Published in final edited form as:

Neurosci Lett. 2011 February 1; 489(1): 1–4. doi:10.1016/j.neulet.2010.11.054.

L-DOPA Reverses Motor Deficits Associated with Normal Aging in Mice

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Abstract

We wished to determine whether L-DOPA, a common treatment for the motor deficits in Parkinson's disease, could also reverse the motor deficits that occur during aging. We assessed motor performance in young (2-3 months) and old (20-21 months) male C57BL/6 mice using the challenge beam and cylinder tests. Prior to testing, mice were treated with L-DOPA or vehicle. Following testing, striatal tissue was analyzed for phenotypic markers of dopamine neurons: dopamine, dopamine transporter, and tyrosine hydroxylase. Although the dopaminergic markers were unchanged with age or L-DOPA treatment, L-DOPA reversed the motor deficits in the old animals such that their motor coordination was that of a young mice. These findings suggest that some of the locomotor deficits that accompany normal aging are responsive to L-DOPA treatment and may be due to subtle alterations in dopaminergic signaling.

Keywords

Behavior; dopamine; striatum

Aging is usually accompanied by a decline in motor function, a phenomenon that can be observed in laboratory animals as well as in humans. For example, rodents typically exhibit a decline in spontaneous motor activity and motor coordination [1-3,7]. Such motor functions appear to be heavily influenced by the dopamine (DA) system [17]. One important piece of evidence for this conclusion comes from studies of Parkinson's disease (PD). In that condition, many of the motor deficits are strongly correlated with the loss of the DA neurons of the nigrostriatal projection [5].

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a) The authors have no actual or potential conflicts of interest.

b) All animals were maintained in temperature-controlled rooms in the barrier facility maintained by the Division of Laboratory Animal Resources (DLAR) at the University of Pittsburgh. Animals were housed in accordance with *Guidelines for the Care and Use of Animals*, at 23°C with lights on between 1900 and 0700 hr. Animals were fed and watered *ad libitum*, and the woodchips in their cages changed every other day. Full-time trained animal care personnel performed all animal handling and maintenance. The director of the DLAR, a trained veterinarian, supervised animal care. All animal care was overseen by the Institutional Animal Care and Use Committee (IACUC).

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L-DOPA, which increases the availability of DA in striatum, is able to temporarily improve motor function in PD patients [15,16]. This is another source of support for the role of DA deficiency in motor deficits. Moreover, the efficacy of L-DOPA in PD suggests the possibility that the drug would also be effective in motor dysfunction that occurs with normal aging, a clinical problem for which there is no standard treatment. To test this hypothesis, the effects of L-DOPA on balance, motor coordination and spontaneous activity were examined in young and old male mice using a challenge beam test that provides very sensitive measures of motor performance and cylinder test, respectively [6-8]. Then, immediately following behavioral testing, DA and DA metabolite content were determined in the striata of these animals. In a separate group of animals, the expression of tyrosine hydroxylase (TH) and the high affinity DA transporter (DAT) were determined in the striata one week following behavioral testing.

Young (2.5 mo) and old (20.5 mo) male C57BL/6 mice (Harlan Laboratories, Indianapolis, IN) were housed 3 per cage and maintained on a 12 h light:dark cycle with ad libitum access to food and water. The animals were utilized in compliance with all applicable laws and regulations as well as principles expressed in the National Institutes of Health, University of Pittsburgh, and the Guide for the Care and Use of Laboratory Animals.

The mice were trained to traverse an elevated beam (4 segments, 25 cm long by 3.5 cm narrowing to 0.5 cm by 1 cm; [8]). After two training sessions, wire mesh with 1 cm² openings was placed over the beam and the animals were videotaped and timed making their traversals. Spontaneous activity was measured using a transparent cylinder. 40 min prior to these tests of motor performance, all mice were given benserazide (Sigma, St Louis, MO; 12.5 mg/kg; i.p.) to block peripheral decarboxylation of the L-DOPA followed 10 min later by L-DOPA (Sigma; 15 mg/kg, i.p.) or an equivalent volume of saline vehicle. Videotapes were viewed for number of steps, errors and rears by an investigator blind to the treatment. Mice were decapitated 30 minutes or one week later, and the striatum rapidly dissected, frozen on dry ice, and stored at - 80°C until assay.

Samples were sonicated in 0.1 N perchloric acid (20 µl/mg wet tissue weight) for analysis of DA levels or in ice cold lysis buffer for analysis of two key phenotypic markers of DA neurons, TH, and DAT. The lysis buffer consisted of 20 mM Tris, pH 6.8, 137 mM NaCl, 25 mM β-glycerophosphate, pH 7.14, 2 mM NaPPi, 2 mM EDTA, 1 mM Na₃VO₄, 1% Triton X-100, 10% glycerol, 5 µg/ml leupeptin, 5 µg/ml aprotinin, 2 mM benzamidine, 0.5 mM DTT and 1 mM PMSF.

Protein content was assessed by bicinchoninic acid assay (Pierce) and 20 µg of protein was loaded on an SDS PAGE gel and transferred to an Immobilon-FL PVDF membrane (Millipore). Membranes were washed in Tris-buffered saline with 0.1% Tween (TTBS) and blocked for 1 h in a fish-serum based blocking solution (LI-COR Biosciences) at room temperature. Membranes were then incubated overnight at 4°C in monoclonal rat anti-DAT (1:2000; Chemicon MAB369), monoclonal mouse anti-TH (1:20,000, Chemicon MAB318), or monoclonal mouse anti-α-tubulin (1:50,000, Sigma Clone B512 T5168). Binding was visualized with infrared secondary antibodies fluorescing at 700 nm (goat anti-rat for DAT; goat anti-mouse for tubulin) or 800 nm (goat anti-mouse for TH; Odyssey Imaging; LI-COR) applied for 1 h at room temperature (1:10,000 in blocking solution). Blots were scanned on an Odyssey Imager and quantified with Odyssey software, using background subtraction above and below each band. Grayscale images were used for quantification, and the images were pseudo-colored red (700 nm) and green (800 nm) to delineate DAT and TH using distinct fluorescent wavelengths.

Striatal tissue was analyzed by HPLC for DA, DOPAC, and HVA content as previously described [21]. A 20 μ l aliquot of each sample was injected onto an ESA C18 column (2.1 $\times$ 150 mm, ESA, Inc., Chelmsford, MA). The mobile phase consisted of 75 mM H_2NaPO_4 , 1.7 mM octanesulfonic acid, 25 mM Na_2EDTA 0.00001% triethylamine (v/v), and 10% acetonitrile (v/v), pH 3.0 and was pumped through the system at a rate of 0.3 ml/min using an ESA LC-10AD pump (ESA Inc., Chelmsford, MA). Samples were detected coulometrically using an ESA Coulochem Model 4100A detector, an ESA Model 5010 conditioning cell, and an ESA Model 5014B microdialysis cell (ESA Inc., Chelmsford, MA). The settings for detection were $E_1 = -0.26$ V, $E_2 = +0.28$ V, guard cell = +0.4 V. The limits of detection were in the femtomole range. Quantification was done using standard curves for DA, DOPAC and HVA.

All statistical tests were performed using Graph Pad Prism. The data in figures 1 and 2 were analyzed using a two-way ANOVA ($p < 0.05$) followed by *post hoc* comparison using a Bonferroni multiple comparison test. The data in figure 3 were analyzed using an unpaired t-test with a 95% confidence interval.

To determine if old animals showed a decline in motor function, we compared motor performance of the young, saline and L-DOPA treated animals to the old, saline and L-DOPA treated animals using a challenge beam test and cylinder test. The overall ANOVA revealed a significant effect of age ($F = 9.430$, $p = 0.0043$; Fig. 1A) in the challenge beam test but not the cylinder test. Post-hoc analysis indicated that aged animals made 46.08% more errors on the beam than the young group ($t = 4.863$, $p < 0.001$) suggesting that motor function and coordination declines with age. However, when analyzing number of times the animals reared in the cylinder, there was no significant difference between young and old animals, with or without L-DOPA treatment (Fig. 1B).

The effect of L-DOPA on motor function was also examined in both young and old animals. The overall ANOVA revealed a significant interaction of drug treatment $\times$ age ($F = 6.474$, $p = 0.0160$; Fig. 1A). Post-hoc analysis indicated that the old L-DOPA treated mice made 46.36% fewer errors than the old, saline group ($t = 2.239$, $p < 0.01$). However, L-DOPA had no significant effect in young animals. These data suggest that L-DOPA reverses the age-related decline in motor coordination but has no significant effect on motor behavior in younger mice. Furthermore, in this study, spontaneous activity as measured by the number of rears the animals make was not significantly changed with age or L-DOPA treatment. However, the old animals showed a trend towards decreased activity as noted by the decline in the number of rears (Fig. 1B)

Since L-DOPA acts by increasing the availability of DA in brain, we examined the effect of aging on DA, DOPAC, and HVA concentrations in the striatum of young and old saline and L-DOPA treated animals. Interestingly, we found no significant alterations in the concentrations of DA or DA metabolites in the aged animals as compared to the young mice (Fig. 2), and that the ratio of DOPAC and DOPAC plus HVA to DA, a measure of DA turnover, also remained unchanged with age. Moreover, L-DOPA treatment did not alter the levels of DA, DOPAC or HVA in young or old mice. These data suggest that motor decline in our paradigm is not related to comparable decreases in DA content or turnover in the striatum of our aged animals and that L-DOPA was not acting by temporarily reversing a reduction in DA stores or its synthesis in our paradigm.

Although we did not observe a change in DA, DOPAC, or HVA concentrations, it was possible that there were age-related alterations in other components of the DA neuronal system. Thus, we performed Western blot analysis of DAT and TH expression in the striatum of mice of the two age groups. Yet, as in the case of DA content, DAT and TH

expression were unchanged with age or L-DOPA treatment (Figs. 3A and B). These data suggest that alterations in DAT and TH expression are also not related to the motor decline observed in the old animals in this paradigm and L-DOPA was not acting to increase TH or DAT expression (Fig. 3A and 3B).

In the present study, we examined the effects of L-DOPA on motor deficits observed with normal aging. We observed that L-DOPA significantly decreased the errors made by the old animals on a novel challenge beam test for motor coordination and balance, whereas it had no effect on the errors made by young mice as they traversed the beam. One explanation for this effect of L-DOPA was that it reversed a loss of DA in the aged mice. Indeed, striatal DA content has been shown to decrease with age in some reports [3,4], as has striatal TH [12], DAT [11,19], and the number of DA neurons in the substantia nigra [13,1]. However, in the present study, decline in motor function was not accompanied by age-related alterations in DA, DOPAC, or HVA striatal content. Moreover, TH and DAT levels also were unchanged with age. Similar results have been reported by others [2,22,21], indicating that significant strain differences exist among rodents with regard to the impact of age on DA neurons. Another possible explanation for the effect of L-DOPA on motor coordination in the aged animals is that it alters noradrenergic function. Rommelfanger and colleagues (2007) found that dopamine b-hydroxylase knockout mice (Dbh^{-/-}), which lack norepinephrine but have normal DA levels in the striatum, have a significant loss of motor coordination as compared to heterozygous litter mates (Dbh^{+/-}) as measured by the same challenge beam test used in this study. Therefore, it is possible that in our study L-DOPA increases norepinephrine levels and signaling in the aged animals. This will be examined in future studies.

Interestingly, our aged animals did not show a significant decline in spontaneous activity (Fig. 1B). This may be due to the specific test used (ie. cylinder test) or the measurements observed (ie. # of rears). However, similarly, other measurements of spontaneous activity (ie. forelimb and hindlimb steps) did not decline with age (data not shown). Although we expected a decline in spontaneous activity with age, these results further support the loss of motor coordination observed with aging in that the data from the challenge beam test is not confounded by a loss in spontaneous activity in the old animals.

Our finding that age-related motor deficits were reversed with L-DOPA could also indicate that age is associated with a decline in dopaminergic signaling. Although this decline was not associated with a decrease in any of the phenotypic markers that we assessed, other possibilities remain. These include a decrease in DA release that was not associated with a modification of DA synthesis or turnover, and a decline in the capacity of the target neurons to respond to DA. Another possibility is that by increasing the availability of DA, L-DOPA is overcoming some other deficit in CNS signaling.

Whatever the mechanism underlying the actions of L-DOPA in our model, these results raise the possibility that L-DOPA therapy may be an effective intervention for the motor deficits that frequently accompany aging. Some years ago, a report appeared in which the effects of L-DOPA were examined in 10 healthy elderly volunteers and no effect on movement velocity, reaction time or tremor was observed [14]. However, the number of people in this study was very limited and the investigators did not measure motor coordination. A more recent study has shown that L-DOPA improves upper limb function in otherwise normal aged rhesus monkeys [10]. Furthermore, in a study of motor memory performance in the elderly, L-DOPA enhanced the effects of training on motor memory formation and DA release in the caudate nucleus [9].

L-DOPA has been used for more than 40 years in the treatment of PD. It appears to be well tolerated and, whereas side effects typically appear in the later stages of PD, these are likely to be a consequence of the progressive loss of DA neurons that accompanies the disease and thus may not be a factor in normal aging. Therefore, on the basis of our data, the few related findings that have appeared in the literature, and the apparent lack of side effects of the drug in otherwise normal adults, we suggest that with further investigation L-DOPA could be considered as a useful treatment for aged-related motor decline.

Research Highlights

- Old animals make more errors indicating a loss of motor coordination and balance.
- L-dopa reverses the age-related loss of motor coordination.
- A loss of motor function with age is not accompanied by a loss of dopamine, TH or DAT.

Acknowledgments

These studies were funded by the following sources: NINDS (NS19608), NIA (AG25848), NIA (AG024827-05), and DOD (ERMS 03281022).

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Fig. 1. L-DOPA treatment reverses age-related deficits in motor coordination. A) Old animals made significantly more errors than their younger counterparts when traversing the challenge beam (* $p < 0.05$; $n = 9-12$ in saline groups). L-DOPA significantly reduced the errors made by old animals (** $p < 0.05$; $n = 6$ in L-DOPA groups) such that the number of errors was similar to that in the young animals. B) Young and old animals made similar numbers of rears in the cylinder test. Although there is a trend for a decrease in rears in the old animals, this was not significant. Furthermore, L-DOPA had no effect on the number of rears in either young or old animals.

Fig. 2. Striatal DA and DA metabolite levels did not change with age or L-DOPA administration. Moreover, the ratio of DOPAC and/or HVA to DA was unaltered with age or L-DOPA treatment indicating no change in DA turnover. (data not shown)

Fig. 3. Phenotypic markers of DA neurons are unaltered with age and L-DOPA treatment. A) Striatal DAT expression was not changed with age or L-DOPA administration. B) The expression of TH, the rate limiting enzyme in DA synthesis, was not changed with age or L-DOPA administration.