

SHORT COMMUNICATION

Comparison of murine behavioural and physiological responses after forced exercise by electrical shock versus manual prodding

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Abstract

Animal models of exercise have been useful to understand underlying cellular and molecular mechanisms. Many studies have used methods of exercise that are unduly stressful (e.g., electrical shock to force running), potentially skewing results. Here, we compared physiological and behavioural responses of mice after exercise induced using a prodding technique that avoids electrical shock versus a traditional protocol using electrical shock. We found that exercise performance was similar for both techniques; however, the shock group demonstrated significantly lower locomotor activity and higher anxiety-like behaviour. We also observed divergent effects on muscle pain; the prodding group showed hyperalgesia immediately after exercise, whereas the shock group showed hypoalgesia. Corticosterone concentrations were elevated to a similar extent for both groups. In conclusion, mice that were exercised without shock generated similar maximal exercise performance, but postexercise these mice showed an increase in locomotor activity, less anxiety-like behaviour and altered muscle pain in comparison to mice that exercised with shock. Our data suggest that running of mice without the use of electrical shock is potentially less stressful and might be a better technique to study the physiological and behavioural responses to exercise.

KEYWORDS

anxiety-like behaviour, forced exercise, mice, muscle pain

1 | INTRODUCTION

Exercise is increasingly appreciated as a means to improve human health. A well-prescribed and supervised exercise regimen can significantly enhance the benefits of exercise, whereas a poorly prescribed regimen can have deleterious results (Vina et al., 2012). To understand the physiological mechanisms that underlie the beneficial effects of exercise, animal models have been used. These models commonly force animals to exercise using aversive stimuli as motivation (e.g., electrical shock, blasts of air, tail tapping). Evidence

suggests that the physiological and emotional stress associated with these techniques can significantly impact the physiological and behavioural effects of exercise (Moraska et al., 2000; Pitcher, 2018).

Among these aversive techniques, forced running using electrical shock might be particularly stressful. Independent of exercise, electrical foot shock is one of the most common means for producing measured amounts of discomfort and stress in rodents and is regularly used to generate models of human psychiatric disease (Bali & Jaggi, 2015). In addition, stress associated with electrical stimulation has been shown to increase motor output variability during isometric

contraction in humans, which can impair locomotor performance (Noteboom et al., 2001). Moreover, Knab et al. (2009) found that forced running with electrical shock caused high intra-mouse variability in exercise capacity, whereas distances of voluntary wheel running were highly consistent.

Although voluntary wheel running protocols might avoid some of the potential stress associated with forced exercise, voluntary exercise is difficult to standardize. In contrast, forced protocols allow for precise and uniform control of variables (velocity, incline, duration, etc.) and allows for the testing of maximal exercise capacity. Here, we developed a non-electrical shock technique to induce mice to run on a treadmill (manual prodding) and compared it with a standard technique using electrical shock by assessing behavioural and physiological responses immediately after exercise.

2 | METHODS

2.1 | Ethical approval

All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of the University of Iowa (protocol no. 1990903) and conformed to the national guidelines set by the Association for Assessment and Accreditation of Laboratory Animal Care. All experiments were carried out according to the guidelines described by Grundy (2015), and we have taken all steps to minimize the pain and suffering of the animals.

2.2 | Animals

Eleven-week-old female C57BL/6J mice (Jackson Laboratory, Sacramento, CA) were acclimated to the new environment for a week before beginning the experiments in a temperature-controlled room (22°C) with a 12 h–12 h light–dark cycle and had access to standard mouse food and water *ad libitum*. We studied female mice because they exercise more readily than male mice (De Bono et al., 2006) and they develop fatigue-induced hyperalgesia more readily than male mice (Gregory et al., 2013). Mice were killed with CO₂ according to AVMA guidelines.

2.3 | Experimental design

Separate groups of mice were used in three different protocols. In each protocol, mice were randomly assigned to exercise with electrical shock (shock), exercise without electrical shock (prodding) and control groups. Shock and prodding groups underwent an incremental maximal exercise protocol, and the control group was placed on a non-moving treadmill for approximately the same amount of time as exercise groups (35 min). In protocol 1, mice underwent open field testing immediately after exercise ($n = 30$). In protocol 2, corticosterone measurements were obtained 5 min before and immediately and

New Findings

- **What is the central question of this study?**

Forced treadmill exercise using electrical shock is the most common technique in rodent exercise studies. Here, we examined how the use of electrical shock during forced treadmill exercise affects behavioural and physiological responses in comparison to a novel non-electrical shock technique.

- **What is the main finding and its importance?**

In comparison to mice that underwent traditional treadmill running induced by electrical shock, mice that underwent forced running using a novel technique involving gentle prodding to induce running showed: (i) higher locomotor activity; (ii) less anxiety-like behaviour; and (iii) altered exercise-induced muscle pain immediately after exercise.

1 h after exercise ($n = 18$). In protocol 3, mice underwent muscle withdrawal threshold (MWT) testing 1 day before (baseline) and immediately after exercise ($n = 20$). Mice in protocols 1 and 3 were analysed for exercise performance on treadmill exercise testing.

2.4 | Treadmill exercise protocols

All groups were acclimated to the treadmill (Columbus Instruments, Columbus, OH) for 5 days for 30 min/day, with gradually increasing speed and incline as previously described (Khataei et al., 2020), with electrical shock grids (1 mA, 1 Hz, 200 ms duration) at the rear of the treadmill to encourage continued ambulation. For maximal exercise testing, mice were placed individually on a non-moving treadmill [20° incline throughout; 15–25° incline is best to achieve maximal O₂ uptake in mice (Kemi et al., 2002)] for 10 min, followed by 10 min warm-up at 6 m/min with shock bars on. For the shock group, the electrical shock was left on throughout the exercise protocol. For the prodding group, we turned off the shock grids at the beginning of testing and instead used gentle prodding with a tongue depressor to encourage running as needed (Conner et al., 2014). After warm-up, the speed was increased to 8 m/min, and then increased by 2 m/min every 3 min (accelerations were 2 m/min²) until exhaustion. For the shock group, exhaustion was defined as the point at which mice stayed on the shock grids for >10 s and did not resume running. For the prodding group, exhaustion was defined when mice stayed at the end of treadmill and did not resume running after 10 consecutive proddings in 10 s.

2.5 | Muscle withdrawal threshold

The MWT was measured by applying force-sensitive tweezers to the belly of the gastrocnemius muscle as previously described (Skyba et al., 2005), whereby lower thresholds indicate greater sensitivity to mechanical stimuli. Briefly, mice were acclimated to the testing protocol in two 5 min sessions on 3 days. The mice were gently restrained in a gardener's glove, the hindlimb was held in extension and the muscle squeezed with progressive force with the force-sensitive tweezers until the mouse withdrew its hindlimb. Three trials for each hindlimb were averaged.

2.6 | Corticosterone radioimmunoassay

Adrenocortical activity was measured by obtaining blood samples from the tail vein (Anderson et al., 2014, 2019). A small longitudinal incision was made at the distal tip of the tail with a sterile blade while mice were restrained. Blood samples (~20 μ l) were collected into chilled plastic microfuge tubes containing EDTA and aprotinin, centrifuged, and fractionated for storage of plasma at -80°C until assayed. Silver nitrate was applied to achieve haemostasis. Plasma corticosterone was measured without extraction, using an antiserum raised in rabbits against a corticosterone-bovine serum albumin conjugate, with ^{125}I -corticosterone-bovine serum albumin as the tracer (MP Biomedicals). The sensitivity of the assay was 8 ng/ml; intra- and inter-assay coefficients of variation were 5 and 10%, respectively.

2.7 | Open field test

For the assessment of locomotor activity and anxiety-like behaviour, mice were subjected to the open field test immediately after exercise. Horizontal movements and vertical (rearing) movements were quantified as the number of photobeam breaks during 30 min in a lighted chamber (40.6 cm wide \times 40.6 cm deep \times 36.8 cm high; 1500 lux; 16 \times 16 photobeams to record horizontal movements and 16 rearing photobeams, with 2.54 cm photobeam spacing; San Diego Instruments). Centre activity was defined as the percentage of horizontal beam breaks occurring in the centre of the open field (25.5 cm \times 25.5 cm) per total horizontal beam breaks.

2.8 | Statistical analysis

GraphPad Prism (San Diego, California, US; v.8.0) was used to analyse data statistically. Bar graphs represent mean values \pm SD. One-way and two-way ANOVA, Student's unpaired *t* tests and *F*-tests were used to analyse the results. If significant differences were observed, the indicated post hoc tests were performed as described in the figure legends. Sample sizes for each experimental protocol were determined based on the effect sizes for changes in the variable from our previous

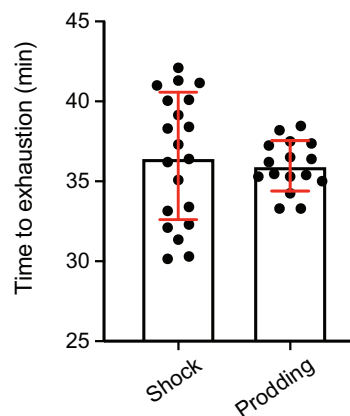


FIGURE 1 Comparison of maximal exercise capacity using electrical shock or prodding methods. Time to exhaustion was measured in shock and prodding groups during incremental treadmill exercise. Although there was no difference between the means of groups (Welch's *t* test; $P > 0.05$, $n \geq 16$), the variances of two groups were significantly different (*F*-test; $F = 6.402$, $n_1 = 20$, $n_2 = 16$, $P < 0.001$). n_1 , number of mice in Shock group; n_2 , number of mice in Prodding group

and pilot studies [$\alpha = 0.05$, $\beta = 0.8$; G*Power v.3.1 software (Faul et al., 2007)].

3 | RESULTS

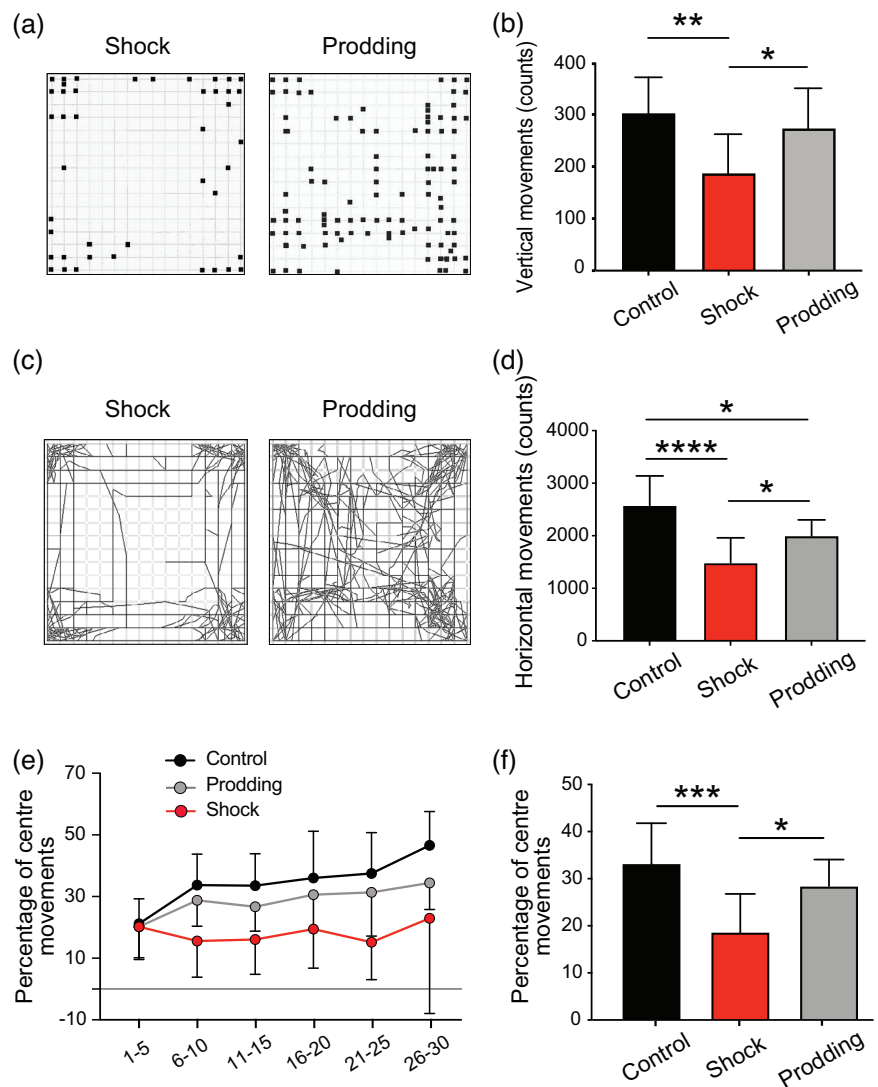
3.1 | Exhaustive exercise with manual prodding elicited similar exercise performance to electrical shock

In pilot studies, we observed that running mice on a treadmill using a standard technique with an electrical shock grid at the back of the treadmill to induce running caused signs of stress (increased freezing behaviour, urination and defecation, vocalization and escape behaviour during and immediately after running). Therefore, we used a novel protocol whereby we turned the shock bars off and, instead, used gentle prodding with a tongue depressor to induce running (Conner et al., 2014; Khataei et al., 2020). We first compared the maximal exercise capacity using either the electrical shock (shock group) or the prodding technique (prodding group) to induce running and found no difference in the time to exhaustion between groups (Figure 1). Interestingly, the variance from the mean was significantly less for the prodding group compared with the shock group.

3.2 | Exercise with shock caused less locomotor activity and increased anxiety-like behaviour after exercise in comparison to exercise with prodding

Immediately after exercise, we used an open field assay to record the locomotor activity of the mice. Control mice were placed on a

FIGURE 2 Locomotor activity during 30 min in an open field immediately after exhaustive exercise. (a) Representative vertical movement (rearing) zone maps of single mice from the shock and prodding groups. Each dot represents a rearing event. (b) Summary data for vertical activity. One-way ANOVA revealed a significant difference between groups ($F_{2,27} = 6.55, P < 0.01, n \geq 9$). Tukey's test found that the shock group showed less rearing compared with the control ($^{*}P < 0.01$) and prodding ($^{*}P < 0.05$) groups. (c) Representative zone maps of horizontal movements of single mice from the shock and prodding groups. (d) Summary data for horizontal activity. One-way ANOVA revealed a significant difference between groups ($F_{2,27} = 14.10, P < 0.0001, n \geq 9$). Tukey's test showed that both shock ($^{****}P < 0.0001$) and prodding ($^{*}P < 0.05$) groups made fewer movements compared with the control group, and the shock group made fewer movements than the prodding group ($^{*}P < 0.05$). (e) Centre relative to total horizontal movements per 5 min interval. (f) Total (percentage) horizontal centre activity. One-way ANOVA showed a significant difference between groups ($F_{2,27} = 9.80, P < 0.001, n \geq 9$). Tukey's test found that the shock group showed less centre activity compared with the control ($^{***}P < 0.001$) and prodding ($^{*}P < 0.05$) groups



non-moving treadmill before open field recording. To assess post-exercise fatigue, we measured vertical (rearing) and horizontal activity for 30 min in an open field chamber (Huang et al., 2018; Kobayashi et al., 2008). The prodding group of mice exhibited significantly more vertical movements compared with the shock group and were similar to the control group (Figure 2a,b). The prodding group also exhibited more horizontal movements than the shock group (Figures 2c,d).

As a measure of anxiety-like behaviour, we tracked the percentage of horizontal movements in the centre relative to the total movements immediately after exercise. The tendency of mice to avoid the brightly lit centre of an open field and remain near the walls is an index of anxiety (Crawley, 1985). Similar to control mice, the prodding group began to habituate to the novelty of the chamber during the 30 min period and exhibited increasing centre movements (Figures 2e,f). In contrast, the shock group continued to avoid the centre. These results suggest that the prodding group of mice were less fatigued and showed less anxiety-like behaviour after exercise than the shock group.

In separate groups of mice, we tested whether exercise with electrical shock versus prodding would generate differential increases in plasma corticosterone, the main glucocorticoid in mice (Coleman

et al., 1998). In the control group, corticosterone concentrations were not significantly altered immediately and 1 h after 35 min exposure to a non-moving treadmill (Figure 3). In contrast, corticosterone was increased significantly and to a similar extent immediately after exhaustive exercise and remained elevated 1 h later in both shock and prodding groups compared with their respective baseline measurements.

3.3 | Exercise with or without shock produced opposite effects on immediate exercise-induced muscle pain

The peaks of exercise, particularly that involving high-intensity muscle contractions, are commonly associated with muscle pain, referred to here as immediate exercise-induced muscle pain (IEIP; Khataei et al., 2020). We measured IEIP after exhaustive exercise in the shock, prodding and control groups of mice. As a measure of muscle pain, we recorded the MWT, whereby force-sensitive tweezers were applied to the gastrocnemius muscle until the limb was withdrawn (a lower MWT

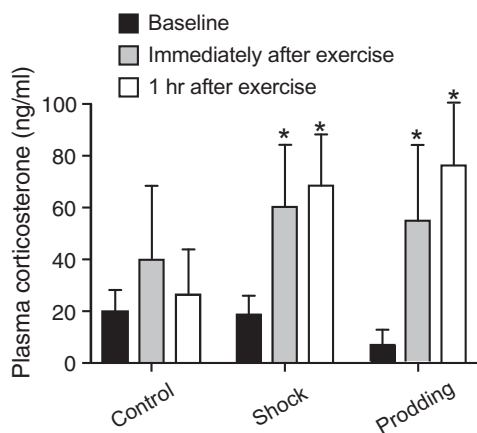


FIGURE 3 Plasma corticosterone concentrations after exhaustive exercise. Corticosterone was measured before (baseline) and immediately and 1 h after exercise. Two-way repeated-measures ANOVA showed that there was a significant group effect ($F_{2,15} = 5.18$, $P < 0.05$), time effect ($F_{1,71,25,64} = 18.59$, $P < 0.0001$) and interaction between time and group ($F_{4,30} = 3.09$, $P < 0.05$, $n = 6$). Tukey's test revealed that corticosterone increased immediately and 1 h after exercise for experimental groups compared with their respective baseline ($^*P < 0.05$), but was unchanged for the control group over time, and there were no differences between shock and prodding groups

indicates increased pain, or hyperalgesia), at baseline (1 day before) and immediately after exhaustive exercise. In comparison to the control group, we found significant and divergent differences between the shock and prodding groups after exercise. The MWT was increased immediately after exercise for the shock group, and it was lower for the prodding group compared with their baseline values (Figure 4). These results indicate that the prodding group developed muscle hyperalgesia associated with intense exercise (IEIP), whereas exercise with shock caused a paradoxical muscle hypoalgesia.

4 | DISCUSSION

4.1 | Forced running and electrical shock are stressful to mice

Ours is the first study to compare the effects of manual prodding versus electrical shock as a means to force treadmill exercise in mice. Despite achieving the same level of exercise, the two groups demonstrated significantly different locomotor activity patterns in an open field assay immediately after exercise. The shock group demonstrated fewer vertical and horizontal movements and spent less time in the centre of the chamber than either the control or the prodding group of mice. Although open field behaviour is driven by multiple different motivations, avoidance of the chamber centre is a validated means to measure anxiety in mice (Crawley, 1985; Treit & Fundytus, 1988). In addition, both exercise groups had equally increased concentrations of corticosterone after exercise.

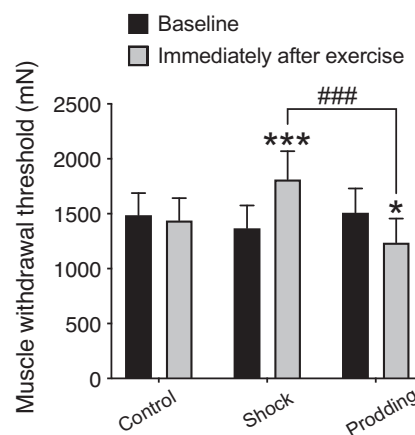


FIGURE 4 Muscle withdrawal threshold (MWT) immediately after exhaustive exercise. The MWT was measured 1 day before (baseline) and immediately after exercise. Two-way repeated-measures ANOVA showed a significant group effect ($F_{2,17} = 3.86$, $P < 0.05$) and an interaction between group and time ($F_{2,17} = 36.07$, $P < 0.0001$, $n \geq 6$). Sidak's test found that MWT was higher after exercise compared with baseline for the shock group ($***P < 0.0001$), lower compared with baseline for the prodding group ($^*P < 0.01$), and there was a significant difference between shock and prodding groups after exercise ($####P < 0.0001$)

Our results are consistent with others showing that forced running is stressful for rodents, as measured by both physiological markers and assays of behaviour. Compared with voluntary exercise on a running wheel, forced exercise of mice and/or rats diminished the time spent in the centre of an open field, increased defecation and increased faecal corticosterone (Leasure & Jones, 2008; Svensson et al., 2016). Moreover, the stress associated with forced exercise can be maladaptive, thus potentially overriding the benefits of exercise. For example, rats that underwent voluntary exercise had better motor recovery after stroke compared with rats that underwent forced exercise (Ke et al., 2011).

Although diminished locomotor activity might be another sign of increased stress and anxiety, rearing and horizontal movements within an open field assay have also been used as measures of fatigue after exercise (Huang et al., 2018; Kobayashi et al., 2008). Although the two exercise groups of mice exercised to the same extent, the diminished movements of the shock group after exercise suggest that they experienced greater muscle fatigue. Although some degree of stress can enhance exercise performance, excessive physiological stress has been shown to increase central fatigue, alter motor neural output and diminish motor function (Marker et al., 2016; Noteboom et al., 2001). Additionally, stress induced by electrical stimulation increases the variability of motor output during isometric contraction, which can impair locomotor performance (Noteboom et al., 2001). Interestingly, we found greater variability of exercise performance in the shock group compared with the prodding group, although we recognize that the very different means to determine exhaustion might have contributed to the differences in variability.

If the shock group of mice experienced greater stress/anxiety than the prodding group, why was corticosterone elevated equally (~10-fold) in both groups after exercise? Although corticosterone is a commonly measured marker of pathological stress, acute exercise also increases cortisol/corticosterone (CORT) concentrations in humans and rodents (Chen et al., 2017; Viru, 1992). In fact, corticosterone concentrations are increased in rats immediately after voluntary wheel running, suggesting that exercise increases corticosterone concentrations independent of the stress associated with forced exercise (Griesbach et al., 2012). Moreover, exhaustive treadmill running in rats (using light air puffs to induce running) generated higher corticosterone concentrations (~5-fold increase) compared with 60 min of stress-inducing immobilization (~3-fold increase) (Hand et al., 2002). It is interesting to speculate why increases in CORT associated with chronic stress, including administration of exogenous CORT, are detrimental to neural adaptation/function and predictive of negative health outcomes in a variety of disease contexts, whereas the increase in CORT that occurs with exercise is generally believed to be an adaptive response and is associated with improvement in cognitive function and health (Adlard & Cotman, 2004; Chen et al., 2017).

4.2 | Stress- and exercise-induced hypo- versus hyperalgesia

Strenuous exercise is associated with muscle pain during and immediately after cessation of exercise (Miles & Clarkson, 1994; Park & Rodbard, 1962), which we termed IEIP (Khataei et al., 2020). The mechanism involves activation of type III and IV muscle afferents by metabolites and other chemicals that accumulate within exercising muscle and, perhaps, mechanical injury during eccentric contractions (Katz et al., 1935; Rodbard & Pragay, 1968). We previously showed that forced maximal treadmill exercise using manual prodding causes IEIP in mice as measured by a reduction in MWT. Moreover, we found that the acid-sensing ion channels (ASICs) are required for IEIP; mice with a genetic lack of the subunit ASIC3 experienced no change in MWT immediately after exercise (Khataei et al., 2020). Here, we found that mice that underwent forced maximal treadmill exercise induced by shock had the opposite result. The shock group had an increase in MWT compared with their baseline, suggesting that they experienced an increase in pain threshold, or hypoalgesia, immediately after exercise.

Although exercise training is an effective therapy for chronic pain conditions, and even a single bout of exercise can increase pain thresholds in humans, termed exercise-induced hypoalgesia (Naugle et al., 2012; Rice et al., 2019), most of these studies measure pain at a site that is remote from the contracting muscle, and they generally do so with some time delay from the cessation of exercise. Thus, the mechanisms that underlie exercise-induced hypoalgesia are most certainly from those that cause IEIP. Moreover, given that both shock and prodding groups achieved the same level of exercise on the treadmill, it is unlikely that the degree of exercise accounts for the

differences in MWT immediately after exercise. Thus, we do not believe that exercise-induced hypoalgesia, as defined by the mechanisms described in previous studies, accounts for the increase in MWT in the shock group. Instead, we suspect that this hypoalgesia was caused by the additional stress associated with electrical shock.

Although repeated and prolonged exposure to stressors over extended periods of time tends to cause a maladaptive exacerbation of pain responses (stress-induced hyperalgesia), if the aversive stimulus is acute, pain sensation is generally suppressed (stress-induced analgesia or hypoalgesia). This hypoalgesia is believed to be an adaptive response to acute stress as part of the fight-or-flight response (Bolles & Fanselow, 1980). There are a number of studies in the literature reporting stress-induced hypoalgesia in rodents elicited by foot shock (Ferdousi & Finn, 2018). In fact, one group has used forced exercise using shock to induce treadmill walking as a means to induce stress-induced analgesia in mice (Furuta et al., 2003). Thus, we believe that the normal IEIP caused by strenuous exercise (as seen in the prodding group) was overridden by stress-induced hypoalgesia in the shock group.

4.3 | Implications for animal models of exercise research

Although exercise has been used successfully as a preventive or therapeutic strategy to treat various conditions, including chronic pain (Law & Sluka, 2017), chronic fatigue syndrome (Larun et al., 2017), cardiac diseases (Zhang et al., 2016) and neurodegenerative diseases (Campos et al., 2016), a poorly prescribed exercise regimen can exacerbate such conditions. Whether exercise is beneficial or not depends upon the intensity, type and duration of exercise and, perhaps, the associated stress (Pitcher, 2018; Sluka et al., 2018). Both exercise and stress activate the sympathetic/adrenergic and corticosteroid systems. Interestingly, when stress responses are activated by aversive, prolonged and unescapable stimuli, they tend to cause maladaptive alterations of homeostasis, whereas exercise generally promotes beneficial adaptive responses. The use of animal models of exercise is crucial to our understanding of these underlying mechanisms. Forced running is inherently stressful to rodents; however, it has the advantage over voluntary wheel running in that the degree of exercise can be prescribed and uniform within a group. Our data here show that different exercise techniques to run mice on a treadmill can cause profoundly different behavioural responses immediately after exercise, including fatigue, anxiety and pain and, most certainly, other behavioural and physiological responses.

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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

T.K. and C.J.B. conceived and designed the research and drafted the manuscript. T.K., S.A.-R. and A.M.S.H. performed the experiments. T.K. analysed the data and prepared the figures. T.K., J.J.R. and C.J.B. interpreted the results of experiments and edited and revised the manuscript. All authors approved the final version of manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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