BRAIN RESEARCH 1621 (2015) 187–196

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Research Report

The motirod: a novel physical skill task that enhances motivation to learn and thereby increases neurogenesis especially in the female hippocampus

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ARTICLE INFO

Article history:
Accepted 23 November 2014
Available online 24 December 2014

Keywords:
Adult neurogenesis
Dentate gyrus
Hippocampus
Sex differences
Physical skill learning

ABSTRACT

Males and females perform differently on a variety of training tasks. In the present study we examined performance of male and female rats while they were trained with a gross motor skill in which they learn to maintain their balance on an accelerating rotating rod (the accelerating rotarod). During training, many animals simply step off the rod, thus terminating the training. This problem was addressed by placing cold water below the rod. We termed the new training procedure "motirod" training because the trained animals were apparently motivated to remain on the rod for longer periods of time. Groups of male and female adult Sprague-Dawley rats were trained on either the standard accelerating rotarod or the motirod for four trials per day on four consecutive days. Latency to fall from the rod (in seconds) was recorded. The motivating feature increased performance especially in females ($p = .001$). As a consequence of enhanced performance, females retained significantly more new cells in the dentate gyrus of the hippocampus than those trained on the accelerating rotarod or those that received no training. In addition, individuals that learned well retained more new cells, irrespective of sex or task conditions. Previous studies have established that new cells rescued from death by learning remain in the hippocampus for months and mature into neurons (Leuner et al., 2004a; Shors, 2014). These data suggest that sex differences in physical skill learning can arise from sex differences in motivation, which thereby influence how many new neurons survive in the adult brain.

This article is part of a Special Issue entitled SI: Brain and Memory.

1. Introduction

In partnership with the Office of Research on Women's Health (ORWH) the NIH has developed a strategic plan entitled *A Vision for 2020 for Women's Health Research*. These policies were implemented in phases beginning in October 2014. The primary objective of this strategic plan is to increase awareness and gain a better understanding of conditions and diseases that specifically affect women's health and quality of life. The plan will also benefit men, by encouraging rigorous research into the

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http://dx.doi.org/10.1016/j.brainres.2014.11.045
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roles that sex and gender play in disease risk. With this change in policy we, as scientists, will confront a number of striking sex differences in our research programs and these differences will surface in perhaps unexpected ways. Over the past 15 years, our laboratory has reported a number of rather dramatic stress differences in rodent models of learning. For example, we reported that female rats can learn a classically conditioned eyeblink response faster than males. However, exposure to a stressful event can suppress the same type of learning in females while actually enhancing performance in males (Wood and Shors, 1998; Wood et al., 2001; Shors, 2001). However, during operant conditioning tasks, females often outperform males and do not express the performance deficits typically observed in males, a phenomenon referred to as "learned helplessness," a putative model of depression (Dalla et al., 2008; Shors et al., 2007). These sex differences in performance and learning have consequences for numerous measures of plasticity and brain function. In the case of classical eyeblink conditioning, females retained more new neurons in their hippocampus than did males, simply as a result of sex differences in learning (Dalla et al., 2008). In the present experiment, we report yet another example of a sex difference in learning and performance. In this particular instance, the sex difference only emerges as the task demands change, thereby illustrating the importance of attending to sex differences while remaining aware of their dynamic response to changing experimental conditions.

Learning increases the number of new neurons in the adult brain by rescuing them from death (Shors, 2014). These cells are generated in the hippocampus of the adult brain. Under "normal" conditions, most of the new cells die, even before they have fully matured into neurons. Recently, we reported that acquisition of new physical skills required to perform the accelerating rotarod procedure increases the number of these cells that survive (Curlik et al., 2013). Despite the positive effects of rotarod training on cell survival, there were performance outcomes that diminished our enthusiasm for using this particular task. Specifically, many rats would simply step off the rod once it began to accelerate. Since there was no consequence for this behavior we hypothesized that the rats were not motivated to perform the task. To address this problem we placed a shallow pool of cold water under the rod. It was hypothesized that training with the addition of this motivating feature, which we refer to as Motirod Training, would enhance performance and thereby increase the number of surviving cells in the hippocampus. In line with the recent provision for sexes, we examined performance in both males and females and observed robust sex differences, as described below. Moreover, the sex differences in learning produced significant consequences for the survival of new cells in the dentate gyrus of the hippocampus.

2. Results

2.1. Physical skill learning

Repeated-measures analysis of variance of performance during physical skill training revealed a significant 3-way interaction among trial, sex, and rotarod conditions ($F_{(15,525)}=2.21$, $p<.001$) with a main effect of trial ($F_{(15,525)}=25.91$, $p<0.001$) and training condition ($F_{(1,35)}=12.76$, $p<0.01$). Separate repeated-measures ANOVA's conducted for each sex revealed main effects of trial for both males ($F_{15,\ 240)}=10.39$, $p<0.001$) and females ($F_{(15,285)}=17.5$, $p<0.001$). These results suggest that both sexes increased their latency to fall from the rod as training progressed. Separate repeated measures analysis of variance indicated that the latency to fall from the rod as training progressed over the 16 trials in all four trained groups: male rotarod ($F_{(15,\ 135)}=5.14$, $p<0.001$), male motirod ($F_{(15,105)}=5.56$, $p<0.001$), female rotarod ($F_{(15,150)}=1.81$, $p<0.05$) and female motirod ($F_{(15,135)}=21.22$, $p<0.001$). These results suggest that each of the four groups successfully learned the motor skill task.

To detect sex differences in learning, we compared the performance of males versus females during training on the two tasks. Separate repeated-measures ANOVA's revealed a significant interaction between trial and rotarod condition for females ($F_{(15,285)}=5.68$, $p<0.001$; Fig. 1D), but not for males ($F_{(15,240)}=0.72$, $p>0.05$; Fig. 1E). These results indicate that females trained on the motirod outperformed females trained on the rotarod. However, there was no difference in performance in males trained on the rotarod compared to the motirod. Therefore, the addition of the motivating stimulus had a pronounced enhancing effect on learning in females.

Body weights differed significantly between male and female rodents. To determine whether these differences accounted for the performance differences during training, we conducted a Pearson correlation analysis between individual body weights and latency to fall from the rod. There was no correlation between average latency to fall from the rod and body weight in females ($r=-.22$, $p>.05$), males ($r=-.47$, $p>.05$), or in both sexes combined ($r=-.29$, $p>.05$). These results suggest that individual differences in body weight did not account for individual differences in performance during training with the motor skill tasks.

2.2. Neurogenesis: effect of training on cell survival in females

Animals were sacrificed three weeks following the BrdU injection, a time point when most cells that would have died would have already done so. The remaining cells tend to survive for at least several months. Analysis of cell survival was performed separately for males and females due to differences in volume and density of their hippocampi. In the first analysis, we examined the effects of training versus no training on the number of surviving cells in either sex. We used a one-away analysis of variance, with training condition (no training, rotarod, motirod) as the independent measure, and the number of surviving BrdU+ cells in the total dentate gyrus as the dependent measure. Results indicated a significant interaction between the number of BrdU+ cells and training condition ($F_{2,28}=15.32$, $p<0.001$; Fig. 2A). Post-hoc Tukey comparisons revealed that females trained on the motirod retained significantly more cells than females that were not trained ($p<0.001$) and those trained on the rotarod ($p<0.05$). Females trained on the rotarod retained more BrdU+ cells than the untrained females ($p<0.05$). These differences among the three female training conditions were observed in both the GCL ($F_{2,28}=10.27$, $p<0.001$) and the hilus ($F_{2,28}=5.47$, $p<0.05$). Taken together, these results suggest that