Progressive Impairment of Motor Skill Learning in a D-Galactose-Induced Aging Mouse Model

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Abstract.- Chronic administration of D-galactose (D-gal) has been reported to cause behavioral deterioration in mice similar to what is observed in the aging process, but the effect of D-gal on motor skill learning has not been examined. In the present study, mice were treated with D-gal (100 mg/kg/day) for a period ranging from 1 to 9 weeks, and motor skill learning was assessed using the rotarod test. D-gal-treated mice exhibited deficits in performance, including a shorter latency to fall and a decrease in intersession improvement compared to controls. Notably, motor skill deficiencies in mice subjected to short-term D-gal treatment (2–4 weeks) were rescued through repeated training, while there was no comparable improvement in mice receiving D-gal over a long term (≥ 5 weeks). The decline in rotarod performance reached a plateau at 7 weeks of D-gal exposure, suggesting that there is a ceiling effect. These results provide evidence that D-gal impairs motor learning capacity in a time-dependent manner, and demonstrate that chronic administration of D-gal is a reliable model for the behavioral decline associated with aging.

Keywords: D-galactose, motor skill learning, rotarod, mouse, aging model.

INTRODUCTION

D-Galactose (D-gal) is a monosaccharide present in small quantities in organisms, which is converted to glucose during metabolism (Schadewaldt et al., 2000; Kaleem et al., 2008; Wu et al., 2008; Park et al., 2013). An excess of D-gal can cause oxidative damage to various tissues through the production of reactive oxygen species and advanced glycation end-products (Lu et al., 2007, 2010; Park et al., 2013), which can also occur during normal aging. Several studies in mammals and Drosophila have reported that chronic, systemic exposure to D-gal results in the acceleration of senescence in various tissues such as brain, kidney, liver, ovary, and blood cells; this paradigm has therefore been used as an experimental model for aging (Park and Choi, 2012; Chen et al., 2006; Cui et al., 2006). Moreover, some behavioral manifestations associated with aging have also been observed in conjunction with chronic D-gal exposure, such as deficits in learning and memory (Cui et al., 2006; Chen et al., 2006; Wei et al., 2005; Tian et al., 2011; Yoo et al., 2012; Chiu et al., 2011; Zhang et al., 2007; Lu et al., 2006; Parameshwaran et al., 2010; Wu et al., 2008), a decline in cognitive function (Wang et al., 2009; Kumar et al., 2010, 2011; Chen et al., 2008; Lu et al., 2006), the impairment of locomotor function (Gu et al., 2013; Kumar et al., 2010; Banji et al., 2013; Parameshwaran et al., 2010), as well as a decrease in immune regulation (Lu et al., 2007, 2010).

Although it is a fundamental adaptive mechanism, motor skill learning in this animal model of aging has yet to be examined. In this study, the effect of D-gal on motor skill acquisition, as assessed by performance in the rotarod test, was examined in mice. The findings indicate that chronic exposure to D-gal leads to deficits in motor learning, and confirm that this model can be used to study the behavioral dimensions of the normal aging process.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice (N = 220; age: 8 weeks; 20±2 g) were used in this study. Animals were housed five per cage in a controlled room (22±2°C; 60±5% humidity; 12:12 h light-dark cycle) with
food and water *ad libitum*. After 1 week of acclimatization to the home cage, mice were randomly assigned to one of 11 groups (n = 20 per group). One group (W0) received no injections, while a second group (W9+0) received daily subcutaneous injections of saline for 9 weeks; both groups served as controls. D-gal was purchased from Sigma-Aldrich (St. Louis, MO, USA) and prepared in saline (0.9% w/v NaCl), and mice were injected at 100 mg/kg. Mice in the nine D-gal treatment groups (W1–W9) were injected daily with D-gal for 1 week, 2 weeks, etc., for up to 9 weeks. The injections were performed between 17:00 and 19:00. All animals were weighted before the behavioral test in order to evaluate the health status and avoid body weight interference.

Animal housing and all experimental procedures were in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication 80-23, revised 1996) and followed the requirements of the Provisions and General Recommendations of Chinese Experimental Animal Administration Legislation.

**Motor skill learning**

The accelerated rotarod task is widely used as a test of motor skill learning in rodents (Buitrago *et al.*, 2004; Jones and Roberts, 1968). Mice were tested on a computer-controlled rotarod apparatus (Rotamex-5; Columbus Instruments, Columbus, USA) next day after the cessation of D-gal treatment. The animals were first acclimated on the rod at a rotation of 4 rpm for 30 s; the rod was then accelerated to 40 rpm over 60 s (*i.e.*, 0.6 rpm/s). The latency for animals to fall from the rotating rod was recorded automatically with infrared sensors; times longer than 60 s were recorded as 60 s. Each mouse underwent three trials per session with a 180 s rest period between trials, and two sessions per day for 4 consecutive days. The mean latency of the three trials in each session was calculated. All behavioral testing was performed between 9:00–11:00 and 14:00–16:00.

**Statistical analysis**

The data are presented as mean ± S.E.M. One-way and repeated-measures two-way ANOVA were used, with post hoc comparisons where required, to determine mean differences between groups. *P*<0.05 were considered statistically significant.

**RESULTS**

**Effects of D-gal on latency to fall and intersession improvement in performance**

Mice showed no statistical difference in the body weight among groups (W1–W9, D-gal-treated groups; W0 and W9+0, controls; data not shown). However, a progressive improvement in the latency to fall was observed for all groups until the sixth training session, after which there was no further substantial improvement (Fig. 1A). A two-way ANOVA with repeated measures revealed significant effects for training (*F*\(_{7,1463} = 2221.174, \ P < 0.01\)), treatment (*F*\(_{10,209} = 28.639, \ P < 0.01\), and the training × treatment interaction (*F*\(_{70,1463} = 2.476, \ P < 0.01\), indicating that motor skill learning was affected by training and D-gal treatment.

A significant effect of training on latency to fall was revealed by one-way ANOVA in both the control groups (W0: *F*\(_{7,152} = 199.624, \ P < 0.01\); W9+0: *F*\(_{7,152} = 169.729, \ P < 0.01\) and the D-gal treatment groups (W1: *F*\(_{7,152} = 117.065, \ P < 0.01\); W2: *F*\(_{7,152} = 138.289, \ P < 0.01\); W3: *F*\(_{7,152} = 163.871, \ P < 0.01\); W4: *F*\(_{7,152} = 148.002, \ P < 0.01\); W5: *F*\(_{7,152} = 113.107, \ P < 0.01\); W6: *F*\(_{7,152} = 60.882, \ P < 0.01\); W7: *F*\(_{7,152} = 60.012, \ P < 0.01\); W8: *F*\(_{7,152} = 43.890, \ P < 0.01\); W9: *F*\(_{7,152} = 73.731, \ P < 0.01\). Logistic regression analysis demonstrated that intersession improvement in each group followed an asymptotic curve that was approximated by a binomial equation (Fig. 1A and Table I). A strong linear correlation was found between the parameters in these equations, indicating that a learning component for rotarod performance existed in all groups.

D-gal treated mice displayed shorter latencies to fall compared to control animals (*i.e.*, the curve shifted down). The cumulative latency to fall scores were significantly different among groups (*F*\(_{10,209} = 28.638, \ P < 0.01\); Fig. 1B). Compared to control groups, total scores in the D-gal-treated groups showed a progressive decline from W1 to W9 with a
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D-Galactose impairs motor skill learning

Fig. 1. Latency to fall in the rotarod test following D-gal treatment; A, The latency to fall over eight training sessions for control (W0, W9+) and D-gal treatment (W1–W9) groups; B, Cumulative latency to fall scores over eight training sessions. Note, performance declined progressively for treatment periods longer than 2 weeks (W2). W0, without injection; W1–W9, with daily D-gal injections for 1–9 weeks; W9+, with daily saline injections for 9 weeks.

n.s., not significant; *P < 0.05; **P < 0.01.

Gradual increase in the reduction percentage (Fig. 1B and Table II).

Table I-
Binomial equations for the motor skill learning curve in each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Binomial equations</th>
</tr>
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<tbody>
<tr>
<td>W0</td>
<td>y = -0.563x^2 + 7.826x + 13.06; R^2 = 0.982</td>
</tr>
<tr>
<td>W1</td>
<td>y = -0.552x^2 + 7.364x + 10.71; R^2 = 0.987</td>
</tr>
<tr>
<td>W2</td>
<td>y = -0.543x^2 + 7.245x + 9.247; R^2 = 0.993</td>
</tr>
<tr>
<td>W3</td>
<td>y = -0.526x^2 + 7.173x + 8.922; R^2 = 0.994</td>
</tr>
<tr>
<td>W4</td>
<td>y = -0.472x^2 + 7.027x + 8.256; R^2 = 0.999</td>
</tr>
<tr>
<td>W5</td>
<td>y = -0.414x^2 + 6.420x + 8.322; R^2 = 0.999</td>
</tr>
<tr>
<td>W6</td>
<td>y = -0.355x^2 + 5.768x + 8.267; R^2 = 0.999</td>
</tr>
<tr>
<td>W7</td>
<td>y = -0.327x^2 + 5.454x + 8.137; R^2 = 0.999</td>
</tr>
<tr>
<td>W8</td>
<td>y = -0.308x^2 + 5.280x + 7.535; R^2 = 0.999</td>
</tr>
<tr>
<td>W9</td>
<td>y = -0.318x^2 + 5.375x + 6.997; R^2 = 0.999</td>
</tr>
<tr>
<td>W9+0</td>
<td>y = -0.560x^2 + 7.608x + 12.34; R^2 = 0.979</td>
</tr>
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</table>

Rotarod performance improved with each training session. The greatest improvement was observed between sessions 1 and 2 (i.e., Δ1), with the degree of improvement between consecutive sessions diminishing gradually, suggesting a ceiling effect on performance (Fig. 2). While intersession improvement in D-gal-treated groups decreased for Δ1 and Δ2, an increase was observed for Δ3 through Δ7 (Fig. 2). These results demonstrate that motor skill learning decreased as a result of prolonged exposure to D-gal.

Effects of D-gal on latency to fall in each session

A significant effect of D-gal on latency to fall was observed in each session (Session 1: F_{10,209} =
9.682, \(P < 0.01\); Session 2: \(F_{10,209} = 20.373, P < 0.01\); Session 3: \(F_{10,209} = 27.983, P < 0.01\); Session 4: \(F_{10,209} = 24.645, P < 0.01\); Session 5: \(F_{10,209} = 23.005, P < 0.01\); Session 6: \(F_{10,209} = 16.118, P < 0.01\); Session 7: \(F_{10,209} = 11.376, P < 0.01\); Session 8: \(F_{10,209} = 10.907, P < 0.01\); Session 9: \(F_{10,209} = 10.209, P < 0.01\); Session 10: \(F_{10,209} = 9.682, P < 0.01\). Differences in performance scores among the D-gal treatment groups (W1–W9) were observed starting from the first training session, indicating that the degree to which motor skill acquisition is inhibited is directly proportional to the time of exposure to D-gal. Moreover, the consistently inferior performance of the long-term D-gal-treated animals compared to controls for the duration of the study implies that the inhibitory effects of D-gal on motor skill learning persist over time.

Table II. Scores for the latency to fall in each group and the reduction percentage compared to the control group (means ± SEM).

<table>
<thead>
<tr>
<th>Group</th>
<th>Latency to fall (s)</th>
<th>Reduction percentage</th>
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<tbody>
<tr>
<td>W0</td>
<td>245.57 ± 2.26</td>
<td>----</td>
</tr>
<tr>
<td>W1</td>
<td>238.10 ± 3.85</td>
<td>3.03% (^1)</td>
</tr>
<tr>
<td>W2</td>
<td>234.27 ± 3.29</td>
<td>4.60% (^*)</td>
</tr>
<tr>
<td>W3</td>
<td>229.40 ± 2.90</td>
<td>6.58% (^*)</td>
</tr>
<tr>
<td>W4</td>
<td>222.73 ± 2.94</td>
<td>9.30% (^*)</td>
</tr>
<tr>
<td>W5</td>
<td>213.22 ± 3.99</td>
<td>13.17% (^*)</td>
</tr>
<tr>
<td>W6</td>
<td>204.47 ± 5.40</td>
<td>16.74% (^*)</td>
</tr>
<tr>
<td>W7</td>
<td>194.62 ± 5.30</td>
<td>20.75% (^*)</td>
</tr>
<tr>
<td>W8</td>
<td>187.38 ± 6.44 s</td>
<td>24.89% (^*)</td>
</tr>
<tr>
<td>W9</td>
<td>184.45 ± 4.43 s</td>
<td>24.89% (^*)</td>
</tr>
<tr>
<td>W9+0</td>
<td>241.37 ± 2.76 s</td>
<td>1.71% (^*)</td>
</tr>
</tbody>
</table>

\(^1\) \(P < 0.05\); \(^*\) \(P < 0.05\); \(^\ast\) \(P < 0.01\) vs W0

In each session, the performance score for the W1 group was not significantly different from that of the W0 and W9+0 control groups, indicating that short-term (i.e., 1 week) exposure to D-gal does not significantly affect motor learning capacity (Fig. 3). Interestingly, in sessions 1–3, the difference between the W2 group and controls was significant (Fig. 3A–C; \(P < 0.05\) or \(< 0.01\)); in session 4, the difference was significant only in mice treated for 3 weeks (W3) or longer (Fig. 3D; \(P < 0.05\) or \(< 0.01\) vs. W0); and in sessions 5–8, the effects of D-gal were observed only in mice treated for 5 or more weeks (W5–W9) (Fig. 3E–H; \(P < 0.05\) or \(< 0.01\) vs. W0). These results imply that the impairment of performance caused by intermediate-term (i.e., 2–4 weeks) D-gal treatment can be overcome though training, but that long-term (i.e., \(\geq 5\) weeks) exposure to D-gal produces irreversible deficits in motor learning capacity. Furthermore, there were no observable differences between the W7, W8, and W9 groups in terms of performance (Fig. 3A–H; \(P > 0.05\)), demonstrating that saturation is reached by 7 weeks, and no further deterioration in performance is induced by extending the period of D-gal administration.

DISCUSSION

In this study, motor skill learning in mice was examined on an accelerated rotarod over eight training sessions. Test performance steadily improved until a plateau was reached (Fig. 1), as previously reported (Buitrago et al., 2004). Although short-term D-gal treatment had no effect on motor learning capacity, longer-term exposure resulted in significant declines in performance (Figs. 1, 2). This suggests that the degree to which motor skill acquisition is inhibited depends on the time of exposure to D-gal. Performance deficits induced by intermediate-term, but not by long-term, D-gal administration were ameliorated by repeated training. Additionally, there were no differences in performance between the W7, W8, and W9 groups, indicating that the maximal effect of D-gal was produced by a 7-week treatment period (Fig 3). This time course correlates with the onset of neurotoxicity resulting from chronic D-gal exposure (Chiu et al., 2011; Cui et al., 2006; Gu et al., 2013). The D-gal-induced effects on motor skill acquisition reflect the changes in motor performance that occur during the normal aging process, in which a progressive decline is observed with the advancement of age (Altun et al., 2007).

Although chronic administration of D-gal is widely used to mimic aging, the underlying mechanism is unclear. One possibility is that chronic D-gal exposure causes a substantial rise in oxidative stress (Banji et al., 2013), which leads to cellular damage wrought by free radicals and protein and lipid oxidation (Lu et al., 2007, 2010; Cui et al., 2006; Hsieh et al., 2011; Kumar et al., 2010; Parameshwaran et al., 2010). D-gal has been shown to alter the activities of superoxide dismutase,
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![Fig. 3](image)

Fig. 3. Latency to fall in the rotarod test following D-gal treatment in each of eight training sessions: A–C, In sessions 1–3, significant differences in performance were observed for D-gal treatment periods of 2 weeks (W2) or longer (W3–W9) compared to controls (W0, W9+0); D, In session 4, significant differences were only observed for treatment periods ≥ 3 weeks (W3); E–H, In sessions 5–8, differences were only observed for treatment periods ≥ 5 weeks (W5). In all sessions, no differences were observed between the W7, W8, and W9 treatment groups. n.s., not significant; *P < 0.05; **P < 0.01.

catalase, glutathione, hydroxyproline, cholinesterase, and monoamine oxidase in a dose-dependent manner (Tian et al., 2011; Wang et al., 2009, 2012, Chen et al., 2010; Cui et al., 2006; Chiu et al., 2011; Zhang et al., 2007), mirroring changes that are observed during senescence. Furthermore, D-gal induces the morphological and functional deterioration of neurons (Cui et al., 2006; Chen et al., 2006; Liu et al., 2010), perturbs neurotransmitter balance (Gu et al., 2013) and cellular homeostasis (Park and Choi, 2012; Liu et al., 2010; Gu et al., 2013) to examine the effects of D-gal on motor skill learning in mice, and determine the time course that most closely parallels the normal aging process.

The duration of D-gal treatment has ranged considerably in different aging models. This study followed the most commonly used protocol of D-gal administration (100 mg/kg/day subcutaneously) (Wang et al., 2012; Cui et al., 2006; Park and Choi, 2012; Parameshwaran et al., 2010; Liu et al., 2010; Gu et al., 2013) to examine the effects of D-gal on motor skill learning in mice, and determine the time course that most closely parallels the normal aging process. Treatment regimens longer than 5 weeks induced irreversible behavioral impairment, while no further deterioration was observed for D-gal exposure lasting longer than 7 weeks. These results demonstrate that chronic exposure to D-gal at 100 mg/kg/day over a 7-week period provides a reliable model to study the behavioral consequences of
aging in mice. Future studies can determine whether the age-related deterioration in motor learning performance can be explained by the neurotoxic effects of chronic D-gal exposure.

ACKNOWLEDGEMENTS

This work was supported by grants from the Natural Science Foundation of China (No. 31272168), Natural Science Foundation of Henan Provincial Education Bureau (No. 13A180107) and the Funding Project for Young Leading Teachers by Henan Province (No. 2011GGJS-163)

Conflict of interest  None.

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(Received 13 November 2013, revised 13 December 2013)