Behavioral and neuronal interactions between exercise and alcohol: Sex and genetic differences

Winona C. Booher1,2 | Guillermo J. Reyes Martínez1,2 | Marissa A. Ehringer1,2

1Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado
2Department of Integrative Physiology, University of Colorado, Boulder, Colorado

Correspondence
Dr. Marissa A. Ehringer, University of Colorado, Institute for Behavioral Genetics, 447 UCB, Boulder, Colorado 80309.
Email: marissa.ehringer@colorado.edu

Abstract
Alcohol use disorders (AUDs) lead to early death and many devastating consequences for individuals, families and society. Currently, few effective treatments are available, but emerging research suggests exercise might be beneficial in some individuals. To develop the most effective exercise treatment program, more research on intensity, type, timing, stage of addiction, drug involved, sex of subject and subject population is needed. This review highlights the complexity of the interaction between alcohol behaviors and exercise, with a focus on the role of sex and genetics. Moreover, we describe a variety of rodent models used to investigate the neuronal physiology changes that underlie alcohol consumption and exercise. Specifically, current data indicate that moderate exercise may ameliorate neuronal damage caused by alcohol consumption. Additionally, we describe studies of rodent models in the context of hedonic substitution to draw broad conclusions about shared underlying neurobiological mechanisms. Until recently, most studies in rodents were performed only in males, and few studies have utilized different genetic strains of mice or rats. Comparing similar behavioral paradigms across sex and strain, it has become clear that major sex and genetic differences exist for each behavioral context alone (alcohol consumption and exercise) and combined. Therefore, future research in this area should be developed with careful study design and attention to address both of these factors.

Keywords
addiction, alcohol, drugs, environmental enrichment, exercise, genetics, mouse, rat, sex differences, wheel running

1 | INTRODUCTION

Alcohol Use Disorder (AUD) is defined as “a chronic relapsing brain disease characterized by an impaired ability to stop or control alcohol use despite social, occupational or health consequences.” In 2016, an estimated 488,000 adolescents aged 12 to 17, 3.7 million young adults aged 18 to 25 and 10.9 million adults aged 26 or older had suffered from an AUD in the past year. Heavy alcohol use negatively affects the health of a person’s brain, heart, liver, pancreas, immune system and increases a person’s risk of cancer and mental health problems. Although there has been progress in the field, the risk factors underlying the transition from controlled alcohol use to AUD are still poorly understood, and prevention and treatment approaches are limited.

In contrast to the long list of negative health consequences alcohol consumption causes, overwhelming evidence suggests that lifelong exercise increases health span and delays the onset of numerous chronic conditions/diseases. This review discusses progress from...
recent work examining possible positive effects of exercise on pathological consequences as a result of alcohol consumption, including behavioral and neurophysiological effects. It is broadly organized to discuss how sex and genetic background influence outcomes related to either concurrent, intermittent or successive exposure to alcohol and exercise. The central topics focus on rodent studies, but a few examples of human studies are included to provide relevance to the human condition. We present a summary of evidence from papers listed in Tables 1 and 2 demonstrating that: (a) both moderate alcohol consumption and moderate exercise are rewarding, (b) exercise can ameliorate pathological neuronal consequences caused by excessive or chronic alcohol consumption, (c) chronic levels of alcohol intake and intense exercise can lead to hypoactivity of reward function, and a variety of other neuronal adaptations, (d) effects of exercise on alcohol behaviors is dependent on multiple factors, including timing of exposure to the wheel, whether the wheel is locked, and timing of exposure to alcohol and (e) there is evidence that interaction effects between alcohol and exercise exposure show differential responses due to sex and underlying genetics. Throughout the review, sex differences will be highlighted, since many studies only examined one sex. Furthermore, none of the studies used experimental designs to examine the effects of gonadal hormones, so we cannot draw any conclusions about how these may fluctuate under testing conditions that combine alcohol and exercise. It is beyond the scope of this review to summarize all of the individual studies examining sex hormones using models of alcohol-related phenotypes or models of exercise, but we conclude with a discussion about future directions in this area.

2 | MODERATE ALCOHOL CONSUMPTION AND MODERATE EXERCISE

2.1 | Alcohol and exercise are rewarding

There is a well-documented correlation between exercise intensity and alcohol consumption in both men and women,5-10 likely due to the activation of the mesolimbic dopaminergic reward pathway.11-19 Briefly, dopaminergic neurons in the ventral tegmental area (VTA) project to the nucleus accumbens (NAc), a key brain region involved in the processing of reward response.20,21 Both alcohol consumption and exercise activate the mesolimbic dopaminergic reward system through increases in dopamine concentrations and dopamine receptor binding.11-19,22-26 Dopamine signaling is widely used as a "marker" for measuring feelings of reward.27 One approach to measuring mesolimbic dopaminergic reward system activation is to quantify levels of tyrosine hydroxylase (TH), which is the rate-limiting factor in dopamine synthesis.28 For example, both rats with access to voluntary wheel running and zebrafish exposed to alcohol exhibited increased TH mRNA levels in the VTA, suggesting both alcohol and exercise increase VTA dopamine concentrations.11,25 Furthermore, rats with access to voluntary wheel running and alcohol-dependent rats exhibited increased NAc Fos expression, suggesting both alcohol and exercise increase NAc neuronal activity.11,26 However, administration of alcohol directly into the NAc of a rat had no effect on extracellular dopamine levels.29,30 Together, these studies suggest that alcohol works by facilitating the firing of dopaminergic neurons from the VTA to the NAc via a disinhibitory mechanism involving the reduction of potassium currents in the VTA.20,21,30,31 Human imaging studies have measured dopamine receptor binding to confirm that the euphoric feeling experienced during alcohol consumption is a result of endogenous dopamine release in the NAc.16-18 Additionally, physically active adults exhibited reduced age-related striatal dopamine D2 receptor loss.32 In summary, these studies provide support that both moderate alcohol consumption and moderate exercise activate the mesolimbic dopaminergic reward system. For this reason, moderate exercise may be beneficial when trying to reduce alcohol consumption or combat alcohol cravings.

2.2 | Evidence that exercise ameliorates neuronal damage due to alcohol consumption

As noted above, there is a well-documented relationship between exercise intensity and alcohol consumption in both men and women.5-10 Mesolimbic dopaminergic reward pathway activation from both behaviors may contribute to this interaction; however, Leasure et al33 speculated about other factors that may be involved. They focused on four motivating reasons why people who consume high levels of alcohol may also exercise intensely: "work hard, play hard," celebration, body image and guilt. This hypothesis posits that people attempt to use exercise to offset the pathological effects of alcohol consumption. In the context of the brain, there is evidence to support the idea that these behaviors result in several counteracting effects on synaptic plasticity related to neuronal cell death and proliferation.34-39

For example, heavy alcohol consumption causes neuronal damage by increasing oxidative stress and neuroinflammation.37,40-42 After ingestion, alcohol metabolism generates two toxic by-products: acetaldehyde and reactive oxygen species. This process leads to a reduction of antioxidant enzymes and eventually oxidative tissue damage.37 Conversely, both aerobic and anaerobic exercise upregulate the expression of antioxidant enzymes and therefore counteract the oxidative damage caused by stress, such as alcohol consumption.43 Using a variety of behavioral paradigms in rodent models, several groups have demonstrated that physical exercise can increase neurogenesis in the hippocampus, basal forebrain and cerebellum by mitigating the neuroinflammation and oxidative damage caused by binge alcohol exposure.34,36,37,39,42,44 In contrast, a study conducted with female Long-Evans rats reported that binge-alcohol exposure increased and caused a prolonged effect on the morphology of medial prefrontal cortex microglia, regardless of exercise.45 In a follow up study, it was demonstrated that this effect was sex-dependent, and only observed in female rats.46 These studies also highlight a critical sex disparity in this body of literature, since only a handful included female rodents.36,44,46 This is especially relevant given reported sex
<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects and sex</th>
<th>Exercise schedule</th>
<th>Alcohol schedule</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maynard &amp; Leasure, Plos One, 2013</td>
<td>Female Long-Evans rats</td>
<td>28 days of voluntary wheel running following 1 or 2 cycles of binge alcohol exposures</td>
<td>1 or 2 cycles of binge alcohol exposure (1 cycle = 4 days of 5 g/kg alcohol liquid diet via gavage every 8 hours)</td>
<td>Exercise restored alcohol-induced hippocampal damage</td>
</tr>
<tr>
<td>Helfer et al Brain Research, 2016</td>
<td>Adolescent male Long-Evans rats</td>
<td>Unlimited access to voluntary wheel running on postnatal days 30-72</td>
<td>Gastric infusion of 5.25 g/kg alcohol on postnatal days 4-9</td>
<td>Adolescent wheel running increased cell proliferation and neurogenesis in the hippocampus of alcohol-exposed rats</td>
</tr>
<tr>
<td>Barton et al, Neuroscience, 2017</td>
<td>Female Long-Evans rats</td>
<td>1 week after the last alcohol exposure, 28 days of voluntary wheel running</td>
<td>4 days of 5 g/kg alcohol liquid diet via gavage every 8 hours</td>
<td>Binge alcohol treatment, regardless of exercise, exerts a prolonged effect on morphology of mPFC microglia</td>
</tr>
<tr>
<td>Barton et al, Brain Science, 2017</td>
<td>Male and female Long-Evans rats</td>
<td>6 days after the last alcohol exposure, 11 days of voluntary wheel running</td>
<td>4 days of 5 g/kg alcohol liquid diet via gavage every 8 hours</td>
<td>Voluntary wheel running, following binge alcohol treatment, exerts sex specific effects on microglia (increased MHC II in the female medial prefrontal cortex and hippocampus, no effect in males)</td>
</tr>
<tr>
<td>Vetreno et al, Frontiers in Neuroscience, 2018</td>
<td>Adolescent male Wistar rats</td>
<td>Paired housing, unlimited access to voluntary wheel running on postnatal days 24-80</td>
<td>5 g/kg alcohol intragastric delivery (2 days on, 2 days off) during postnatal days 25-55</td>
<td>Voluntary wheel running prevented alcohol-induced loss of neurogenesis markers in the hippocampus, blocked increased expression of cell death marker cleaved caspase 3 and prevented the increase of hippocampal pNF-κB p65 and induction of neuroimmune NF-κB target genes, including TNFα and IκBα</td>
</tr>
<tr>
<td>Vetreno &amp; Crews., Plos One, 2018</td>
<td>Adolescent male and female Wistar rats</td>
<td>Paired housing, unlimited access to voluntary wheel running on postnatal days 24-80</td>
<td>5 g/kg alcohol intragastric delivery (2 days on, 2 days off) during postnatal days 25-55</td>
<td>Voluntary wheel running from prevented alcohol-induced cholinergic neuron shrinkage and loss of cholinergic neuron markers as well as the increase of pNF-κB p65 in the adult basal forebrain</td>
</tr>
<tr>
<td>Vetreno et al Addiction Biology, 2018</td>
<td>Adolescent male Wistar rats</td>
<td>Paired housing, unlimited access to voluntary wheel running on postnatal days 56-95</td>
<td>5 g/kg alcohol intragastric delivery (2 days on, 2 days off) during postnatal days 25-55</td>
<td>Voluntary wheel running restored cognitive deficits, and normal levels of H3K9me2 and DNA methylation at promoter regions of Chat and H3K9me2 of Trkα</td>
</tr>
<tr>
<td>Lamarão-Vieira et al Oxidative Medicine and Cellular Longevity, 2018</td>
<td>Adolescent male Wistar rats</td>
<td>30 minute sessions of treadmill running, 5X/week for 4 weeks</td>
<td>4 cycles of binge-alcohol exposure by gavage (1 cycle = 3 g/kg for 3 days, off for 4 days)</td>
<td>Physical exercise attenuated oxidative stress and morphofunctional cerebellar damages</td>
</tr>
<tr>
<td></td>
<td>Adolescent male Wistar rats</td>
<td></td>
<td>4 cycles of binge-alcohol exposure by gavage</td>
<td>Physical exercise promoted neuroprotective effects in</td>
</tr>
</tbody>
</table>
differences in the response to binge-like alcohol consumption and metabolism, in which hormonal factors may contribute to the apparent selective vulnerability of the female brain to alcohol. For example, alcohol consumption causes females to display greater susceptibility to innate oxidative stress, neuroinflammation and anatomical and histological alterations due to alcohol consumption. While more research is needed in the female population, together these studies suggest that physical exercise may be used as a preventative and therapeutic tool, particularly in females, to ameliorate some of the pathological neuronal effects caused by alcohol consumption.

The long-term consequences of repeated neuroinflammation and oxidative stress include a decline in cognitive function. In contrast, regular exercise has been shown to increase reaction time, nonspatial memory, object recognition, spatial learning and overall cognitive performance. As mentioned above, exercise may be able to prevent and reverse a portion of the neuroinflammation and oxidative stress caused by alcohol consumption. Thus, there is an emerging body of work supporting the hypothesis that physical exercise may be able to repair cognitive deficits caused by heavy alcohol consumption. In several studies of adolescent and adult male Wistar and Long-Evans rats (females not tested), those exposed to physical activity following binge-alcohol exposure demonstrated improved cognitive function, motor activity and short-term recognition memory. Again, there has been an imbalance in study across sexes in this body of literature, especially given the knowledge that females display greater susceptibility to acute and long-term memory alterations due to alcohol consumption. While more research is needed in females, these results imply that physical exercise can improve cognitive deficits, and therefore may provide positive treatment benefits among those consuming moderate amounts of alcohol.

3 | Behavioral Models Investigating the Interaction Between Wheel Running and Alcohol Consumption

Numerous studies in rodents have provided evidence for an interaction between voluntary wheel running and alcohol consumption, In a recent review, Hallgreen et al pointed out that, "physical exercise is widely regarded as "medicine" for the prevention and treatment of myriad somatic health conditions." However, the effectiveness of physical activity to treat AUDs remains understudied. Additional trials are needed to validate the effectiveness of physical exercise to treat different aspects of AUDs (eg, consumption, cravings, withdrawal), but physical exercise remains as a safe and free alternative that may also improve comorbid health problems.

3.1 Concurrent models of alcohol consumption and exercise

Several studies have investigated the effect of voluntary exercise on 2-bottle choice (2BC) and 2-hour limited access drinking in the dark (DID) alcohol paradigms using unlimited access to voluntary wheel running. In an early study, Crews et al used a typical 12 day 10% alcohol 2BC paradigm and reported no effect of voluntary wheel running on male C57BL/6J alcohol consumption (females were not tested). Conversely, Pichard et al reported a decrease in male C57BL/6J 10% alcohol consumption with access to voluntary wheel running but no change in male DBA/2J alcohol consumption. In these experiments, the 2BC paradigm was much longer (51 days) than typical 2BC protocols and unlimited access to voluntary wheel running did not begin until day 17. Furthermore, in the 2BC alcohol intake model, DBA/2J mice consume very little, therefore may not be the best model to examine the effect of exercise on voluntary alcohol consumption. In a different series of studies, Ehringer et al used separate cohorts of male and female C57BL/6J mice to investigate the effect of voluntary wheel running on both 10% and 20% alcohol 2BC and DID (20% alcohol). Notably, C57BL/6J mice are virtually identical to C57BL/6J mice, a strain known to consume and prefer large quantities of alcohol. The difference between the strains is the C57BL/6J colonies were bred and maintained at the University of Colorado Boulder, Institute for Behavioral Genetics (IBG) for several years without bringing in breeders from an outside vendor. Access to a running wheel significantly reduced female C57BL/6J alcohol consumption and preference in both 10% and 20% alcohol 2BC. However, this effect was not observed in male C57BL/6J mice or in either sex during the DID paradigm. The 10% alcohol 2BC result has been replicated in three additional studies, which also expanded to examine the effects of each environment (wheel running, alcohol 2BC or both) on gene expression. Darlington et al examined mRNA expression levels of several alcohol- and exercise-related genes in various regions of the brain. Importantly, Bdnf mRNA showed increased expression in the hippocampus due to exercise, but decreased expression due to alcohol, supporting previous work described above. In addition, expression of the dopamine receptor D1 (Drd1) mRNA was reduced in the striatum due to exercise, and dopamine receptor genes have recently emerged from large human genome-wide association studies. Furthermore, mRNA expression for Slc18a2, a monoamine transporter protein, showed an
<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects and sex</th>
<th>Exercise schedule</th>
<th>Alcohol schedule</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barr, Pharmacology Biochemistry Behavior, 1988</td>
<td>Male Sprague-Dawley rats</td>
<td>Unlimited access to voluntary wheel running for 8 weeks</td>
<td>Alcohol liquid diet, 5-35% of energy content, increased in 5% increments each week</td>
<td>Alcohol consumption did not affect wheel running</td>
</tr>
<tr>
<td>McMillan et al, Drug and Alcohol Dependence, 1995</td>
<td>Male alcohol-preferring (P) and nonalcohol-preferring (NP) rats</td>
<td>No wheel on days 1–10, unlimited access to voluntary wheel running on days 11-20, locked wheel on days 21-25, unlimited voluntary wheel running on days 26-31</td>
<td>3 rounds (different rats), 2-bottle choice, 5% alcohol for first and second round, 10% alcohol for third round</td>
<td>Initial access to voluntary wheel running decreased alcohol intake in P rats, no effect in NP rats. Second access to voluntary wheel running had no effect in P or NP rats</td>
</tr>
<tr>
<td>Werme et al, Behavioral Brain Research, 2002</td>
<td>Male Lewis rats</td>
<td>Unlimited access to voluntary wheel running for 5 weeks of alcohol consumption, withdrawal and reinstatement</td>
<td>2-bottle choice with 10% alcohol for 5 weeks, followed by a 1, 2 or 4-week withdrawal period and then alcohol reinstatement</td>
<td>Voluntary wheel running during the 1 and 2 week withdrawal periods resulted in increased alcohol preference during reinstatement, no difference during the 4 week withdrawal period</td>
</tr>
<tr>
<td>Crews et al, Alcohol, 2004</td>
<td>Male C57BL/6J mice</td>
<td>Unlimited access to voluntary wheel running for 12 days</td>
<td>No access on days 1-4, 2-bottle choice on days 5-12 with escalating alcohol concentrations from 2 to 10%</td>
<td>Access to voluntary wheel running had no effect on alcohol intake</td>
</tr>
<tr>
<td>Ozburn et al, Alcohol, 2008</td>
<td>Female C57BL/6J mice</td>
<td>Intermittent access to voluntary wheel running</td>
<td>2-bottle choice with 10% alcohol for 93 days, alcohol removed on days 80-86</td>
<td>Wheel access did not significantly alter alcohol intake, alcohol deprivation increased wheel running, resumption of alcohol reduced wheel running</td>
</tr>
<tr>
<td>Ehringer et al, Alcohol, 2009</td>
<td>Male and female C57BL/6 mice</td>
<td>Unlimited access to voluntary wheel running for 13 days, locked wheel control included</td>
<td>2-bottle choice with alcohol concentration escalating from 3 to 10%</td>
<td>Voluntary wheel running, not a locked wheel, reduced female alcohol consumption and preference, no effect in males</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unlimited access to voluntary wheel running for 14 days, locked wheel control included</td>
<td>2-bottle choice with alcohol concentration escalating from 3-20%</td>
<td>Voluntary wheel running, not a locked wheel, reduced female alcohol consumption and preference, no effect in males</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unlimited access to voluntary wheel running on days 8-18, locked wheel control included</td>
<td>No access to alcohol on days 1-14, 2-hour access to 20% alcohol during dark cycle on days 15-18</td>
<td>No differences in alcohol consumption for both male and females</td>
</tr>
<tr>
<td>Pichard et al, Alcohol, 2009</td>
<td>Male C57BL/6J &amp; DBA/2J mice</td>
<td>No wheel access on days 1-16, unlimited access to voluntary wheel running on days 17-23, forced wheel running on days 24-44, unlimited access to voluntary wheel running on days 45-51</td>
<td>2-bottle choice with alcohol concentration escalating from 0 to 10% during days 1-16, 10% alcohol on days 17-51</td>
<td>Forced wheel running resulted in increased alcohol intake in C57BL/6J mice, no effect in DBA/2J mice</td>
</tr>
<tr>
<td>Reference</td>
<td>Subjects and sex</td>
<td>Exercise schedule</td>
<td>Alcohol schedule</td>
<td>Results</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------</td>
<td>------------------</td>
<td>-----------------</td>
<td>---------</td>
</tr>
<tr>
<td>No wheel access on days 1-16, unlimited access to voluntary wheel running on days 17-51</td>
<td>2-bottle choice with alcohol concentration escalating from 0 to 10% during days 1-16, 10% alcohol days 17-51</td>
<td>C57BL/6J mice with voluntary wheel access reduced alcohol intake, no effect on DBA/2J mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brager &amp; Hammer., Physiology &amp; Behavior, 2012</td>
<td>Male aged Syrian Hamsters</td>
<td>60 day experiment with unlimited access to voluntary wheel running days 11-40, locked wheel control included</td>
<td>2-bottle choice with 20% alcohol</td>
<td>Access to voluntary wheel running, not a locked wheel, reduced alcohol consumption and preference</td>
</tr>
<tr>
<td>McCulley et al, Pharmacology Biochemistry and Behavior, 2012</td>
<td>Male Charles River (CR) rats</td>
<td>25-27 days of voluntary wheel running</td>
<td>Liquid diet with 6% vol/vol alcohol on days 11-24</td>
<td>Voluntary exercise attenuated alcohol withdrawal-induced increases in seizure susceptibility</td>
</tr>
<tr>
<td>Devaud et al., Pharmacology Biochemistry and Behavior, 2012</td>
<td>Male and female Charles River (CR) rats</td>
<td>25–27 days of voluntary wheel running</td>
<td>Liquid diet with 6% vol/vol alcohol on days 11-24</td>
<td>Voluntary exercise attenuated alcohol withdrawal-induced increases in seizure susceptibility in both males and females</td>
</tr>
<tr>
<td>Piza-Palma et al, Alcoholism: Clinical and Experimental Research, 2014</td>
<td>Male and female C57BL/6J mice</td>
<td>Unlimited access to voluntary wheel running on days 1-14, running wheel was locked for 6 hours on days 15-20</td>
<td>2-bottle choice with 12% alcohol on days 1-14, days 15-20 alcohol was removed for 3 hours (first half of locked wheel period)</td>
<td>Females increased their alcohol consumption and preference when their running wheels were locked, no effect in males</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unlimited access to voluntary wheel running on days 1-15 or intermittent locked wheel on days 6-15</td>
<td>2-hour access to 20% alcohol during dark cycle</td>
<td>Females increased their alcohol consumption and preference when their running wheels were locked, no effect in males</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unlimited access to voluntary wheel running on days 1-5, intermittent locked wheel on days 6-15</td>
<td>2-hour access to 20% alcohol during dark cycle</td>
<td>Females increased their alcohol consumption and preference when their running wheels were locked, no effect in males</td>
</tr>
<tr>
<td>Darlington et al, Behavioural Brain Research, 2014</td>
<td>Female C57BL/6J mice</td>
<td>Unlimited access to voluntary wheel running for 16 days</td>
<td>2-bottle choice with alcohol concentrations escalating from 3 to 10%</td>
<td>Voluntary wheel running decreased female alcohol consumption and preference</td>
</tr>
<tr>
<td>Gallego et al, Physiology &amp; Behavior, 2015</td>
<td>Adolescent male and female C57BL/6Lbg mice</td>
<td>Unlimited access to voluntary wheel running for 21 days</td>
<td>2-bottle choice with 10% alcohol for 21 days</td>
<td>Voluntary wheel running decreased female alcohol consumption and preference</td>
</tr>
<tr>
<td>Darlington et al, Genes, Brain, and Behavior, 2016</td>
<td>Female C57BL/6Lbg mice</td>
<td>Unlimited access to voluntary wheel running for 16 days</td>
<td>2-bottle choice with alcohol concentration escalating from 3 to 10%</td>
<td>Voluntary wheel running decreased female alcohol consumption and preference</td>
</tr>
<tr>
<td>McGonigle et al, Alcohol, 2016</td>
<td>Female C57BL/6J, β-endorphin knock-out (βE-KO) and β-endorphin heterozygote (βE-HT)</td>
<td>Unlimited access to voluntary wheel running every other day for 10 days</td>
<td>2 hour access to 20% alcohol during dark cycle</td>
<td>βE-KO and βE-HT mice increased alcohol consumption when wheel running was prevented</td>
</tr>
</tbody>
</table>
interaction effect due to exercise and alcohol. In cages without a wheel, mice with alcohol showed increased *Slc18a2* expression compared with water only mice. In cages with a wheel, mice with alcohol showed decreased expression of *Slc18a2* compared with water/wheel mice. This work was extended in a follow-up study using RNA sequencing to identify differentially expressed genes and gene networks in the striatum due to wheel running, alcohol 2BC or both. Several genes were identified showing differential expression due to the interaction between alcohol consumption and exercise, including multiple potassium channel genes, *Oprm1*, *Prkcg*, *Stxbp1*, *Cshr1*, *Gabra3*, *Slc6a13*, *Stx1b*, *Pomc*, *Rassf5* and *Camta2*. In both of these gene expression studies, only female C57BL/6Jb mice were used since the behavioral effect had not been observed in males. Gallego et al. conducted similar experiments using adolescent C57BL/6Jb male and female mice. The differential sex effect observed in the initial study of adults was replicated, since the female adolescent mice showed decreased voluntary alcohol intake with the wheel, while the males did not. Differences in *Bdnf* mRNA and BDNF protein expression in the hippocampus due to exercise during adolescence was also confirmed, but in both sexes, leaving in question what other factors may contribute to the combined interaction that may be different in males and females. Furthermore, this is one of the few studies that reported a linear correlation between distance traveled via wheel running and change alcohol consumption due to exercise. However, this effect of distance traveled was observed only in the females, leading the authors to speculate that sex hormones may contribute to the interaction effects. Taken together, the results of these studies, using fairly simple behavioral paradigms, suggest that exercise (unlimited access to voluntary wheel running) may serve as a hedonic substitution for alcohol consumption. However, length of the experiment and sex both affect the results.

### 3.2 Effect of locked wheel on alcohol consumption

Although many of these studies suggest that unlimited access to wheel running reduces alcohol consumption and preference, it is possible that exercise (wheel-running) is simply a form of environmental enrichment, and the observed effects are not specific to exercise. Ehringer et al. addressed this possibility by locking the running wheel as an additional cage condition, which was compared with the no wheel and unlocked wheel conditions. Female C57BL/6Jb mice reduced their 10% alcohol preference from ~80% (alcohol/sedentary) to ~50% (alcohol/running) but did not show reduced consumption under the locked wheel condition. Conversely, male C57BL/6Jb mice reduced their 10% alcohol preference from ~66% (alcohol/sedentary) to ~40% (alcohol/locked wheel), showing an "intermediate" reduction in alcohol preference which a wheel that did not move. Notably, neither the presence of a free nor a locked wheel affected male or female C57BL/6Jb alcohol consumption. In a separate series of studies of two more moderately drinking strains, interesting differences due to sex and genetics were observed with regard to a locked wheel compared with a free running wheel and 10% alcohol preference. In 129/SvEvTac mice, both sexes showed reduced alcohol consumption with both a locked wheel and a free running wheel. However, a sex difference was observed in C3H/Ibg mice, where the males showed a decrease in alcohol consumption with a locked wheel and free wheel, but the C3H/Ibg females showed no differences in

#### Table 2 (Continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects and sex</th>
<th>Exercise schedule</th>
<th>Alcohol schedule</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somkuwar et al, Brain, Behavior, and Immunity, 2016</td>
<td>Male Wistar rats</td>
<td>Paired housing, access to voluntary wheel running throughout the entire experiment</td>
<td>2–3 weeks of 10% alcohol self administration via lever presses, followed by another 7 weeks of self administration or concurrent self administration and chronic intermittent ethanol (CIE) vapor</td>
<td>Voluntary wheel running enhanced alcohol seeking during abstinence in rats not exposed to CIE, voluntary wheel running reduced alcohol reinstatement in both CIE and non CIE exposed rat</td>
</tr>
<tr>
<td>Lynch et al, Physiology &amp; Behavior, 2019</td>
<td>Adolescent male Long-Evan rats</td>
<td>30 minute access to voluntary wheel running for 24 sessions</td>
<td>After running sessions were completed, rats were given intermittent access to 20% alcohol for 4 weeks</td>
<td>Access to voluntary exercise preceded greater alcohol intake/preference in adulthood</td>
</tr>
<tr>
<td>Booher et al, Alcohol, 2019</td>
<td>Male and female 129/SvEvTac &amp; C3H/Ibg mice</td>
<td>Unlimited access to voluntary wheel running for 13 days, locked wheel control included</td>
<td>2-bottle choice with alcohol concentrations escalating from 3 to 10%</td>
<td>Voluntary wheel running and a locked wheel reduced male and female 129/SvEvTac and male C3H/Ibg alcohol consumption, no effect in female C3H/Ibg mice</td>
</tr>
</tbody>
</table>
alcohol intake among any of the three groups. Finally, Brager and Hammer demonstrated that aged Syrian male hamsters (24 months of age) with access to a free running wheel, as opposed to a locked wheel, will significantly decrease their 20% alcohol consumption and preference. In summary, these studies suggest that exercise, not just environmental enrichment, is a key component for the reduction of alcohol consumption. Importantly, its efficacy is modulated by both sex and genotypic background.

3.3 | Intermittent access models of alcohol consumption and exercise

Another commonly used method to investigate the effect of exercise on alcohol consumption is intermittent wheel access during 2BC and DID alcohol paradigms. For example, McMillan et al reported that initial access to voluntary wheel running decreased 5% and 10% 2BC alcohol consumption in male alcohol-prefering (P) rats, which were selectively bred for high alcohol consumption, but had no effect on their male nonalcohol-prefering (NP) counterparts. However, this effect disappeared during the rats’ second access to voluntary wheel running, but this has not been studied further, so the neurobiological differences that may underlie the difference in response upon the second access to a wheel, compared with the first, are completely unknown. In female C57BL/6J mice, Ozburn et al utilized an extensive 93 day 10% alcohol 2BC behavioral paradigm to examine the effects of environmental enrichment, wheel running and alcohol deprivation. They provided alternating access to a locked and free running wheel on a weekly basis, in the presence of 2BC alcohol, and no differences in alcohol intake were observed. However, following the 79 days of unlimited alcohol access with weekly alternating locked and free wheel, alcohol was removed but free wheel access remained. The alcohol deprivation led to a significant increase in the distance run, followed by a significant decrease in distance run when the alcohol was reinstated. This increase in distance run during alcohol deprivation supports the hypothesis that the female C57BL/6J mice may be using the hedonic substitution of exercise for alcohol to help alleviate withdrawal symptoms. In fact, it has been demonstrated in both humans and mice that exercise can mitigate withdrawal symptoms. In a similar but shorter behavioral paradigm, Piza-Palma et al used both male and female C57BL/6J mice to investigate the effect of intermittent wheel running access on 12% 2BC (wheel access for 6 hours a day) and DID (20% alcohol; wheel access every other day) alcohol consumption. During both 2BC and DID, female, but not male, C57BL/6J mice increased their alcohol consumption and preference when their running wheels were locked, again substantiating the idea that female mice may be using wheel running as a substitute for alcohol consumption. Finally, McGonigle et al examined the effect of intermittent wheel access on DID (20% alcohol) using female C57BL/6J, β-endorphin knock-out (βE-KO) and β-endorphin heterozygote (βE-HT) mice. Both βE-KO and βE-HT significantly increased their alcohol consumption when wheel running was prevented. As seen in the Ozburn et al study, this effect was not observed in the female C57BL/6J strain. The behavioral protocols used for these intermittent wheel access in combination with alcohol 2BC share similar features, but there are differences in study designs that limit ability to draw overarching conclusions. Collectively, they do reiterate the same points highlighted above regarding the important differences due to sex and genetics with regard to response and possible interactions between intermittent exercise and alcohol consumption.

3.4 | Other models of combined alcohol and exercise behaviors

Finally, there have been a few other studies using unique behavioral paradigms in rodents to investigate the interaction of exercise and alcohol consumption. For example, using a voluntary liquid diet with or without alcohol (5-35%, increasing by 5% weekly), Barr observed no differences in either voluntary wheel running or voluntary alcohol consumption in outbred male Sprague-Dawley rats. This is the only study that used an outbred strain and a liquid diet, and has not been replicated, which makes it difficult to draw any conclusions about how or why this model differs from animals that receive standard chow. Another important question to consider is whether the ability to choose exercise or not plays a role in whether voluntary alcohol consumption will decrease. Richard et al examined this using a forced wheel running protocol in a separate cohort of C57BL/6J and male DBA/2J mice. As described above, the C57BL/6J mice showed decreased 10% alcohol intake with voluntary wheel running, but forced wheel running led to increased 10% alcohol consumption. Forced wheel running is considered a stressor and previous studies have demonstrated that forced wheel running results in increased alcohol consumption. Thus, the most parsimonious explanation of the Pichard et al study is that the stress from forced wheel running resulted in opposite (increased) alcohol consumption when compared with the other voluntary wheel running studies. Again, since no effects of wheel running were observed in the male DBA/2J mice, underlying genetics are important too. This is especially since DBA/2J mice are known to show a greater conditioned place preference to alcohol compared with C57BL/6J mice, even though they show very low levels of voluntary consumption in 2BC paradigms. In both of these studies, the animals were subjected to either forced alcohol consumption or forced wheel running, both of which are extremely stressful to the animal and may explain the lack of difference or increased alcohol consumption. More recently, Lynch et al investigated the effects of adolescent exposure to exercise and its possible effects on adulthood alcohol behaviors. This study found that adolescent male Long-Evans rats with access to 24, 30 minute sessions of voluntary wheel running exhibited increased alcohol consumption and preference in adulthood. Together, these studies highlight the complexity of the field and again draw attention to the lack of study among females compared with males.

A few investigators have tried to evaluate whether exercise may be beneficial in the context of ameliorating or reducing withdrawal-
related behaviors. Werme et al tested male Lewis rats to investigate the effect of voluntary wheel running during alcohol withdrawal on 10% alcohol reinstatement. This study reported that access to voluntary wheel running during a 1 or 2 week withdrawal period led to significantly increased alcohol preference and intake when alcohol was reintroduced. These findings converge with the neurobiological effects discussed above showing similar effects on the dopaminergic reward pathways, which suggest that exercise may "prime" individuals for increased intake of alcohol. However, these effects appear to be complicated since they are different dependent on timing of withdrawal experience. Additionally, Somkuwar et al tested the effect of voluntary wheel running during alcohol abstinence and reinstatement in male Wistar rats. All of the pair-housed rats were trained to lever press for 10% alcohol and half of the rats experienced concurrent chronic intermittent ethanol (CIE) vapor. Interestingly, voluntary wheel running enhanced alcohol seeking during abstinence in rats not exposed to CIE, indicating an alcohol deprivation effect. However, contrary to the Werme et al study, voluntary wheel running reduced alcohol reinstatement in both CIE and non-CIE exposed rats, indicating a protective effect. Finally, two studies investigated the effect of voluntary wheel running on alcohol withdrawal-induced seizures. Using an identical paradigm, Devaud et al and McCulley et al demonstrated that voluntary wheel running during 2 weeks of a 6% alcohol liquid diet attenuated the increased susceptibility to alcohol withdrawal-induced seizures in both male and female Charles River (CR) rats. This suggests that regular exercise may exert a protective effect during alcohol consumption, as well as benefit an individual during alcohol-withdrawal. While the variety of behavioral paradigms limits our ability to draw an overarching conclusion, most studies suggest voluntary exercise results in reduced alcohol consumption.

4 | ROLE OF GENETICS IN AUDS AND EXERCISE

As mentioned, one possible mechanism to explain the behavioral interaction between alcohol consumption and exercise could be related to their common action on similar neuronal pathways, which may be due to shared genes. Numerous twin, adoption and family studies have established the important role of genetics in AUDs, with heritabilities estimated between 49 and 60%. Recent large-scale human genome-wide association studies (GWAS) have identified various loci associated with alcohol behaviors. In addition to the widely-replicated associations with genes encoding alcohol-metabolizing enzymes, neuronal genes such as and DRD2 also emerged as significant hits. For exercise, twin studies investigating leisure-time physical activity (LTPA), physical activity (PA), physical inactivity (PI) and sedentary behaviors have yielded heritability estimates ranging from ~25 to 85% in humans. In addition, recent genome-wide association studies of exercise behaviors identified a few genes reaching significance. These include genes highly expressed in the mesolimbic system of the brain, including DRD2. SLC9A9 and GABRG3. DRD2 codes for the dopamine D2 receptor and was identified as significant in genome-wide association studies of both alcohol and exercise behaviors. Dopamine is essential for reward related behaviors and thus the results of these studies provide support that the mesolimbic dopaminergic system is involved in both alcohol consumption and exercise.

In addition, rodent models of both alcohol and exercise related behaviors have been developed that validate a genetic component to both behaviors. In two independent screens of standard laboratory mouse strains, sizable differences in voluntary alcohol consumption were revealed. For example, when housed in identical environments, the inbred strains discussed in these reports demonstrated a wide range of levels of voluntary alcohol consumption. The C57 substrains showed the highest levels of consumption, the C3H and 129 substrains exhibited moderate levels of consumption and the DBA substrains displayed the lowest levels of consumption. Interestingly, a study performed by Lerman et al compared voluntary running duration (hours/night), distance (km/night) and average speed (m/min) of seven different male inbred strains of mice. This study revealed the significant effect genetic background has on baseline wheel running behavior. An independent synthesis of the results of these papers suggests a trend for higher alcohol consuming strains of mice to voluntarily run longer distances, when compared with the strain patterns for alcohol preference. Furthermore, many different selected and inbred strains of mice have been generated to model various aspects of alcohol-related behaviors, including self-administration, reinstatement and withdrawal (reviewed in ). In complementary studies, High Runner lines of mice have been selected for high levels of daily wheel running, and nonselected controls have been maintained for many years (reviewed in ). Although these animals have not been tested in the context of addictive drugs, which is an important area for future research, transcriptional profiling revealed differential gene expression patterns in neuronal networks also implicated in addiction, including differences in Drd2 expression.

Because alcohol and exercise both activate the mesolimbic dopaminergic reward pathway, it can be hypothesized that greater alcohol consuming strains of mice will run farther distances than low or moderate consuming strain of mice. Results from the separate studies of voluntary 2BC and voluntary wheel running by Belknap et al, Yoneyama et al, and Lerman et al support this hypothesis. Similarly, findings from the three strains (male and female C7BL/6Jbg, C3H/1bg and 129/SvEvTac) examined in the Ehringer et al and Booher et al studies provide general support for this idea. The female C57BL/1bg mice consumed the greatest amount of alcohol and ran the farthest distance. This pattern was followed with the remainder of the mice except for the male C57BL/6Jbg mice, which consumed more alcohol than the C3H/1bg and 129/SvEvTac strains of mice but only ran farther than the 129/SvEvTac mice. Collectively, there remain very few studies that have carefully examined both sex and genetics within the context of a single behavioral paradigm, so additional research is needed to clarify the relative importance of each factor. It is clear there are
common differences due to sex, where in most cases females are more responsive to the presence of a running wheel. However, underlying genetic differences in predisposition to both behaviors is likely to be an important factor as well.

### 5 SUMMARY

Here we present a summary of evidence suggesting that various levels of alcohol consumption, exercise intensity and their interactions result in differential behavioral and neuronal consequences. In addition, the collection of studies thus far suggests there are important sex and genetic differences that contribute to the complex individual behaviors relating to alcohol intake and exercise. When these two behaviors are combined, the complexity is amplified. For example, evidence suggests both moderate alcohol consumption and moderate exercise create feelings of euphoria via mesolimbic dopaminergic reward system activation. Thus, we postulate that moderate exercise may help reduce cravings and ultimately reduce alcohol intake of moderate consumers. In addition, moderate exercise has been demonstrated to ameliorate specific pathological neuronal consequences and improve cognitive deficits caused by alcohol consumption. Together, the results of these studies suggest that moderate exercise may be useful to cease or reverse negative neuronal consequences caused by moderate alcohol intake. Conversely, due to neuroadaptations that resemble those caused by heavy alcohol consumption, such as reward system hypofunction, chronic levels of intense exercise may increase an individual's susceptibility to addiction. Therefore, while exercise has many health-related benefits, it remains unclear whether it may always benefit for treatment of AUDs. This discrepancy is also observed in the rodent models described above. For example, the effect of exercise on alcohol behaviors is dependent on time of exposure to the wheel, whether the wheel is locked, and timing of exposure to alcohol. The inconsistency of results and paradigms highlight an important area for future research. In addition, the interaction effect of gonadal hormones on alcohol consumption and exercise is a crucial area of research lacking from the field. To our knowledge, only two studies investigating testicular and cardiovascular health have begun to tease apart this effect. Both studies demonstrated that exercise can reduce ethanol-induced testicular and cardiovascular damage in male rats, and again females were not tested. Finally, a recent survey by Beery and Zucker reported that the ratio of male-only to female-only studies ranges from 3.7:1 in physiology and 5:1 in pharmacology and neuroscience. When both sexes were studied, the rodent models used to study the effects of exercise on alcohol behaviors clearly demonstrate sex differences. However, a majority of the studies were performed on only one sex, usually males. Therefore, as the field advances toward understanding details about the role of specific exercise intensity, type, timing, stage of addiction, drug involved, gonadal hormones and genetic factors, it will be crucial to include both sexes for future research.

### CONFLICT OF INTEREST

The authors declare they have no conflicts of interest, financial or otherwise, that might influence their objectivity regarding this work.

### DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

### ORCID

Marissa A. Ehringer https://orcid.org/0000-0003-0388-7375

### REFERENCES


56. Squeglia LM, Schweinsburg AD, Pulido C, Tapert SF. Adolescent binge drinking linked to abnormal spatial working memory brain...
64. Ehringer MA, Hoft NR, Zunhammer M. Reduced alcohol consumption in mice with access to a running wheel. Alcohol. 2009;43: 443-452.

How to cite this article: Booher WC, Reyes Martínez GJ, Ehringer MA. Behavioral and neuronal interactions between exercise and alcohol: Sex and genetic differences. Genes, Brain and Behavior. 2020:e12632. https://doi.org/10.1111/gbb.12632