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Voluntary wheel running attenuates urinary bladder hypersensitivity and dysfunction following neonatal maternal separation in female mice

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Abstract

Aims—Patients with interstitial cystitis/painful bladder syndrome (IC/PBS) commonly suffer from widespread pain and mood disorder, which has been attributed to improper functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Voluntary exercise has been shown to improve HPA axis function, therefore we are determining whether voluntary wheel running can attenuate urological pain and dysfunction following neonatal maternal separation (NMS) in female mice.

Methods—Mice underwent NMS for 3 h/day from postnatal Day 1–21, were caged with free access to running wheels at 4 weeks of age, and assessed 4 weeks later for bladder sensitivity, micturition, reward behavior, mast cell degranulation, and HPA axis-related in vitro analysis.

Results—Increased bladder sensitivity, void frequency, and mast cell degranulation was observed in adult sedentary (–Sed) NMS mice, compared to naïve-Sed controls. Sucrose preference was increased in NMS-Sed mice and corticotropin-releasing factor receptor 1 (CRF₁) and glucocorticoid receptor mRNA levels were significantly reduced in the hippocampus. Exercise normalized bladder sensitivity, micturition output, and increased brain-derived neurotrophic factor (BDNF) mRNA levels in the hippocampus of NMS mice. Mast cell degranulation was also normalized in NMS bladders following exercise.

Conclusions—Voluntary exercise normalized behavioral outcomes resulting from NMS in female mice, increased hippocampal BDNF mRNA levels, and decreased mast cell degranulation in the bladder. Together these results provide novel insight into the efficacy of voluntary exercise to attenuate comorbid outcomes resulting from exposure to early life stress.

Keywords

brain-derived neurotrophic factor; corticotropin-releasing hormone; mast cell; pain; pituitary-adrenal system

1 | INTRODUCTION

Patients with interstitial cystitis/painful bladder syndrome (IC/PBS) often suffer from additional chronic pain syndromes and/or mood disorders.^{1,2} This comorbidity has been clinically linked with childhood maltreatment and IC/PBS patients with a history of sexual abuse reported greater pain during a pelvic exam than those with no history of abuse.³ Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is a common outcome following exposure to stress or adversity in early life.⁴ Rodent models of neonatal maternal separation (NMS)⁵ have recapitulated this effect in a laboratory setting, including our studies demonstrating urogenital hypersensitivity in both male⁶ and female^{7,8} NMS mice.

The main hormone driving HPA axis signaling is corticotropin-releasing factor (CRF), which signals both locally within the HPA axis to drive glucocorticoid release⁹ and peripherally on immune and urothelial cells.¹⁰ The hippocampus and other limbic structures provide regulatory feedback onto the HPA axis, mediated through the glucocorticoid receptor (GR) and CRF receptors, CRF₁ and CRF₂.^{9,11} Models of NMS have largely shown a decrease in hippocampal GR and CRF receptor expression, representing decreased inhibition onto the HPA axis.^{5,12,13} Mast cells residing in visceral tissues can degranulate in response to HPA axis activation, which has been observed in tissue biopsies from IC/PBS patients.¹⁴ Release of mast cell contents can sensitize nearby sensory nerve terminals and heighten bladder sensitivity and output.^{13,15}

Voluntary exercise favorably influences plasticity within regions of the brain that are negatively affected by NMS,¹⁶ including increased BDNF expression and associated neurogenesis in the hippocampus.^{17,18} Although increased physical activity can ameliorate some of the negative effects of NMS,^{12,19} the impact of voluntary exercise on stress-associated visceral hypersensitivity has not been reported. Therefore, we are testing the hypothesis that voluntary exercise can normalize altered urological sensitivity, micturition, and reward behavior resulting from NMS. Molecular and cellular changes in the hippocampus and bladder were also investigated to determine whether these regulatory and downstream regions, respectively, were responsive to exercise intervention.

2 | MATERIALS AND METHODS

2.1 | Animals

Female C57Bl/6 mice (Charles River, Wilmington, MA), born and housed in the Research Support Facility at the University of Kansas Medical Center, were used for all experiments. Mice received water and food ad libitum and were housed on a 12-h light cycle (600-1800 h). All research was approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee (protocol numbers 2013-2150 and 2016-2344) in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2 | Experimental groups and ages

Three separate cohorts of naïve and NMS mice were used to avoid confounding effects of additional stressors. In the first group, mice underwent urinary bladder distension (UBD) at

8 weeks of age, were euthanized immediately afterward, and tissue was taken for mRNA and protein analysis. In the second group, mice underwent micturition patterning at 8 weeks of age, followed by sucrose preference testing, and were sacrificed approximately one week later upon confirmation of estrus and tissue was taken for mast cell visualization. In the third group, serum was collected from 8-week-old mice that underwent no additional testing for this study.

2.3 | Neonatal maternal separation

Pregnant dams (Charles River, Wilmington, MA) were delivered to the animal facility during the last week of gestation. On postnatal days 1–21, NMS litters were removed and placed en masse into clean glass beakers, containing small amounts of their home cage bedding material, and held at 34°C and 50% humidity for 3 h.⁶ Naïve mice were similarly born and housed, without handling outside of normal husbandry care, and all mice were weaned on P22 and caged with same-sex littermates.

2.4 | Exercise

At 4 weeks of age, female NMS and naïve mice were pairhoused in cages equipped with a stainless-steel running wheel (Mini Mitter; Bend, OR). Sedentary mice remained in standard cages with IACUC-compliant enrichment standards. Running distance was monitored by Vital View Acquisition and Analysis software (Harvard Apparatus, Holliston, MA).

2.5 | Vaginal lavage

The vaginal canal was gently irrigated with 100 µl of phosphate buffered saline (PBS) and suspended cells were examined under light microscopy. Estrous cycle stage was estimated by determining the ratios of nucleated epithelial cells, cornified epithelial cells, and leukocytes and verification of estrus was made following at least 4 consecutive days of observation. No correlations between the estimated stage of estrous cycle and the results from behavioral assays or histological and molecular analysis were observed (data not shown).

2.6 | Urinary bladder distension

Urinary bladder distension (UBD) was performed as previously described.⁷ Briefly, under inhaled isoflurane (4% induction, 2% maintenance), wire electrodes (0.003" diameter; Grass Technologies, West Warwick, RI) were implanted into the abdominal musculature and the bladder was catheterized intravesically via the urethra. Anesthesia was lowered until hind limb reflexes, but not escape behaviors, were present (~0.9% isoflurane). Body temperature was maintained at approximately 37°C. Gas flow from a compressed nitrogen tank was manually adjusted and monitored using a dual-stage low delivery pressure regulator (Matheson-Linweld, Kansas City, MO) and delivered via a custom-made distension control device (The University of Iowa Medical Instruments, Iowa City, IA). Following verification of two measurable evoked responses to 60 mmHg, each pressure (15, 30, 45, 60 mmHg) was applied in triplicate for 20 s with a 2-min rest period in between. Electromyographic activity was amplified, filtered, and recorded (Spike 2, Cambridge

Electronic Design, Cambridge, UK) and the visceromotor response (VMR) was quantified and expressed as a percent of baseline activity.

2.7 | Micturition patterning behavior

Micturition was assessed as previously described.⁷ Briefly, mice were placed on a piece of filter paper (Model 583 Gel Dryer, Bio-Rad Laboratories, Hercules, CA) and enclosed under a clear Plexiglas container (36 cm length × 20 cm width × 13 cm height) for 1 h duration. Urine spots were visualized under ultraviolet light and counted and measured using NIH Image J software. All assessments took place between 600 and 900 h to avoid confounding effects of diurnal stress hormone release.

2.8 | Sucrose preference

Mice were individually housed in cages equipped with two polysulfone drinking bottles (BioDAQ Liquid Choice Unplugged Allentown cages, Biological Data Acquisition, New Brunswick, NJ). Both bottles were filled with standard drinking water and the mice were acclimated for 24 h. One bottle was then filled with 1% sucrose diluted in drinking water and bottle weights were measured and the position of the bottles were interchanged daily for two consecutive days. Volume of 1% sucrose consumed and the percentage of 1% sucrose consumed were calculated for each mouse.

2.9 | mRNA extraction and qRT-PCR

Total mRNA was isolated from hippocampus using Trizol reagent (Ambion, Austin, TX) and RNeasy micro kit (Qiagen, Valencia, CA) per manufacturer's instructions. A 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) determined concentration and purity and the iScript cDNA synthesis kit (Bio-Rad) was used to synthesize cDNA from total RNA. SsoAdvanced SYBR Green Supermix (Bio-Rad) was used to perform quantitative real-time PCR on 1 µg of total cDNA using a Bio-Rad CFX manager 3.1 real time PCR system with the following 20 µM primers (Integrated DNA Technologies, Coralville, IA): GAPDH forward, 5'-CCCT GCCTTTTCTACGGTGT; GAPDH reverse, 5'-TTCCCC GTAGCCATTGTTTGT; CRF1 forward, 5'-CCCTGCCT TTTTCTACGGTGT, CRF1 reverse, 5'-TTCCCGGTAGCCATTGTTTGT; CRF2 forward, 5'-CCTGTGGACACTTTT GGAGCA; CRF2 reverse, 5'-TGTTGCAGTAGGTGTAGGGAC; GR forward, 5'-GACTCCAAAGAATCCTTAGCT CC; GR reverse, 5'-CTCCACCCCTCAGGGTTTTAT; BD NF forward, 5'-CAGGTTTCGAGAGGTCTGACGA; BDNF reverse, 5'-CGCGTCCTTATGGTTTTCTTCG. Samples were run in triplicate with a negative control for each amplification series. Efficiency values were derived for each individual sample (LinRegPCR software v2012.3) and threshold cycle values were subtracted from GAPDH and percentage of fold change over naïve sedentary was calculated using the Pfaffl method.

2.10 | Serum corticosterone

Blood (20 µl) was collected via tail clip during the early portion of the light-cycle (0800-1100 h), clotted on ice for 1 h, and centrifuged at 10 000 rpm for 10 min at 4°C. The clear supernatant (serum) was removed and stored at -20°C until analysis. An ELISA kit

was used to quantify serum corticosterone (CORT) according to manufacturer's instructions (American Laboratory Products Company, Salem, NH).

2.11 | Mast cell degranulation

Following transcardial perfusion with ice cold 4% paraformaldehyde, whole bladders were dissected and postfixed at room temperature for 1 h. Bladders were cryoprotected overnight in 30% sucrose at 4°C, mounted in Tissue-Tek OTC mounting medium (Sakura Finetek, Torrance, CA), and stored at -20°C. As previously described,⁶ non-serial slide-mounted 7 µm-thick cryostat sections were stained with acidified (pH <1.0) 1% toluidine blue solution, dried overnight, dehydrated with 95% and 100% ethanol, and coverslipped with 1X phosphate buffered saline. Digital images were captured under light microscopy (QIClick digital CCD Camera, QImaging, Surrey, BC, Canada; Nikon eclipse 90i, Nikon Instruments, Inc., Melville, NY) and the total number of non-degranulated mast cells and degranulated mast cells were counted in at least eight separate sections spanning the length of bladder tissue.

2.12 | Protein analysis

As previously described,⁷ total protein was isolated from the bladder using Cell Extraction Buffer containing Halt protease and phosphatase inhibitors (ThermoFisher Scientific, Waltham, MA) and Na₃VO₄. A D_c protein assay (ThermoFisher Scientific) was used to determine protein concentration and samples were reduced, subjected to SDS-PAGE (Criterion 4% to 12% Bis-Tris gels; Bio-Rad), and transferred to Nitrocellulose membrane (Whatman GmbH, Dassel, Germany). Non-specific binding was blocked by incubation in 5% milk in Tris-buffered saline with Tween-20 (TBST) for 1 h. Membranes were then incubated overnight at 4°C with primary antisera to CRF₁ (1:500; Millipore, Billerica, MA), CRF₂ (1:800; Millipore), and GAPDH (1:1000; Cell Signaling Technology, Beverly, MA). Membranes were then washed with TBST and incubated with anti-rabbit secondary antibody (1:10 000; Cell Signaling Technology) for 1 h. Densitometry was performed using Quantity One 4.6.9 software (Bio-Rad).

2.13 | Statistical analysis

Calculations were performed on Microsoft Excel and statistical analyses were performed using two-way analysis of variance (ANOVA, with or without repeated measures) followed by Bonferroni's or Tukey's posttest and Pearson correlation (GraphPad Prism 6, GraphPad Software, Inc., La Jolla, CA; IBM SPSS Statistics, IBM Corporation, Armonk, NY) as denoted in the results section and figure legends. All data are expressed as mean ± SEM and $P < 0.05$ was considered significant.

3 | RESULTS

We previously reported that adult (~16-week-old) female NMS mice exhibited increased bladder sensitivity, as well as altered micturition patterns, and reduced hippocampal gene expression indicative of diminished negative input onto the HPA axis.⁷ In the current study, naïve and NMS mice were housed with free access to running wheels beginning at 4 weeks of age to determine if voluntary physical activity could prevent NMS-induced changes.

3.1 | Urinary bladder sensitivity

Sedentary NMS mice (NMS-Sed) had a significantly greater VMR during UBD than sedentary naïve mice (naïve-Sed; Figure 1A and 1B). Exercised NMS (NMS-Ex) mice had significantly lower VMR than NMS-Sed mice and were not significantly different from either naïve group (Figures 1A and 1B). No impact of exercise was observed on VMR in naïve mice (Figures 1A and 1B).

3.2 | Micturition patterning analysis

A significant increase in void frequency was observed in NMS-Sed mice compared to naïve-Sed mice; however, no difference was present between naïve-Ex and NMS-Ex void frequency (Figure 2A). Void size was significantly smaller in NMS mice with naïve-Ex mice having significantly larger voids compared to both naïve-Sed and NMS-Ex mice (Figure 2B). Fecal output was significantly higher in NMS-Sed mice compared to naïve-Sed and exercise significantly increased fecal output in both naïve and NMS mice (Figure 2C).

3.3 | Reward behaviors

Sedentary NMS mice had a significantly higher sucrose preference than naïve-Sed mice, which was not affected by exercise (Figure 3A). Sucrose preference and void frequency were negatively correlated in NMS-Ex mice and NMS-Sed mice that displayed <5 voids in the 1 h testing period, a level similar to naïve mice (Figure 3B), suggesting a possible mechanistic link between these two observations.

Running distance was calculated over the 4-week period in all groups of mice as a measure of reward behavior. In all mice, a significant impact of age was observed, such that the distance ran was not consistent across all weeks (Figure 3C). NMS mice displayed a trend toward decreased distance ran over the entire period ($P = 0.068$, two-way RM ANOVA), and ran a significantly shorter distance during the 7th week of life compared to naïve mice (Figure 3C).

3.4 | Hippocampal mRNA levels

A significant effect of NMS was observed for CRF₁, GR, and BDNF mRNA levels in the hippocampus, resulting in lower mRNA levels of CRF₁ and GR across both NMS groups (Figure 4A and 4C) and higher mRNA levels of BDNF across both NMS groups (Figure 4D). Exercise significantly increased the mRNA levels of BDNF across both groups, particularly in NMS-Ex hippocampus compared to both NMS-Sed and naïve-Ex levels (Figure 4D). No significant impact of exercise was observed on CRF₁ and GR mRNA levels (Figures 4A and 4C) and CRF₂ mRNA levels were not impacted by NMS or exercise (Figure 4B).

3.5 | Serum corticosterone and mast cell activation

Exercise and NMS had a significant interaction effect on serum corticosterone (CORT) levels (Figure 5A). A trend toward increased serum CORT was observed in NMS-Sed, compared to both naïve-Sed and NMS-Ex mice, although the differences did not reach significance.

Exercise and NMS also had significant effects on histological evidence of mast cell degranulation in the bladder (Figure 5B). A significantly higher percentage of degranulated mast cells was observed in NMS-Sed mice, compared to both naïve-Sed and NMS-Ex mice.

3.6 | Bladder protein levels

Exercise significantly decreased CRF₁ protein levels across NMS and naïve bladder samples (Figure 5C), particularly in naïve-Ex mice. Protein levels of CRF₂ were not significantly impacted by NMS or exercise (Figure 5D).

4 | DISCUSSION

Voluntary exercise has been shown to attenuate molecular and behavioral changes associated with a dysfunctional HPA axis. Open access to running wheels ameliorated anxiety-like and depressive behaviors in NMS rats, as well as normalized hippocampal GR and BDNF mRNA levels.¹² A separate study also reported a significant decrease in depressive behavior in exercised NMS rats compared to sedentary counterparts.²⁰ To date, no published works have reported on the impact of exercise on NMS-induced hypersensitivity; however, exercise has been shown to significantly improve symptom severity in patients with centralized pain disorders.²¹

Here we observed improvements following voluntary exercise on previously established⁷ outcomes of NMS in female mice. Sedentary adult female NMS mice developed significant bladder hypersensitivity measured as increased VMR during UBD at 8 weeks of age. This bladder hypersensitivity was completely absent in NMS mice given voluntary access to running wheels beginning at 4 weeks of age. Voluntary exercise also normalized void frequency in NMS mice, but did not impact void size in NMS mice unlike in naïve mice. Together, these data suggest that access to voluntary exercise can attenuate bladder hypersensitivity and over-activity following NMS.

Voluntary exercise has also normalized behavioral changes in adult rats exposed to NMS.¹² We observed a significant correlation between anhedonia and polyuria in NMS mice, despite an increase in sucrose preference in NMS-Sed mice. This correlation persisted in NMS-Ex mice, indicating that despite normalizing micturition frequency, exercise did not uncouple the comorbidity between anhedonia and voiding. Increased fecal output in NMS-Sed mice likely denoted heightened anxiety, as has been previously reported in NMS rodent models.⁵ A decrease in running distance/pair was observed among NMS mice, indicating a negative impact on reward behavior. Despite running shorter distances, this level of voluntary exercise was sufficient to improve many of the urological consequences of NMS, but did not affect reward behaviors.

Clinically, early life stress has been shown to reduce hippocampal volume,²² and improper hippocampal development can systemically disinhibit the HPA axis.²³ Additionally, chronic glucocorticoid exposure can blunt neurogenesis and spine formation, reduce GR and MR, and diminish BDNF signaling in the hippocampus.²⁴ We observed decreased hippocampal GR and CRF₁ mRNA levels in NMS hippocampus, with no significant impact of exercise on their expression levels. An exercise-induced increase in hippocampal GR was reported in

NMS rats¹²; however, the rats were older (15 weeks), suggesting that a longer exercise intervention in our NMS mice may also affect GR, as well as the CRF receptors. BDNF mRNA levels were not affected by NMS, but were increased by exercise in both naïve and NMS hippocampus. Considering that BDNF promotes neurogenesis and synaptic plasticity within the hippocampus, in opposition to the actions of glucocorticoids,²⁵ this relative change in expression patterns could indicate a restoration of negative hippocampal feedback onto the HPA axis. This observation was also reflected in the significant interaction effect of NMS and exercise on serum CORT levels, suggesting that the exercise-induced decrease in CORT in NMS mice might be secondary to a BDNF-induced increase in neurogenesis or reinstatement of proper negative feedback.

Peripheral expression of CRF receptors has been observed in most pelvic organs, including the urothelial layer,¹⁰ and can be modulated by acute or chronic stress exposure.¹³ While feline IC bladders show increased CRF₁ and CRF₂ expression,¹⁰ we have consistently observed a decrease in CRF₁ expression in the bladder of female NMS mice,^{7,8} indicating a CRF₂-dominant signaling environment, which is required for stress-induced increases in bladder permeability or sensitivity.¹³ Clearly this is not the only mechanism at play, as naïve-Ex mice do not demonstrate bladder hypersensitivity despite a similar increase in CRF₂:CRF₁ expression. The decrease in CRF₁ protein in naïve-Ex bladder may possibly underlie the increase in void size.^{10,13}

Mast cells are a major peripheral source of CRF and are exquisitely responsive to acute stress exposure and activation of the HPA axis.¹⁵ Increased mast cell infiltration and activation has been observed in biopsies of affected tissue from IC/PBS patients.¹⁴ We have previously reported an increase in mast cell degranulation in NMS female bladder,⁷ which was also observed here. Voluntary exercise beginning at 4 weeks of age completely normalized histological evidence of mast cell degranulation in NMS mice. The continual observation of mast cell degranulation correlating with our observed physiological and behavioral changes associated with urological organs following NMS and exercise, both in this and previous studies,^{6,7} further suggest that central changes within the HPA axis, likely resulting from diminished negative feedback from the hippocampus, may be driving these outcomes.

5 | CONCLUSIONS

Voluntary exercise normalized behavioral outcomes resulting from NMS in female mice, including increased bladder sensitivity, micturition frequency, and sucrose preference. Hippocampal BDNF mRNA levels were significantly increased and mast cell degranulation was normalized in the bladder of NMS-Ex mice. Taken together, voluntary exercise may prevent many of the deleterious outcomes affecting the urogenital system resulting from exposure to early life stress.

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References

1. Bullones Rodriguez MA, Afari N, Buchwald DS. National institute of D, digestive, kidney diseases working group on urological chronic pelvic P. evidence for overlap between urological and nonurological unexplained clinical conditions. *J Urol*. 2013; 189:S66–S74. [PubMed: 23234637]
2. Krieger JN, Stephens AJ, Landis JR, et al. Relationship between chronic nonurological associated somatic syndromes and symptom severity in urological chronic pelvic pain syndromes: baseline evaluation of the mapp study. *J Urol*. 2014; 193:1254–1262. [PubMed: 25444992]
3. Seth A, Teichman JM. Differences in the clinical presentation of interstitial cystitis/painful bladder syndrome in patients with or without sexual abuse history. *J Urol*. 2008; 180:2029–2033. [PubMed: 18804240]
4. Maniam J, Antoniadis C, Morris MJ. Early-life stress, HPA axis adaptation, and mechanisms contributing to later health outcomes. *Front Endocrinol*. 2014; 5:73.
5. O'Mahony SM, Hyland NP, Dinan TG, Cryan JF. Maternal separation as a model of brain-gut axis dysfunction. *Psychopharmacology*. 2011; 214:71–88. [PubMed: 20886335]
6. Fuentes IM, Pierce AN, O'Neil PT, Christianson JA. Assessment of perigenital sensitivity and prostatic mast cell activation in a mouse model of neonatal maternal separation. *J Vis Exp*. 2015; 102:e53181.
7. Pierce AN, Di Silvestro ER, Eller OC, Wang R, Ryals JM, Christianson JA. Urinary bladder hypersensitivity and dysfunction in female mice following early life and adult stress. *Brain Res*. 2016; 1639:58–73. [PubMed: 26940840]
8. Pierce AN, Ryals JM, Wang R, Christianson JA. Vaginal hypersensitivity and hypothalamic-pituitary-adrenal axis dysfunction as a result of neonatal maternal separation in female mice. *Neuroscience*. 2014; 263:216–230. [PubMed: 24462609]
9. Bale TL, Vale WW. CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu Rev Pharmacol Toxicol*. 2004; 44:525–557. [PubMed: 14744257]
10. Hanna-Mitchell AT, Wolf-Johnston A, Roppolo JR, Buffington TC, Birder LA. Corticotropin-releasing factor family peptide signaling in feline bladder urothelial cells. *J Endocrinol*. 2014; 222:113–121. [PubMed: 24829219]
11. Herman JP, Ostrander MM, Mueller NK, Figueiredo H. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Prog Neuropsychopharmacol Biol Psychiatry*. 2005; 29:1201–1213. [PubMed: 16271821]
12. Maniam J, Morris MJ. Voluntary exercise and palatable high-fat diet both improve behavioural profile and stress responses in male rats exposed to early life stress: role of hippocampus. *Psychoneuroendocrinology*. 2010; 35:1553–1564. [PubMed: 20594764]
13. Pierce AN, Christianson JA. Stress and chronic pelvic pain. *Prog Mol Biol Transl Sci*. 2015; 131:509–535. [PubMed: 25744684]
14. Theoharides TC, Sant GR, el-Mansoury M, Letourneau R, Ucci AA Jr, Meares EM Jr. Activation of bladder mast cells in interstitial cystitis: a light and electron microscopic study. *J Urol*. 1995; 153:629–636. [PubMed: 7861501]
15. Theoharides TC, Donelan JM, Papadopoulou N, Cao J, Kempuraj D, Conti P. Mast cells as targets of corticotropin-releasing factor and related peptides. *Trends Pharmacol Sci*. 2004; 25:563–568. [PubMed: 15491778]
16. Dishman RK, Berthoud HR, Booth FW, et al. Neurobiology of exercise. *Obesity*. 2006; 14:345–356. [PubMed: 16648603]
17. Park SS, Stranahan AM, Chadwick W, et al. Cortical gene transcription response patterns to water maze training in aged mice. *BMC Neurosci*. 2011; 12:63. [PubMed: 21714909]
18. Stranahan AM, Lee K, Martin B, et al. Voluntary exercise and caloric restriction enhance hippocampal dendritic spine density and BDNF levels in diabetic mice. *Hippocampus*. 2009; 19:951–961. [PubMed: 19280661]

19. Liu M, Kuhel DG, Shen L, Hui DY, Woods SC. Apolipoprotein E does not cross the blood-cerebrospinal fluid barrier, as revealed by an improved technique for sampling CSF from mice. *Am J Physiol Regul Integr Comp Physiol*. 2012; 303:R903–R908. [PubMed: 22933021]
20. Marais L, Stein DJ, Daniels WM. Exercise increases BDNF levels in the striatum and decreases depressive-like behavior in chronically stressed rats. *Metab Brain Dis*. 2009; 24:587–597. [PubMed: 19844781]
21. Kaleth AS, Saha CK, Jensen MP, Slaven JE, Ang DC. Effect of moderate to vigorous physical activity on long-term clinical outcomes and pain severity in fibromyalgia. *Arthritis Care Res*. 2013; 65:1211–1218.
22. Gorka AX, Hanson JL, Radtke SR, Hariri AR. Reduced hippocampal and medial prefrontal gray matter mediate the association between reported childhood maltreatment and trait anxiety in adulthood and predict sensitivity to future life stress. *Biol Mood Anxiety Disord*. 2014; 4:12. [PubMed: 25408863]
23. Taliaz D, Loya A, Gersner R, Haramati S, Chen A, Zangen A. Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. *J Neurosci*. 2011; 31:4475–4483. [PubMed: 21430148]
24. Suri D, Vaidya VA. Glucocorticoid regulation of brain-derived neurotrophic factor: relevance to hippocampal structural and functional plasticity. *Neuroscience*. 2013; 239:196–213. [PubMed: 22967840]
25. Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature*. 2011; 476:458–461. [PubMed: 21814201]

Figure 1.

Urinary bladder sensitivity was measured at 8 weeks of age following neonatal maternal separation (NMS) and voluntary exercise. A, At 8 weeks of age, sedentary (-Sed) NMS mice ($n = 24$) displayed significantly increased visceromotor response (VMR) during urinary bladder distension ($P = 0.0038$, RM two-way ANOVA) and at 15, 30, and 60 mmHg, compared to naïve-Sed mice ($n = 20$). Exercised (-Ex) NMS mice ($n = 6$) that were given running wheels at 4 weeks of age displayed a significantly reduced VMR compared to NMS-Sed ($P = 0.0168$, RM two-way ANOVA). The VMR of naïve-Ex mice ($n = 6$) was not significantly different from either naïve-Sed or NMS-Ex. B, Analysis of the area under the curve (AUC) for the entire UBD procedure revealed a significant effect of exercise and significantly higher VMR in NMS-Sed mice compared to either naïve-Sed or NMS-Ex mice. Bracket indicates a significant impact of exercise ($^{\dagger} P < 0.05$), two-way ANOVA. *, ** $P < 0.05$, 0.01 NMS-Sed versus naïve-Sed; #, ## $P < 0.05$, 0.01 NMS-Ex versus NMS-Sed; Fisher's LSD posttest

Figure 2.

Urine and fecal output were measured at 8 weeks of age following neonatal maternal separation (NMS) and voluntary exercise. A, Micturition patterning analysis revealed that NMS-Sed mice ($n = 16$) had significantly more voids compared to naïve-Sed mice ($n = 18$); however, NMS-Ex mice ($n = 6$) did not display a significantly different void frequency compared to naïve-Ex mice ($n = 6$). B, The average void size was significantly larger in naïve-Ex mice compared to both naïve-Sed and NMS-Ex mice. C, Fecal output was significantly higher in NMS-Sed mice, compared to naïve-Sed. Exercise increased fecal output in both naïve and NMS mice, compared to sedentary counterparts. Brackets indicate a

significant impact of NMS (§ $P < 0.05$) or exercise (†††† $P < 0.0001$), two-way ANOVA. *, **, *** $P < 0.05, 0.01, 0.001$ versus naïve; #, ##, ### $P < 0.05, 0.01, 0.001$ versus sedentary; Fisher's LSD posttest

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Figure 3.

Behaviors indicative of reward were measured in mice at 8 weeks of age following neonatal maternal separation (NMS) and voluntary exercise. A, In a two-choice preference test, sedentary (-Sed) NMS mice ($n = 15$) had a significantly higher preference for sucrose than naïve-Sed mice ($n = 15$). Exercise had no effect on sucrose preference in either group ($n = 6$ for each). B, A significant correlation was observed between sucrose preference and void frequency in NMS-Ex mice ($r^2 = 0.7595$, $P = 0.0237$) and NMS-Sed mice who had <5 voids ($r^2 = 0.6707$, $P = 0.0069$), a similar output to naïve mice, such that reduced sucrose preference was correlated with greater void frequency. C, Distance ran per Day was

measured on a weekly basis for each pair of mice ($n = 6$ pairs for all groups). A significant impact of time was observed within each group and NMS mice ran significantly fewer km than naïve mice during the seventh week of life. Bracket indicates a significant impact of time ($^{\alpha\alpha} P < 0.01$), two-way ANOVA; $*P < 0.05$ versus naïve, Tukey's posttest

Figure 4.

The mRNA levels of the corticotropin-releasing factor (CRF) receptors, CRF₁ and CRF₂, the glucocorticoid receptor (GR), and brain-derived neurotrophic factor (BDNF) were measured in the hippocampus of 8-week-old mice following neonatal maternal separation (NMS) and voluntary exercise. Relative mRNA levels are expressed as a percentage of sedentary (-Sed) naïve levels. A, A significant effect of NMS was observed on CRF₁ mRNA levels. B, The mRNA levels of CRF₂ were not significantly impacted by NMS or exercise. C, A significant effect of NMS was observed on GR mRNA levels, particularly in NMS-Sed hippocampus. D, The mRNA levels of BDNF were significantly impacted by both NMS and exercise. BDNF mRNA levels in NMS-Ex hippocampus was significantly higher than both NMS-Sed and naïve-Ex. Brackets indicate a significant impact of NMS (§ $P < 0.05$) or exercise (†† $P < 0.01$), two-way ANOVA. * $P < 0.05$ versus naïve; ## $P < 0.01$ versus sedentary; Fisher's LSD posttest. All groups ($n = 6$) except NMS-Sed ($n = 4$)

Figure 5.

Serum corticosterone (CORT) and bladder mast cell degranulation and corticotropin-releasing factor (CRF) receptor protein levels were measured following neonatal maternal separation (NMS) and voluntary exercise. A, A significant interaction effect of NMS and exercise was observed, such that a trend toward increased CORT was indicated in sedentary (-Sed) NMS compared to naïve-Sed ($P = 0.120$, $n = 3$) and NMS-Ex ($P = 0.074$, $n = 5$). B, A significant impact of NMS, exercise, and an interaction effect of both was observed on histological evidence of mast cell degranulation in the bladder. NMS-Sed mice ($n = 3$) exhibited a significant increase in the percentage of degranulated mast cells in the bladder, compared to naïve-Sed ($n = 4$) counterparts. NMS-Ex mice ($n = 5$) had a significantly lower percentage of degranulated mast cells than NMS-Sed and were not different from naïve-Sed or naïve-Ex ($n = 4$) percentages. Representative Western blots are shown for CRF₁ (C), CRF₂ (D), and corresponding GAPDH with bands at 55, 47, and 35 kD, respectively. Protein levels are expressed as a percentage of naïve-Sed levels. C, A significant impact of exercise was observed on CRF₁ protein levels, particularly in naïve mice. No significant impact of NMS or exercise was observed on bladder levels of CRF₂ (D). Brackets indicate a

significant impact of NMS ($^{\S} P < 0.05$), exercise ($^{\dagger}, ^{\dagger\dagger\dagger} P < 0.05, 0.001$), and an NMS/Ex interaction ($^{\&}, ^{\&\&} P < 0.05, 0.01$), two-way ANOVA. $^{**} P < 0.01$ versus naïve; $^{\#}, ^{\#\#\#} P < 0.05, 0.001$ versus sedentary; Fisher's LSD posttest. $n = 6$ unless otherwise noted

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