Voluntary Wheel Running Does Not Affect Lipopolysaccharide-Induced Depressive-Like Behavior in Young Adult and Aged Mice

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Key Words

Aging · Depressive-like behavior · Proinflammatory cytokines · Sickness behavior · Voluntary wheel running

Abstract

Objective(s): Peripheral stimulation of the innate immune system with lipopolysaccharide (LPS) causes prolonged depressive-like behavior in aged mice that is dependent on indoleamine 2,3 dioxygenase (IDO) activation. Regular moderate-intensity exercise training has been shown to exert neuroprotective effects that might reduce depressive-like behavior in aged mice. The purpose of this study was to test the hypothesis that voluntary wheel running (VWR) would attenuate LPS-induced depressive-like behavior and brain IDO gene expression in 4- and 22-month-old C57BL/6J mice.

Methods: Mice were housed with a running wheel (VWR) or no wheel (standard) for 30 (young adult mice) or 70 days (aged mice), after which they were intraperitoneally injected with LPS (young adult mice: 0.83 mg/kg; aged mice: 0.33 mg/kg). **Results:** Young adult VWR mice ran on average 6.9 km/day, while aged VWR mice ran on average 3.4 km/day. Both young adult and aged VWR mice increased their forced exercise tolerance compared to their respective standard control groups. VWR had no effect on LPS-induced anorexia, weight loss, increased immobility in the tail suspension test and decreased sucrose preference in either young adult or

aged mice. Four (young adult mice) and 24 h (aged mice) after injection of LPS, mRNA transcripts for TNF- α , IL-1 β , IL-6, and IDO were upregulated in the whole brain independently of VWR. **Conclusion:** Prolonged physical exercise has no effect on the neuroinflammatory response to LPS and its behavioral consequences in young adult and aged mice.

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Introduction

Major depressive disorders are a multifactorial set of psychiatric disorders with varying symptomatology and, presumably, etiologies [1]. Common symptoms include: depressed mood, reduced energy, loss of pleasure (anhedonia), dysregulated sleep and appetite, and feelings of guilt and/or suicide [1, 2]. The worldwide prevalence of depressive disorders is ~15% in adults and upwards of ~20% in adults over the age of 65 years [2–4]. Further compounding the age-associated risk of major depressive disorders, older adults exhibit longer durations of depressive symptoms compared to young adults, and often suffer less dramatic depressive symptoms that go undiagnosed as merely a ‘consequence’ of aging, yet significantly reduce social interactions and quality of life [5–7].

The mechanisms of age-related depression are multifaceted and not well understood, but several lines of evi-

dence implicate inflammation as a potential driving factor [8]. Aging is associated with chronic low-level inflammation, a condition known as ‘inflammaging’ [9]. Indeed, apparently healthy subjects >65 years exhibit significantly elevated circulating C-reactive protein levels compared to healthy young adults [9, 10]. The relationship between chronic inflammation and increased prevalence of depression is highlighted by the strong correlations between symptoms of depression and C-reactive protein and IL-6 levels in older adults [9, 10]. In addition to inducing a chronic low level of systemic inflammation, aging sensitizes the brain to subsequent immune stimuli such as infections and inflammatory stimuli [11]. Microglial cells from aged mice are primed in the sense they exhibit exaggerated and prolonged proinflammatory cytokine production following stimulation with lipopolysaccharide (LPS). This age-associated priming results in numerous behavioral consequences [11]. For example, peripheral injection of LPS lengthens the duration of sickness behavior (i.e. anorexia, weight loss, and lethargy) in aged mice compared to young adult mice, and this is associated with an exaggerated and prolonged upregulation of brain IL-1 β , TNF- α , and IL-6 [12, 13].

Aged mice also exhibit greater sensitivity to inflammation-induced depressive-like behavior, which is related to the activity of the tryptophan-degrading enzyme indoleamine 2,3 dioxygenase (IDO). IDO is the initial and rate-limiting enzyme of tryptophan catabolism through the kynurenine pathway [8, 14]. Activation of IDO is a required step in the development of inflammation-induced depressive-like behavior [15–17]. Aging is associated with prolonged duration of IDO activation in response to inflammatory stimuli, which results in protracted depressive-like behavior [18]. The same results are obtained in aged mice inoculated with *Bacillus Calmette-Guérin* [19].

The increased prevalence and burden of major depressive disorders is partially due to suboptimal treatment options [20]. The current protocol for treating age-related depression involves antidepressant therapies (e.g. tricyclic antidepressants and serotonin-selective reuptake inhibitors), which are moderately effective, but do not completely address the underlying inflammatory pathophysiology [21, 22]. Furthermore, a significant percentage of patients are nonresponders to currently available drugs [23]. Thus, there continues to be a major need to identify safe, alternative approaches for treating depression and reducing inappropriate neuroinflammation. Regular, moderate-intensity aerobic exercise may be one approach to alleviate depressive symptom burden in older adults. The mood-enhancing benefits of cardiovascular exercise

have been well documented in the literature and through the anecdotal testimony of thousands of exercisers. Cross-sectional studies demonstrate an association between a high amount of physical activity and a low amount of depressive symptoms in both middle-aged and aged populations [24]. For example, we found that depressive symptoms were related to low physical activity, low aerobic fitness, high C-reactive protein, and adiposity in elderly women [25]. Furthermore, physical exercise might be especially efficient in reducing depressive symptoms among patients with mild-to-moderate depression, which is important because a large fraction of older adults (10–20%) have clinically significant depressive symptoms that do not meet the criteria for major depression [26]. It is plausible that exercise interventions could be utilized in these individuals, in the absence of pharmacological therapy, to mitigate depressive symptoms. In 2006, Sjosten and Kivela [24] conducted a meta-analysis of published randomized clinical trials to determine the strength of an effect of regular exercise on depression in older adults, and concluded exercise was effective in treating depression among those suffering from minor or major depression and reducing depressive symptoms in those with significant depressive symptoms at baseline. In addition to human studies, rodent studies have also demonstrated antidepressant effects of regular moderate intensity exercise training [27]. Moon et al. [28] found 4 weeks of voluntary wheel training in young mice significantly reduced basal forced swim test immobility compared to an untrained cohort, while Duman et al. [29] demonstrated voluntary wheel running (VWR) increased sucrose consumption in chronically stressed mice. There are many unsubstantiated but potential ways in which exercise may improve mood and reduce depressive symptoms, including increased brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF), hippocampal neurogenesis, and anti-inflammatory effects [30–34]. We previously reported that voluntary wheel training does not attenuate LPS-induced sickness behavior in aged mice; however, we did not assess depressive-like behavior or IDO activation, and it is possible that VWR could affect sickness behavior independently of depressive-like behavior and IDO [13]. Furthermore, no additional studies have examined whether exercise training can attenuate inflammation-induced depressive-like behavior in young adult and aged mice using a defined inflammatory stimulus.

Therefore, we sought to examine the influence of VWR on LPS-induced depressive-like behavior and IDO activation in young adult and aged mice. We hypothesized that VWR would induce anti-inflammatory effects and

attenuate LPS-induced depressive-like behavior and brain proinflammatory cytokine and IDO gene expression in both young adult and aged mice.

Materials and Methods

Due to significant phenotypic differences between young adult and aged mice, such as the training duration necessary to induce metabolic adaptations and their endogenous sensitivity to LPS [12, 18, 19, 35], and the fact that the young adult and aged mice were obtained from two different sources, we conducted two separate and different experiments in the young and aged mice. Therefore, the data from the young adult and aged mice cannot be directly compared. Our purpose for this study design was not to examine the aging effect, which is already well documented [12, 18, 19], but rather to determine whether regular exercise can attenuate LPS-induced depressive-like behavior under the optimal experimental conditions for each age category. For this paper, we designate these two experiments as experiment 1 [young (4-month-old) adult mice] and experiment 2 [aged (19-month-old) mice].

Animals

All experiments were conducted under the guidelines of the Institutional Animal Care and Use Committee of the University of Illinois at Urbana-Champaign.

Experiment 1 (Young Adult Mice). Four-month-old male C57BL/6J mice ($n = 40$) were obtained from the Jackson Laboratories (Bar Harbor, Me., USA) and singly housed in cages with corn cob bedding in a temperature (23°C)- and humidity (45–55%)-controlled environment with a 12-hour dark/light cycle (lights off 09:00–21:00). Mice were allowed ad libitum access to food and water for the entire duration of the study and were given 2 weeks to acclimate to the housing conditions prior to study commencement. All young adult mice were subjected to the treadmill running test, tail suspension test (TST), and sucrose preference test after the training intervention.

Experiment 2 (Aged Mice). Nineteen-month-old male C57BL/6 mice ($n = 40$) were obtained from the National Institute of Aging (Bethesda, Md., USA) and singly housed in cages with corn cob bedding in a temperature (23°C)- and humidity (45–55%)-controlled environment with a 12-hour dark/light cycle (lights off 09:00–21:00). Mice were allowed ad libitum access to food and water for the entire duration of the study and were given 2 weeks to acclimate to the housing conditions prior to study commencement. All aged mice were subjected to the treadmill running test, TST, and sucrose preference test after the training intervention.

Voluntary Wheel Training

Experiment 1 (Young Adult Mice). Following acclimation, mice were randomized to VWR or 'normal' (standard) housing condition for a duration of 30 days, which is sufficient time to induce training-associated metabolic adaptations [unpubl. data]. The VWR mice were individually housed in plexiglass cages ($36\text{ L} \times 20\text{ W} \times 15\text{ H cm}$) that contained a stainless steel running wheel (diameter 22.86 cm; Mini-Mitter Repironics, Bend, Oreg., USA). Wheel revolutions were relayed via telemetry to a computer in the facility. Standard mice were housed in smaller cages ($30\text{ L} \times 19\text{ W} \times 13\text{ H cm}$) without any type of environmental enrichment. Follow-

ing training, all young adult mice were removed from their respective housing condition and singly housed in clean cages ($30\text{ L} \times 19\text{ W} \times 12\text{ H cm}$) for a 24-hour period prior to LPS treatment in order to washout any acute effects of the last wheel training session as acute exercise has been shown to affect inflammatory responses to LPS [36, 37]. The young adult mice remained in these standard cages for the duration of the study.

Experiment 2 (Aged Mice). Exercise training adaptations occur more slowly in aged mice compared to younger mice due to the lower volume of daily running and metabolic differences [35]. Therefore, aged mice were randomized to VWR or standard housing condition for a duration of 70 days, which is sufficient time to induce training-associated metabolic adaptations in aged mice [13]. All other aspects of the intervention were identical to experiment 1. Following training, all aged mice were removed from their respective housing condition cage and singly housed in clean cages ($30\text{ L} \times 19\text{ W} \times 12\text{ H cm}$) for a 24-hour period prior to LPS treatment in order to washout any acute effects of the last wheel training session as acute exercise has been shown to affect inflammatory responses to LPS [36, 37]. The aged mice remained in these standard cages for the duration of the study.

Treadmill Running Test

Experiment 1 (Young Adult Mice). To assess VWR-induced training adaptations, we measured forced exercise fatigability on day 26 of the 30-day VWR intervention. Mice ran until exhaustion on a motor-driven treadmill at 5% grade at gradually increasing speeds from 10–26 m/min. Exhaustion was defined as the point at which mice refused to run despite prompting by mild prodding with the hand for a period of 10 s; electric shock was not used in this test.

Experiment 2 (Aged Mice). To assess VWR-induced training adaptations in aged mice, we measured forced exercise fatigability on day 65 of the 70-day VWR intervention. Mice ran until exhaustion on a motor-driven treadmill at gradually increasing speeds from 6–21 m/min. As above, exhaustion was defined as the point at which the mouse refused to run despite prompting by mild prodding with the hand for a period of 10 s; electric shock was not used in this test.

LPS Administration

Given that aged mice exhibit a greater sensitivity to LPS-induced neuroinflammation and behavioral disturbances [12, 18, 19], we used different LPS doses for experiments 1 and 2. Based upon our preliminary data (not shown), both doses represent the minimum effective dose capable of inducing depressive-like behavior in the respective age cohorts of C57bl/6 mice. As rationale, we reasoned that if an exercise effect would be found it would be found at the minimum effective dose of LPS.

Experiment 1 (Young Adult Mice). After 30 days of VWR training, mice were randomized and injected intraperitoneally with saline or *Escherichia coli* LPS (lot 3129, serotype 0127:B8; Sigma, St. Louis, Mo., USA; dissolved in sterile saline) at a dose of 0.83 mg/kg. Following injection, mice were placed back into their home cages.

Experiment 2 (Aged Mice). Following 70 days of VWR training, aged mice were randomized and injected intraperitoneally with saline or *E. coli* LPS (lot 3129, serotype 0127:B8; Sigma; dissolved in sterile saline) at a dose of 0.33 mg/kg. Following injection, mice were placed back into their home cages.

Measurement of Sickness Response

To ensure LPS had the intended effect, physiological sickness responses were assessed by changes in body weight and food intake. Decreased food intake and the resulting body mass loss are sensitive measures of sickness in animals, and dependent on proinflammatory cytokine expression [38].

Experiment 1 (Young Adult Mice). Body weight and food intake were measured 24 h after injection and calculated as the change from pre-injection baseline.

Experiment 2 (Aged Mice). Body weight and food intake were measured 24 and 48 h after injection and calculated as the change from pre-injection baseline.

Measurement of Depressive-Like Behavior

Tail Suspension Test. Given that aged mice exhibit prolonged depressive-like behavior compared to young mice [18], we measured TST immobility 24 h after injection in experiment 1 and 48 h after injection in experiment 2. The TST, a standardized test of depressive-like behavior in which depression is inferred from increased duration of immobility, was conducted as previously described [15]. Briefly, mice were taken from their home cage and hung from their tail on a hook connected to a strain gauge for a period of 6 min. A computerized system for processing the force exerted on the gauge (Mouse Tail Suspension Package, MED-TSS-MS; Med Associates, St. Albans, Vt., USA) collected and analyzed the movements of each individual mouse. An immobility threshold was determined by establishing an activity level that would exclude all movements and only encompass immobility. Time below this threshold indicated the time of immobility.

Sucrose Preference Test. Anhedonia is a key component of depression and can be measured in mice by their preference to consume a sweetened solution. A sucrose preference test was performed following a 3-day acclimation program during the 24-hour period following LPS administration. As LPS-induced decreased sucrose preference resolves by 24 h in both young adult and aged mice [unpubl. data], this was the only time period measured for both experiment 1 and experiment 2. Mice were presented with two identical volumetric drinking tubes containing either water or a 1% sucrose solution. Drinking tubes were weighed before and after the 24-hour period to measure fluid consumption of each respective solution. Drinking tube position (right vs. left) was alternated each training period to reduce potential bias from place preference. Sucrose preference was calculated using the following formula: [sucrose intake/(water intake + sucrose intake)] $\times$ 100.

Tissue Collection

Experiment 1 (Young Adult Mice). A separate group of mice ($n = 39$) underwent an identical training intervention and LPS treatment as experiment 1, but instead of behavioral testing, mice were killed by CO₂ exposure 4 h after injection for tissue collection to examine proinflammatory cytokine and IDO gene expression. This time point was chosen based on previous data indicating it as the peak of proinflammatory cytokine and IDO gene expression [39]. Tissues were dissected out after transcardial perfusion with ice-cold PBS saline.

Experiment 2 (Aged Mice). A separate group of mice ($n = 38$) underwent an identical training intervention and LPS treatment as experiment 2, but instead of behavioral testing, mice were killed by CO₂ exposure 24 h after injection for tissue collection to examine proinflammatory cytokine and IDO gene expression. This time

point was chosen based on data by Godbout et al. [18] indicating it as the critical time point of prolonged proinflammatory cytokine and IDO gene expression, differentiating the aged and young adult neuroinflammatory response.

RNA Extraction and Reverse Transcription and Real-Time RT-PCR

Total RNA from whole brain was extracted with Qiagen RNeasy mini kits (Valencia, Calif., USA). Reverse transcription reactions were completed in an Eppendorf Mastercycler Thermocycler (Hamburg, Germany) using an Applied Biosystem (Foster City, Calif., USA) high-capacity reverse transcriptase kit with 2,000 ng total RNA and random primers for each reaction. Quantitative real-time reverse transcription PCR was performed on an Applied Biosystems Prism 7900 using TaqMan gene expression assays for TNF- α (Mm0043258_m1), IL-1 β (Mm00434228_m1), IL-6 (Mm00446190_m1), IL-10 (Mm00439616_m1), BDNF (Mm01334042_m1), IDO (Mm00492586_m1), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Mm999999_g1) purchased from Applied Biosystems. Reactions were performed in duplicate according to the manufacturer's instructions. Relative quantitative measurement of target gene expression was conducted using the $\Delta\Delta C_T$ method with GAPDH as the endogenous housekeeping gene and standard + saline-treated mice were used as the reference group. We chose to analyze TNF- α , IL-1 β , IL-6, IL-10, and IDO because they are critical mediators of inflammation-induced sickness and depressive-like behavior in mice [40–42]. BDNF is a critical neurogenic and anti-inflammatory growth factor that is highly influenced by exercise [43]. Our group has shown that inflammatory stimuli such as LPS can reduce brain BDNF [44, 45].

Statistical Analysis

Data from experiments 1 and 2 were analyzed independently using SPSS v18 (Chicago, Ill., USA). All data were normally distributed, as determined by the Shapiro-Wilk test. Exercise-induced differences in body weight and fatigability were detected using an independent sample t test (VWR vs. standard). Exercise-induced differences in behavioral and gene expression responses to LPS were detected using a 2 (VWR vs. standard) $\times$ 2 (saline vs. LPS) ANOVA with repeated measures when necessary. Any reference to 'groups' implies the following: (VWR + saline, VWR + LPS, standard + saline, and standard + LPS). Data are expressed as means $\pm$ SEM. The α level was set at $p \leq 0.05$ and all tests were two tailed. When appropriate, between-group differences were determined using Fisher's least significant difference post hoc multiple pairwise comparisons.

Results

Effects of VWR on Body Weight and Fatigability

Experiment 1 (Young Adult Mice). There were no statistically significant differences in baseline body weights between exercise conditions ($t_{36} = -0.31$, $p = 0.76$; table 1). Young adult VWR mice ran an average of 6.91 ± 0.25 km per day (table 1), and there were no differences

Table 1. Intervention-induced adaptations

	Distance run, km/day	Pre-intervention body weight, g	Post-intervention body weight, g	Body weight change, %	Forced exercise time-to-fatigue, min
<i>Experiment 1: young adult mice</i>					
Voluntary wheel	6.91±0.25	24.72±0.56	24.58±0.38	0.09±1.9 ^a	58.5±2.7 ^a
Standard	–	24.92±0.23	26.45±0.24	6.11±0.47	47.29±2.86
<i>Experiment 2: aged mice</i>					
Voluntary wheel	3.4±0.4	33.07±0.42	32.22±0.49	–1.43±1.20 ^b	75.7±8.50 ^b
Standard	–	33.23±0.50	33.30±0.29	1.35±0.77	33.34±2.58

Means ± SEM; n = 9–10/group. ^a p < 0.05 vs. young adult standard housing conditions. ^b p < 0.05 vs. aged standard housing conditions.

in daily running distance between young adult VWR + saline and young adult VWR + LPS groups ($t_{18} = 1.4$, $p = 0.18$). As a result of increased energy expenditure due to VWR, young adult VWR mice gained less body weight compared to young adult standard mice (time × exercise: $F_{1,36} = 15.8$, $p = 0.00$; table 1). There were no differences in body weight change ($t_{18} = -0.06$, $p = 0.96$) between VWR + saline and VWR + LPS mice. To assess VWR-induced improvements in muscle endurance, we subjected all young adult mice to a forced treadmill exercise test to exhaustion. Young adult VWR mice ran the longest before reaching exhaustion, running approximately 50% longer than young adult standard mice, indicating that the 30-day VWR protocol induced the expected metabolic adaptations responsible for improved endurance performance (intervention: $t_{33} = -2.7$, $p = 0.01$; table 1). There were no differences in forced exercise performance ($t_{18} = -0.80$, $p = 0.44$) between young adult VWR + saline and young adult VWR + LPS mice.

Experiment 2 (Aged Mice). There were no statistically significant differences in baseline body weights between exercise conditions ($t_{33} = -0.24$, $p = 0.81$; table 1). Aged VWR mice ran an average of 3.40 ± 0.40 km per day (table 1), and there were no differences in daily running distance between aged VWR + saline and aged VWR + LPS groups ($t_{16} = -0.98$, $p = 0.33$). Aged VWR mice lost more body weight compared to aged standard mice (time × exercise: $F_{1,33} = 15.1$, $p = 0.00$; table 1) and ran significantly longer before reaching exhaustion, indicating the efficacy of the 70-day VWR protocol (intervention: $t_{33} = -4.7$, $p = 0.00$; table 1). There were no differences in body weight change ($t_{16} = 0.64$, $p = 0.53$) or forced exercise performance ($t_{16} = 0.92$, $p = 0.37$) between aged VWR + saline and aged VWR + LPS mice.

Effects of VWR on LPS-Induced Brain Gene Expression

Experiment 1 (Young Adult Mice). We investigated whether VWR could mitigate LPS-induced gene expression changes in the brains of young mice 4 h after injection (fig. 1a). LPS administration resulted in a significant increase in whole-brain TNF- α , IL-1 β , IL-6, and IFN- γ mRNA (treatment: $F_{1,32} = 100.0$, $p = 0.00$; $F_{1,32} = 76.7$, $p = 0.00$; $F_{1,32} = 60.8$, $p = 0.00$; $F_{1,32} = 32.6$, $p = 0.00$ for TNF- α , IL-1 β , IL-6, and IFN- γ , respectively), which was not attenuated by VWR (intervention × treatment interaction: $F_{1,32} = 0.54$, $p = 0.47$; $F_{1,32} = 0.69$, $p = 0.41$; $F_{1,32} = 0.0$, $p = 0.99$; $F_{1,32} = 0.89$, $p = 0.35$ for TNF- α , IL-1 β , IL-6, and IFN- γ , respectively; fig. 1a). Similarly, LPS significantly upregulated IDO gene expression (treatment: $F_{1,32} = 27.3$, $p = 0.00$), which was also not altered by VWR (intervention × treatment: $F_{1,32} = 1.2$, $p = 0.28$). VWR, as applied in this study, could not attenuate the LPS-induced reduction in brain BDNF mRNA expression (intervention × treatment: $F_{1,32} = 1.98$, $p = 0.17$). There were no intervention main effects (p values for intervention main effects = 0.50, 0.41, 0.99, 0.28, 0.99, and 0.36 for TNF- α , IL-1 β , IL-6, IDO, IFN- γ , and BDNF, respectively). These data support our findings of a lack of effect of VWR on sickness and depressive-like behavior induced by LPS in young adult mice.

Experiment 2 (Aged Mice). At 24 h after injection, LPS administration resulted in a significant increase in whole-brain TNF- α , IL-1 β , and IL-6 mRNA (treatment: $F_{1,36} = 36.2$, $p = 0.00$; $F_{1,36} = 46.3$, $p = 0.00$; $F_{1,36} = 9.2$, $p = 0.005$ for TNF- α , IL-1 β , and IL-6, respectively), none of which were attenuated by VWR (intervention × treatment interaction: $F_{1,36} = 0.63$, $p = 0.43$; $F_{1,36} = 0.30$, $p = 0.59$; $F_{1,32} = 0.07$, $p = 0.80$ for TNF- α , IL-1 β , and IL-6, respectively;

Fig. 1. Effects of VWR on LPS-induced changes in sickness behavior, depressive-like behavior, and brain gene expression in young adult mice. LPS administration resulted in significant (*) upregulation of TNF- α , IL-1 β , IL-6, IDO, and IFN- γ , and a significant (*) downregulation of BDNF 4 h after injection (a). There were no significant intervention main effects or intervention by treatment interaction effects for any gene measured. LPS administration resulted in significant (*) reductions in food intake (b) and body weight (c), increased 24-hour TST immobility (d), and reduced 24-hour sucrose preference (e), but there were no intervention main effects or intervention by treatment interactions. Means $\pm$ SEM; $n = 9$ –10/group.

fig. 2a). LPS significantly upregulated brain IDO (treatment: $F_{1,36} = 12.0$, $p = 0.01$), which was not affected by VWR (intervention $\times$ treatment: $F_{1,36} = 0.004$, $p = 0.98$). LPS did not significantly increase whole-brain IFN- γ 24 h after injection (treatment: $F_{1,36} = 1.6$, $p = 0.21$). Additionally, VWR, as applied in this study, could not attenuate the LPS-induced reduction in brain BDNF mRNA expression (intervention $\times$ treatment: $F_{1,36} = 376$, $p = 0.54$). There were no intervention main effects (p values for intervention main effects = 0.74, 0.58, 0.68, 0.95, and 0.51 for TNF- α , IL-1 β , IL-6, IDO, and BDNF, respectively). Interestingly, however, there was a main effect for IFN- γ

in that VWR training reduced whole-brain IFN- γ mRNA expression (intervention: $F_{1,36} = 4.34$, $p = 0.04$). Collectively, these data support our findings of a lack of effect of VWR on sickness and depressive-like behavior induced by LPS in aged mice.

Effects of VWR on LPS-Induced Sickness Responses

Experiment 1 (Young Adult Mice). As expected, LPS injection resulted in a significant reduction in food intake compared to young adult saline-injected mice 24 h after injection (time $\times$ treatment: $F_{1,32} = 43.7$, $p = 0.00$), but there were no statistically significant differences be-

Fig. 2. Effects of VWR on LPS-induced changes in sickness behavior, depressive-like behavior, and brain gene expression in aged mice. LPS administration resulted in significant (*) upregulation of TNF- α , IL-1 β , IL-6, and IDO and a significant (*) downregulation of BDNF 4 h after injection. There were no significant intervention main effects or intervention by treatment interaction effects for any gene measured (**a**). LPS administration resulted in significant (*) reductions in food intake (**b**) and body weight (**c**), increased 48-hour TST immobility (**d**), and reduced 24-hour sucrose preference (**e**), but there were no intervention main effects or intervention by treatment interactions. Means $\pm$ SEM; $n = 9$ –10/group.

tween groups (time $\times$ exercise $\times$ treatment: $F_{1,32} = 0.08$, $p = 0.78$; fig. 1b). There was no difference in food intake between young adult saline-injected VWR and young adult saline-injected standard mice (time $\times$ intervention: $F_{1,32} = 0.58$, $p = 0.45$). As a result of LPS-induced anorexia, all young adult LPS-injected mice lost statistically significant amounts of body weight during the 24 h after LPS (time $\times$ treatment: $F_{1,32} = 36.8$, $p = 0.00$), and there were no significant differences between VWR and standard housed young adult mice in response to LPS (time $\times$ intervention $\times$ treatment: $F_{1,32} = 0.19$, $p = 0.67$; fig. 1c). There was no difference in body weight changes

between young adult saline-injected VWR and young adult standard mice (time $\times$ intervention: $F_{1,32} = 0.44$, $p = 0.51$).

Experiment 2 (Aged Mice). Like young adult LPS-injected mice, aged LPS-injected mice exhibited a significant reduction in food intake 24 and 48 h after injection compared to aged saline-injected mice (time $\times$ treatment: $F_{2,60} = 36.9$, $p = 0.00$), but there was no significant effect of VWR training (time $\times$ intervention $\times$ treatment: $F_{2,60} = 0.1$, $p = 0.93$; fig. 2b). There was no difference in food intake between aged saline-injected VWR and aged standard mice (time $\times$ exercise: $F_{2,60} = 0.43$, $p = 0.65$), nor was

there an exercise main effect (exercise: $F_{1,30} = 0.5$, $p = 0.49$). Aged LPS-injected mice lost significant body weight compared to aged saline-injected mice (time $\times$ treatment: $F_{2,60} = 36.1$, $p = 0.00$), and there were no significant differences between aged VWR and aged standard housed mice in response to LPS (time $\times$ exercise $\times$ treatment: $F_{2,60} = 0.03$, $p = 0.97$; fig. 2c). There was no difference in body weight changes between aged saline-injected VWR and aged standard mice (time $\times$ exercise: $F_{2,60} = 0.1$, $p = 0.93$), nor was there an exercise main effect (intervention: $F_{1,30} = 0.04$, $p = 0.83$).

Effects of VWR on LPS-Induced Depressive-Like Behavior

Experiment 1 (Young Adult Mice). We assessed depressive-like behavior in young adult mice 24 h after LPS injection via the TST and sucrose preference test. LPS significantly increased duration of immobility in the TST (treatment: $F_{1,32} = 6.3$, $p = 0.02$), but there was no interaction (exercise $\times$ treatment interaction: $F_{1,32} = 0.10$, $p = 0.75$; fig. 1d). There were no differences in baseline sucrose preference between groups ($F_{3,32} = 0.15$, $p = 0.93$). LPS significantly decreased sucrose preference compared to baseline (time $\times$ treatment interaction: $F_{1,32} = 5.97$, $p = 0.02$), but exercise did not affect this (time $\times$ exercise $\times$ treatment interaction: $F_{1,32} = 0.18$, $p = 0.67$; fig. 1e). There were no exercise main effects (p values for exercise main effects = 0.86 and 0.83 for TST and SPT, respectively). Taken together, these data indicate VWR training had no effects on LPS-induced depressive-like behavior in young adult mice.

Experiment 2 (Aged Mice). We assessed depressive-like behavior in aged mice via the decreased sucrose preference that is apparent 24 h after injection and the increased duration of immobility that is measured in the TST 48 h after injection. LPS significantly increased the duration of immobility (treatment: $F_{1,32} = 19.2$, $p = 0.00$), but there was no interaction (exercise $\times$ treatment interaction: $F_{1,32} = 0.00$, $p = 0.96$; fig. 2d). There were no differences in baseline sucrose preference between groups ($F_{3,34} = 0.83$, $p = 0.49$). LPS significantly decreased sucrose preference compared to baseline (time $\times$ treatment interaction: $F_{1,32} = 8.9$, $p = 0.006$), but exercise did not affect this (time $\times$ exercise $\times$ treatment interaction: $F_{1,32} = 0.28$, $p = 0.60$; fig. 2e). There were no exercise main effects (p values for exercise main effects = 0.97 and 0.22 for TST and sucrose preference tests, respectively). Taken together, these data indicate VWR training had no effects on LPS-induced depressive-like behavior in aged mice.

Discussion

We investigated whether a VWR intervention could attenuate LPS-induced depressive-like behavior in young adult and/or aged mice. Thirty days of VWR in young adult mice and 70 days of VWR in aged mice induced the expected training adaptations, including body weight loss and increased forced treadmill exercise performance. Independent of the intervention type, young adult mice injected with LPS exhibited a significant reduction in food intake, resulting in weight loss 24 h after injection. Similarly, aged mice injected with LPS exhibited a significant reduction in food intake, resulting in body weight loss 24 and 48 h after injection, which was also not affected by the VWR training. These data confirm our previous research demonstrating no beneficial effects of VWR on LPS-induced sickness behavior across multiple LPS doses in aged mice [13]. Contrary to our hypothesis, the measures of depressive-like behavior did not differ according to the exercise condition in either the young adult or aged mice. To further support the lack of an exercise effect on LPS-induced depressive-like behavior, we analyzed brain pro-inflammatory cytokine and IDO gene expression, and found no significant wheel running effects in either young adult or aged mice. Taken together, these data show clearly that prolonged voluntary exercise in mice has no effect on the neuroinflammatory and behavioral response to LPS and this lack of effect is the same in both young adult and aged mice.

A potential explanation for our lack of significant findings could be an insufficient study sample size to detect between-group differences. However, we conducted an a priori power analysis (type I and II errors set at 5 and 80%, respectively) to determine the minimum sample size necessary to observe a significant intervention $\times$ treatment interaction for our most variable measure, sucrose preference. Based upon a thorough investigation of the literature examining the effects of exercise training on depressive-like behavior in rodent models, we determined that we would need 10 mice per group to detect a ~40–50% (effect size ~0.65) exercise-induced difference in the sucrose preference test [46]. Therefore, we believe our data reflect a lack of VWR effect rather than insufficient statistical power. An alternative interpretation of our findings could be that our respective LPS doses were too high and masked any subtle benefits of VWR. However, as stated in the Methods section, we utilized the minimum effective LPS dose necessary to induce depressive-like behavior in the respective mouse models. We previously examined the effects of VWR training on LPS-induced sickness

behavior at a very low LPS dose (0.02 mg/kg) and observed no protective effects of VWR [13], which lends further credence to the lack of effect within our current experiments.

To the best of our knowledge, this is the first study to utilize a defined inflammatory challenge (i.e. LPS) to examine the effects of exercise training on inflammation-induced depressive-like behavior. Several groups have investigated the effects of exercise training on unpredictable chronic mild stress-induced depressive-like behavior and neuroinflammation, and found promising results. Solberg et al. [47] found 6 weeks of VWR of mice in conjunction with 6 weeks of chronic stress increased sucrose preference and decreased forced swim test immobility compared to mice that were exposed to chronic stress but without prior wheel training. These results were similar to those of Zheng et al. [48], who demonstrated 4 weeks of wheel training in conjunction with chronic mild stress recovered sucrose preference, which was associated with decreased hippocampal corticosterone expression and increased hippocampal BDNF expression. Exactly how BDNF may be mediating depressive-like behavior is unclear, but one possibility is that BDNF may be improving survival of neurons affected by chronic stress. Duman et al. [29] proposed an alternative molecular mechanism; these authors discovered 4 weeks of wheel training increased prefrontal cortex IGF-1, and this increase was associated with a protected sucrose preference and immobility in the forced swim test. While this response appears to be inherently different than the BDNF hypothesis, data by Park et al. [44, 45] indicate IGF-1 protects brain BDNF signaling during an inflammatory challenge. Therefore, it is plausible that exercise-induced IGF-1 could be mediating its antidepressant effects via BDNF regulation. These studies support much of the recent work on the neuroprotective effects of exercise, which have focused on BDNF regulation within the hippocampus. BDNF is a neurotrophin that acts predominantly in the hippocampus, cortex, and basal forebrain to promote neurogenesis, neuron survival, and synaptic plasticity. Like LPS, chronic stress reduced BDNF via an IL-1 β -dependent mechanism causing suppressed hippocampal neurogenesis and neuron survival [49]. Numerous studies have demonstrated that exercise training upregulates hippocampal BDNF gene and protein expression, and this is associated with improved behavioral outcomes during inflammatory challenge [29–31, 46, 50, 51]. We observed an LPS-induced reduction in whole-brain BDNF, but failed to detect a VWR-induced increase in whole-brain BDNF gene expression in both our young adult and aged mice. This

suggests the exercise-induced upregulation of BDNF demonstrated in the literature is not a global event, but rather a spatially dependent phenomenon primarily observed in the hippocampus.

More relevant to our specific aims, a recent study by Liu et al. [46] examined the effects of swim training on depressive-like behavior and IDO activity in rats submitted to chronic mild stress. Following 4 weeks of swim training in combination with chronic stress, swim-trained rats exhibited increased sucrose preference and decreased forced swim test immobility compared to chronically stressed rats that did not swim. Moreover, these behavioral results were associated with reduced prefrontal cortex IFN- γ , TNF- α , and IDO gene expression, indicating the protective effects of exercise training in this chronic stress model may be mediated by inhibiting IDO activation and metabolism. In relation to our results, these data indicate that depressive-like behavior in response to chronic mild stress is amenable to the beneficial effects of VWR, whereas LPS-induced depressive-like behavior is not. Again, it is important to note that we utilized the minimum LPS dosage which induces depressive-like behavior in our models. What is unknown is whether other inflammatory stimuli, perhaps those acting more chronically, are equally insensitive to VWR.

A potential explanation for the differential findings between our study and those using chronic mild stress is that LPS-induced depressive-like behavior is a well-defined acute phenomenon dependent on activation of the IDO pathway [15], while chronic mild stress-induced depression is a chronic paradigm dependent on numerous cellular and molecular mechanisms (e.g. hypothalamic-pituitary-adrenal axis dysregulation, aberrant monoamine signaling, and reduced growth factor synthesis), which synergize to induce a depressive-like behavior phenotype [52]. Under basal conditions, IDO is expressed at low levels in microglial cells and astrocytes, with the majority of IDO activity occurring in astrocytes, and the primary end product being kynurenic acid, a neuroprotective NMDA antagonist [8, 14, 53]. However, following immune activation (e.g. LPS), proinflammatory cytokines TNF- α and IFN- γ induce IDO activation in microglial cells, shifting kynurenine metabolism towards the production of 3-hydroxykynurenine and quinolinic acid, which are implicated in inflammation-induced depressive-like behavior [54]. An alternative explanation could be due to temporal differences in the administration of exercise and ‘inducer’ of behavioral disturbance. In chronic mild stress studies, exercise was performed chronically during the administration of the stressors,

whereas in our study exercise was performed prior to LPS administration. Mice were not allowed wheel access after LPS as it has been shown that they do not run during this acute inflammatory challenge [55]. This hypothesis concurs with the BDNF literature, which indicates exercise-induced BDNF improves neuron survival during stress rather than protecting against acute inflammatory injury [51].

Regarding the effects of exercise training on age-related neuroimmune alterations, several reports have demonstrated exercise may attenuate microglial priming [30, 31, 50, 56]. Vukovic et al. [56] found VWR training reduced hippocampal microglial expression of major histocompatibility complex II and increased neuronal fractalkine (CX3CL1) in young mice. Fractalkine binds to its receptor (CX₃CR) on microglia and maintains the cell in a quiescent state. Following immune activation, fractalkine signaling is critical for attenuating the microglial inflammatory response and restoring brain inflammation to baseline levels. Several studies indicate that aging reduces basal levels of fractalkine, which may partially explain microglial priming [11, 57–59]. Furthermore, following an LPS challenge, microglia from aged mice exhibited a prolonged downregulation of the fractalkine receptor compared to young mice, which may contribute to the prolonged proinflammatory cytokine response and protracted behavioral disturbances observed in aged mice [58]. Kohman et al. [50] demonstrated similar antipriming effects of exercise in hippocampal microglia of aged mice. Eight weeks of VWR in aged Balb/c mice reduced microglial proliferation and microglial IGF-1 gene expression [50]. Barrientos et al. [31] further addressed the effects of exercise on microglial priming by isolating primary hippocampal microglia from the wheel-trained and sedentary aged rats. Following stimulation with numerous doses of LPS (0–100 ng), the microglia from the VWR rats exhibited significantly reduced TNF- α and IL-1 β gene expression compared to microglia from rats housed in locked wheels, indicating that exercise training may ‘reverse’ age-induced microglial priming and subsequent neuroinflammation. Interpreting this study in the context of ours is difficult, as *ex vivo* microglial stimulation occurs under tightly controlled and optimal experimental conditions. The researchers did not report the upper-limit LPS concentration where they observed no differences in microglial priming between wheel running and locked-wheel rats. It is quite possible that the minimum effective LPS doses we utilized to induce depressive-like behavior corresponded to a higher ‘stimulus’ than 100 ng LPS applied to hippocampal microglia *in vitro*, thus abolishing

any protective effect of exercise. Additionally, similar to the BDNF literature, these studies only examined the effects of exercise on hippocampal microglial priming, the brain region most affected by exercise training. Our lack of observed exercise-induced neuroprotection in whole-brain tissue further supports our hypothesis that the protective effects of exercise are not a global event, but rather a brain region-dependent phenomenon primarily observed in the hippocampus.

While our study clearly demonstrates no effect of VWR on LPS-induced depressive-like behavior in young adult and aged mice, we recognize certain limitations. We did not include a ‘locked’-wheel control group for the voluntary wheel intervention. It is plausible that any observed VWR effects could have been due to environmental enrichment rather than adaptations induced by the exercise component of wheel running. However, we have previously reported no differences between VWR, locked-wheel, and standard housing interventions on LPS-induced sickness behavior in aged mice [13]. Furthermore, Kobil et al. [60] elegantly demonstrated the act of running, rather than environmental enrichment, is responsible for neurotrophic effects of VWR. A second potential limitation is that we conducted our forced treadmill test to fatigue 5–6 days prior to LPS administration and behavioral testing, which, given the differential central nervous system effects induced by forced versus voluntary running, could have influenced our results. However, Carmichael et al. [61] previously demonstrated that plasma creatine kinase, brain IL-1 β , and VWR were normalized in adult mice 24 h after prolonged uphill treadmill running. Additionally, we observed no reductions in daily VWR distances in the 5- to 6-day post-treadmill running tests in both our young adult and aged mice, indicating our treadmill testing protocol induced minimal stress and fatigue in our mice. A final limitation is that our study design did not assess brain cytokine and IDO gene expression across numerous time points, which would strengthen our conclusion that VWR does not affect LPS-induced IDO induction and the subsequent depressive-like behavior. We chose to assess brain gene expression at the selected time points based on observations that 4 h after injection inflammation peaked in young mice, and 24 h after injection was the critical time point of prolonged proinflammatory cytokine and IDO gene expression, differentiating the aged and young adult neuroinflammatory responses [12, 18]. Because we did not conduct a time course analysis of brain cytokine and IDO gene expression, we cannot definitively conclude that VWR had no effects on neuroinflammation at the given

doses of LPS, and it is possible that subtle inflammatory effects persist in the brain that are not reflected by behavioral outcomes. Similarly, we recognize whole-brain analysis does not reveal potential exercise-induced neuroprotective regional differences, and thus can only speculate that the protective effects of exercise occur in a region-specific manner. Given that depressive-like behavior was our primary outcome and there were no observed VWR-induced changes in depressive-like behavior, measuring brain cytokine and IDO gene expression at numerous time points and across numerous brain regions did not seem worthwhile in the context of our experiments.

In conclusion, we demonstrate that voluntary wheel exercise training does not affect LPS-induced depressive-like behavior, nor does it influence LPS-induced brain cytokine or IDO gene expression 4 or 24 h after injection,

in young adult and aged mice, respectively. The lack of neuroprotective effects occurred despite using the minimum effective doses and optimal behavioral time points in our respective mouse models. Collectively, these data indicate the antidepressant and neuroprotective effects of exercise are sensitive to the inflammatory stimulus utilized (i.e. LPS vs. chronic mild stress), and further research is necessary to elucidate the complex effects of exercise training on inflammation-induced depressive-like behavior.

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