Pharmacological Reports 71 (2019) 1020–1024

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Short communication

Voluntary exercise ameliorates anxiogenic effects of acute methamphetamine exposure in Swiss-Webster mice

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ARTICLE INFO

Article history:
Received 14 December 2018
Received in revised form 18 May 2019
Accepted 5 June 2019
Available online 6 June 2019

Keywords:
Anxiety
Exercise
Methamphetamine
Mouse
Open-field task

ABSTRACT

Background: The present experiment examined the ability of voluntary exercise (*i.e.*, home-cage wheel running; HCWR) to ameliorate anxiety-like behavior associated with acute methamphetamine exposure in male, Swiss-Webster mice.
Methods: Mice were permitted access to home-cage running wheels (Exercise), locked home-cage running wheels or no home-cage running wheels (Sedentary) for 6 weeks and then exposed to different methamphetamine doses (vehicle, 0.25, 0.5, or 1.0 mg/kg) once weekly during an 8 h open-field session for 4 weeks. Group differences in hypothalamic-pituitary-adrenal (HPA) activity also were assessed by weighing adrenal glands.
Results: It was found that HCWR ameliorated anxiety-like behavior after an injection of either the 0.5 or 1.0 mg/kg methamphetamine dose. Adrenal weights did not differ between Exercise and Sedentary mice.
Conclusion: Taken together, these results suggest that voluntary exercise ameliorates the anxiogenic effects of methamphetamine depending on the dose, perhaps *via* a non-HPA mechanism.

Introduction

Methamphetamine use disorder has been shown to be comorbid with emotional disorders (*e.g.*, anxiety) [1]. Furthermore, a feature of methamphetamine withdrawal in users is anxiety [2]. The anxiogenic effects of methamphetamine have been linked to its interaction with the hypothalamic-pituitary-adrenal (HPA) axis [3]. Dysregulation of the anti-reward circuits (*e.g.*, HPA) by addictive drugs has been suggested to induce negative-affective states (*e.g.*, anxiety) that motivate drug-taking behavior in people [4].

Repeated methamphetamine exposure has been shown to induce anxiety-like behavior in mice, especially associated with withdrawal [5]. While much less research has examined the effects of *acute* methamphetamine exposure, studies have found that low doses (1–4 mg/kg) induce anxiogenic effects in mice [6,7]. Acute methamphetamine exposure too has been shown to increase HPA activity [8].

Voluntary exercise has been evaluated as a non-pharmacological treatment for methamphetamine use disorder [9]; however, the mechanism(s) to account for its efficacy is unknown. Preclinical animal studies have shown that voluntary exercise, defined as home-cage wheel running (HCWR), produces anxiolytic effects in mice [10–12], and these anxiolytic effects are linked to changes in HPA functioning [13].

The present experiment, building on previous work in the laboratory showing that acute methamphetamine exposure induces anxiety-like behavior in mice [6], as assessed by the open-field task [14], evaluated the effects of HCWR on ameliorating the anxiogenic effects of acute methamphetamine exposure. Because adrenal weights, as a measure of HPA activity, have been shown to distinguish between Exercise and Sedentary mice [13], adrenal weights also were assessed.

Material and methods

Male, adolescent (~21 days of age) Swiss-Webster mice ($N = 30$) were obtained from Charles River Laboratories (Raleigh, N.C) and were singly housed in white polypropylene tubs that measured $32 \times 20.5 \times 17.5$ cm (L × W × H), lined with paper bedding (Care-free Ultra). Upon arrival, Exercise mice ($n = 15$) were placed in tubs containing a store-bought wire-mesh running wheels (Super Pet Hamster 5 ¾ Run-Around ®) for 6 weeks. Running wheels were equipped with bike computers (Cat-Eye Velo 7 Bicycle Computer CC-VL520 ®), attached to the running wheels and calibrated to the wheel's size in order to record distanced traveled (km). Sedentary mice were housed in cages with no home-cage running wheels ($n = 8$) or locked home-cage running wheels ($n = 7$). The experiment conforms to the guidelines established by

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http://dx.doi.org/10.1016/j.pharep.2019.06.001
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the *NIH Guide for the Care and Use of Laboratory Animals* [15]. The Institutional Animal Care and Use Committee at Dickinson College approved the experiment described.

To assess anxiety-like behavior, 15 open-field chambers (27 x 27 cm; MED-OFA-510, Med-Associates, VT), linked to a personal computer (Activity Monitor software; Med-Associates, VT), and placed in a sound-attenuated cubicle (MED-OFA-022, Med-Associates, VT), were used for all sessions. A computer-generated square was placed in the center of the chamber and was used to calculate the time in center. The perimeter area was defined as 3.0 cm from each wall and the center as the remaining area [6].

Following the initial 6 weeks, Exercise and Sedentary mice continued their respective exercise regimens and were subjected to once weekly, 8-hr behavioral-sampling sessions (see Fig. 1). The 8-hr behavioral-sampling session was divided into 3 phases: baseline, stimulant and clearance. Mice were placed in the chambers in the absence of any drug during the first 3 h of the session (Hours 1–3; Baseline). After 3 h, mice were injected subcutaneously, at a volume of 10 ml/kg (body weight), with vehicle (physiological saline) or methamphetamine HCl (Sigma, St. Louis, MO, USA) and returned to the chamber for an additional 5 -h period. Beginning with the hour immediately following the injection (Hour 4; Stimulant Phase), an increase in the mouse's locomotor activity was anticipated. Due to the half-life of methamphetamine in mice (~ 60 min) [16], Hours 5–8 were anticipated to reflect the offset of the methamphetamine (Clearance Phase). Thus, a relatively long behavioral-sampling period permitted evaluation of possible baseline, stimulant and clearance differences between groups. Previous research has used this protocol to assess the anxiogenic effects of methamphetamine in mice [6]. Mice were injected with vehicle or a different methamphetamine dose (vehicle, 0.25, 0.5, or 1.0 mg/kg, salt weight) once a week for 4 weeks. (Due to a computer malfunction, the data for 7 and 8 mice in the Sedentary and Exercise groups, respectively, were lost on the 1.0 mg/kg dose session). Previous work has shown that a repeated behavioral-testing procedure at 1-week intervals does little to diminish anxiety-like behavior as assessed by the open-field task in mice and is an efficient within-subjects' procedure for examining dose-response curves [17]. To control for drug-dose order effects, the experiment was conducted in two replications: half of the mice underwent an escalating regimen (vehicle→0.25→0.5→1.0; Replication 1) and the other half of the mice underwent a deescalating regimen (1.0→0.5→0.25→vehicle; Replication 2). HCWR for Exercise mice and mouse body weights were recorded weekly. Upon completion of the experiment, the adrenal glands (left and right) were weighed.

Three dependent measures of anxiety-like behavior were measured: general activity (distance traveled), rearing (vertical counts) and exploration (time in center) [14]. Each dependent measure was subjected separately to an Exercise Regimen (Exercise *vs*. Sedentary) x Dose (Vehicle *vs*. 0.25 *vs*. 0.5 *vs*. 1.0 mg/kg) x Hour (1–8) repeated-measures, analysis of variance (ANOVA; SPSS, IBM Version 25). Because of the lost data at the 1.0 mg/kg dose, and to maintain sample sizes across methamphetamine doses, missing values/imputed data analyses also were conducted on each dependent measure and the estimated data were included in the ANOVAs. *Post-hoc* comparisons involved independent-samples *t* tests. Adrenal weights were subjected to an Exercise Condition x Adrenal Gland (left *vs*. right) repeated-measures ANOVA. Only significant main effects and interactions are reported. Statistical decisions were made at $\alpha = 0.05$.

Results

One mouse died during the course of the experiment and his data were excluded. Furthermore, the no home-cage running wheel and locked home-cage running wheel groups did not differ and were collapsed into one group, Sedentary, and compared to Exercise mice. Finally, a mouse's response to methamphetamine did not differ statistically based on the methamphetamine dose order (escalating *vs*. deescalating); thus, the data were collapsed.

An Exercise Regimen x Week (1–10) ANOVA[1] conducted on the weight data yielded significant main effects of Exercise Regimen, $F(9, 117) = 272.3$, $p < 0.001$, and Week, $F(1, 13) = 8.4$, $p = 0.012$, as well as a significant Exercise Regimen x Week interaction, $F(9, 117) = 3.5$, $p = 0.001$. Subsequent contrasts revealed that Exercise mice weighed less than Sedentary mice, beginning as early as Week 1 and detected during all weeks, $ts(13) > 2.1$, $ps < 0.047$, except Weeks 2, 4 and 5 (Fig. 2A). A one-way ANOVA, conducted on the HCWR data, was significant, $F(9, 63) = 9.73$, $p < 0.001$. Subsequent dependent-samples *t* tests revealed that mice increased HCWR during the first 6 weeks, plateaued at Week 6, and then decreased with the start of methamphetamine exposure and reliably so by Week 10, $t(7) = 4.47$, $p = 0.003$ (Fig. 2B).

The 3-way ANOVA, conducted on general activity, yielded significant main effects of Exercise Regimen, $F(1, 27) = 6.48$, $p = 0.017$, Hour, $F(7, 189) = 106.62$, $p < 0.001$, and Dose, $F(3, 81) = 20.86$, $p < 0.001$. The Exercise Regimen x Hour, $F(7, 189) = 4.73$, $p < 0.001$, Dose x Hour, $F(21, 567) = 27.0$, $p < 0.001$, and Exercise Regimen x Dose x Hour, $F(21, 567) = 1.79$, $p = 0.017$, interactions also were significant. Subsequent contrasts revealed that Exercise and Sedentary mice did not differ during any hour on vehicle or 0.25 mg/kg dose sessions; however, Exercise mice were more active during Hour 3 and Hours 6–8 on the 0.5 mg/kg dose session, $ts(27) \geq 2.26$, $ps \leq 0.032$, and Hours 5–8 after administration of the 1.0 mg/kg dose, $ts(27) \geq 2.12$, $ps \leq 0.04$ (Fig. 3A–D).

The 3-way ANOVA, conducted on the rearing data, yielded significant main effects of Exercise Regimen, $F(1, 27) = 7.19$,

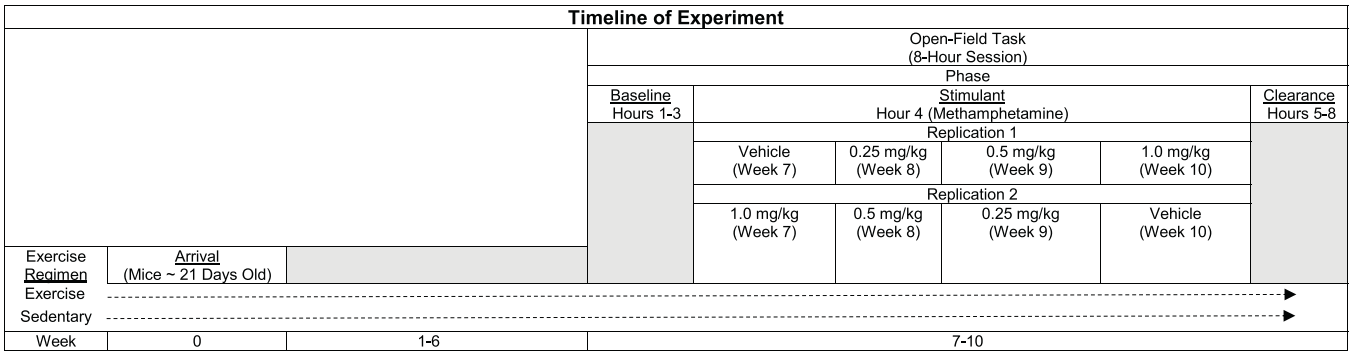

Fig. 1. Timeline of Experiment.

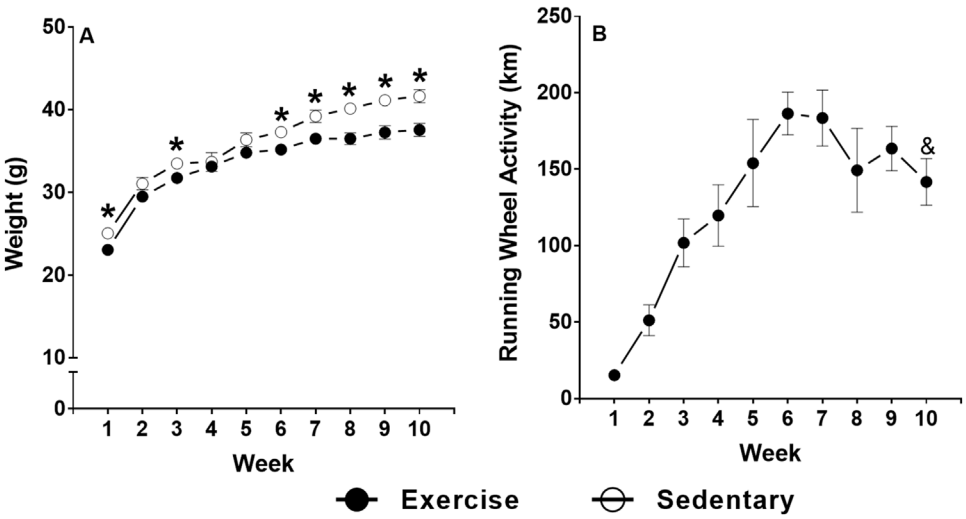

Fig. 2. Mean weights (g) of Exercise and Sedentary mice during the 10 weeks of the experiment (A). Mean home-cage wheel running (km) during each week of the experiment for Exercise mice (B). Error bars represent ± 1 SEM. * = significant difference between Exercise and Sedentary mice, *ps* < 0.05. & = significant difference between Week 6 compared to Week 10 for the Exercise mice, $p < 0.05$.

$p = 0.012$, and Hour, $F(7, 189) = 69.70$, $p < 0.001$. The Exercise Regimen x Hour, $F(7, 189) = 2.34$, $p = 0.026$, and Dose x Hour, $F(21, 567) = 10.49$, $p < 0.001$, interactions also were significant. The statistically significant Exercise Regimen x Hour interaction, combined with the non- significant Exercise Regimen x Dose x Hour interaction, suggests that Exercise mice reared more than Sedentary mice regardless as to the methamphetamine dose (Fig. 3E–H).

The 3-way ANOVA, conducted on the exploration data, yielded significant main effects of Hour, $F(7, 189) = 17.57$, $p < 0.001$, and Dose, $F(3, 81) = 7.01$, $p < 0.001$. The Dose x Hour, $F(21, 567) = 9.18$, $p < 0.001$, and Exercise Regimen x Dose x Hour, $F(21, 567) = 2.24$, $p = 0.01$, interactions also were significant. Subsequent contrasts revealed that Exercise mice did not differ from Sedentary mice with respect to the amount of time spent in the center during any hour on the vehicle or 0.25 mg/kg dose sessions; however, Exercise mice spent more time in the center during Hour 3 of the 0.5 mg/kg dose session, and spent more time in the center during Hours 7 and 8 after administration of the 0.5 mg/kg and 1.0 mg/kg doses, $ts(27) \geq 2.19$, $ps \leq 0.035$ (Fig. 3I–L).

The 2-way ANOVA, conducted on the adrenal weights, revealed no significant main effects or interaction (Fig. 4).

Discussion

The present report yielded several findings of primary interest. First, Exercise mice were more active, reared more and spent more time in the center compared to Sedentary mice during occasional baseline periods (see Fig. 3A–L), consistent with the anxiolytic effects of HCWR in mice [10–12]. Yet, these occasional group differences were gone by Hour 4 (Stimulant Phase) for the general activity and exploration measures; thus, not complicating later group differences (see below). Second, Exercise mice showed an overall increase in rearing compared to Sedentary mice regardless as to the methamphetamine dose, complicating the interpretation of group differences in rearing following the 1.0 mg/kg dose. That said, the failure to observe group differences on the general activity and exploration measures during Hour 4 suggests that HCWR did not robustly alter the locomotor-activating effects of methamphetamine. Third, and most importantly, Exercise mice were more active and spent more time in the center compared to Sedentary mice following clearance from the 0.5 and 1.0 mg/kg doses, indicating that HCWR ameliorated the effects of methamphetamine at doses previously reported to induce anxiety-like behavior in mice [6]. Admittedly, others have found that acute methamphetamine exposure has anxiolytics effects in rats [18]. Species differences perhaps account for the discrepancy. Finally, the failure to find a difference between the locked home-cage running wheel and no home-cage running wheel groups suggests that the anxiolytic effects of HCWR are not due simply to environmental enrichment.

HCWR increased and plateaued over the first 6 weeks then decreased by the end of methamphetamine exposure. One possible explanation of this last finding is that the stimulant properties of methamphetamine may have interfered with a mouse's general activity during the dark phase of the mouse's light-dark (sleep-wake) cycle. Indeed, Kitanaka et al. showed that methamphetamine exposure during the light phase decreased HCWR during the dark phase that same day [19]. HCWR also resulted in weight loss in Exercise mice over the course of the experiment, a result not observed in mice perhaps due to the limited-duration exercise regimen previously used [11].

Unlike a previous study [13], adrenal weights did not reliably differ between Exercise and Sedentary mice, suggesting that the anxiolytic effects of HCWR are not due to changes in HPA activity. This conclusion, however, should be tempered, as adrenal weights may not have been sensitive in detecting the impact of HCWR on HPA activity, a limitation of the present experiment. Indeed, corticosterone levels, another indicator of HPA activity, have been found to increase several hours after acute methamphetamine exposure [8], temporally mirroring the anxiety-like behavior observed in the present report. Moreover, HCWR has been shown to alter corticosterone levels in mice [13]. Thus, a future experiment should assess the effects of HCWR on corticosterone levels following anxiogenic doses of methamphetamine.

Preclinical animal studies have found that voluntary exercise reduces anxiety-like behavior associated with morphine withdrawal [20]. Furthermore, previous work has suggested that the methamphetamine clearance phase is associated with anxiety-like behavior similar to withdrawal [6]. Thus, the primary finding of the present report provides preclinical evidence that voluntary exercise reduces anxiety-like behavior associated with acute methamphetamine withdrawal. Clinically speaking, such a result may indicate that by ameliorating negative affective states associated with methamphetamine withdrawal, states that have been proposed to motivate compulsive drug-taking behavior in people [4], voluntary exercise may serve as a non-pharmacological treatment for methamphetamine use disorder.

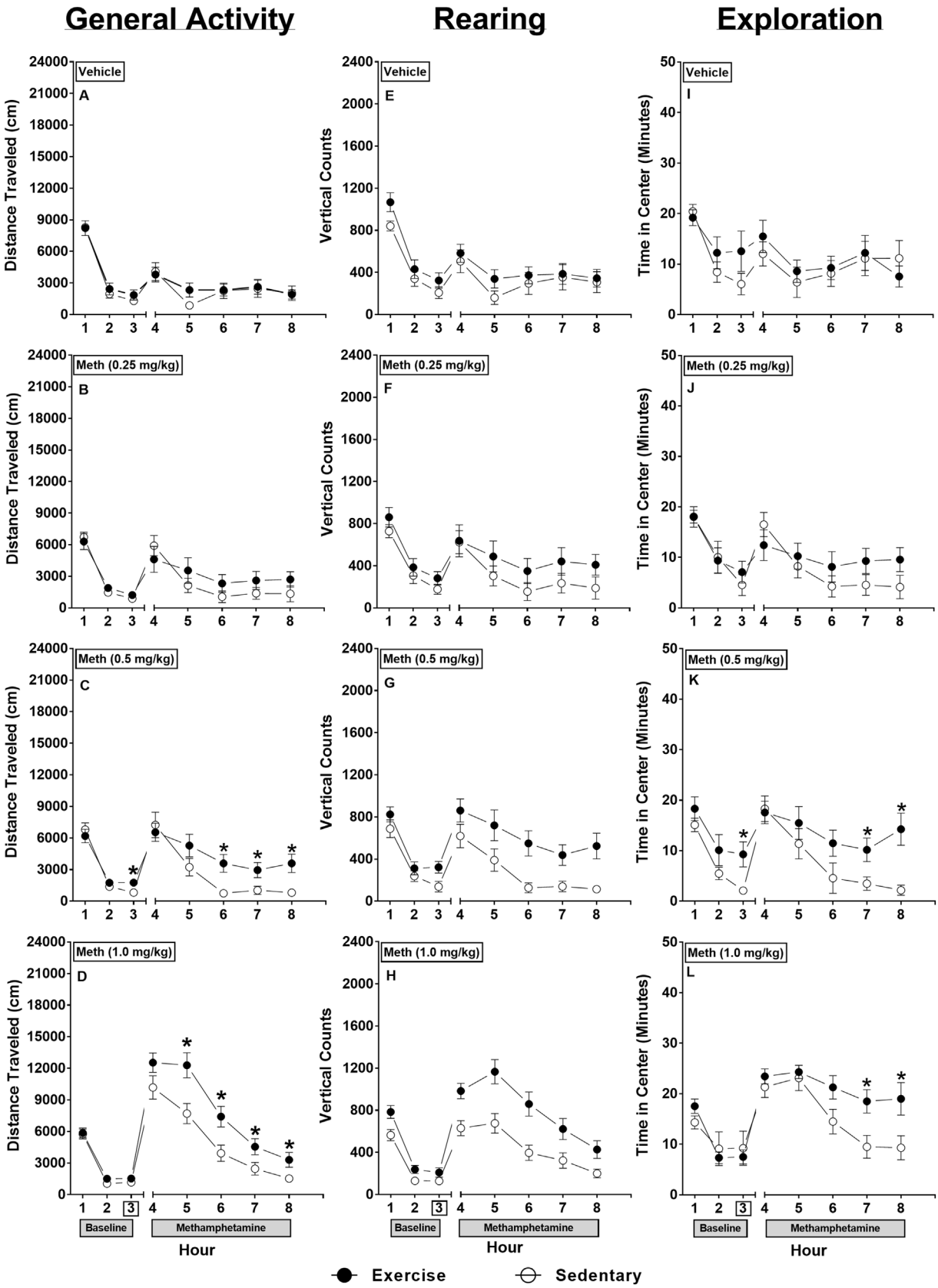

Fig. 3. Mean distance traveled (A–D), vertical counts (E–H) or time in center (I–L) as a function of methamphetamine dose during each 8h session for Exercise and Sedentary mice. Error bars represent ± 1 SEM. * = a significant difference between Exercise and Sedentary mice, *ps* < 0.05.

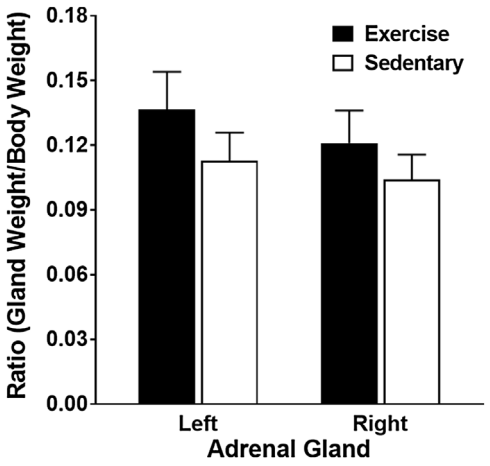

Fig. 4. Mean adrenal (left *vs.* right) weights (expressed as a ratio = adrenal weight/body weight to correct for differences in body weight) for Exercise and Sedentary mice.

Footnotes

[1]Weights and home-cage running wheel data were collected only during the second replication of Experiment 1 and reflect the data of only 7 and 8 mice from the Sedentary and Exercise groups, respectively.

Funding

The author wishes to thank the Department of Psychology at Dickinson College for its financial support, and Alex Vouga and Elise Guma for their assistance with the experiment.

Conflict of interest

There are no perceived conflicts of interest associated with this research.

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