in Fig. 2B. For DOPAC/DA ratios, statistically significant effects were obtained for both the age ($F_{2,41} = 6.99$, $p < 0.01$) and genotype ($F_{1,41} = 9.19$, $p < 0.01$) factors. Post hoc analysis showed that for both the +/+ and +/− DAT mice, DOPAC/DA ratios of the 4–10 month old mice were significantly lower than their respective 11–17 and 18–24 month old mice groups. In addition, DOPAC/DA ratios of the +/− DAT mice were significantly greater than the +/+ DAT mice at the 18–24 month old age period.

The present results reveal a number of interesting aspects of interactions between age and genetic mutation of the DAT upon NSDA function. The most salient finding being that the aging NSDA system is differentially affected by the genotypic condition of the DAT. Interestingly, with regard to both locomotor activity and striatal DA, +/− DAT mice give the appearance of diminished, if not absent, age-related deficits. In these +/− DAT mice, neither locomotor activity (Fig. 1) nor striatal DA concentrations (Fig. 2A) show statistically significant deceases over the three age ranges sampled. Such findings contrast markedly with that obtained from wild type controls where significant decreases in locomotor activity are present in the 18–24 month old mice as compared with their 4–10- and 11–17-month old cohorts; and significant decreases in striatal