Title: Access to running wheels decreases social motivation in adult C57BL/6J female mice

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Abstract

Physical activity offers myriad benefits to health and well-being, in humans and other animals as well. In rodents, voluntary wheel running can attenuate the effects of both physical and social stressors on rodent social behavior. Whether wheel running affects rodent social behaviors per se remains less well understood. We conducted the current study to test whether home cage access to running wheels impacts the social behaviors of adult, group-housed C57BL/6J female mice during same-sex interactions with novel females. Group-housed females were either given continuous home cage running wheel access or a standard paper hut starting at weaning, and as adults, the social behaviors of females from these two groups were measured during interactions with novel female mice. In two cohorts of females, we found that 5 weeks of running wheel access during adolescence reduced the time that female subjects spent engaged in social investigation of a novel female and also tended to reduce total ultrasonic vocalizations produced during interactions. These effects were not reversed by a 2-week period in which running wheels were removed. Finally, we found that a 2-week period of running wheel access in adulthood recapitulates the effects of 5 weeks of running wheel access on female social investigation. Overall, we find that running wheel access has an enduring inhibitory influence on female social behavior during same-sex interactions, a finding that has implications for the design of studies that include same-sex interactions between female mice.

Keywords: wheel running, social motivation, ultrasonic vocalization, female, mouse

Introduction

Physical activity (i.e., exercise) promotes both physical and mental well-being. For example, exercise reduces the risk of chronic diseases, including cardiovascular disease, diabetes, and cancer (1–8). Exercise is also associated with improved cognitive function (9,10), reduced depressive symptoms (4,11–15), and better health-related quality of life (16–18).

Similar to findings in humans, studies in rodents also support the idea that exercise conveys health benefits, including cardiovascular benefits (19,20), increased cognitive ability and hippocampal neurogenesis (21,22), and antidepressant and anxiolytic effects on behavior (19,23–25). The most common form of exercise provided to laboratory rodents is voluntary wheel running, in which rodents are given free access to a running wheel in their home environment (20,26). In fact, both laboratory and wild rodents run spontaneously when given access to running wheels (20,26,27), indicating that rodents find wheel running to be rewarding (28–30). Within the vast literature examining the effects of voluntary wheel running on rodent health and behavior, there is a particularly rich body of work showing that wheel running can ameliorate stress-induced changes in social behavior. For example, wheel running attenuates the effects of chronic social defeat stress on sociability and aggression in male mice (31–33), attenuates the effects on sociability of inescapable tail shock in female and male rats (34,35), reduces the effects of chronic social isolation on sociability and aggression in male mice (36), and reduces the severity of anxiety-like and depressive-like behaviors in female prairie voles subjected to a combination of social isolation and chronic mild stress (37).

Although past work robustly supports the idea that voluntary wheel running can attenuate the effects of stressors on rodent social behavior, much less is known about whether wheel running impacts rodent social behaviors per se. This gap in knowledge stems from the fact that studies employing voluntary wheel running typically include single-housed subjects with free access to a running wheel that electronically tracks rotations, in order to longitudinally track wheel running for a single animal. Social isolation itself is a stressor that can substantially alter mouse social behaviors (38–43), which complicates the use of the standard voluntary wheel running paradigm to investigate the effects of wheel running on social behavior. Thus, whether wheel running affects the social behaviors of unstressed, socially-housed rodents remains understudied.

To provide mice with exercise enrichment, our lab routinely places (non-electronic) running wheels in mouse home cages at the time of weaning, and cages of mice in our colony subsequently have continuous running wheel access throughout adolescence and adulthood.

Given our lab's interest in the behavioral and social contexts that influence affiliative social behaviors of female mice (42–44), we designed the current study to test whether free access to running wheels impacts the social behaviors of adult, group-housed females. Because we provided groups of females with non-electronic running wheels, we use the term "running wheel access" to distinguish our experimental design from the standard voluntary wheel running paradigm. In Experiment 1, same-sex groups of female siblings were either given continuous home cage running wheel access or a standard paper hut starting at weaning (3 weeks of age), and as adults (8 weeks of age), the social behaviors of females from these two groups were measured during interactions with novel female mice. In follow-up experiments, we replicated our findings from Experiment 1 in a second cohort of females (Experiment 2a) and tested whether the effects of 5 weeks of running wheel access on female social behavior could be reversed by a 2-week period in which running wheels were removed (Experiment 2b). Finally, we tested whether a 2-week period of running wheel access in adulthood could recapitulate the effects of 5 weeks of running wheel access throughout adolescence on female adult social behaviors (Experiment 2c). We find that running wheel access overall has an inhibitory influence on female social motivation during same-sex interactions, a finding that has implications for the design of studies that include same-sex interactions between female mice.

Materials and Methods

Further information and requests for resources should be directed to the corresponding author, Katherine Tschida (kat227@cornell.edu).

Ethics Statement: All experiments and procedures were conducted according to protocols approved by the Cornell University Institutional Animal Care and Use Committee (protocol #2020-001).

Subjects: Adult (> 8 weeks old) female C57BL/6J (Jackson Laboratories, 000664) mice were group-housed with same-sex siblings from weaning (postnatal day 21) until the start of experiments. At weaning, females were assigned to either the hut condition (control) or the wheel condition (experimental). Hut condition cages included a paper hut and other standard enrichment (crinkle paper, nestlets, and care fresh bedding) but no running wheel (Fig. 1, left). Wheel condition cages included most standard enrichment (crinkle paper, nestlets, and care fresh bedding) but did not contain a paper hut and instead contained a combined shelter and running wheel (Innodome + Innowheel, from Innovive) (Fig. 1, right). Mice were kept on a 12h:12h reversed light/dark cycle and given ad libitum food and water for the duration of the experiment. Subjects did not have social interactions with novel mice prior to the start of experiments.

Figure 1. Home cage contents for cages in hut vs. wheel conditions. Home cage contents are shown for a cage in the hut condition (left, control) and a cage in the wheel condition (right, experimental). Food and water were provided at libitum but are not shown.

Experiment 1 design (Fig. 2, top)**:** At the time of weaning (postnatal day 21), female siblings from a given litter were placed into a home cage and housed together until adulthood. In Experiment 1, each female sibling group was assigned to either the wheel or the hut condition, and cages were maintained in their respective enrichment conditions until adulthood. All cages assigned to the wheel condition had continuous access to a running wheel, but running wheel usage by individual mice within the cage was not tracked. After 5 weeks in either the wheel or the hut condition (~8 weeks of age), subject females were given a social interaction with a novel, group-housed female mouse. On the day of the social interaction test, the home cage of the subject female was placed into a sound-attenuating recording chamber (Med Associates) and siblings were moved temporarily to a clean cage. A custom lid (with tall sides and no top) was placed on the cage to allow video and audio recordings, and the mouse shelter (either running wheel or paper hut) was removed from the cage. The recording chamber was equipped with an infrared light source (Tendelux) and a webcam (Logitech), with the infrared filter removed to

enable video recording under infrared lighting. A novel, group-housed adult female was then placed in the cage with the subject and allowed to interact for 10 minutes. Novel females were re-used across multiple social interaction tests, but the same novel female was never used in more than one social interaction test per day.

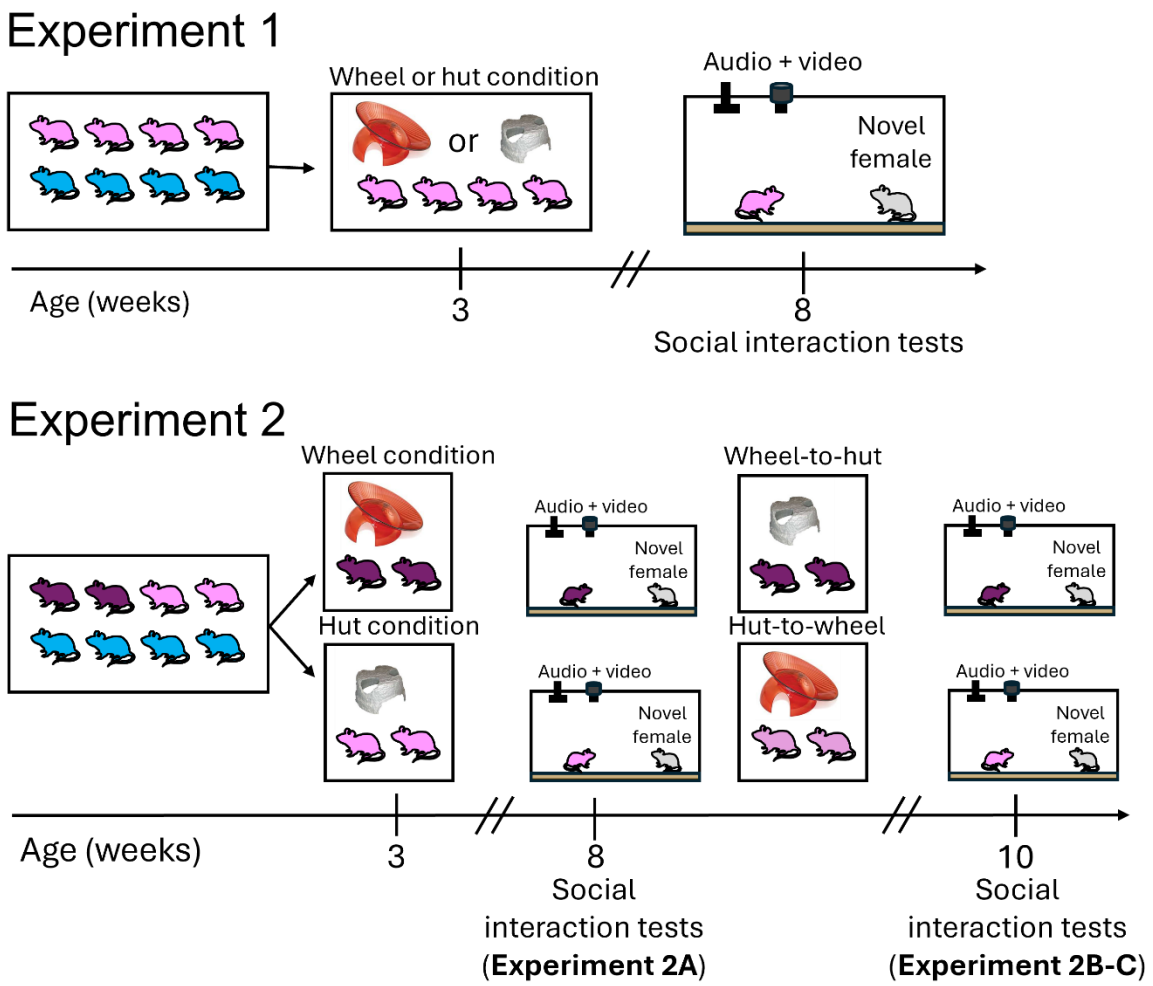


Figure 2. Study design. Schematic of the study design is shown for Experiment 1 (top) and Experiment 2 (bottom). Female subjects are shown in light and/or dark purple, male siblings are shown in blue, and novel females used in social interaction tests are shown in gray.

Experiment 2 design (Fig. 2, bottom)**:** The goals of Experiment 2 were two-fold. First, we aimed to replicate the findings of Experiment 1 and to account for potential contributions of litter effects to the results of Experiment 1. To this end, Experiment 2 employed a design in which females

from a given litter were divided into two groups at weaning, with one group of female siblings placed in a home cage and assigned to the wheel condition, and the remaining female siblings placed in another home cage and assigned to the hut condition. As a result, only litters that included at least 4 female siblings were used in Experiment 2, such that each female cage contained 2-3 siblings. Second, we wanted to test whether removing running wheels from cages of adult females from the wheel condition would reverse the effects of 5 weeks of running wheel access on female social behavior. Conversely, we wanted to evaluate whether a shorter period of running wheel access in adulthood could mimic the effects on female social behavior of 5 weeks of running wheel access from weaning until adulthood. To this end, Experiment 2 females were assigned to the wheel or hut condition at weaning and then given a 10-minute-long social interaction with a novel, group-housed female at 8 weeks of age as described for Experiment 1. Then, each female cage's condition was reversed for 2 weeks (i.e., running wheels were removed from wheel condition cages and replaced with paper huts, and paper huts were removed from hut condition cages and replaced with running wheels), and female subjects were given a second 10-minute-long social interaction test at 10 weeks of age. Social interaction tests at 8 weeks and 10 weeks were conducted as described in Experiment 1, and each subject female was tested with a different (novel) female at the 8-week and 10-week social interaction tests.

Scoring of social investigation from video recordings: Trained observers blinded to experimental group assignments used BORIS v.8.13 to score times in webcam videos during which the subject female engaged in social investigation (i.e., sniffing or following) of the novel female. Times marked by mutual social investigation (mutual sniffing and/or circling) were also included in the total amount of social investigation time for the subject female. We did not observe any instances of mounting or fighting in our dataset.

USV recording and detection: USVs were recorded with an ultrasonic microphone (Avisoft, CMPA/CM16), amplified (Presonus TubePreV2), and digitized at 250 kHz (Spike 7, CED). USVs were detected with custom Matlab codes (Tschida et al., 2019) using the following parameters: mean frequency > 45 KHz; spectral purity > 0.3; spectral discontinuity < 1.0; minimum USV duration = 5 ms; minimum inter-syllable interval = 30 ms). Because we did not use methods to assign USVs to individual signalers (44–48), total USVs reported in social interaction tests represent the total number of USVs produced by each pair of females.

Statistical analyses: The Shapiro-Wilk test was used to evaluate the normality of data distributions, and non-parametric two-sided comparisons were used for non-normally distributed data (alpha = 0.05). No statistical methods were used to pre-determine sample size.

Data availability: All source data generated in this study will be deposited in a digital data repository, and this section will be modified prior to publication to include the persistent DOI for the dataset.

Results

Experiment 1: Running wheel access throughout adolescence decreases social investigation in adult C57BL/6J female mice during interactions with novel females

After 5 weeks of continuous home cage access to either a running wheel or a paper hut, each group-housed adult (8-week-old) female subject was given a 10-minute-long social interaction test with a novel, group-housed female mouse (Fig. 2; see Methods). Given prior literature on the benefits of voluntary wheel running in rodents, we hypothesized that long-term running wheel access would promote social motivation in adult female mice and in turn would enhance rates of social investigation and ultrasonic vocalizations (USVs) during affiliative social interactions with novel females. Contrary to our hypothesis, we found that female subjects from the wheel condition spent significantly less time engaged in social investigation of novel, visitor females compared to female subjects in the hut condition (Fig. 3A, left; Mann Whitney U test: $z = 3.1$, $p = 0.002$). Total USVs also tended to be lower in pairs that included females from the wheel condition, although this difference was not statistically significant (Fig. 3A, right; Mann Whitney U test: $z = 1.9$, $p = 0.06$). In summary, running wheel access throughout adolescence decreased social investigation in adult female mice during interactions with novel female mice and also tended to decrease total USVs produced by the pair.

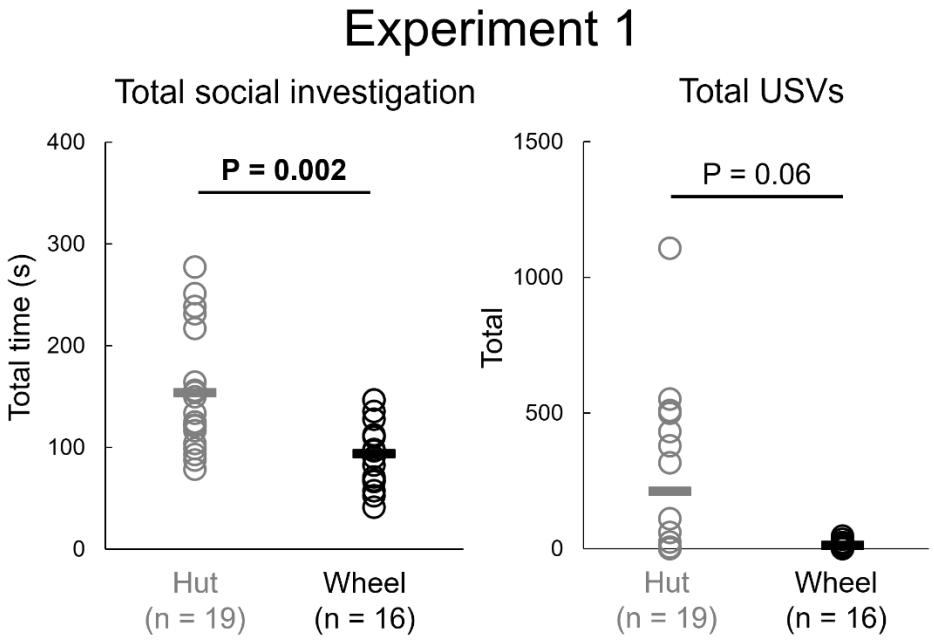


Figure 3. Running wheel access throughout adolescence decreases social investigation in adult female mice during interactions with novel females. (A) Left plot shows total time that female subjects spent engaged in social investigation of a novel female. Right, same as left, for total USVs recorded from female-female pairs. Black and gray lines show mean values.

Experiment 2a: In a second cohort of adult C57BL/6J females, running wheel access throughout adolescence decreases social investigation and total USVs during interactions with novel females.

One caveat to the study design in Experiment 1 is that all female siblings from a given litter were assigned to either the wheel condition or to the hut condition. Thus, differences in social behavior between litters might have contributed to the differences in social behavior between females in the wheel and hut conditions. To better account for any contribution of litter effects, we conducted a second set of experiments with a modified design, in which female siblings from each litter were divided, with a subset (≥ 2 female siblings) assigned to the wheel condition and the remaining female siblings (≥ 2) assigned to the hut condition (Fig. 2).

Similar to the results of Experiment 1, we found that females from the wheel condition spent significantly less time engaged in social investigation of novel females compared to females in the hut condition (Fig. 4, left; Mann Whitney U test: $z = -4.45$, $p < 0.0001$). Total USVs were also significantly lower in pairs that included females from the wheel condition (Fig. 4, right; Mann Whitney U test: $z = -2.34$, $p = 0.02$). Alongside the results of Experiment 1, these findings support the idea that running wheel access throughout adolescence decreases social motivation in adult female mice during same-sex interactions.

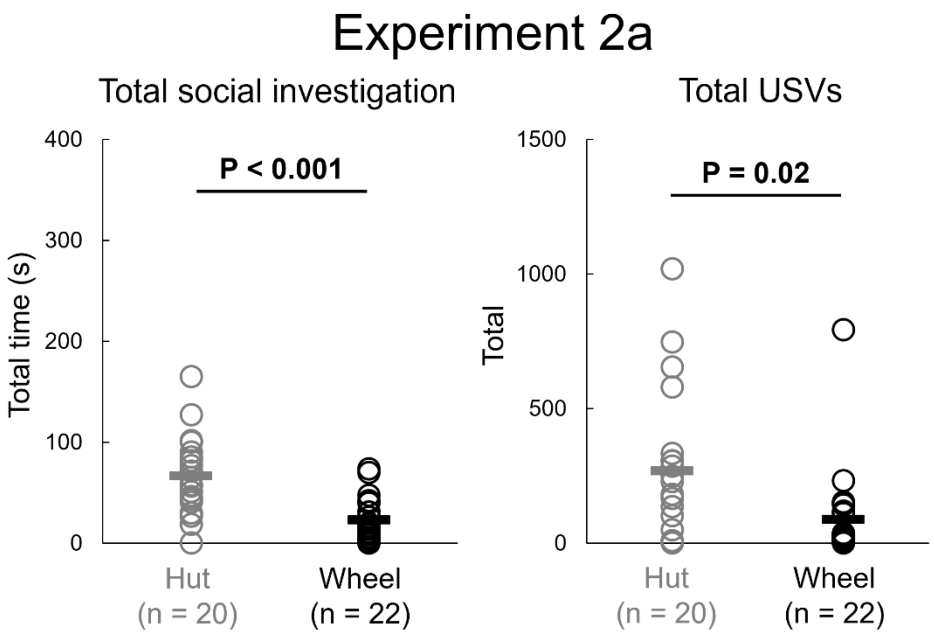


Figure 4. In a second cohort of females, running wheel access throughout adolescence decreases social investigation and total USVs during interactions with novel females. Left plot shows total time that female subjects spent engaged in social investigation of a novel female. Right, same as left, for total USVs recorded from female-female pairs. Black and gray lines show mean values.

Experiment 2b: Can removal of running wheels in adulthood reverse the effects of running wheel access throughout adolescence on adult female social behavior?

Given the robust effects of long-term running wheel access on the social behaviors of adult female mice, we next tested whether these effects could be reversed by removing the running wheel. To this end, after their 8-week social interaction tests, females (n = 22) from Experiment 2a that had been raised with a running wheel since weaning had the running wheels removed from their home cages and replaced with paper huts. After 2 weeks (10-week time point), the wheel-to-hut females were given a second social interaction test with a novel female (Fig. 2, bottom). We found that removal of running wheels for 2 weeks failed to significantly increase either social investigation or total USVs (Fig. 5; Wilcoxon signed-rank tests to compare 8-week vs. 10-week behaviors; $z = -1.18$, $p = 0.24$ for social interaction; $z = -1.34$, $p = 0.18$ for USVs).

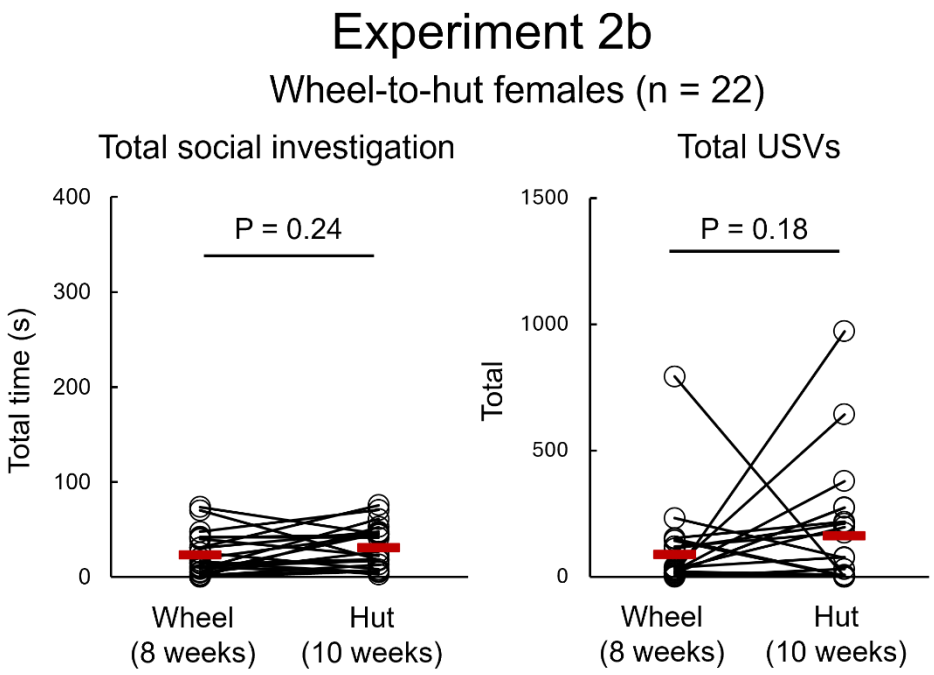


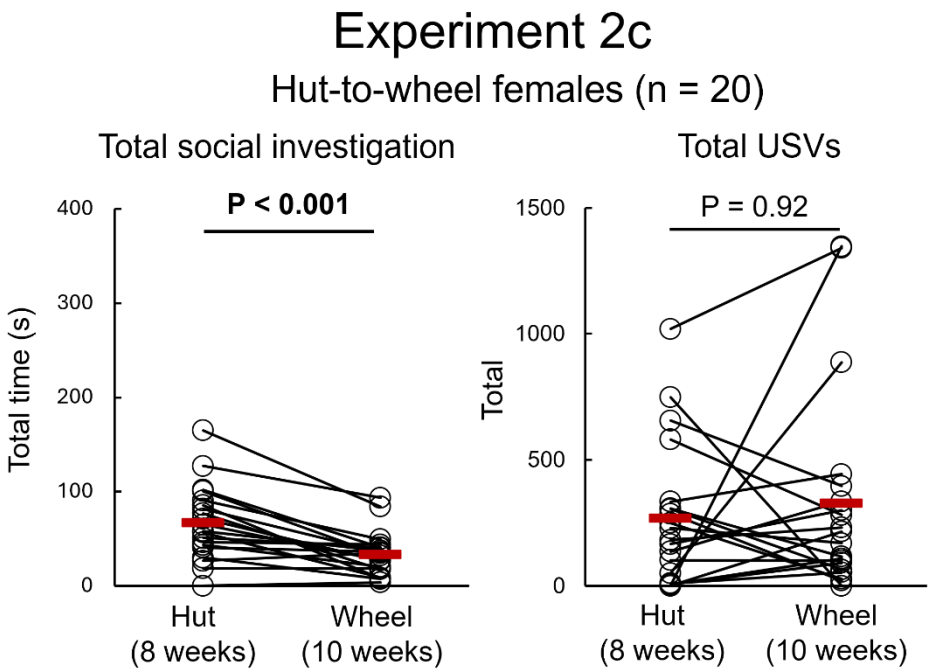
Figure 5. Two weeks without running wheel access does not reverse the effects of running wheel access throughout adolescence on adult female social behavior. Left plot shows total time that female subjects spent engaged in social investigation of a novel female, after 5 weeks of running wheel access (8-week time point) and again after 2 weeks with the running wheel removed and replaced with a paper hut (10-week time point). Right, same as left, for total USVs. Red symbols show mean values.

Experiment 2c: Can 2-weeks of running wheel access in adulthood mimic the effects of running wheel access throughout adolescence on adult female social behavior?

Multiple factors could contribute to the inhibitory effects of running wheel access on adult female social behavior observed in Experiments 1 and 2a. One possibility is that the timing of running wheel access during adolescence is a critical factor. Another possibility is that the total duration (~5 weeks) of running wheel access is important, and that shorter durations of running wheel access, regardless of when they occurred during development, would fail to impact female social behavior. On the other hand, it could be that even a shorter duration of running wheel access in adulthood is enough to attenuate female social behavior during interactions with novel females. To test this last idea, after their 8-week social interaction test, females (n = 20) from the hut condition in Experiment 2a had their paper huts removed and replaced with running wheels. After

238 2 weeks (at 10-weeks of age), the hut-to-wheel females were given a second social interaction
239 test with a novel female (Fig. 2, bottom).

240 Two weeks of running wheel access significantly decreased the total time that females engaged
241 in social investigation of a novel female (Fig. 6, left; Wilcoxon signed-rank tests to compare 8-
242 week vs. 10-week total social investigation; $z = -3.57$, $p < 0.001$). Notably, total social investigation
243 in the hut-to-wheel females (after 2 weeks of running wheel access in adulthood) was not
244 significantly different from total social investigation in the Experiment 2A females that had 5 weeks
245 of running wheel access throughout adolescence (Mann Whitney U test: $z = -1.62$, $p = 0.10$). In
246 contrast to the decrease in social investigation, there was no significant change in total USVs
247 recorded from pairs that included the hut-to-wheel females (Fig. 6, right; Wilcoxon signed-rank
248 tests to compare 8-week vs. 10-week total USVs; $z = -0.1$, $p = 0.92$). We conclude that at least
249 with regard to time spent engaged in social investigation, the effects of longer-term running wheel
250 access during adolescence can be recapitulated by a shorter period of running wheel access in
251 adulthood.



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253 **Figure 6. Two weeks of running wheel access in adulthood decreases social investigation**
254 **but not USVs during interactions same-sex interactions with novel females.** Left plot shows
255 total time that female subjects spent engaged in social investigation with a novel female after 5

weeks of paper hut access (8-week time point) and again after 2 weeks of running wheel access (10-week time point). Right, same as left, for total USVs. Red symbols show mean values.

Discussion

Across two cohorts of female mice, we find evidence that 5 weeks of running wheel access throughout adolescence decreases social motivation in adulthood during interactions with novel females. What mechanisms can account for the effects of running wheel access on female social behavior? The finding that a 2-week period of running wheel access in adulthood mimics the effects of longer-term, adolescent running wheel access suggests that the critical factor is not developmental in nature (Fig. 6). One possibility is that females with access to running wheels are simply more stimulated in their home cage environment (i.e., less bored) and are correspondingly less motivated to interact with novel social partners. On the other hand, the effects of running wheel access are at least somewhat long-lasting, given that removing running wheels for 2 weeks in the wheel-to-hut females did not reverse the effects of 5 weeks of running wheel access on female social behaviors. This finding contradicts somewhat with the idea that running wheel access exerts an inhibitory effect on female social behavior solely via short-term impacts on affective state.

An alternative explanation for why running wheel access decreases female social motivation relates to past work showing that rodents find wheel running to be highly rewarding. For example, rodents will lever press for running wheels (29,49), and wheel running can drive conditioned place preference (28,50). Of particular note are past studies showing that wheel running decreases the motivation for other reinforcers, such as sucrose (51), cocaine (52,53), and amphetamine (54). Based on these findings, one possibility is that wheel running alters sensitivity to other rewards, potentially including social rewards. Future work could test this idea more specifically by examining the effects of running wheel access on the strength of social conditioned place preference in female mice (55,56) or on performance in operant conditioning tasks designed to measure social reward (57,58).

Prior work using pairs of male mice found that social hierarchy influenced wheel running, and that the dominant mouse spent more time running on the wheel than the subordinate (59). Whether social hierarchy influences running wheel behavior in group-housed female mice remains an interesting topic for future study. Although we did not track running wheel usage in the current

study, either overall or by individual, future work could employ methods to track running wheel usage for individual females within a cage, in order to test whether differences in running wheel usage relates to or predicts the magnitude of later effects on social motivation. Future work should also consider whether the effects observed in the current study are specific to wheel running or, alternatively, generalize to other types of environmental enrichment. Finally, whether wheel running affects the social behaviors of male mice, either during same-sex interactions with other males or during courtship interactions with females, remains an important topic for future investigation.

In summary, our results show that home cage running wheel access can exert unanticipated consequences on the social behaviors of female mice. Although there is ample evidence that wheel running offer important welfare benefits for laboratory rodents, our findings nonetheless have important implications for the design of studies that include measures of social behavior in female mice.

Acknowledgements

We thank the CARE staff for their excellent mouse husbandry.

Competing Interests

No competing interests declared.

References

1. Bonen A. Benefits of exercise for type II diabetics: convergence of epidemiologic, physiologic, and molecular evidence. Can J Appl Physiol. 1995 Sept;20(3):261–79.

2. Helmrich SP, Ragland DR, Paffenbarger RS. Prevention of non-insulin-dependent diabetes mellitus with physical activity. Med Sci Sports Exerc. 1994 July;26(7):824–30.

3. O'Connor GT, Hennekens CH, Willett WC, Goldhaber SZ, Paffenbarger RS, Breslow JL, et al. Physical exercise and reduced risk of nonfatal myocardial infarction. Am J Epidemiol. 1995 Dec 1;142(11):1147–56.

4. Penedo FJ, Dahn JR. Exercise and well-being: a review of mental and physical health benefits associated with physical activity. Curr Opin Psychiatry. 2005 Mar;18(2):189–93.

5. Schnohr P, Scharling H, Jensen JS. Changes in leisure-time physical activity and risk of death: an observational study of 7,000 men and women. Am J Epidemiol. 2003 Oct 1;158(7):639–44.

6. Shephard RJ, Shek PN. Cancer, immune function, and physical activity. Can J Appl Physiol. 1995 Mar;20(1):1–25.

7. Tran ZV, Weltman A, Glass GV, Mood DP. The effects of exercise on blood lipids and lipoproteins: a meta-analysis of studies. Med Sci Sports Exerc. 1983;15(5):393–402.

8. Whelton SP, Chin A, Xin X, He J. Effect of Aerobic Exercise on Blood Pressure. Ann Intern Med. 2002 Apr 2;136(7):493–503.

9. Colcombe S, Kramer AF. Fitness effects on the cognitive function of older adults: a meta-analytic study. Psychol Sci. 2003 Mar;14(2):125–30.

10. Erickson KI, Hillman CH, Kramer AF. Physical activity, brain, and cognition. Curr Opin Behav Sci. 2015 Aug 1;4:27–32.

11. Babyak M, Blumenthal JA, Herman S, Khatri P, Doraiswamy M, Moore K, et al. Exercise treatment for major depression: maintenance of therapeutic benefit at 10 months. Psychosom Med. 2000;62(5):633–8.

12. Camacho TC, Roberts RE, Lazarus NB, Kaplan GA, Cohen RD. Physical activity and depression: evidence from the Alameda County Study. Am J Epidemiol. 1991 July 15;134(2):220–31.

13. Farmer ME, Locke BZ, Mościcki EK, Dannenberg AL, Larson DB, Radloff LS. Physical activity and depressive symptoms: the NHANES I Epidemiologic Follow-up Study. Am J Epidemiol. 1988 Dec;128(6):1340–51.

14. Galper DI, Trivedi MH, Barlow CE, Dunn AL, Kampert JB. Inverse association between physical inactivity and mental health in men and women. Med Sci Sports Exerc. 2006 Jan;38(1):173–8.

340 15. Lampinen P, Heikkinen RL, Ruoppila I. Changes in intensity of physical exercise as
341 predictors of depressive symptoms among older adults: an eight-year follow-up. Prev Med.
342 2000 May;30(5):371–80.

343 16. Anokye NK, Trueman P, Green C, Pavey TG, Taylor RS. Physical activity and health related
344 quality of life. BMC Public Health. 2012 Aug 7;12:624.

345 17. Bize R, Johnson JA, Plotnikoff RC. Physical activity level and health-related quality of life in
346 the general adult population: A systematic review. Prev Med. 2007 Dec 1;45(6):401–15.

347 18. Rejeski WJ, Brawley LR, Shumaker SA. Physical activity and health-related quality of life.
348 Exerc Sport Sci Rev. 1996;24:71–108.

349 19. Bilu C, Einat H, Zimmet P, Vishnevskia-Dai V, Schwartz WJ, Kronfeld-Schor N. Beneficial
350 effects of voluntary wheel running on activity rhythms, metabolic state, and affect in a
351 diurnal model of circadian disruption. Sci Rep. 2022 Feb 14;12(1):2434.

352 20. Manzanares G, Brito-da-Silva G, Gandra PG. Voluntary wheel running: patterns and
353 physiological effects in mice. Braz J Med Biol Res. 2018 Dec 10;52(1):e7830.

354 21. Huang YQ, Wu C, He XF, Wu D, He X, Liang FY, et al. Effects of Voluntary Wheel-Running
355 Types on Hippocampal Neurogenesis and Spatial Cognition in Middle-Aged Mice. Front Cell
356 Neurosci. 2018 June 26;12:177.

357 22. van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and
358 neurogenesis in the adult mouse dentate gyrus. Nat Neurosci. 1999 Mar;2(3):266–70.

359 23. Cunha MP, Oliveira Á, Pazini FL, Machado DG, Bettio LEB, Budni J, et al. The
360 antidepressant-like effect of physical activity on a voluntary running wheel. Med Sci Sports
361 Exerc. 2013 May;45(5):851–9.

362 24. Duman CH, Schlesinger L, Russell DS, Duman RS. Voluntary Exercise Produces
363 Antidepressant and Anxiolytic Behavioral Effects in Mice. Brain Res. 2008 Mar
364 14;1199:148–58.

365 25. Pagliusi M, Bonet IJM, Brandão AF, Magalhães SF, Tambeli CH, Parada CA, et al.
366 Therapeutic and Preventive Effect of Voluntary Running Wheel Exercise on Social Defeat
367 Stress (SDS)-induced Depressive-like Behavior and Chronic Pain in Mice. Neuroscience.
368 2020 Jan 21;428:165–77.

369 26. Greenwood BN, Fleshner M. Voluntary wheel running: a useful rodent model for
370 investigating mechanisms of stress robustness and exercise motivation. Curr Opin Beh Sci.
371 2019 Aug 1;28:78–84.

372 27. Meijer JH, Robbers Y. Wheel running in the wild. Proc Biol Sci. 2014 July
373 7;281(1786):20140210.

374 28. Greenwood BN, Foley TE, Le TV, Strong PV, Loughridge AB, Day HEW, et al. Long term
375 voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward
376 pathway. Behav Brain Res. 2011 Mar 1;217(2):354–62.

29. Kagan J, Berkun M. The reward value of running activity. J Comp Physiol Psychol. 1954 Apr;47(2):108.

30. Novak CM, Burghardt PR, Levine JA. The use of a running wheel to measure activity in rodents: relationship to energy balance, general activity, and reward. Neurosci Biobehav Rev. 2012 Mar;36(3):1001–14.

31. Calpe-López C, Martínez-Caballero MÁ, García-Pardo MP, Aguilar MA. Resilience to the short- and long-term behavioral effects of intermittent repeated social defeat in adolescent male mice. Pharmacol Biochem Behav. 2023 June 1;227–228:173574.

32. Mul JD, Soto M, Cahill ME, Ryan RE, Takahashi H, So K, et al. Voluntary wheel running promotes resilience to chronic social defeat stress in mice: a role for nucleus accumbens ΔFosB. Neuropsychopharmacology. 2018 Aug;43(9):1934–42.

33. Zhang J, He ZX, Wang LM, Yuan W, Li LF, Hou WJ, et al. Voluntary Wheel Running Reverses Deficits in Social Behavior Induced by Chronic Social Defeat Stress in Mice: Involvement of the Dopamine System. Front Neurosci. 2019;13:256.

34. Tanner MK, Hohorst AA, Mellert SM, Loetz EC, Baratta MV, Greenwood BN. Female rats are more responsive than are males to the protective effects of voluntary physical activity against the behavioral consequences of inescapable stress. Stress. 2023 Nov;26(1):2245492.

35. Tanner MK, Fallon IP, Baratta MV, Greenwood BN. Voluntary exercise enables stress resistance in females. Behav Brain Res. 2019 Sept 2;369:111923.

36. Ren L, Tai F. Voluntary wheel running ameliorates abnormalities in social behavior induced by social isolation: Involvement of neural and neurochemical responses. Neurosci Lett. 2023 May 29;806:137241.

37. Watanasriyakul WT, Wardwell J, McNeal N, Schultz R, Woodbury M, Dagner A, et al. Voluntary physical exercise protects against behavioral and endocrine reactivity to social and environmental stressors in the prairie vole. Soc Neurosci. 2018 Oct;13(5):602–15.

38. Valzelli L. The “isolation syndrome” in mice. Psychopharmacologia. 1973 Aug 3;31(4):305–20.

39. Weiss IC, Pryce CR, Jongen-Rêlo AL, Nanz-Bahr NI, Feldon J. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav Brain Res. 2004 July 9;152(2):279–95.

40. Wiberg GS, Grice HC. Long-term isolation stress in rats. Science. 1963 Oct 25;142(3591):507.

41. Zelikowsky M, Hui M, Karigo T, Choe A, Yang B, Blanco MR, et al. The Neuropeptide Tac2 Controls a Distributed Brain State Induced by Chronic Social Isolation Stress. Cell. 2018 May 17;173(5):1265-1279.e19.

42. Zhao X, Chae Y, Smith D, Chen V, DeFelipe D, Sokol JW, et al. Short-term social isolation acts on hypothalamic neurons to promote social behavior in a sex- and context-dependent manner. eLife. 2025 Mar 4;13:RP94924.

43. Zhao X, Ziobro P, Pranic NM, Chu S, Rabinovich S, Chan W, et al. Sex- and context-dependent effects of acute isolation on vocal and non-vocal social behaviors in mice. PLoS One. 2021;16(9):e0255640.

44. Malone CA, Ziobro P, Khinno J, Tschida KA. Rates of female mouse ultrasonic vocalizations are low and are not modulated by estrous state during interactions with muted males Sci Rep. 2025 Feb 26;15(1):6841.

45. Oliveira-Stahl G, Farboud S, Sterling ML, Heckman JJ, van Raalte B, Lenferink D, et al. High-precision spatial analysis of mouse courtship vocalization behavior reveals sex and strain differences. Sci Rep. 2023 Mar 30;13(1):5219.

46. Sangiamo DT, Warren MR, Neunuebel JP. Ultrasonic signals associated with different types of social behavior of mice. Nat Neurosci. 2020 Mar;23(3):411–22.

47. Sterling ML, Teunisse R, Englitz B. Rodent ultrasonic vocal interaction resolved with millimeter precision using hybrid beamforming. Bathellier B, King AJ, Bathellier B, Shea SD, Ey E, editors. eLife. 2023 July 26;12:e86126.

48. Waidmann EN, Yang VHY, Luo E, Doyle WC, Jarvis ED. Mountable miniature microphones to identify and assign mouse ultrasonic vocalizations. Cell Reports Methods [Internet]. 2025 June 16 [cited 2025 Sept 18];5(6). Available from: https://www.cell.com/cell-reports-methods/abstract/S2667-2375(25)00117-1

49. Iversen IH. Techniques for establishing schedules with wheel running as reinforcement in rats. J Exp Anal Behav. 1993 July;60(1):219–38.

50. Lett BT, Grant VL, Koh MT. Naloxone attenuates the conditioned place preference induced by wheel running in rats. Physiol Behav. 2001 Feb;72(3):355–8.

51. Satvat E, Eikelboom R. Dissociation of conditioned and unconditioned factors in the running-induced feeding suppression. Physiol Behav. 2006 Oct 30;89(3):428–37.

52. Cosgrove KP, Hunter RG, Carroll ME. Wheel-running attenuates intravenous cocaine self-administration in rats: sex differences. Pharmacol Biochem Behav. 2002 Oct;73(3):663–71.

53. Smith MA, Schmidt KT, Iordanou JC, Mustroph ML. Aerobic Exercise Decreases the Positive-Reinforcing Effects of Cocaine. Drug Alcohol Depend. 2008 Nov 1;98(1–2):129–35.

54. Kanarek RB, Marks-Kaufman R, D'Anci KE, Przypek J. Exercise attenuates oral intake of amphetamine in rats. Pharmacol Biochem Behav. 1995 Aug;51(4):725–9.

55. Granza AE, Amaral IM, Monteiro DG, Salti A, Hofer A, El Rawas R. Social Interaction Is Less Rewarding in Adult Female than in Male Mice. Brain Sci. 2023 Oct 10;13(10):1445.

56. Harda Z, Chrószcz M, Misiołek K, Klimczak M, Szumiec Ł, Kaczmarczyk-Jarosz M, et al. Establishment of a social conditioned place preference paradigm for the study of social reward in female mice. Sci Rep. 2022 July 4;12(1):11271.

57. Hu RK, Zuo Y, Ly T, Wang J, Meera P, Wu YE, et al. An amygdala-to-hypothalamus circuit for social reward. Nat Neurosci. 2021 June;24(6):831–42.

58. Ramsey LA, Holloman FM, Hope BT, Shaham Y, Venniro M. Waving through the window: a model of volitional social interaction in female mice. Biol Psychiatry. 2022 June 1;91(11):988–97.

59. Vargas-Pérez H, Sellings L, Grieder T, Díaz JL. Social dominance rank influences wheel running behavior in mice. Neurosci Lett. 2009 July 3;457(3):137–40.