Received: 19 April 2022 | Revised: 27 September 2022 | Accepted: 15 October 2022

DOI: 10.1002/dev.22347

Developmental Psychobiology WILEY

RESEARCH ARTICLE

# Chronic running-wheel exercise from adolescence leads to increased anxiety and depression-like phenotypes in adulthood in rats: Effects on stress markers and interaction with BDNF Val66Met genotype

Emily J. Jaehne[1] | Jessica N. Kent[1] | Nikki Lam[1] | Lina Schonfeld[1] | Jereme G. Spiers[2] | Veronica Begni[3] | Federico De Rosa[3] | Marco A. Riva[3,4] | Maarten van den Buuse[1,5]

[1]Department of Psychology, Counselling and Therapy, School of Psychology and Public Health, La Trobe University, Melbourne, Australia

[2]Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Australia

[3]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy

[4]Biological Psychiatry Laboratory, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy

[5]Department of Pharmacology, University of Melbourne, Melbourne, Australia

**Correspondence**
Prof Maarten van den Buuse, School of Psychology and Public Health, La Trobe University, Bundoora, Melbourne, Victoria 3086, Australia.
Email: m.vandenbuuse@latrobe.edu.au

## Abstract

Exercise has been shown to be beneficial in reducing symptoms of affective disorders and to increase the expression of brain-derived neurotrophic factor (BDNF). The BDNF Val66Met polymorphism is associated with reduced activity-dependent BDNF release and increased risk for anxiety and depression. Male and female Val66Met rats were given access to running wheels from 3 weeks of age and compared to sedentary controls. Anxiety- and depression-like behaviors were measured in adulthood using the elevated plus maze (EPM), open field (OF), and forced swim test (FST). Expression of BDNF and a number of stress-related genes, the glucocorticoid receptor (*Nr3c1*), serum/glucocorticoid-regulated kinase 1 (*Sgk1*), and FK506 binding protein 51 (*Fkbp5*) in the hippocampus were also measured. Rats given access to running wheels developed high levels of voluntary exercise, decreased open-arm time on the EPM and center-field time in the OF, reduced overall exploratory activity in the open field, and increased immobility time in the FST with no differences between genotypes. Chronic exercise induced a significant increase in *Bdnf* mRNA and BDNF protein levels in the hippocampus with some of these effects being genotype specific. Exercise decreased the expression of *Nr3c1* and *Sgk1*, but increased the expression of *Fkbp5*. These results suggest that chronic running-wheel exercise from adolescence increased anxiety and depression-like phenotypes in adulthood, independent of BDNF Val66Met genotype. Further studies are required to confirm that increased indices of anxiety-like behavior are independent from reduced overall locomotor activity.

KEYWORDS
anxiety, brain-derived neurotrophic factor, depression, exercise, rat model, Val66Met

## 1 | INTRODUCTION

Anxiety disorders are the most common mental health disorders in children and adolescents, affecting around 7% of children every year (Merikangas et al., 2010). The median age of onset of childhood anxiety disorders is 11 years, and anxiety disorders have been found to be present in 1.5% of preschoolers (Wichstrøm et al., 2012). Effective early interventions to reduce incidence and symptoms of affective disorders at a young age are therefore urgently needed and have become an important policy focus (Larun et al., 2006).

A number of randomized controlled trials have provided evidence of the efficacy of exercise as a treatment in reducing symptoms of affective disorders across all age groups and genders (Stubbs et al., 2017; Wipfli et al., 2008). Exercise routines can also have various positive effects on general health and are self-sustaining in that individuals can independently maintain exercise routines once the basic skills have been learnt (Abu-Omar et al., 2017). Several preclinical studies also demonstrate anxiolytic and antidepressant effects of exercise in rodent models (Duman et al., 2008; Morgan et al., 2018; Salam et al., 2009).

Given the purported beneficial effects of exercise on mental and physical health, it is surprising that little research to date has examined the effects of chronic exercise from adolescence as an intervention for anxiety disorders. A Cochrane systematic review examining the effectiveness of exercise in reducing or preventing anxiety in children aged 6–20 years (Larun et al., 2006) found that exercise significantly decreased anxiety symptoms. However, the small number of studies available showed heterogeneity regarding population, intervention, and measurement instruments used. It therefore remains unclear what the exact cause/effect relationship is between exercise, age, and genetic factors versus treating or preventing anxiety and depression.

One mechanism by which exercise might cause beneficial effects on mental health is by increasing the expression of brain-derived neurotrophic factor (BDNF) in the brain (Walsh & Tschakovsky, 2018). BDNF has been implicated in psychiatric disorder development due to its role in neuronal development, maturation, and plasticity (Notaras & van den Buuse, 2019). Voluntary exercise promotes BDNF expression in the rodent brain (Johnson et al., 2003; Neeper et al., 1995) and, in human studies, upregulates circulating BDNF levels (Rasmussen et al., 2009; Szuhany et al., 2015). In individuals with mental health disorders such as anxiety disorders and depression, this was associated with improvements in mental health symptoms (DeBoer et al., 2012; Mata et al., 2010; Toups et al., 2011).

A single-nucleotide polymorphism (SNP) of the BDNF gene, Val66Met (rs6265), results in deficient BDNF translocation and secretion and thereby may contribute to psychiatric disorder development (Notaras et al., 2015). Different lines of evidence implicate the Val66Met SNP as a candidate risk factor in anxiety disorders (Notaras et al., 2015). This is supported by various association studies (Gratacòs et al., 2007; Zai et al., 2012) that have shown that the Met allele may be the risk allele for psychiatric disorders given that Met carriers exhibit the greatest reduction of activity-dependent secretion of BDNF in the brain (Egan et al., 2003; Tsai, 2018). Evidence suggests that exercise may improve the deficient activity-dependent release of BDNF associated with the Val66Met SNP, although different studies have shown variable results. For example, Helm et al. (2017) showed that both Met and Val carriers had increased serum BDNF after short bursts of high intensity cycling, whereas Nascimento et al. (2015) showed that only Met carriers exhibited significant improvements in peripheral BDNF levels after exercise. However, Lemos et al. (2016) demonstrated that BDNF was increased in Val/Val, but not Val/Met participants following 4 months of aerobic exercise. Another study failed to find an association between BDNF genotype, physical activity, and depressive symptoms (Gujral et al., 2014). In mice carrying the human BDNF Val66Met SNP (Ieraci et al., 2016), lower baseline BDNF levels in the hippocampus and a higher level of depression-like behavior in adult male Met/Met mice were not recovered following running wheel exercise. However, no previous studies in BDNF deficiency models have investigated the effect of chronic exercise from adolescence in both males and females, and the effects in the brain remain unclear.

**TABLE 1** Number of rats assigned to control and exercise conditions by genotype and sex

| | Val/Val | | Val/Met | | Met/Met | |
|---|---|---|---|---|---|---|
| | Males | Females | Males | Females | Males | Females |
| Control | 16 | 12 | 15 | 16 | 15 | 14 |
| Exercise | 10 | 10 | 10 | 10 | 10 | 10 |

In this study, we therefore aimed to investigate the effects of voluntary exercise from adolescence, via access to running wheels in the home cage, on anxiety- or depression-like behaviors in adulthood. We used a novel rat model of the Val66Met polymorphism with a valine to methionine substitution (Val68Met) in the rat *Bdnf* gene (Jaehne et al., 2022; Mercado et al., 2021). Although we have shown that this model is not associated with altered anxiety-like behavior at baseline (Jaehne et al., 2022), we expected differential sensitivity to prolonged exercise between the genotypes. We therefore compared behavioral changes in adulthood in males and females of all three genotypes and also measured expression of BDNF and a number of stress-related genes in the hippocampus. These included the glucocorticoid receptor (*Nr3c1*) and serum/glucocorticoid-regulated kinase 1 (*Sgk1*), which are activated by glucocorticoids, and FK506 binding protein 51 (*Fkbp5*), which regulates sensitivity of the glucocorticoid receptor.

## 2 | METHODS

### 2.1 | Animals

A total of 148 male and female Sprague–Dawley rats with the BDNF Val68Met polymorphism were used for this study to determine the effect of chronic exercise from adolescence on anxiety- and depression-like behaviors (Table 1).

The rats were bred, weaned, and housed at La Trobe University Bundoora Campus within the La Trobe Animal Research and Training

Facility (LATRF) as previously described (Jaehne et al., 2022). The animals were kept on a 12-h light/dark cycle (lights on 7 a.m. to 7 p.m.; 115 lux) with ad libitum access to pellet food and tap water and housing and testing room temperatures controlled at 21 ± 2°C. Behavioral testing and general handling occurred during the light hours between 8 a.m. and 2 p.m. under the same environmental conditions as housing rooms. Experimental procedures and animal care were carried out in accordance with the ARRIVE and National Health and Medical Research Council of Australia animal ethics guidelines and were approved by the La Trobe University Animal Experimentation Ethics Committee (AEC 18042).

At three weeks of age, rats were weaned and randomly assigned to control or exercise conditions. Rats in the control condition were housed in groups of two-four of the same sex but various genotypes in individually ventilated cages (IVC; Tecniplast, Italy). Rats in the exercise condition were housed in groups of two of the same sex and genotype in exercise wheel cages (Lafayette Instruments, Indiana, USA). These rats were allowed continuous 24-h access to the exercise wheels with the exception of brief regular weighing, cage cleaning, or behavior testing. A computer monitoring system was attached to the exercise cages which recorded the total number of full wheel revolutions within 1-h intervals (Scurry Activity Monitoring software; Lafayette Instrument, UK). A single full wheel revolution was equivalent to approximately 90 cm distance traveled. IVC and exercise cages were kept in separate rooms. In a separate experiment (Supporting Information Methods and Results), an additional group of control rats were kept in the exercise wheel cages with the wheel locked. At eight weeks of age, behavioral testing started and included elevated plus maze (EPM), open field (OF), and forced swim test (FST). Rats were undisturbed for at least three days between behavioral tests. All rats remained housed in their assigned cages until euthanized for tissue collection following the completion of behavioral testing at 10 weeks of age.

## 2.2 | Behavioral testing

### 2.2.1 | Elevated plus maze

The animals were tested on the EPM for 5 min as previously described (Jaehne et al., 2022). The apparatus consisted of two closed arms with 50 cm high walls and two open arms without walls. Video tracking software was used to analyze duration and number of entries to the open and closed arms (see Supporting Information Methods and Results for details).

### 2.2.2 | Open field

Rats were placed into an open enclosure approximately 100 cm × 100 cm in size close to one of the walls and left to explore for 10 min (Jaehne et al., 2022). Video tracking software was used to analyze total duration and number of entries into the 50 × 50 cm square center zone (see Supporting Information Methods and Results for details).

### 2.2.3 | Forced swim test

On 2 consecutive days, rats were placed in a clear Perspex cylinder (20 cm W × 50 cm H) filled up to 30 cm with water at 23 ± 2°C. On the test day, the rats were in the apparatus for 5 min, and their behavior was recorded for analysis of immobility and climbing behavior (see Supporting Information Methods and Results for details).

## 2.3 | Organ collection and brain analysis

One to two days following the final behavioral test, rats were deeply anesthetized with $CO_2$ and decapitated for collection of brain, pituitary, adrenal, and heart. Because of significant differences in body weights between the groups, organ weights were expressed as percentage of final body weight. Pituitary and adrenal gland weights were used as an indirect basic physiological measure of hypothalamic–pituitary–adrenal (HPA) activity (Kessing, Willer, & Knorr, 2011). A random selection of brains ($n$ = 6–8/group) had dorsal hippocampus dissected for gene expression analysis. In previous studies on the effects of stress in adolescence/young adulthood, we observed that stress-induced deficits in learning and memory were associated with altered exon-specific BDNF gene expression in the dorsal, but not ventral hippocampus in rats (Hill et al., 2014). Functional pathways and expression of stress markers have been shown to differ between dorsal and ventral hippocampus, necessitating separate analysis of these subregions (Lee et al., 2017). The remaining ventral hippocampus was dissected for BDNF ELISA. Hippocampus samples were rapidly frozen on dry ice and stored at −80°C until further analysis.

## 2.4 | Gene expression analysis

RNA was isolated from dorsal hippocampus using PureZol RNA isolation reagent (Bio-Rad Laboratories) and used for quantitative real-time polymerase chain reaction (qRT-PCR) (CFX384 Real-Time system, Bio-Rad Laboratories) following the ΔΔCT method with $\beta$-actin as the endogenous control (Anacker et al., 2013; Brivio et al., 2021; Guidotti et al., 2013). Data are presented as % compared to the male Val/Val IVC control group (set at 100%). See Jaehne et al. (2022) and Supporting Information Methods and Results for details and primer sequences.

## 2.5 | BDNF ELISA

BDNF protein concentrations in tissue lysates from the ventral hippocampus were determined using a commercially available ELISA kit (Biosensis Cat#: BEK-2211). Sample concentrations were determined

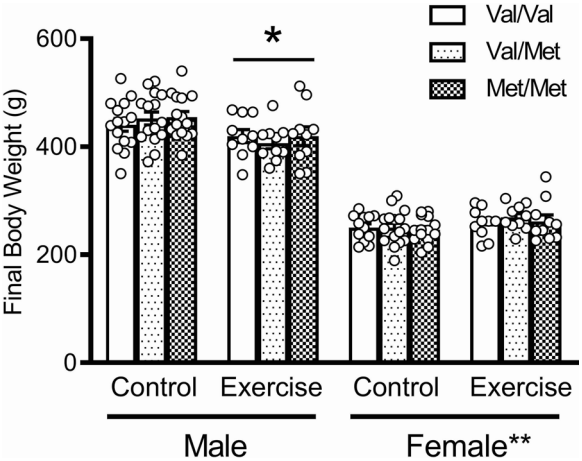


**FIGURE 1** Final body weight of rats following the completion of behavioral testing. Females were significantly smaller than males (**$p < .001$). Housing with access to exercise wheels led to significantly lower body weight in male rats only (*$p < .01$). No effects of genotype were seen. Values represent mean ± SEM ($n = 10–16$/group).

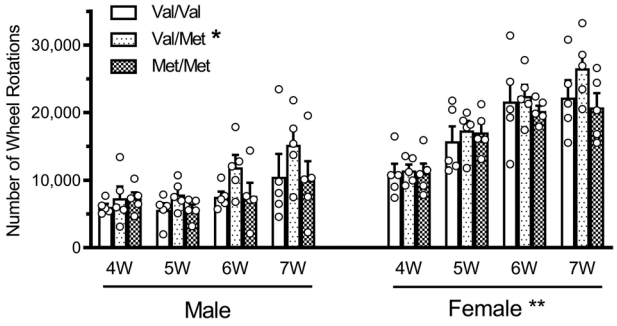


**FIGURE 2** Average daily number of wheel rotations from age 4–7 weeks (W). Females had significantly higher number of wheel rotations than males (**$p < .001$). Irrespective of sex of the animals, Val/Met rats completed significantly more wheel rotations than Val/Val and Met/Met rats at 5, 6 and 7 weeks of age (*$p < .05$). Values represent mean ± SEM of $n = 5$ groups of two. Rats had access to running wheels from weaning at 3 weeks of age until completion of behavioral testing at 10 weeks of age.

from a BDNF standard curve, corrected for dilution and protein concentration, and expressed as ng/mg protein. See Jaehne et al. (2022) and Supporting Information Methods and Results for details.

### 2.6 | Data analysis

Statistical analyses were conducted using IBM Statistical Package for the Social Sciences (SPSS) version 26. Data were screened for outliers using box plots. Normality was checked using skewness and kurtosis standardized *z*-scores, and visual examination of histograms. Homogeneity of variance was assessed using Levene's Test for Equality of Variance. In the case of violations of this assumption, data were suitably transformed for analysis, but raw data are presented in figures. Sphericity was tested using Mauchly's Test of Sphericity, and the Greenhouse–Geisser correction was used where necessary. Differences between groups were analyzed using a mixed ANOVA with condition (IVC control, exercise), genotype, and sex as between-subjects factors, and weeks of age (four, five, six, seven) as within-subjects factor. Mean number of wheel rotations was obtained during the dark hours from a total of 3 undisturbed days from weeks of age four to seven. Statistical significance was assumed at $p < .05$. As a measure of effect size, partial eta squared ($\eta_p^2$) values were used, with cut-offs being ≥0.01 small, ≥0.06 medium, and ≥0.14 large (see Supporting Information Methods and Results for further details).

## 3 | RESULTS

### 3.1 | Body weight

Analysis of final body weights (Figure 1) revealed a significant interaction between condition and sex ($F(1,135) = 14.8$, $p < .001$, $\eta_p^2 = 0.099$) with a medium effect. Moreover, there was a large significant main effect of sex ($F(1,135) = 798.8$, $p < .001$, $\eta_p^2 = 0.86$) reflecting that, as expected, males had larger final body weights than females. Analysis of sexes separated showed that exercise males, but not exercise females, had significantly lower final body weights than sex-matched controls ($F(1,69) = 10.8$, $p = .002$, $\eta_p^2 = 0.14$). No interaction or main effects of genotype were found.

### 3.2 | Exercise

Analysis of number of wheel rotations over time (Figure 2) showed a main effect of age ($F(3,162) = 119.3$, $p < .001$, $\eta_p^2 = 0.69$), with wheel running increasing over time, and significant interactions between age and sex ($F(3,162) = 28.1$, $p < .001$, $\eta_p^2 = 0.34$) and between age and genotype ($F(6,162) = 3.75$, $p = .010$, $\eta_p^2 = 0.12$), with large and medium effects, respectively. Therefore, further analyses were conducted for each week of age separately. A series of two-way between-subjects analyses of variance (ANOVAs) on total wheel revolutions during the dark phase in each week of age found no significant interactions between genotype and sex in any of the 4 weeks. A significant main effect of sex was found in all 4 weeks (week 4: $F(1,54) = 40.4$, $p < .001$, $\eta_p^2 = 0.43$; week 5: $F(1,54) = 201.3$, $p < .001$, $\eta_p^2 = 0.79$; week 6: $F(1,54) = 146.9$, $p < .001$, $\eta_p^2 = 0.73$; week 7: $F(1,54) = 63.4$, $p < .001$, $\eta_p^2 = 0.54$), in which females were exercising significantly more than males, all large effects. Additionally, a significant main effect of genotype with sexes combined was observed in weeks five to seven (week 5: $F(2,54) = 3.31$, $p = .044$, $\eta_p^2 = 0.11$; week 6: $F(2,54) = 4.73$, $p = .013$, $\eta_p^2 = 0.15$; week 7: $F(2,54) = 5.74$, $p = .005$, $\eta_p^2 = 0.18$) that was not shown in week four ($F(2,54) = 0.69$, $p = .51$, $\eta_p^2 = 0.025$), with a medium effect in week five, and large effects in weeks six and seven. Bonferroni post hoc tests demonstrated that Val/Met rats completed slightly, but significantly more wheel rotations than both Val/Val and Met/Met rats during weeks five to seven (Figure 2; $p < .05$) irrespective of sex.

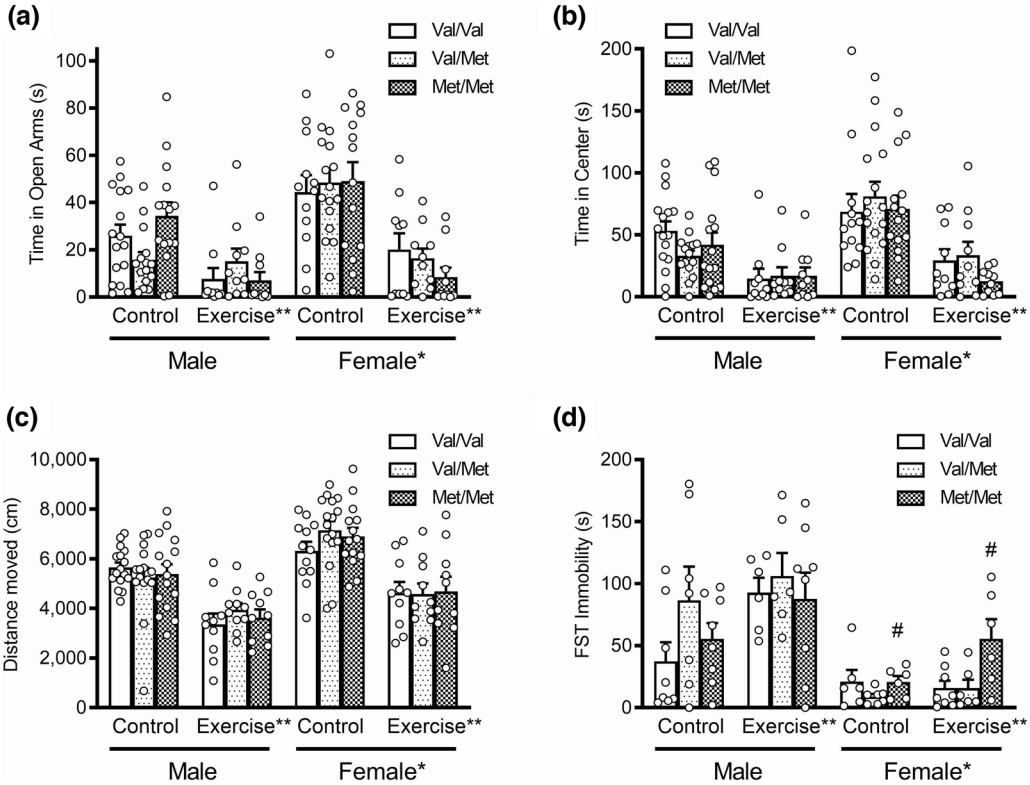


**FIGURE 3** Time spent in open arms of the elevated plus maze (EPM) (a), time spent in center (b) and distance traveled in the open field (c) ,and immobility time in the forced swim test (FST) (d). Exercise rats showed significantly less time in open arms, time in center and distance traveled and higher immobility compared to individually ventilated cage (IVC) control housed rats (**$p < .001$; FST $p < .05$). Female rats had higher time in open arms, time in center and distance traveled and lower immobility compared to males (* $p < .001$). Most effects were independent of genotype. Female Met/Met rats displayed higher immobility than female Val/Met rats (#$p < .05$). Values represent mean ± SEM ($n = 10–16$/group; FST $n = 6–8$/group).

## 3.3 | Behavioral testing

### 3.3.1 | Elevated plus maze

Analysis of total duration of time spent in open arms (Figure 3a) found a significant medium main effect of sex ($F(2,136) = 13.9$, $p < .001$, $\eta_p^2 = 0.09$) where males showed shorter duration on the open arms than females. There was also a significant large main effect of condition ($F(1,136) = 61.8$, $p < .001$, $\eta_p^2 = 0.31$), with exercise rats spending less time in the open arms, and a significant interaction between genotype and condition ($F(2,136) = 3.22$, $p = .043$, $\eta_p^2 = 0.05$) with a small effect, although there was no main effect of genotype ($F(2,136) = 0.29$, $p = .75$, $\eta_p^2 = 0.004$). Bonferroni-adjusted post hoc pairwise comparisons of the genotype × condition interaction with sexes combined revealed that, despite this significant interaction, rats of all genotypes in the exercise condition displayed more anxiety-like behavior than the controls (Val/Val $p = .0004$, Val/Met $p = .019$, Met/Met $p < .0001$).

To ensure the change in the duration of time spent in the open arms does not merely reflect an overall reduction of activity, we also calculated this measure as a percentage of the total duration of time spent in the open arms and closed arms combined (Figure S1a). This % ratio was significantly lower in rats in the exercise condition compared to controls (large main effect of condition, $F(1,136) = 53.3$, $p < .001$, $\eta_p^2 = 0.28$). Females had significantly higher % time in open arms than males independent of genotype or condition ($F(1,136) = 14.0$, $p < .001$, $\eta_p^2 = 0.093$) with a medium effect. We also scored the number of entries in open arms and closed arms and, as another measure of anxiety-like behavior independent of overall locomotor activity (Pellow et al., 1985), calculated the % number of open arm entries over the total number of arm entries (Figure S1b). Again, this % ratio was significantly lower in rats in the exercise condition compared to controls (large effect, $F(1,136) = 35.9$, $p < .001$, $\eta_p^2 = 0.21$) and significantly higher in females compared to males (small effect, $F(1,136) = 6.39$, $p = .013$, $\eta_p^2 = 0.045$).

### 3.3.2 | Open field

*Center field duration and number of visits*: ANOVA on the time spent in the center of the open field (Figure 3b) found a large significant main effect for condition ($F(1,136) = 62.87$, $p < .001$, $\eta_p^2 = 0.32$), with rats in the exercise condition showing lower time in center than controls. A significant main effect of sex was also found ($F(1,136) = 14.03$, $p < .001$, $\eta_p^2 = 0.09$) with a medium effect, reflecting lower time in center observed in males compared to females. No main effect was found for genotype ($F(2,136) = 0.62$, $p = .54$, $\eta_p^2 = 0.01$), and no significant

interaction effects were seen. We also calculated the % number of entries into the center of the open field (Figure S1c). ANOVA of the percentage of center field entries versus total number of entries in center and outer zones combined showed that this % ratio was significantly lower in rats in the exercise condition compared to controls ($F(1,136) = 35.9$, $p < .001$, $\eta_p^2 = 0.21$) with a large effect.

*Total distance traveled*: An ANOVA performed on total distance traveled (Figure 3c) found a significant main effect for condition ($F(1,136) = 75.48$, $p < .001$, $\eta_p^2 = 0.36$) with a large effect, indicating that rats in the exercise condition had lower exploratory activity than controls. There was also a large significant main effect of sex ($F(1,136) = 26.80$, $p < .001$, $\eta_p^2 = 0.17$), where females showed more activity than males. The main effect of genotype was nonsignificant ($F(2,136) = 0.40$, $p = .67$, $\eta_p^2 = 0.01$), and all interaction effects were also nonsignificant.

### 3.3.3 | Forced swim test

Analysis of immobility time in FST (Figure 3d) found a significant interaction of genotype and sex ($F(2,65) = 3.78$, $p = .028$, $\eta_p^2 = 0.10$) with a medium effect. Bonferroni-adjusted post hoc pairwise comparisons demonstrated that in females, Met/Met rats displayed more immobility than Val/Met rats ($p = .012$). Additionally, a medium significant main effect of condition was found ($F(1,68) = 5.36$, $p = .024$, $\eta_p^2 = 0.073$) in which rats in the exercise condition displayed more immobility behavior than controls. Furthermore, a large significant main effect of sex was found ($F(1,68) = 40.9$, $p < .001$, $\eta_p^2 = 0.38$), reflecting more immobility time in males than females. Additional preliminary analysis of climbing behavior showed no significant main effect of condition or genotype, and no significant interactions between these factors (Figure S1d). Female rats showed significantly more climbing behavior than male rats ($F(1,68) = 6.68$, $p = .012$, $\eta_p^2 = 0.089$) with a medium effect.

## 3.4 | Organ weights

*Pituitary gland*: ANOVA conducted on pituitary gland weights (Figure 4a) showed a significant main effect of sex ($F(1,84) = 453.7$, $p < .001$, $\eta_p^2 = 0.84$) with a large effect, where females had larger pituitary gland percentages compared to males. There was also a significant main effect of condition ($F(1,84) = 40.7$, $p < .001$, $\eta_p^2 = 0.33$) and a significant interaction between condition and sex ($F(1,84) = 12.5$, $p = .001$, $\eta_p^2 = 0.13$) with a medium effect, although exercise led to lower weights in both males ($p = .003$) and females ($p < .001$). There was no main effect of or interactions with genotype.

*Adrenal gland*: ANOVA of adrenal gland weights (Figure 4b) showed a significant main effect of sex ($F(1,85) = 356.0$, $p < .001$, $\eta_p^2 = 0.81$) where females had larger relative adrenal weights than males. There were also main effects of both condition ($F(1,85) = 16.1$, $p < .001$, $\eta_p^2 = 0.16$) and genotype ($F(2,85) = 4.11$, $p = .020$, $\eta_p^2 = 0.088$) as well as significant interaction effects between condition and sex ($F(1,85) = 10.6$, $p = .002$, $\eta_p^2 = 0.11$), genotype and condition ($F(2,85) = 5.19$, $p = .007$, $\eta_p^2 = 0.11$), and genotype and sex ($F(2,85) = 3.31$, $p = .041$, $\eta_p^2 = 0.072$) with medium effects. Bonferroni post hoc pairwise comparisons indicated that only in female Val/Met ($p = .003$) and Met/Met ($p = .006$) rats, chronic exercise led to increased adrenal weight compared to controls.

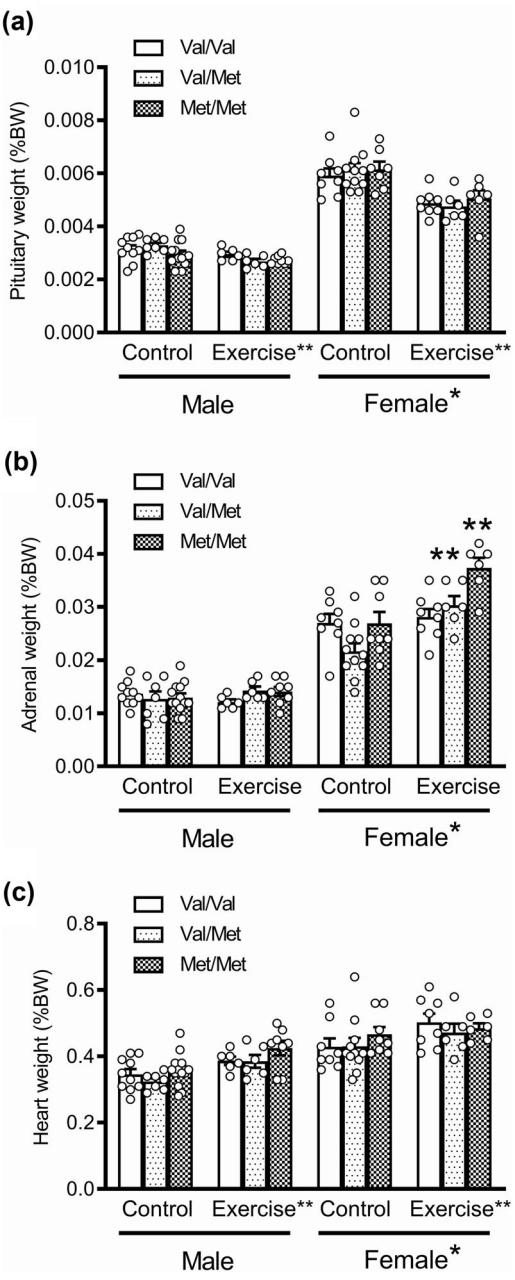


**FIGURE 4** Pituitary (a), adrenal (b), and heart (c) weight expressed as % of final body weight. Exercise rats had significantly smaller pituitary gland weight percentages and significantly larger adrenal glands and hearts compared to individually ventilated cage (IVC) control housed rats (**$p < .01$). For adrenal glands, this difference was found only in female Val/Met and Met/Met rats. Female rats had larger pituitary and adrenal glands and hearts compared to males (*$p < .001$). Values represent mean ± SEM ($n = 6$–13/group).

*Heart*: ANOVA of heart weights (Figure 4c) showed a significant main effect of sex ($F(1,85) = 55.6$, $p < .001$, $\eta_p^2 = 0.40$), with females having larger hearts as a percentage of body weight than males. There was also a main effect of condition ($F(1,85) = 16.3$, $p < .001$, $\eta_p^2 = 0.16$), where exercise led to an increase in heart size. There was no main effect of or interactions with genotype or any other significant interactions.

### 3.5 | Gene expression

RT-qPCR analysis of total *Bdnf* mRNA levels (Figure 5a) revealed a significant effect of exercise condition ($F(1,53) = 4.12$, $p = .040$, $\eta_p^2 = 0.077$) with exercise increasing gene expression. However, there was no significant effect of either genotype ($F(2,53) = 0.067$, $p = .94$, $\eta_p^2 = 0.003$) or sex ($F(1,53) = 1.71$, $p = .20$, $\eta_p^2 = 0.031$) or any significant interactions.

RT-qPCR analysis of glucocorticoid receptor (*Nr3c1*; GR) mRNA levels as well as of other stress-related genes (*Sgk1*, *Fkbp5*) showed varying effects of the exercise condition (Figure 5b–d). As shown by main effects of condition, exercise significantly decreased the expression of *Nr3c1* ($F(1,57) = 5.0$, $p = .029$, $\eta_p^2 = 0.080$) and *Sgk1* ($F(1,51) = 8.0$, $p = .007$, $\eta_p^2 = 0.14$) but increased the expression of *Fkbp5* ($F(1,50) = 15.1$, $p < .001$, $\eta_p^2 = 0.23$). There were no effects of sex or genotype on *Nr3c1* expression, and no significant interactions. There was a significant main effect of sex on *Sgk1* expression ($F(1,51) = 12.0$, $p = .001$, $\eta_p^2 = 0.19$), with females showing higher expression than males, and a significant sex × genotype × condition interaction ($F(2,51) = 3.51$, $p = .037$, $\eta_p^2 = 0.12$). Analysis of data from males and females separated showed a significant effect of exercise in males ($F(1,28) = 5.40$, $p = .028$, $\eta_p^2 = 0.16$) but not females ($F(1,23) = 3.0$, $p = .097$, $\eta_p^2 = 0.12$) with no further effects of or interactions with genotype. There was a significant main effect of sex on *Fkbp5* expression ($F(1,50) = 7.09$, $p = .010$, $\eta_p^2 = 0.12$) where females again showed higher expression than males, while there was also a significant main effect of genotype on expression of this gene ($F(2,50) = 8.12$, $p < .001$, $\eta_p^2 = 0.25$), with no significant interactions. Bonferroni post hoc analysis of the main effect of genotype with sexes and conditions combined showed that Val/Met rats had significantly higher expression of *Fkbp5* than both Val/Val ($p = .0064$) and Met/Met ($p = .011$) rats.

### 3.6 | ELISA

ANOVA analysis of BDNF protein levels in the ventral hippocampus (Figure 5e) showed a significant effect of condition ($F(1,60) = 53.2$, $p < .001$, $\eta_p^2 = 0.47$), where exercise resulted in increased levels of BDNF compared to controls. There was a significant main effect of sex ($F(1,60) = 9.37$, $p = .003$, $\eta_p^2 = 0.14$), with males showing higher levels than females. There was also a significant main effect of genotype ($F(2,60) = 4.34$, $p = .017$, $\eta_p^2 = 0.13$) and significant interactions between sex and condition ($F(1,60) = 6.24$, $p = .015$, $\eta_p^2 = 0.094$) and sex, genotype, and condition ($F(2,60) = 11.9$, $p < .001$, $\eta_p^2 = 0.29$). Bonferroni post hoc analysis showed that exercise significantly increased BDNF levels only in male Val/Val ($p < .001$) and Met/Met ($p < .001$) rats and female Val/Met rats ($p < .001$).

## 4 | DISCUSSION

This study in rats showed that voluntary access to wheel running from weaning leads to significantly higher anxiety and depression-like behavioral measures, demonstrated by decreased open-arm time on the EPM, decreased OF center time and overall exploratory activity, and increased immobility time in the FST. There were no differences between Val66Met genotypes in the effect of exercise on behavior. It was also shown that exercise rats had significantly smaller pituitary glands and significantly larger adrenal glands and hearts compared to controls, while in males exercise led to a decrease in total body weight. Finally, exercise led to a significant increase in *Bdnf* mRNA levels as well as BDNF protein levels in the hippocampus. Exercise decreased the expression of the glucocorticoid receptor, *Nr3c1*, and *Sgk1* while increasing the expression of *Fkbp5*.

### 4.1 | Duration and severity of exercise

Exercise is widely considered to have beneficial antidepressant-like and anxiolytic-like effects (see Section 1). However, it has also been suggested that excessive exercise, such as that associated with exercise addiction or overtraining syndrome, may be psychologically harmful (Weinstein et al., 2015), and the prevalence of depressive symptoms is reported to be higher in athletes than nonathletes (Frank et al., 2013; Ostapiuk-Karolczuk et al., 2015). There may be a dose-dependent relationship between exercise and affective behavior, whereby too much exercise may have detrimental psychological effects. In exercise addiction, an individual develops an obsession with exercise causing features such as withdrawal symptoms and mood modification (Peluso & Guerra de Andrade, 2005; Weinstein et al., 2015). Although previous studies in rats have used different methods of reporting distance run or number of wheel revolutions over different time periods (Fan et al., 2022; Ferguson et al., 2020; Ferguson et al., 2021; Ishikawa et al., 2014; O'Leary et al., 2019; O'Leary et al., 2019), the amount of exercise experienced by the rats in our study generally appears high by comparison. This may be a possible explanation for the increase in anxiety- and depression-like behaviors seen in our exercise rats. However, it is difficult to draw direct comparisons between our study and human athletes who are also exposed to high levels of stress due to environmental factors such as travel and competition.

### 4.2 | Forced versus voluntary exercise and age of the animals

While many studies have shown a strong and consistent trend for anxiolytic effects of voluntary exercise in rodents (Binder et al., 2004; Duman et al., 2008; Morgan et al., 2018), some have demonstrated

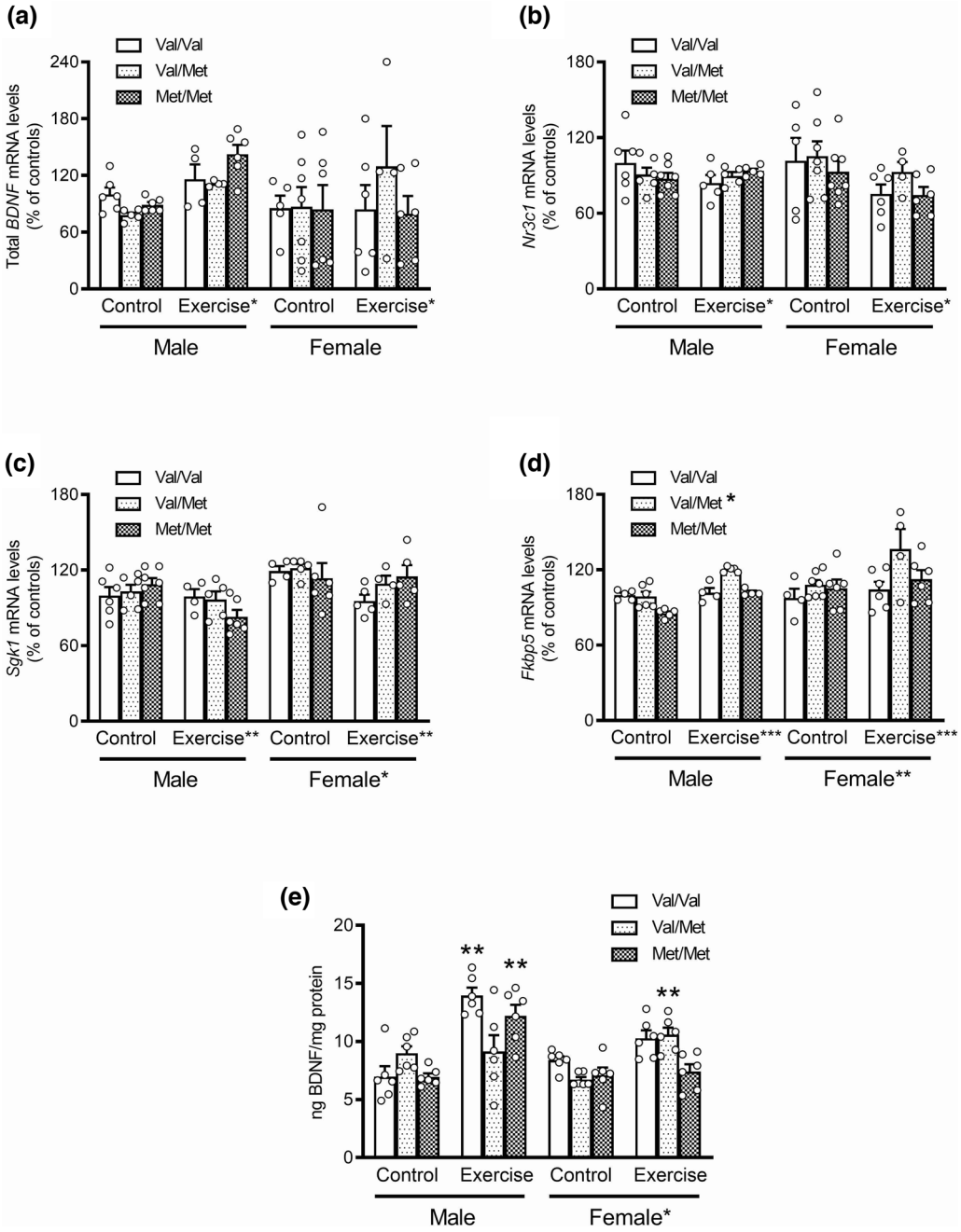


**FIGURE 5** mRNA levels of total *Bdnf* (a), *Nr3c1* (b), *Sgk1* (c), and *Fkbp5* (d) in the dorsal hippocampus. Exercise led to an overall increase in Bdnf and Fkbp5, and a significant decrease in Nr3c1 and Sgk1 expression (*$p < .05$, **$p < .01$, ***$p < .001$). Females showed higher expression of *Sgk1* and *Fkbp5* compared to males (*$p < .05$, **$p < .01$), while Val/Met rats had higher expression of *Fkbp5* than both Val/Val and Val/Met rats (*$p < .05$). Brain-derived neurotrophic factor (BDNF) protein levels in the ventral hippocampus as measured by ELISA (e). Females had lower BDNF levels than males (*$p < .01$). Exercise led to a significant increase in BDNF levels in male Val/Val and Met/Met and female Val/Met rats only (**$p < .001$). Values represent mean ± SEM ($n = 6–7$/group) and with gene expression data presented as % of controls (male Val/Val control rats).

anxiogenic effects of exercise (Burghardt et al., 2004; Morgan et al., 2018) or an increase in FST immobility (Singhal et al., 2019). An explanation for this discrepancy may be whether exercise is forced or voluntary. Burghardt et al. (2004) showed that forced treadmill exercise had no effect on measures of anxiety-like behavior in male Sprague–Dawley rats, but voluntarily exercising rats showed heightened anxiety-like behaviors. Morgan et al. (2018) showed reduced anxiety-like behaviors after 6 months of voluntary wheel running, and also showed some signs of increases in anxiety-like behavior in younger mice that underwent voluntary exercise for only 1–2 months. This suggests that the age of the animals may also play a role. Several studies comparing wheel running in rats starting in adulthood compared to adolescence or younger have shown rats starting at a younger age do tend to show higher levels on voluntary running (Hopkins et al., 2011;

O'Leary, Hoban, Cryan, et al., 2019; O'Leary, Hoban, Murphy, et al., 2019; Purohit et al., 2020). Although these studies did not show an increase in anxiety-like behavior as seen in our study, one reported exercise induced increased freezing in a fear memory task (O'Leary, Hoban, Cryan, et al., 2019). It may be the unique combination of early commencing age, voluntary access, and duration of exercise that explains the current findings.

### 4.3 | Role of the adolescent HPA axis

Adolescent rats exhibit greater and more prolonged corticosterone secretion, that is, an exaggerated HPA axis response, following restraint stress compared to adult rats (Gomez et al., 2002; Romeo et al., 2006). This is an important further consideration to explain discrepancies between our study and previous studies mostly done in adult rats or mice. Voluntary exercise is known to activate components of the HPA axis, including at the level of corticotrophin releasing factor in the brain, pituitary ACTH secretion, and glucocorticoid receptors in pituitary and hippocampus (Stranahan et al., 2008). At moderate levels of exercise, a complex interplay of these factors in hippocampus, hypothalamus, and frontal cortex maintains homeostasis (Duclos & Tabarin, 2016; Stranahan et al., 2008). However, excessive exercise may cause HPA axis dysfunction, increase resting cortisol levels, and affect psychological outcomes (Duclos & Tabarin, 2016; Luger et al., 1987; O'Connor et al., 1989). This dysregulation of the HPA axis during stress caused by excessive exercise may contribute to the cognitive deficits and behavioral impairments seen in depression and anxiety (Stone et al., 2008).

HPA axis hyperactivity in mood disorders may lead to physiological outcomes such as altered pituitary and adrenal gland volume (Krishnan, 1993). In patients with depression, a significant 38%–50% adrenal volume increase was found compared to controls (Kessing et al., 2011). This adrenal hypertrophy reflects chronic HPA axis activation leading to hypercortisolism, confirmed by elevated plasma cortisol levels in depressed patients compared to controls (Rubin et al., 1996). Pituitary volumes were similarly increased in patients with depression compared to controls (Kessing et al., 2011). This may reflect both an increase in number and size of corticotrophic cells that secrete ACTH (Kessing et al., 2011; Krishnan, 1993). The finding of larger relative adrenal glands in our rats suggests that exercise likely resulted in stress, and in turn adrenal hyperactivity, leading to adrenal hyperplasia. Although it was expected that increased ACTH secretion would also cause hypertrophy of the pituitary gland, this was not observed and rats in the exercise condition actually had smaller relative pituitary weights compared to sedentary controls. This could indirectly suggest reduced ACTH release from the pituitary gland after exposure to exercise stress, potentially as a feedback response to chronically elevated corticosterone levels. Reduced pituitary size in our study is contradictory to findings of larger pituitary gland volumes in patients with major depressive disorder (Kessing et al., 2011; Krishnan, 1993). Further studies should include direct measures of corticosterone and ACTH during exercise to confirm these findings.

Analysis of gene expression in the dorsal hippocampus also suggested altered HPA axis activity, leading to feedback changes in the brain. Specifically, chronic exercise decreased the expression of GR and *Sgk1* but increased the expression of *Fkbp5*. It has been suggested that exposure to chronic stress in early life and adulthood in rodents can lead to reduced expression of GR, with increased expression of *Sgk1* and *Fkbp5* (Anacker et al., 2013; Cattaneo & Riva, 2016; Guidotti et al., 2013). However, different types of exercise and stress have been shown to lead to different changes in the brain in terms of these stress markers. Previous studies have shown similar decreases in hippocampal GR gene expression following voluntary wheel running in adult rodents compared to forced treadmill running (Mojtahedi et al., 2020), while exposure to potentially more stressful combined aerobic and resistance exercise training protocols has been shown to result in increases in GR protein levels in the hippocampus (Rostami et al., 2021). *Sgk1* expression has been shown to be increased in the hippocampus following 3 weeks of corticosterone administration (Li et al., 2015), decreased in the hypothalamus in mice following social isolation housing (Berry et al., 2021), and not changed in the hippocampus of male rats following 7 weeks of chronic mild stress (Brivio et al., 2021). Further studies have shown that chronic mild stress in rats leads to increased gene expression of GR and *Sgk1* in the prefrontal cortex but not hippocampus, while *Fkbp5* was increased in both regions (Wei et al., 2016). An 18-day voluntary wheel running protocol has also been shown to increase *Fkbp5* expression in the medial prefrontal cortex and hippocampus of female but not male rats (Yang et al., 2020). From the gene expression analysis, it is therefore unclear if the changes we have seen in our exercise rats indicate a level of stress or not. Interestingly, FKBP5-overexpressing mice have been shown to have lower basal levels of corticosterone and demonstrate increased depressive-like behavior (Criado-Marrero et al., 2020), while showing an increased anxiety phenotype following maternal separation stress (Criado-Marrero et al., 2019).

### 4.4 | No genotype differences in behavior despite differential levels of exercise and gene expression

There were no differences between genotypes in any behavioral measures, although Val/Met rats completed significantly greater numbers of wheel rotations compared to Val/Val and Met/Met genotypes, and there were some genotype-specific findings in gene expression, organ weights, and hippocampus BDNF levels. Val/Met rats had higher expression of *Fkbp5* than both Val/Val and Met/Met rats, although this effect was seen in rats from both conditions. If *Fkbp5* is higher in Val/Met rats throughout their lifetime, it may play a possible role in these rats displaying higher wheel running, although other factors are also likely to be involved. While there was no effect of genotype on pituitary weights, only female Val/Met and Met/Met rats had increased adrenal weight compared to controls following exercise. This may reflect a role of sex of the animals in the relationship between Met carriers and the effect of exercise or stress on adrenal weight. Previous studies have shown that women with the Val/Val genotype showed a

larger cortisol response during a mental stress protocol than Met carriers, with no change seen in men (Jiang et al., 2017). BDNF protein levels in the ventral hippocampus were increased following exercise as expected (Johnson et al., 2003; Neeper et al., 1995), but the increase was particularly pronounced in male Val/Val and Met/Met and female Val/Met rats. BDNF gene expression in the dorsal hippocampus was also increased following exercise, although there were no significant effects of or interactions with genotype or sex as seen for protein levels. It is unclear if these results fit with the stress phenotype indicated by other measures in our study, or why the increase in protein levels was seen in different genotypes in either sex. Previous mouse studies have shown that female Val66Met mice show greater gene expression activation than males after acute stress in the CA3 region of the hippocampus (Marrocco et al., 2017); however, no previous studies have investigated the Val66Met genotype in exercise between males and females. Exercise at an intensity that increases glucocorticoid levels, indicating stress, was shown not to increase hippocampal BDNF levels (Anderson & Shivakumar, 2013; Basso & Suzuki, 2017), suggesting the beneficial effects of exercise on BDNF were reversed when exercise was stressful. Increased anxiety-like behaviors after stressful exercise were associated with increased hippocampal and prefrontal cortex BDNF levels after both stressful and nonstressful exercise (Uysal et al., 2015). In our experiments, it is possible that despite some genotype differences in exercise levels and gene expression, the degree of wheel running displayed by these rats was high enough to result in the same behavioral outcomes in all genotypes.

### 4.5 | Limitations

Rats in the exercise condition were housed in a different cage type than controls, with exercise cages having open tops to allow room for the wheels, and controls being housed in IVC cages which are standard to our facility. Exercise cages also contained only two rats of the same genotype in each cage, while IVC cages housed three to four rats of mixed genotypes. It has been shown that cage type can have a significant effect on behavioral measures in rodents (Logge et al., 2014). We therefore completed a small cohort of rats in the open top exercise wheel cages with the wheels locked in place to prevent activity (see Supporting Information Methods and Results). This showed no significant difference in behaviors between locked-wheel and IVC controls (Figures S2 and S3), therefore confirming the increases in anxiety- and depression-like behaviors seen were due to the running-wheel activity, not the different cage type.

Biochemical measures were obtained from hippocampal tissue sampled from the rats 1–2 days after the last behavioral test. It cannot be excluded that the repeated behavioral testing, which includes a degree of acute stress, may have had lasting effects on the molecular markers we assayed. Therefore, to confirm our results, future studies should include a cohort which undergoes the same exercise protocol but is not subjected to behavioral testing. In addition, analysis of HPA axis markers should be expanded to other tissues, including ventral hippocampus, pituitary, and blood.

The high levels of wheel-running of the rats may have prevented some of the social interaction the rats would normally have engaged in. Social deprivation and social isolation may interfere with normal adolescent/young adult development in both humans and rats (Orben et al., 2020) and single housing, a form of extreme social interaction deprivation, is known to cause increased anxiety-like behavior in rats (Hall, 1998). Thus, we cannot exclude that reduced social interaction may have contributed to the results in this study.

Rats in the exercise condition had lower overall exploratory activity than controls. We speculate that this represents another indication of enhanced anxiety-like behavior (Carola et al., 2002), resulting in the animals being less likely to explore the open field. It has been shown that different anxiety testing paradigms may reveal different aspects of anxiety (van Gaalen & Steckler, 2000). However, while anxiety-like measures on the plus maze were independent from overall number of arms visited, it cannot be excluded that exercise effects on locomotor activity may be contributing to performance differences in the open field. Therefore, before final conclusions about the effect of chronic exercise on anxiety-like behavior can be drawn, further studies are required to dissociate effects of chronic exercise on locomotor activity from those on anxiety-like behavior. This may include other testing models of this behavior, such as the light–dark box, acoustic startle, novelty-suppressed feeding, or analysis of home-cage approach behavior (Kumar et al., 2013). This may also include treatment with anxiolytic drugs to reverse the anxiety-like phenotype.

It should also be noted that immobility in the FST as a measure of depression-like behavior has been questioned (Yankelevitch-Yahav et al., 2015), and it has been suggested that this immobility behavior may reflect an effort to preserve energy until opportunity for escape as rodents learn that they will eventually be removed from the water; therefore, the most efficient solution would be to remain passive until rescue (Yankelevitch-Yahav et al., 2015). Future studies should employ other commonly used measures of depression-like behavior with validity in rodents such as the sucrose preference test.

## 5 | CONCLUSION

This study showed that continuous voluntary exercise from weaning at 3 weeks of age leads to increases in anxiety-like behavior as well as immobility in the FST. These behavioral findings are accompanied by smaller pituitary glands and significantly larger adrenal glands as well as decreased gene expression of GR and *Sgk1* but increased expression of *Fkbp5*. This increase in anxiety and depression-like phenotypes was independent of BDNF Val66Met genotype. It is important in future studies to compare different exercise protocols to differentiate between beneficial effects and possibly stressful effects.

### AUTHOR CONTRIBUTIONS

Investigation, formal analysis, data curation, methodology, project administration, supervision, writing original draft, and writing—review and editing: Emily J. Jaehne. Investigation, formal analysis, and writing original draft: Jessica N. Kent. Investigation, formal analysis, and

writing original draft: Nikki Lam. Investigation, formal analysis, and writing original draft: Lina Schonfeld. Investigation and formal analysis: Jereme G. Spiers. Investigation, formal analysis, and writing—review and editing: Veronica Begni. Investigation and formal analysis: Federico De Rosa. Funding acquisition, methodology, resources, and writing—review and editing: Marco A. Riva. Conceptualization, funding acquisition, methodology, resources, data curation, project administration, supervision, and writing—review and editing: Maarten van den Buuse.

## ACKNOWLEDGMENTS

These study was supported in part by a Research Focus Area grant from La Trobe University, a Manchester Metropolitan University and La Trobe University Collaborative Research Grant (2019), internal grant support from the School of Psychology and Public Health, La Trobe University, internal student support from the School of Psychology and Public Health, La Trobe University, and an Ideas Grant from the National Health and Medical Research Council of Australia (APP1187652). The authors gratefully acknowledge Drs Caryl Sortwell and Timothy Collier from Michigan State University, MI, USA, for making available their Val68Met rat model.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Raw data are available from the authors upon request. BDNF rs6265 Met/Met breeding pairs can be shared by request to Caryl E. Sortwell, PhD, Michigan State University, sortwell@msu.edu.

## ORCID

*Emily J. Jaehne* https://orcid.org/0000-0003-0532-1623
*Jereme G. Spiers* https://orcid.org/0000-0001-5872-8983
*Veronica Begni* https://orcid.org/0000-0003-2244-1782
*Federico De Rosa* https://orcid.org/0000-0003-3678-5244
*Marco A. Riva* https://orcid.org/0000-0002-1699-5060
*Maarten van den Buuse* https://orcid.org/0000-0002-7555-3799

## REFERENCES

Abu-Omar, K., Rütten, A., Burlacu, I., Schätzlein, V., Messing, S., & Suhrcke, M. (2017). The cost-effectiveness of physical activity interventions: A systematic review of reviews. *Journal of Preventive Medicine Reports*, *8*, 72–78.

Anacker, C., Cattaneo, A., Musaelyan, K., Zunszain, P. A., Horowitz, M., Molteni, R., Luoni, A., Calabrese, F., Tansey, K., Gennarelli, M., Thuret, S., Price, J., Uher, R., Riva, M. A., & Pariante, C. M. (2013). Role for the kinase SGK1 in stress, depression, and glucocorticoid effects on hippocampal neurogenesis. *Proceedings of the National Academy of Science USA*, *110*, 8708–8713.

Anderson, E., & Shivakumar, G. (2013). Effects of exercise and physical activity on anxiety. *Frontiers in Psychiatry*, *4*, 27.

Basso, J. C., & Suzuki, W. A. (2017). The effects of acute exercise on mood, cognition, neurophysiology, and neurochemical pathways: A review. *Brain Plasticity*, *2*, 127–152.

Berry, A., Collacchi, B., Capoccia, S., D'Urso, M. T., Cecchetti, S., Raggi, C., Sestili, P., Aricò, E., Pontecorvi, G., Puglisi, R., Ortona, E., & Cirulli, F. (2021). Chronic isolation stress affects central neuroendocrine signaling leading to a metabolically active microenvironment in a mouse model of breast cancer. *Frontiers in Behavioural Neuroscience*, *15*, 660738.

Binder, E., Droste, S. K., Ohl, F., & Reul, J. M. (2004). Regular voluntary exercise reduces anxiety-related behaviour and impulsiveness in mice. *Behavioural Brain Research*, *155*, 197–206.

Brivio, P., Sbrini, G., Tarantini, L., Parravicini, C., Gruca, P., Lason, M., Litwa, E., Favero, C., Riva, M. A., Eberini, I., Papp, M., Bollati, V., & Calabrese, F. (2021). Stress modifies the expression of glucocorticoid-responsive genes by acting at epigenetic levels in the rat prefrontal cortex: Modulatory activity of Lurasidone. *International Journal of Molecular Sciences*, *22*, 6197.

Burghardt, P. R., Fulk, L. J., Hand, G. A., & Wilson, M. A. (2004). The effects of chronic treadmill and wheel running on behavior in rats. *Brain Research*, *1019*, 84–96.

Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., & Renzi, P. (2002). Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. *Behavioural Brain Research*, *134*, 49–57.

Cattaneo, A., & Riva, M. A. (2016). Stress-induced mechanisms in mental illness: A role for glucocorticoid signalling. *Journal of Steroid Biochemistry and Molecular Biology*, *160*, 169–174.

Criado-Marrero, M., Gebru, N. T., Gould, L. A., Smith, T. M., Kim, S., Blackburn, R. J., Dickey, C. A., & Blair, L. J. (2019). Early life stress and high FKBP5 interact to increase anxiety-like symptoms through altered AKT signaling in the dorsal hippocampus. *International Journal of Molecular Sciences*, *20*, 2738.

Criado-Marrero, M., Smith, T. M., Gould, L. A., Kim, S., Penny, H. J., Sun, Z., Gulick, D., Dickey, C. A., & Blair, L. J. (2020). FKBP5 and early life stress affect the hippocampus by an age-dependent mechanism. *Brain, Behavior & Immunity - Health*, *9*, 100143.

DeBoer, L. B., Powers, M. B., Utschig, A. C., Otto, M. W., & Smits, J. A. J. (2012). Exploring exercise as an avenue for the treatment of anxiety disorders. *Expert Review of Neurotherapeutics*, *12*, 1011–1022.

Duclos, M., & Tabarin, A. (2016). Exercise and the Hypothalamo-Pituitary-Adrenal Axis. *Frontiers of Hormone Research*, *47*, 12–26.

Duman, C. H., Schlesinger, L., Russell, D. S., & Duman, R. S. (2008). Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. *Brain Research*, *1199*, 148–158.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., & Dean, M. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*, *112*, 257–269.

Fan, Y., Zhang, L., Kong, X., Liu, K., & Wu, H. (2022). Different exercise time on 5-HT and anxiety-like behavior in the rat with vascular dementia. *American Journal of Alzheimers Disease and Other Dementias*, *37*, 15333175221082743.

Ferguson, L., Giza, C. C., Serpa, R. O., Greco, T., Folkerts, M., & Prins, M. L. (2020). Recovery from repeat mild traumatic brain injury in adolescent rats is dependent on pre-injury activity state. *Frontiers in Neurology*, *11*, 616661.

Ferguson, L., Giza, C. C., Serpa, R. O., Greco, T., Robert, H., Folkerts, M., & Prins, M. L. (2021). Sex differences in neurophysiological changes following voluntary exercise in adolescent rats. *Frontiers in Neurology*, *12*, 685822.

Frank, R., Nixdorf, I., & Beckmann, J. (2013). Depression in elite athletes: Prevalence and psychological factors. *German Journal of Sports Medicine*, *64*, 320–326.

Gomez, F., Houshyar, H., & Dallman, M. F. (2002). Marked regulatory shifts in gonadal, adrenal, and metabolic system responses to repeated restraint stress occur within a 3-week period in pubertal male rats. *Endocrinology*, *143*, 2852–2862.

Gratacòs, M., González, J. R., Mercader, J. M., de Cid, R., Urretavizcaya, M., & Estivill, X. (2007). Brain-derived neurotrophic factor Val66Met

and psychiatric disorders: Meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. *Biological Psychiatry*, *61*, 911–922.

Guidotti, G., Calabrese, F., Anacker, C., Racagni, G., Pariante, C. M., & Riva, M. A. (2013). Glucocorticoid receptor and FKBP5 expression is altered following exposure to chronic stress: Modulation by antidepressant treatment. *Neuropsychopharmacology*, *38*, 616–627.

Gujral, S., Manuck, S. B., Ferrell, R. E., Flory, J. D., & Erickson, K. I. (2014). The BDNF Val66Met polymorphism does not moderate the effect of self-reported physical activity on depressive symptoms in midlife. *Psychiatry Research*, *218*, 93–97.

Hall, F. S. (1998). Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences. *Critical Reviews in Neurobiology*, *12*, 129–162.

Helm, E. E., Matt, K. S., Kirschner, K. F., Pohlig, R. T., Kohl, D., & Reisman, D. S. (2017). The influence of high intensity exercise and the Val66Met polymorphism on circulating BDNF and locomotor learning. *Neurobiology of Learning and Memory*, *144*, 77–85.

Hill, R. A., Klug, M., Kiss Von Soly, S., Binder, M. D., Hannan, A. J., & van den Buuse, M. (2014). Sex-specific disruptions in spatial memory and anhedonia in a "two hit" rat model correspond with alterations in hippocampal brain-derived neurotrophic factor expression and signaling. *Hippocampus*, *24*, 1197–1211.

Hopkins, M. E., Nitecki, R., & Bucci, D. J. (2011). Physical exercise during adolescence versus adulthood: Differential effects on object recognition memory and brain-derived neurotrophic factor levels. *Neuroscience*, *194*, 84–94.

Ieraci, A., Madaio, A. I., Mallei, A., Lee, F. S., & Popoli, M. (2016). Brain-derived neurotrophic factor Val66Met human polymorphism impairs the beneficial exercise-induced neurobiological changes in mice. *Neuropsychopharmacology*, *41*, 3070–3079.

Ishikawa, J., Ogawa, Y., Owada, Y., & Ishikawa, A. (2014). Hyperlocomotor activity and stress vulnerability during adulthood induced by social isolation after early weaning are prevented by voluntary running exercise before normal weaning period. *Behavioural Brain Research*, *264*, 197–206.

Jaehne, E. J., Kent, J. N., Antolasic, E. J., Wright, B. J., Spiers, J. G., Creutzberg, K. C., de Rosa, F., Riva, M. A., Sortwell, C. E., Collier, T. J., & van den Buuse, M. (2022). Behavioural phenotyping of a rat model of the BDNF Val66Met polymorphism reveals selective impairment of fear memory. *Translational Psychiatry*, *12*, 93.

Jiang, R., Babyak, M. A., Brummett, B. H., Siegler, I. C., Kuhn, C. M., & Williams, R. B. (2017). Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism interacts with gender to influence cortisol responses to mental stress. *Psychoneuroendocrinology*, *79*, 13–19.

Johnson, R. A., Rhodes, J. S., Jeffrey, S. L., Garland, T., & Mitchell, G. S. (2003). Hippocampal brain-derived neurotrophic factor but not neurotrophin-3 increases more in mice selected for increased voluntary wheel running. *Neuroscience*, *121*, 1–7.

Kessing, L. V., Willer, I. S., & Knorr, U. (2011). Volume of the adrenal and pituitary glands in depression. *Psychoneuroendocrinology*, *36*, 19–27.

Krishnan, K. (1993). Pituitary and adrenal changes in depression. *Psychiatric Annals*, *23*, 671–675.

Kumar, V., Bhat, Z. A., & Kumar, D. (2013). Animal models of anxiety: A comprehensive review. *Journal of Pharmacological and Toxicological Methods*, *68*, 175–183.

Larun, L., Nordheim, L. V., Ekeland, E., Hagen, K. B., & Heian, F. (2006). Exercise in prevention and treatment of anxiety and depression among children and young people. *Cochrane Database System Reviews*, *19*, CD004691.

Lee, A. R., Kim, J. H., Cho, E., Kim, M., & Park, M. (2017). Dorsal and ventral hippocampus differentiate in functional pathways and differentially associate with neurological disease-related genes during postnatal development. *Frontiers in Molecular Neuroscience*, *10*, 331.

Lemos, J. R., Alves, C. R., de Souza, S. B. C., Marsiglia, J. D. C., Silva, M. S. M., Pereira, A. C., Teixeira, A. L., Vieira, E. L. M., Krieger, J. E., Negrão, C. E., Alves, G. B., de Oliveira, E. M., Bolani, W., Dias, R. G., & Trombetta, I. C. (2016). Peripheral vascular reactivity and serum BDNF responses to aerobic training are impaired by the BDNF Val66Met polymorphism. *Physiological Genomics*, *48*, 116.

Li, Y. C., Wang, L. L., Pei, Y. Y., Shen, J. D., Li, H. B., Wang, B. Y., & Bai, M. (2015). Baicalin decreases SGK1 expression in the hippocampus and reverses depressive-like behaviors induced by corticosterone. *Neuroscience*, *311*, 130–137.

Logge, W., Kingham, J., & Karl, T. (2014). Do individually ventilated cage systems generate a problem for genetic mouse model research? *Genes, Brain, and Behavior*, *13*, 713–720.

Luger, A., Deuster, P. A., Kyle, S. B., Gallucci, W. T., Montgomery, L. C., Gold, P. W., Loriaux, D. L., & Chrousos, G. P. (1987). Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise. Physiologic adaptations to physical training. *New England Journal of Medicine*, *316*, 1309–1315.

Marrocco, J., Petty, G. H., Ríos, M. B., Gray, J. D., Kogan, J. F., Waters, E. M., Schmidt, E. F., Lee, F. S., & McEwen, B. S. (2017). A sexually dimorphic pre-stressed translational signature in CA3 pyramidal neurons of BDNF Val66Met mice. *Nature Communication*, *8*, 808.

Mata, J., Thompson, R. J., & Gotlib, I. H. (2010). BDNF genotype moderates the relation between physical activity and depressive symptoms. *Health Psychology*, *29*, 130.

Mercado, N. M., Stancati, J. A., Sortwell, C. E., Mueller, R. L., Boezwinkle, S. A., Duffy, M. F., Fischer, D. L., Sandoval, I. M., Manfredsson, F. P., Collier, T. J., & Steece-Collier, K. (2021). The BDNF Val66Met polymorphism (rs6265) enhances dopamine neuron graft efficacy and side-effect liability in rs6265 knock-in rats. *Neurobiology of Disease*, *148*, 105175.

Merikangas, K. R., He, J., Burstein, M., Swanson, S. A., Avenevoli, S., Cui, L., Benjet, C., Georgiades, K., & Swendsen, J. (2010). Lifetime prevalence of mental disorders in US adolescents: Results from the National Comorbidity Survey Replication–Adolescent Supplement (NCS-A). *Journal of the American Academy of Child and Adolescent Psychiatry*, *49*, 980–989.

Mojtahedi, S., Shabkhiz, F., Ravasi, A. A., Rosenkranz, S., Soori, R., Soleimani, M., & Tavakoli, R. (2020). Voluntary wheel running promotes improvements in biomarkers associated with neurogenic activity in adult male rats. *Biochemical and Biophysical Research Communications*, *533*, 1505–1511.

Morgan, J. A., Singhal, G., Corrigan, F., Jaehne, E. J., Jawahar, M. C., & Baune, B. T. (2018). The effects of aerobic exercise on depression-like, anxiety-like, and cognition-like behaviours over the healthy adult lifespan of C57BL/6 mice. *Behavioural Brain Research*, *337*, 193–203.

Nascimento, C. M. C., Pereira, J. R., Pires de Andrade, L., Garuffi, M., Ayan, C., Kerr, D. S., Talib, L. L., Cominetti, M. R., & Stella, F. (2015). Physical exercise improves peripheral BDNF levels and cognitive functions in mild cognitive impairment elderly with different bdnf Val66Met genotypes. *Journal of Alzheimer's Disease*, *43*, 81.

Neeper, S. A., Góauctemez-Pinilla, F., Choi, J., & Cotman, C. (1995). Exercise and brain neurotrophins. *Nature*, *373*, 109–110.

Notaras, M., Hill, R., & van den Buuse, M. (2015). The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: Progress and controversy. *Molecular Psychiatry*, *20*, 916.

Notaras, M., & van den Buuse, M. (2019). Brain-derived neurotrophic factor (BDNF): Novel insights into regulation and genetic variation. *The Neuroscientist*, *25*, 434–444.

O'Connor, P. J., Morgan, W. P., Raglin, J. S., Barksdale, C. M., & Kalin, N. H. (1989). Mood state and salivary cortisol levels following overtraining in female swimmers. *Psychoneuroendocrinology*, *14*, 303–310.

O'Leary, J. D., Hoban, A. E., Cryan, J. F., O'Leary, O. F., & Nolan, Y. M. (2019). Differential effects of adolescent and adult-initiated voluntary exercise on context and cued fear conditioning. *Neuropharmacology*, *145*, 49–58.

O'Leary, J. D., Hoban, A. E., Murphy, A., O'Leary, O. F., Cryan, J. F., & Nolan, Y. M. (2019). Differential effects of adolescent and adult-initiated exercise on cognition and hippocampal neurogenesis. *Hippocampus*, *29*, 352–365.

Orben, A., Tomova, L., & Blakemore, S. J. (2020). The effects of social deprivation on adolescent development and mental health. *Lancet Child Adolesc Health*, *4*, 634–640.

Ostapiuk-Karolczuk, J., Kasperska, A., & Botwina, R. (2015). Is there a relationship between inflammation and depression in athletes? *TRENDS in Sport Sciences*, *3*, 113–124.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, *14*, 149–167.

Peluso, M. A., & Guerra de Andrade, L. H. (2005). Physical activity and mental health: The association between exercise and mood. *Clinics (Sao Paulo)*, *60*, 61–70.

Purohit, D. C., Mandyam, A. D., Terranova, M. J., & Mandyam, C. D. (2020). Voluntary wheel running during adolescence distinctly alters running output in adulthood in male and female rats. *Behavioural Brain Research*, *377*, 112235.

Rasmussen, P., Brassard, P., Adser, H., Pedersen, M., Leick, L., Hart, E., Secher, N. H., Pedersen, B. K., & Pilegaard, H. (2009). Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Experimental Physiology*, *94*, 1062.

Romeo, R. D., Bellani, R., Karatsoreos, I. N., Chhua, N., Vernov, M., Conrad, C. D., & McEwen, B. S. (2006). Stress history and pubertal development interact to shape hypothalamic-pituitary-adrenal axis plasticity. *Endocrinology*, *147*, 1664–1674.

Rostami, S., Haghparast, A., & Fayazmilani, R. (2021). The downstream effects of forced exercise training and voluntary physical activity in an enriched environment on hippocampal plasticity in preadolescent rats. *Brain Research*, *1759*, 147373.

Rubin, R. T., Phillips, J. J., McCracken, J. T., & Sadow, T. F. (1996). Adrenal gland volume in major depression: Relationship to basal and stimulated pituitary-adrenal cortical axis function. *Biological Psychiatry*, *40*, 89–97.

Salam, J. N., Fox, J. H., Detroy, E. M., Guignon, M. H., Wohl, D. F., & Falls, W. A. (2009). Voluntary exercise in C57 mice is anxiolytic across several measures of anxiety. *Behavioural Brain Research*, *197*, 31–40.

Singhal, G., Morgan, J., Jawahar, M. C., Corrigan, F., Jaehne, E. J., Toben, C., Breen, J., Pederson, S. M., Hannan, A. J., & Baune, B. T. (2019). Short-term environmental enrichment, and not physical exercise, alleviate cognitive decline and anxiety from middle age onwards without affecting hippocampal gene expression. *Cognitive, Affective & Behavioral Neuroscience*, *19*, 1143–1169.

Stone, E. A., Lin, Y., & Quartermain, D. (2008). A final common pathway for depression? Progress toward a general conceptual framework. *Neuroscience and Biobehavioral Reviews*, *32*, 508–524.

Stranahan, A. M., Lee, K., & Mattson, M. P. (2008). Central mechanisms of HPA axis regulation by voluntary exercise. *Neuromolecular Medicine*, *10*, 118–127.

Stubbs, B., Vancampfort, D., Rosenbaum, S., Firth, J., Cosco, T., Veronese, N., Salum, G. A., & Schuch, F. B. (2017). An examination of the anxiolytic effects of exercise for people with anxiety and stress-related disorders: A meta-analysis. *Psychiatry Research*, *249*, 102–108.

Szuhany, K. L., Bugatti, M., & Otto, M. W. (2015). A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *Journal of Psychiatric Research*, *60*, 56–64.

Toups, M. S., Greer, T. L., Kurian, B. T., Grannemann, B. D., Carmody, T. J., Huebinger, R., Rethorst, C., & Trivedi, M. H. (2011). Effects of serum brain derived neurotrophic factor on exercise augmentation treatment of depression. *Journal of Psychiatric Research*, *45*, 1301–1306.

Tsai, S. J. (2018). Critical issues in BDNF Val66Met genetic studies of neuropsychiatric disorders. *Frontiers in Molecular Neuroscience*, *11*, 156.

Uysal, N., Kiray, M., Sisman, A. R., Camsari, U. M., Gencoglu, C., Baykara, B., Cetinkaya, C., & Aksu, I. (2015). Effects of voluntary and involuntary exercise on cognitive functions, and VEGF and BDNF levels in adolescent rats. *Biotechnic & Histochemistry*, *90*, 55–68.

van Gaalen, M. M., & Steckler, T. (2000). Behavioural analysis of four mouse strains in an anxiety test battery. *Behavioural Brain Research*, *115*, 95–106.

Walsh, J., & Tschakovsky, M. (2018). Exercise and circulating BDNF: Mechanisms of release and implications for the design of exercise interventions. *Applied Physiology, Nutrition and Metabolism*, *43*, 1095–1104.

Wei, K., Xu, Y., Zhao, Z., Wu, X., Du, Y., Sun, J., Yi, T., Dong, J., & Liu, B. (2016). Icariin alters the expression of glucocorticoid receptor, FKBP5 and SGK1 in rat brains following exposure to chronic mild stress. *International Journal of Molecular Medicine*, *38*, 337–344.

Weinstein, A., Maayan, G., & Weinstein, Y. (2015). A study on the relationship between compulsive exercise, depression and anxiety. *Journal of Behavioral Addictions*, *4*, 315–318.

Wichstrøm, L., Berg-Nielsen, T. S., Angold, A., Egger, H. L., Solheim, E., & Sveen, T. H. (2012). Prevalence of psychiatric disorders in preschoolers. *Journal of Child Psychology and Psychiatry*, *53*, 695–705.

Wipfli, B. M., Rethorst, C. D., & Landers, D. M. (2008). The anxiolytic effects of exercise: A meta-analysis of randomized trials and dose-response analysis. *Journal of Sport and Exercise Psychology*, *30*, 392–410.

Yang, T. Y., Gardner, J. C., Gao, Z., Pan, Y. X., & Liang, N. C. (2020). Role of glucocorticoid signaling in exercise-associated changes in high-fat diet preference in rats. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *318*, R515–r528.

Yankelevitch-Yahav, R., Franko, M., Huly, A., & Doron, R. (2015). The forced swim test as a model of depressive-like behavior. *Journal of Visualized Experiments*, *2*, 52587.

Zai, C. C., Manchia, M., De Luca, V., Tiwari, A. K., Chowdhury, N. I., Zai, G. C., Tong, R. P., Yilmaz, Z., Shaikh, S. A., Strauss, J., & Kennedy, J. L. (2012). The brain-derived neurotrophic factor gene in suicidal behaviour: A meta-analysis. *The International Journal of Neuropsychopharmacology*, *15*, 1037–1042.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Jaehne, E. J., Kent, J. N., Lam, N., Schonfeld, L., Spiers, J. G., Begni, V., De Rosa, F., Riva, M. A., & van den Buuse, M. (2023). Chronic running-wheel exercise from adolescence leads to increased anxiety and depression-like phenotypes in adulthood in rats: Effects on stress markers and interaction with BDNF Val66Met genotype. *Developmental Psychobiology*, *65*, e22347. https://doi.org/10.1002/dev.22347