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ORIGINAL RESEARCH

Selective Breeding for Increased Home Cage Physical Activity in Collaborative Cross and Hsd:ICR Mice

Jonathan A. Zombeck · Erin K. DeYoung · Weronika J. Brzezinska · Justin S. Rhodes

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Abstract Selective breeding experiments for increased wheel running and open field behavior have identified genetic and neurobiological factors associated with increased voluntary physical activity in mice, but no previous study has directly selected for increased distance traveled in the home cage. Therefore, within-family selection was applied to increase home cage activity as measured by continuous video tracking using two different starting populations, G2:F1 Collaborative Cross (CC) and Hsd:ICR mice. Genetic correlations with distance traveled on running wheels and in the open field were evaluated by mid-parent offspring regression. A significant response to selection was observed in CC but not Hsd:ICR. Wheel running was heritable in both populations but not significantly genetically correlated with home cage activity. Open field was not heritable in either population. We conclude that different genes and neural circuits influence physical activity in the home cage as compared to wheel running or open field. Selective breeding for home cage activity in CC mice warrants further exploration.

Keywords Exercise · Wheel running · Home cage activity · Video tracking · Hsd:ICR · Collaborative cross · Artificial selection · Selective breeding · Repeatability · Heritability · Genetic correlation · Obesity · ADHD · Aging

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J. A. Zombeck · E. K. DeYoung · W. J. Brzezinska · J. S. Rhodes (✉)
Department of Psychology, The Beckman Institute, University of Illinois at Urbana-Champaign, 405 N Mathews Avenue, Urbana, IL 61801, USA
e-mail: jrhodes@illinois.edu

Introduction

Selective breeding is a powerful method for producing animal models of genetic disorders and/or as a tool in experimental evolution (Rhodes and Kawecki 2009; Swallow et al. 2009). The purpose of a selective breeding experiment is to produce extreme values for a specific phenotype of interest in a population of organisms over many generations so that changes in genes and biology underlying the shift in the phenotype can be discovered. The advantage of selective breeding as compared to selecting from preexisting natural genetic variation without selection, for example using a reference population such as BXD (Chesler et al. 2008) or a panel of inbred strains (Rhodes et al. 2007), is that selective breeding can produce extreme phenotypic values that exceed the range in the starting population (Garland 2003).

In our laboratory, we are interested in discovering genetic and neurobiological factors that predispose high levels of physical activity. Genetic predisposition for low levels of physical activity in humans is associated with obesity (Choh et al. 2009) and high levels with attention deficit hyperactivity disorder (ADHD) (Sharp et al. 2009). Therefore, understanding how genes influence development and function of neurobiological circuits that increase levels of physical activity could provide potentially useful information for novel obesity treatments (Nehrenberg et al. 2009; Owen et al. 2010), and/or to understand etiology of hyperactivity features of ADHD (Rhodes et al. 2005). Moreover, understanding the neurobiology of increased voluntary physical activity could be useful for developing novel aging therapeutics (Bronikowski et al. 2006) because maintenance of physical activity during normal aging is critical for maintaining physical and mental health (Colcombe and Kramer 2003; Kramer et al. 2006).

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Selective breeding experiments for increased physical activity have been conducted for voluntary wheel running (Swallow et al. 1998) and open field behavior (DeFries et al. 1978) in mice and forced treadmill running to exhaustion in rats (Koch and Britton 2001). Results suggest that each of these traits involve different suites of genes and neural circuits. For example, the mice bred for high levels of voluntary wheel running did not display increased distance in the open field relative to unselected control mice (Bronikowski et al. 2001). The lack of genetic correlation implies that different neural circuits in the brain influence the two traits although the details of the specificity have not been worked out. Both open field behavior and wheel running appear to involve anxiety (e.g., amygdala, corticotropin releasing hormone) and reward circuits in the brain (e.g., nucleus accumbens, dopamine) (Werka et al. 1978; Saigusa et al. 1999; Werme et al. 2002; Rhodes et al. 2003; Malisch et al. 2009). Treadmill running to exhaustion (Koch and Britton 2001) is different from both open field (DeFries et al. 1978) and wheel running (Swallow et al. 1998) because it is forced. Whole animal exercise physiological traits (e.g., mitochondria in the muscles, VO2max) rather than anxiety or reward circuits in the brain probably make dominant contributions when the phenotype is maximum forced capacity (Henderson et al. 2002). Taken together, results from the wheel running, open field, and treadmill running selection experiments illustrate the genetic complexity of physical activity and underscore the need to analyze different models for a comprehensive understanding of the genetics and neurobiology predisposing increased physical activity.

To the best of our knowledge, no study has performed a selective breeding experiment on one of the most basic physical activity traits that can be measured in laboratory animals, the daily distance they travel in their home cages without running wheels. New video tracking technology now makes this feasible (Zombeck et al. 2010). Here, we report results of selective breeding for increased home cage activity starting from two separate founding populations, Hsd:ICR and Collaborative Cross G2:F1 (CC) mice (Chesler et al. 2008).

Hsd:ICR was chosen because this strain has successfully been used as the founding population for a long term selective breeding experiment for increased voluntary wheel running behavior (Swallow et al. 1998). Hsd:ICR is primarily *Mus domesticus* (Swallow et al. 1998; Aldinger et al. 2009). Harlan Sprague–Dawley (Indianapolis, IN) maintains Hsd:ICR in a large population, greater than 200 breeder pairs. Individual genetic variation in Hsd:ICR is comparable to within a wild population of *Mus domesticus* (Aldinger et al. 2009).

The Collaborative Cross mice were used because they display large genetic diversity among individuals which is useful as leverage for selective breeding. The strains were derived from a systematic intercross of 8 inbred strains (A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/H1LtJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ) chosen specifically to increase genetic diversity. For example, 3 different subspecies of *Mus* are represented in this panel including *musculus, domesticus and castaneous*. Three of the strains are wild-derived (CAST/EiJ, PWK/PhJ, and WSB/EiJ) while the remaining 5 are standard inbred strains from different genealogies or historic origins of laboratory mouse strains (Beck et al. 2000). It was estimated that approximately 90% of the known allelic variation in *Mus* is represented in the 8 strains (Roberts et al. 2007). The breeding arrangement or funnel used to generate the founding members for our study was as shown in Fig. 2 in Chesler et al. (2008). Each individual mouse was a representative of the G2:F1 generation (as shown in Fig. 2 in Chesler et al. 2008) and contained a unique mix of DNA from all 8 strains. Moreover, each mouse was generated by a different breeding combination of the 40,000 possible combinations (see Chesler et al. 2008). To the best of our knowledge, this is the first selective breeding experiment to use Collaborative Cross mice as the founding population.

The purpose of this study was threefold, first to determine whether the home cage activity trait as measured by continuous video tracking would respond to selection. Second, to determine which of the two starting populations, Hsd:ICR or CC would display a stronger response to selection, and third to determine the extent to which home cage activity was genetically related to wheel running and open field behavior, traits that were the target of previous successful selective breeding experiments (DeFries et al. 1978; Swallow et al. 1998).

Methods

Animals

A total of 2,143 animals were used in this study. These animals originated from 3 separate lines maintained in our animal facility for 4 or 5 generations, depending on whether the founding population was Hsd:ICR (Experiment 1) or Collaborative Cross (CC) G2:F1 mice (Experiment 2) (see Chesler et al. 2008). Selective breeding was applied in two of the lines, Hsd:ICR and CC high-active. In Experiment 2, an unselected CC control line was also maintained. The CC high-active and CC control lines are still being maintained and are part of an ongoing project (Generation 5 offspring are currently being born). The Hsd:ICR line was terminated at generation 4. See below for more detailed description by experiment.

Husbandry

At weaning (21 days old) animals received an ear tag (National Band and Tag Co., Newport, KY) for individual identification. Mice were housed in groups of 4 by sex except during phenotyping (see below). Rooms were kept controlled for temperature (21 ± 1°C) and photo-period (12:12 L:D; lights on at 10:00 PM and off at 10:00 AM). Food (Harlan Teklad, 7012) and water were provided ad libitum at all times (except for 5 min tests in the open field for some animals as described below). The Beckman Institute Animal Facility is AAALAC approved. All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee and adhered to NIH guidelines.

Experiment 1: Hsd:ICR

In experiment 1, 16 male and 16 female Hsd:ICR (or CD-1) mice were purchased from Harlan Sprague–Dawley and used as the founding population for a selected line. They arrived in our animal facility at 5 weeks of age (hereafter referred to as generation −1). They were initially housed in groups of 4 by sex for 1 week. Following the 1 week acclimation, they were phenotyped for home cage activity, wheel running and open field activity as described below. Following phenotyping, males and females were paired based on their within-sex rank in home cage activity (the highest with the highest, down to the lowest with the lowest). This was done to increase statistical power for the mid-parent offspring regression. The range on the x-axis limits power in regression and the largest possible range in mid-parent values is produced by breeding this way.

The offspring (Generation 0) from these original 16 pairs of mice were phenotyped for home cage activity, wheel running, and open field (see below). Generation 0–4 consisted of 1 litter per pair. Litter sizes are approximately 8–15 pups for the Hsd:ICR strain.

The top 14 males and top 14 females from Generation 0 for home cage activity (see selection criterion below) were selected as breeders to continue the line with the restriction that 14 different families had to be represented for both males and females. Within family selection for increased home cage activity was applied each generation to produce Generations 1–4 (see selection criterion below). Each generation, the selected males and females were randomly paired with the only restriction that sibling mating was not allowed.

Experiment 2: collaborative cross

In experiment 2, we purchased 19 male and 19 female G2:F1 Collaborative Cross mice from Oak Ridge National Laboratory (mice were as described for G2:F1 in Chesler et al. 2008). These 38 mice were designated as generation −1 for the current experiment. They arrived at the University of Illinois quarantine facility at a mean age of 78 days (±13 days SD) where they were quarantined for 7 weeks. Following quarantine, they were transferred to our Beckman Animal Facility where they acclimated for an additional week before phenotyping for home cage activity, wheel running and open field, as described below. Following phenotyping, males and females were paired based on their within-sex rank in home cage phenotype, highest with highest down to lowest with lowest, to maximize statistical power in the mid-parent offspring regression.

The offspring (Generation 0) from these original 19 pairs of mice were phenotyped for home cage activity, wheel running, and open field (see below). Generation 0–5 consisted of 2 litters per pair. Litter sizes are approximately 4–8 pups for the Collaborative Cross strain.

After phenotyping generation 0, two separate reproductively isolated lines were founded, that will hereafter be referred to as CC high-active and CC control. CC control was founded from a randomly selected male and female from each of 14 randomly selected families in Generation 0. CC high-active was founded from the remaining top 14 males and top 14 females in Generation 0 with the restriction that 14 different families had to be represented for both males and females.

In the CC high-active line, within family selection was applied each subsequent generation to produce Generations 1–5 (see selection criterion below). After selection, animals were randomly paired with the only restriction that sibling mating was not allowed. In the CC control line, mice were randomly selected within families and randomly paired (avoiding breeding siblings) to continue the lines.

Phenotyping

In generations −1 and 0, mice were phenotyped for wheel running, home cage activity and open field. In the remaining generations, mice were phenotyped only for home cage activity.

Generation −1 and 0: Mice were individually housed either in cages with running wheels or in custom-made home cages for video tracking for 6 days. The following week, animals were switched, i.e., those on running wheels went to home cages for video tracking, and those in home cages went on running wheels. The third week, animals were tested for open field on two consecutive days during the dark cycle.

Home cage activity

When mice were approximately 60 (± 7) days old, they were placed individually in custom-made acrylic home

cages (18.5 cm × 33.5 cm × 16 cm) with clear plastic lids conducive for video tracking from above for 6 days (Fig. 1). Food and water were delivered from the side so the mouse was visible in all areas of the cage when viewed from above. A mesh divider (mesh size, 3 openings per 2.5 cm) separated adjacent cages so that animals could interact with one another without interfering with video tracking. Two different types of bedding were used depending on whether the mouse had a white or a dark coat color. Corncob bedding (Harlan 7097) was used for dark mice whereas Sheppard Paperchip® was used for white mice (see Fig. 1).

Eight video cameras mounted on the ceiling of the animal room provided continuous video feed into two separate computers running TopScan video tracking software. The video coverage allows continuous monitoring of 64 animals at a time in 64 individual cages (as described above). White lights were placed under the tables holding the cages to produce diffuse light during lights on and were controlled with a timer for 12:12 L:D cycle. Red lights were placed in various positions in the room overhead to illuminate the cages during the dark phase for continuous video tracking (mice cannot see red light). Distance traveled defined by movement of the center of mass of the animals (1 mm resolution), was always recorded on days 5 and 6 of the 6 day test using Topscan video tracking software (Clever Sys Inc, Reston, VA, USA). Typically, we start video tracking after 2–4 days of acclimation because we are interested in measuring habituated activity levels but data from days 2–4 were also collected periodically during the experiments.

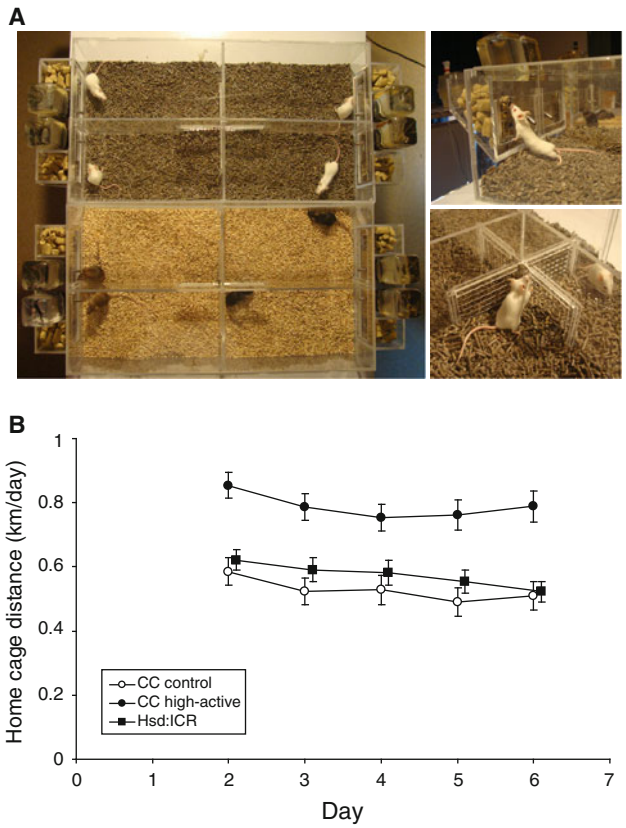

Fig. 1 Home cage activity as measured by continuous video tracking. **a** Left picture shows an overhead view of the custom-made home cages used for video tracking. Note that different bedding is used for tracking white mice as compared to dark mice. Food and water were available from the sides of the cages as shown in the top right photograph. The bottom right photograph shows the mesh divider at the center of the cage allowing the animals to interact in adjacent cages. **b** Mean distance (km/day) ± SE traveled in the home cage across days 2–6 in each of the three separate lines in generation 3

Wheel running

In generation −1 and 0, mice were placed on running wheels for 6 days. Dimensions of running wheel cages were 36 × 20 × 14 cm (L × W × H) with a 23 cm diameter wheel mounted in the cage top (Respironics, Bend, OR). A small amount of Corncob bedding (Harlan 7097) was placed in the cage and water was available ad libitum. Wheel rotations were monitored continuously in 1 min increments via magnetic switches interfaced to a computer running VitalView software (Respironics, Bend, OR). Running distance is calculated as number of rotations multiplied by the circumference of the wheel.

Open field

The open field was constructed of clear Plexiglas, dimensions 67 cm wide ×67 cm long ×31 cm high with white light overhead illumination. In generation −1 and 0, starting at the onset of the dark cycle, mice were placed in the open field for 5 min. This was repeated the following day. The order in which the animals were tested was preserved between days. Distance traveled (mm) was measured continuously by overhead video tracking.

Selection criterion

The selection criterion applies only to the Hsd:ICR line and the CC high-active line described above. Breeders were chosen to populate the subsequent generations based on their home cage activity phenotype (see Home cage activity above). Animals were ranked within families for distance traveled on days 5 and 6 of the 6 day test. The top male and the top female from within each of the 14 families representing the line were chosen as breeders to found the subsequent generation. If fewer than 14 pairs produced offspring, then the second top male and female were chosen within families to produce 14 pairs (a minimum of 10 families were always represented). This selection resulted

in 14 males and 14 females that were randomly paired with the only exception of avoiding sibling mating.

Statistical analysis

Data were analyzed using SAS, or R statistical software. In all analyses, $P \leq 0.05$ was considered statistically significant. Repeatability was estimated as the Pearson's correlation, r, from a simple linear regression of 2 repeated measurements (e.g., distance traveled on day 5 vs. day 6). Phenotypic correlations were estimated as Pearson's r, from simple linear regression of 2 different measurements taken in the same animals (e.g., wheel running distance versus home cage activity).

Narrow-sense heritability, h^2_{narrow}, was estimated as the slope from the simple linear regression of offspring mean phenotypic value against mid-parent value (average value of parents). A weighted regression was also performed where offspring mean values were weighted by the following formula from Falconer and Mackay (1996):

$$W_i = (n_i + n_i T)/(1 + n_i T)$$

where the subscript i refers to the index for a family, and n is the number of individual offspring in the family used to calculate the offspring mean in the regression. T is defined as follows:

$$T = \left[t - 0.5\left(b^2\right)\right]/(1 - t)$$

where t refers to the intra-class correlation (the among-family component of variance divided by the among-family plus the within-family component of variance), and b is the slope of the unweighted mid-parent offspring regression.

Realized heritability, h^2_{realized} was estimated as the response to selection (difference in average trait value between generations) divided by the selection differential (difference in average trait value between selected breeders and population average).

Genetic correlations were estimated using the following formula from Falconer and Mackay (1996):

$$r = (\text{cov}XY + \text{cov}YX)/2\sqrt{\text{cov}XX\text{cov}YY}$$

where covXY is the covariance of mid-parent value for trait X and offspring mean value for trait Y, covYX is the covariance of mid-parent value for trait Y and offspring mean value for trait X, covXX is the covariance of mid-parent value for trait X and offspring mean value for trait X and covYY is the covariance of mid-parent value for trait Y and offspring mean value for trait Y. As recommended by Lynch and Walsh (1998), to evaluate significance of mid-parent offspring estimates of genetic correlations, we generated two P-values, one from offspring Y against mid-parent X and another from offspring X against mid-parent Y.

Results

Home cage activity, as measured by average distance traveled on days 5 and 6, across all animals ($n = 2{,}143$) ranged from 0.09 km/day up to 4.87 km/day. The average across all animals was 0.57 km/day (±0.446 SD). Average level of wheel running ($n = 443$) was 7.25 km/day (±3.444 SD). Average distance traveled in the 5-min open field test ($n = 448$) was 38.1 m (±9.56 SD). For home cage activity, across all populations, we observed a significant sex difference with males moving 77% as far as females ($F_{1,2141} = 42.7$, $P < 0.0001$). For wheel running, males moved 69% as far as females ($F_{1,441} = 82.1$, $P < 0.0001$). For open field, no differences between sexes were observed.

Distance traveled per day in the home cage decreased the first few days, then remained relatively stable for days 4–6 in all 3 lines examined, Hsd:ICR, CC control, and CC high-active (Fig. 1). For the repeated analysis of daily distances traveled, we show results from generation 3, because that generation had the most complete data for days 2–6 in all 3 lines. The pattern was similar for other generations. Referring to the data shown in Fig. 1, the effect of day was significant ($F_{4,1694} = 13.9$, $P < 0.0001$). Line was significant ($F_{2,1694} = 10.9$, $P < 0.0001$), with CC high-active moving more than Hsd:ICR and CC control (both $P < 0.0001$). The interaction between line and day was not significant.

Experiment 1: Hsd:ICR

Circadian rhythm

A qualitative assessment of Fig. 2a shows that the Hsd:ICR mice living in the custom-made home cages with diffuse lighting (see phenotyping home cage activity above) display a normal circadian rhythm that was clear from the continuous overhead video tracking record.

Repeatability

Home cage activity was significantly repeatable in the Hsd:ICR population (Fig. 2b). Considering all the animals phenotyped in generations −1 through 4 (with data for both day 5 and 6, a total $n = 794$) the Pearson's r for the correlation between distance traveled on days 5 and 6 was estimated to be 0.92 ($P < 0.0001$). Note that in the Hsd:ICR population, the highest values for home cage activity were displayed in females (Fig. 2b).

Heritability

Home cage activity in the Hsd:ICR population was not heritable (Fig. 2c). Considering all the animals phenotyped in generations −1 through 4 (a total of 62 families,

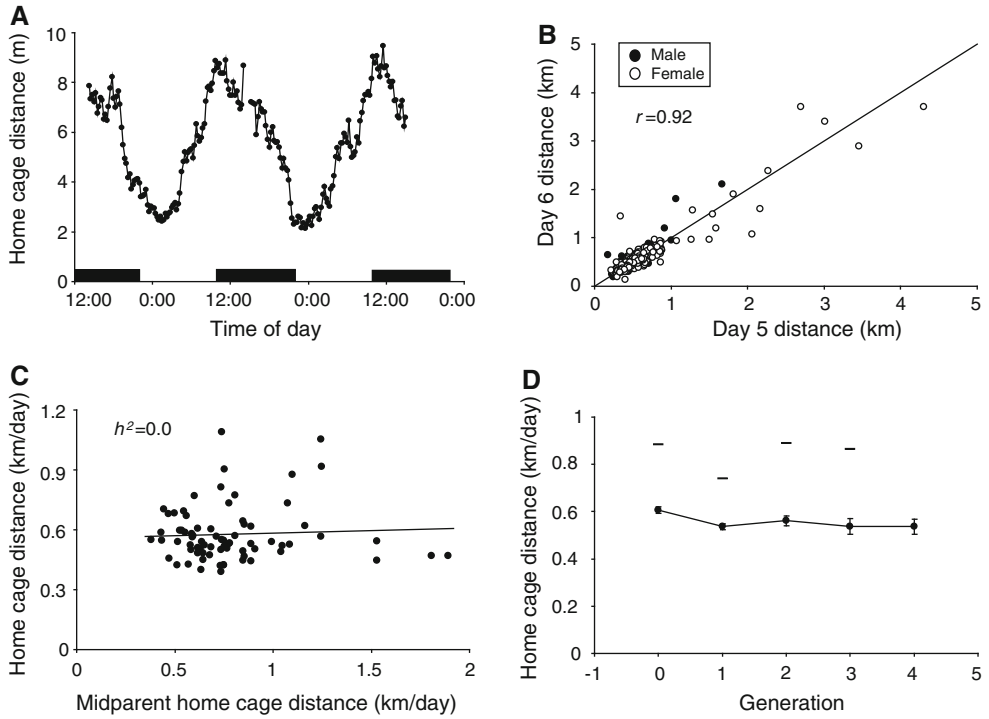

Fig. 2 Home cage activity in Hsd:ICR mice. **a** Circadian rhythm. Average distance traveled (meters) plotted in 15 min intervals for days 5 and 6 for all animals in generation 4. Mice were housed on a 12 h reverse light/dark cycle, lights off at 10:00 AM. The dark periods are represented by black bars at the bottom of the graph. **b** Repeatability. Distance traveled in the home cage on day 6 (km) plotted against day 5 (km). Males are shown as filled circles and females as open circles. The 1:1 line is shown. **c** Heritability. Mean offspring value for home cage activity (distance traveled on days 5 and 6) plotted against the average value for the sire and dam. The least squares simple linear regression line is shown. **d** Response to selection. Circles represent the population means (±SE) from each generation for home cage activity (distance traveled on days 5 and 6 in km/day). The horizontal lines above the population means show the mean activity scores of mice selected as breeders to found the following generation

$n = 812$ animals), the estimate of the slope of the mid-parent offspring regression for distance traveled on days 5 and 6 was 0.004 (±0.045 SE) ($F_{1,60} = 0.01$, $P = 0.92$). The slope of the weighted mid-parent offspring regression was -0.0006 ± 0.044 ($F_{1,60} = 0.00$, $P = 0.99$).

Response to selection

The Hsd:ICR line displayed no response to 4 generations of selection for increased home cage activity. This was observed despite substantial selection differentials applied each generation (see Fig. 2d). See Table 1 for estimates of realized heritability, h^2_{realized}, by generation. The average realized heritability estimate over the 4 generations of selection was 0.03.

Correlations with wheel running and open field

Wheel running was significantly repeatable (Fig. 3a). Considering all the animals phenotyped in generations −1 and 0 (with data for both day 5 and 6, a total $n = 222$) the Pearson's r for the correlation between distance traveled on days 5 and 6 was estimated to be 0.84 ($P < 0.0001$). Open field was also significantly repeatable (Fig. 3b). The Pearson's r for the correlation between distance traveled on day 1 versus 2 was estimated to be 0.64 ($P < 0.0001$).

Table 1 Realized heritability estimates of home cage activity

	Gen 1	Gen 2	Gen 3	Gen 4	Gen 5	Avg.
Collaborative cross	0.16	0.52	0.64	0.05	0.26	0.33
Hsd:ICR	0[a]	0.12	0[a]	0[a]	N/A	0.03

[a] The actual estimates were slightly negative

Wheel running was significantly heritable, in generations −1 and 0 (Fig. 3c). The estimate of the slope of the mid-parent offspring regression was 0.37 ± 0.123 SE ($F_{1,13} = 9.1$, $P = 0.01$). Weighted regression gave the same estimate. Open field was not heritable (Fig. 3d). Estimate of the slope was -0.04 ± 0.237 SE ($F_{1,13} = 0.03$, $P = 0.86$). Weighted regression gave a similar estimate, -0.05 ± 0.232 SE ($F_{1,13} = 0.04$, $P = 0.84$).

Individual phenotypic values for home cage activity (average distance on days 5 and 6) were only weakly correlated with levels of wheel running (see Fig. 4a) ($r = 0.21$, $P = 0.002$) and were uncorrelated with open field activity ($r = 0.09$, $P = 0.17$), in the same individuals (see Fig. 4b).

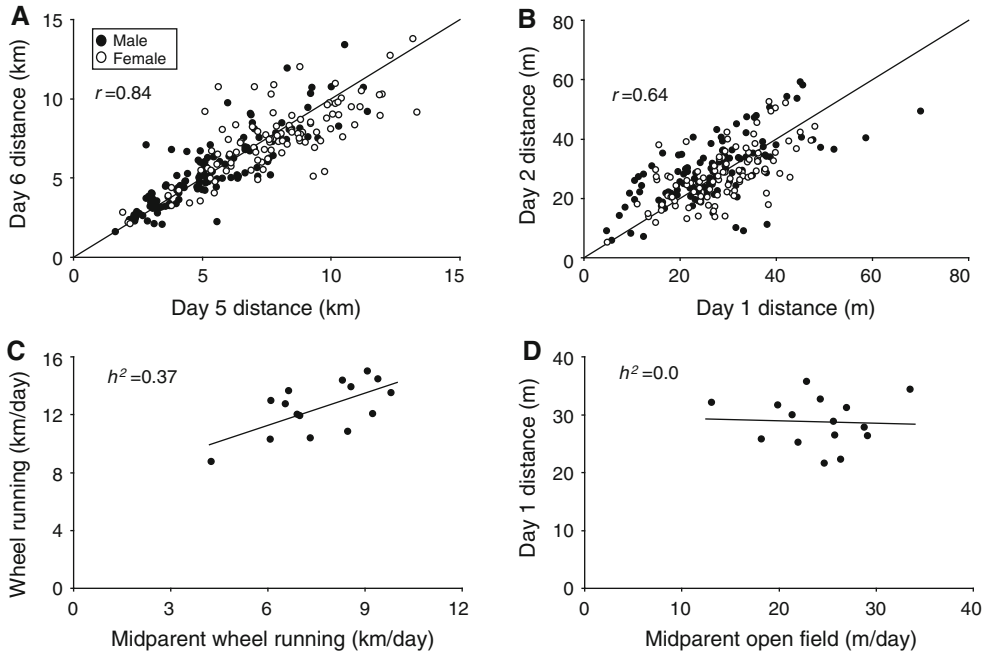

Fig. 3 Wheel running and open field in Hsd:ICR. **a** Repeatability of wheel running. Distance traveled in running wheels on day 6 (km) plotted against day 5 (km). Males are shown as filled circles and females as open circles. The 1:1 line is shown. **b** Repeatability of open field. Similar plot as A except distance traveled in the open field on day 2 (m) is plotted against day 1 (m). **c** Heritability of wheel running. Mean offspring value for wheel running (distance traveled on days 5 and 6 in km) plotted against the average value for the sire and dam. The least squares simple linear regression line is shown. **d** Heritability of open field. Similar plot as C except for distance traveled in the open field on day 1 (m)

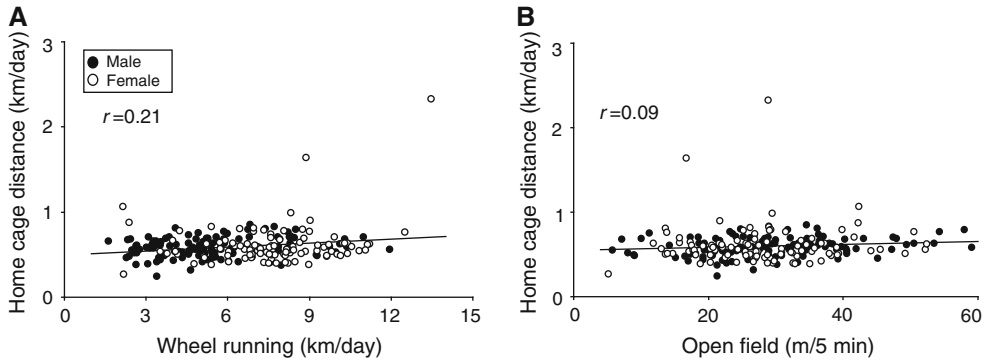

Fig. 4 Phenotypic correlations between home cage activity, wheel running and open field in Hsd:ICR. **a** Phenoptypic correlation between home cage activity and wheel running. Distance traveled in the home cage on days 5 and 6 (km/day) plotted against distance traveled in running wheels on days 5 and 6 (km/day). Males are shown as filled circles and females as open circles. The least squares simple linear regression line is shown. **b** Phenoptypic correlation between home cage activity and open field. Similar plot as A except distance traveled in the home cage is plotted against distance traveled in the open field on day 1 (m/5 min)

Genetic correlations were not evaluated because home cage activity displayed zero heritability (see above).

Experiment 2: collaborative cross

Circadian rhythm

A qualitative assessment of Fig. 5a shows that the CC high-active and CC control mice display normal circadian rhythm under the conditions of the experiment, and that the greater activity displayed by CC high-active versus control predominantly occurs during active phases of the light/dark cycle.

Repeatability

Home cage activity was strongly repeatable in the CC population (Fig. 5b). Considering all the animals

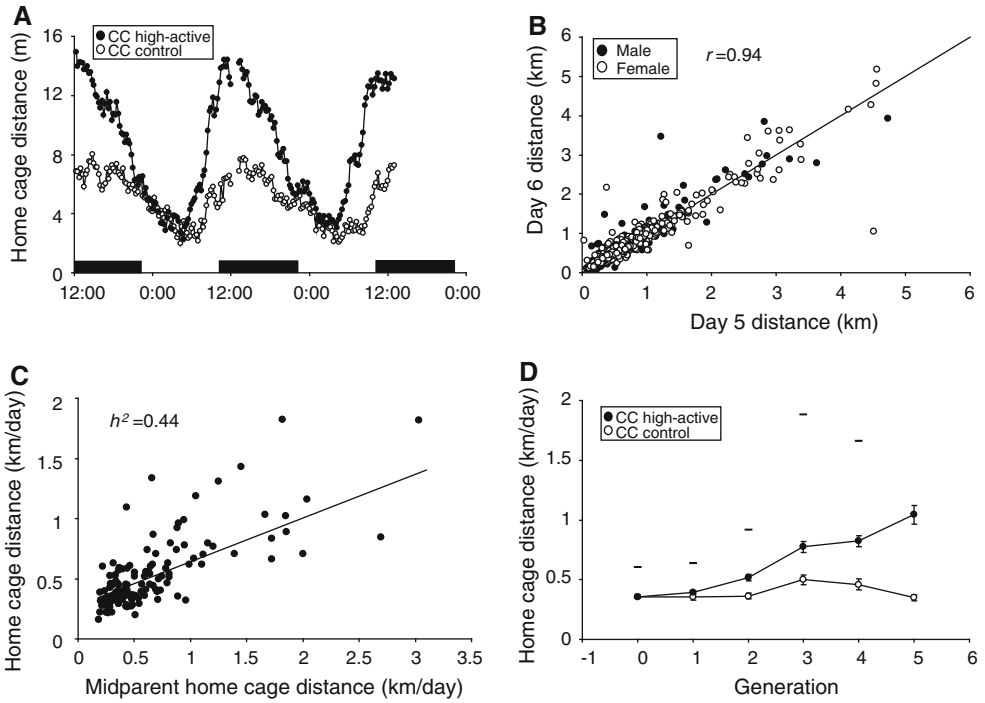

Fig. 5 Home cage activity in CC mice. **a** Circadian rhythm. Average distance traveled (meters) plotted in 15 min intervals for days 5 and 6 for all animals in generation 4. The CC high-active line is plotted separately from CC control. Mice were housed on a 12 h reverse light/dark cycle, lights off at 10:00 AM. The dark periods are represented by black bars at the bottom of the graph. **b** Repeatability. Distance traveled in the home cage on day 6 (km) plotted against day 5 (km). Males are shown as filled circles and females as open circles. The 1:1 line is shown. **c** Heritability. Mean offspring value for home cage activity (distance traveled on days 5 and 6) plotted against the average value for the sire and dam. The least squares simple linear regression line is shown. **d** Response to selection. Circles represent the population means (±SE) from each generation for home cage activity (distance traveled on days 5 and 6 in km/day). The CC high-active line is plotted separately from CC control. The horizontal lines above the population means show the mean activity scores of mice selected as breeders in the CC high-active line to found the following generation

phenotyped in generations −1 through 5 across both CC high-active and control lines (a total $n = 1{,}261$ with data for both days 5 and 6), the Pearson's r for the correlation between distance traveled on days 5 and 6 was estimated to be 0.94 ($P < 0.0001$).

Heritability

Home cage activity in the CC population was significantly heritable (Fig. 5c). Considering all the animals phenotyped in generations −1 through 5 (a total of 130 families, $n = 1{,}260$ animals), the slope of the mid-parent offspring regression, i.e., narrow sense heritability, h^2_{narrow}, was estimated to be 0.44 ± 0.036 SE ($F_{1,128} = 153.4$, $P < 0.0001$). The slope of the weighted mid-parent offspring regression was 0.45 ± 0.035 ($F_{1,128} = 161.4$, $P < 0.0001$).

Response to selection

The CC high-active line displayed a significant response to 5 generations of selection for increased home cage activity (Fig. 5d). A significant response was observed by generation 2 ($F_{1,211} = 12.8$, $P = 0.0004$). By generation 5, CC high-active mice traveled 3 times farther than the unselected control line ($F_{1,160} = 36.1$, $P < 0.0001$). Average distance was 0.35 km/day (±0.026 SE) for control whereas for high-active it was 1.0 km/day (±0.076 SE). See Table 1 for estimates of realized heritability, h^2_{realized}, by generation. The average realized heritability estimate over the 5 generations of selection was 0.33.

Correlations with wheel running and open field

Wheel running was significantly repeatable (Fig. 6a). Considering all the animals phenotyped in generations −1 and 0 (with data for both day 5 and 6, a total $n = 196$) the Pearson's r for the correlation between distance traveled on days 5 and 6 was estimated to be 0.92 ($P < 0.0001$). Open field was also significantly repeatable (Fig. 6b). The Pearson's r for the correlation between distance traveled on day 1 versus 2 was estimated to be 0.61 ($P < 0.0001$).

Wheel running was significantly heritable, in generations −1 and 0 (Fig. 6c). The estimate of the slope of the mid-parent offspring regression was 0.39 ± 0.163 SE

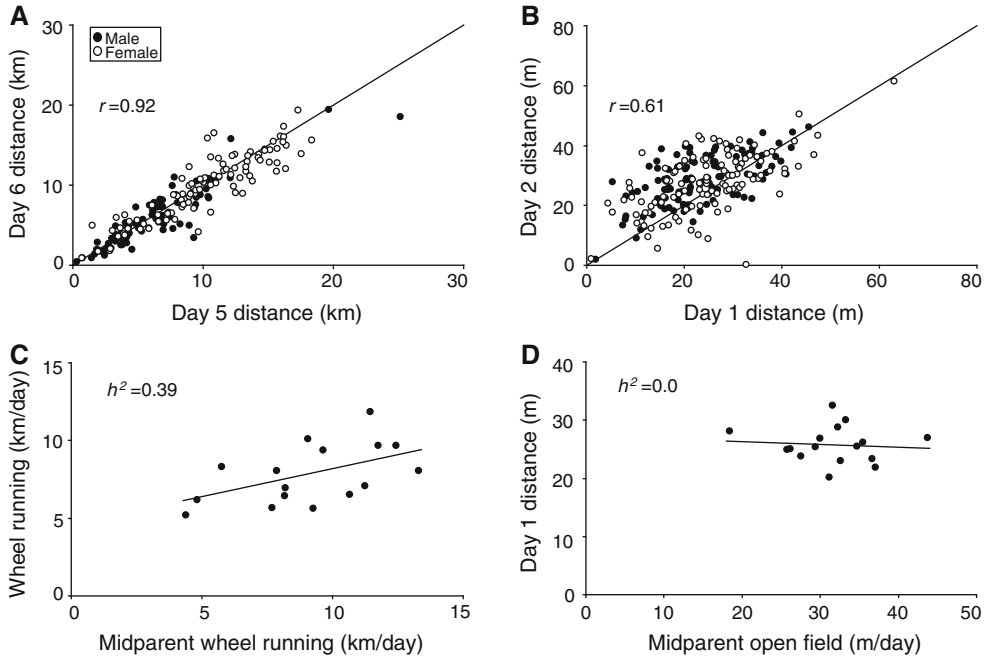

Fig. 6 Wheel running and open field in CC mice. **a** Repeatability of wheel running. Distance traveled in running wheels on day 6 (km) plotted against day 5 (km). Males are shown as filled circles and females as open circles. The 1:1 line is shown. **b** Repeatability of open field. Similar plot as A except distance traveled in the open field on day 2 (m) is plotted against day 1 (m). **c** Heritability of wheel running. Mean offspring value for wheel running (distance traveled on days 5 and 6 in km) plotted against the average value for the sire and dam. The least squares simple linear regression line is shown. **d** Heritability of open field. Similar plot as C except for distance traveled in the open field on day 1 (m)

($F_{1,14} = 5.6$, $P = 0.03$). Using weighted regression, the estimate was 0.38 ± 0.156 SE ($F_{1,14} = 5.9$, $P = 0.03$). Open field was not heritable (Fig. 6d). Estimate of the slope was -0.05 ± 0.145 SE ($F_{1,14} = 0.13$, $P = 0.72$). The weighted estimate was -0.01 ± 0.186 SE ($F_{1,14} = 0.00$, $P = 0.96$).

Individual phenotypic values for home cage activity (average distance on days 5 and 6) were moderately correlated with levels of wheel running (see Fig. 7a) ($r = 0.41$, $P < 0.0001$) and weakly correlated with open field activity ($r = 0.15$, $P = 0.02$) in the same individuals (Fig. 7b). The genetic correlation between home cage activity and wheel running was estimated to be $r = 0.13$, but the mid-parent offspring regressions were not statistically significant (P-values were 0.77 for offspring running against mid-parent cage activity, and 0.13 for offspring cage activity against mid-parent running). Genetic correlations with open field were not evaluated because open field behavior was not significantly heritable (see above).

Discussion

Our results are the first to illustrate the feasibility of selective breeding for increased distance traveled in the home cage using mice as the model organism. The video tracking technology produced highly repeatable measurements of behavior (Figs. 2b, 5b). Significant heritability and response to selection was observed in Collaborative Cross mice (Fig. 5d) but not in Hsd:ICR (Fig. 2d). The success in CC and failure in Hsd:ICR provides critical information for choosing an appropriate strain for future breeding experiments or genetic analyses focused on home cage activity. Hsd:ICR cannot be used because it lacks sufficient genetic variation whereas the CC population is suitable.

The response to selection in the CC population was significant in generations 2 through 5 and inspection of the pattern of change over the generations showed no indication that a selection limit was approaching (Fig. 5D). We are continuing to breed the CC lines, and will report on the selection limit in future generations. By comparison, the CC high active mice at generation 5 displayed approximately twice the level of home cage activity as compared to CAST/EiJ, the strain showing the highest level of activity out of a total of 10 inbred and 2 F1 hybrids recently sampled by our laboratory (Clark et al. 2010). Most previous studies examining genetic variation in home cage activity across laboratory strains of mice have used either photo-beams (Tang et al. 2002; Kliethermes et al. 2005; Tang and Sanford 2005) or infrared sensors (Malisch et al. 2009; Umemori et al. 2009; Nishi et al. 2010; Wada et al.

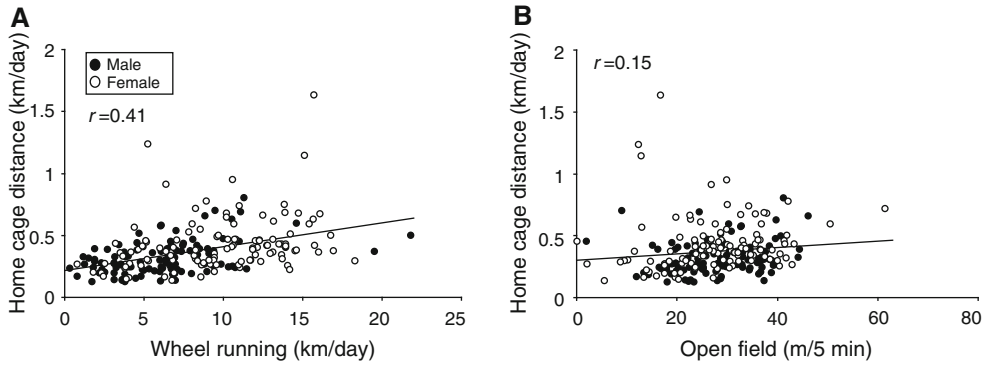

Fig. 7 Phenotypic correlations between home cage activity, wheel running and open field in CC mice. **a** Phenoptypic correlation between home cage activity and wheel running. Distance traveled in the home cage on days 5 and 6 (km/day) plotted against distance traveled in running wheels on days 5 and 6 (km/day). Males are shown as filled circles and females as open circles. The least squares simple linear regression line is shown. **b** Phenoptypic correlation between home cage activity and open field. Similar plot as A except distance traveled in the home cage is plotted against distance traveled in the open field on day 1 (m/5 min)

2010) to measure activity or used different size cages (Kas et al. 2009), therefore the absolute values are difficult to compare between studies.

The lack of significant genetic correlation between home cage activity, wheel running and open field illustrates the complexity of physical activity and suggests that selective breeding for distance traveled in the home cage would make a unique contribution to our understanding of the genetic and neurobiological factors predisposing increased voluntary physical activity in mice. The weak association between distance traveled in the home cage on days 5 and 6 and distance traveled in the open field over the 5-min test (Figs. 4b, 7b) is consistent with previous data reporting lack of genetic correlation between voluntary wheel running and distance traveled in the open field (DeFries et al. 1970; Bronikowski et al. 2001). The open field test is intended to measure exploratory behavior or anxiety in a novel environment (Choleris et al. 2001) whereas wheel running and cage activity are habitual behaviors measured over several days. Genetic variation in wheel running is hypothesized to involve reward circuits in the brain that regulate motivation for running (Rhodes et al. 2001; Werme et al. 2002; Rhodes and Garland 2003; Rhodes et al. 2003). To what extent differences in reward circuits contribute to variation in voluntary physical activity in cages without wheels is not known. If reward circuits are involved, the details are likely to be different from wheel running (e.g., receptor types, neuroanatomical connections etc.) because wheel running and home cage activity were only weakly correlated (Figs. 4a, 7a).

The lack of response to selection and zero heritability in Hsd:ICR for home cage activity was not expected. Hsd:ICR is the strain that was used as the founding population in the selective breeding experiment for increased voluntary wheel running (Swallow et al. 1998). In that experiment by generation 24, the high-running lines displayed increased activity in their home cages (Rhodes et al. 2001; Malisch et al. 2008; Malisch et al. 2009). We confirmed that wheel running was significantly heritable in our founding Hsd:ICR population (Fig. 3c) demonstrating that sufficient genetic variation was present in the founding population for wheel running. Most likely, variation in home cage activity involves different suites of genes compared to wheel running (with only moderate overlap between the two behaviors), and allelic variation in these genes was absent or low in the Hsd:ICR mice. This conclusion is consistent with results in the Collaborative Cross lines where the genetic correlation between home cage physical activity and wheel running was small and not statistically significant.

The high repeatability (Fig. 2b) and zero heritability (Fig. 2c) in Hsd:ICR illustrates the importance of environmental contributions to stable individual differences in physical activity. It would be interesting to discover the relevant environmental influences that cause some animals, especially female Hsd:ICR (see Fig. 2b), to display high levels of activity in absence of genetic influences. Unpublished data from our laboratory suggests that the repeatability of home cage activity in Hsd:ICR is substantially reduced when the time between measurements is increased from days to weeks. Individual levels of physical activity may remain stable over days but can fluctuate greatly over longer periods (e.g., weeks, months). Slowly occurring fluctuations could explain why repeatability is high and heritability zero if measurements on day 5 versus 6 were not representative of the longer-term trends in physical activity in the animal. In the Collaborative Cross lines, repeatability (Fig. 5b) was substantially greater than heritability (Fig. 5c), confirming a strong role for environment in contributing to stable differences in physical activity on repeated days.

One important feature of the phenotype, home cage activity, as reported here, is the construction of the custom-made home cages. As described in the methods section, the

cages were constructed with a mesh divider at the center of adjacent cages (see Fig. 1) so that animals could interact (e.g., sniff and touch each other). The rationale for including this interaction zone is that mice live in groups under natural conditions and we wanted to measure activity levels under normal conditions, not under conditions of stress or anxiety from social isolation (as reviewed in Fone and Porkess 2008). The disadvantage of including the mesh divider, from the point of view of behavior genetics, is that the social interaction potentially introduces noise into estimates of individual differences to the extent that an animal's distance traveled is influenced by the behavior or smells of surrounding animals (Rhodes and Kawecki 2009). Note that animals were assigned to cages so that only same-sex individuals could interact via the common intersection space. This was done to prevent possible, though probably unlikely, breeding via the mesh. Anecdotally, we observed animals often interacting via the wire mesh grid, (e.g., touching, sniffing). Many of the animals also slept curled up next to each other on either side of the wire mesh. Although the degree of social interaction was high, and may have introduced some noise into our estimates, individual differences in distance traveled were significantly heritable in the Collaborative Cross lines (Fig. 5c). This demonstrates that individual genetic contributions to the home cage activity phenotype were robust over and above noise from social interactions.

We strongly believe that a long term replicated selective breeding experiment for increased home cage physical activity starting with the CC founding population would provide a useful resource for biomedicine and evolutionary neuroscience (Rhodes et al. 2005; Rhodes and Kawecki 2009). Such a model would allow comparison of mice from high-active lines with unselected control lines that could lead to insights into the etiology of hyperactivity features of ADHD, novel treatments for obesity and cognitive aging and/or as a basic heuristic model for studying mechanisms of behavioral evolution (Rhodes and Kawecki 2009; Swallow et al. 2009). Results from the correlation analysis suggest that selection for increased home cage activity will make a unique contribution and complement existing selection experiments on wheel running, open field and treadmill running to exhaustion (DeFries et al. 1978; Swallow et al. 1998; Koch and Britton 2001). In future generations, when the activity trait has diverged further, and with replicate high-active and replicate control lines, it would be possible to identify correlated responses to selection at multiple levels of biological organization (e.g., molecules, cells, tissues, physiology, behavior). Such a resource would provide a unique opportunity for a comprehensive analysis of the genetic and neurobiological factors that predispose heightened levels of home cage physical activity in laboratory mice.

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