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Investigation of pre-pubertal sex differences in wheel running and social behavior in three mouse strains

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Abstract

Sex differences in social behaviors exist in mammals during adulthood, and further evidence suggests that sex differences in behavior are present before sexual maturity. In order to model behavioral disorders in animals, it is important to assess baseline sex-related behavioral differences, especially when studying disorders for which sex-related behavioral effects are expected. We investigated the effect of sex on behavior in 3 strains of pre-pubertal mice (C57BL/6, CFW, and CF1) using a wheel-running assay. We found no significant sex differences in latency to run on the wheel or total duration of wheel running within each strain. During the social interaction test, there were no differences between sexes in latency or total duration of contact or following between a subject and novel mouse. We also evaluated behavioral patterns of wheel running and stereotypical behaviors, such as burrowing and grooming. Both sexes showed characteristic wheel running behavior, spending the majority of each trial interacting with the wheel when it was free and more time performing other activities (*e.g.*, stereotypical behaviors, general locomotion) when it was jammed. These results provide evidence that, among various strains of pre-pubertal mice, baseline sex-related behavioral differences are not strong enough to influence the measured behaviors.

Keywords

sexually dimorphic behavior; juvenile mice; wheel running; autism behavioral assay

1. Introduction

To date, neuroscience research remains biased towards male animals, following the assumption that cyclic sex hormones in females may confound results (Cahill, 2012). A 2009 survey showed a male bias across research disciplines, with the ratio of male-only to female-only studies in neuroscience at 5:1 and subject sex omitted in 22–42% of articles (Beery & Zucker, 2011). More disturbingly, investigators often assert that findings from male-only studies lead to conclusions that are applied to both sexes (Cahill, 2012). Researchers across disciplines now recognize the importance of considering sex and sex differences in the design and interpretation of studies (“Putting gender on the agenda,”

2010). Recent suggestions include incorporating females and males in equal numbers with a clear comparison of the two sexes (Prendergast, Onishi, & Zucker, 2014).

The classic Organizational-Activational Hypothesis proposed by Phoenix et al. (1959), which states that gonadal hormones activate brain regions in adulthood previously organized by those hormones early in development, has been expanded to more accurately represent the complexities of sexual development. Sex differences in the brain and behavior result from intricate interactions among steroid hormones synthesized in both the gonads and the brain; sex chromosomes acting at the genetic, cellular, and biochemical level; and the environment from early development throughout the lifespan (Lenz, Nugent, & McCarthy, 2012; Prendergast et al., 2014). Sex differences in the brain occur at many levels, including differences in regional volume and/or cell number, morphology, physiology, molecular signaling, and gene expression (Lenz et al., 2012), leading to differences in learning and memory, fear, anxiety, and nociception (McCarthy, Arnold, Ball, Blaustein, & De Vries, 2012; Prendergast et al., 2014).

Mammalian species tend to exhibit sex differences in social behavior as adults, especially those relating to mating and courtship; however, differences often extend beyond obvious variations in mating behavior (Meaney, Stewart, & Beatty, 1985). Previous studies have reported no apparent sex-related differences in general activity in the mouse (Lamberty & Gower, 1988), nor an effect of estrous cycle on activity in adult mice (Dowse, Umemori, & Koide, 2010; Meziane, Ouagazzal, Aubert, Wietrzyk, & Krezel, 2007). However, other evidence has linked sex steroids to wheel running distance, speed, and duration in both sexes of adult mice (Bowen et al., 2012) and shown that in the Hsd:ICR strain (outbred descendants of the Swiss-Webster strain), females spend more time wheel running and at a faster speed than males (Swallow, Carter, & Garland, 1998). These measures did not differ by sex in lines of mice selectively bred from the Hsd:ICR strain for high wheel running activity (Garland et al., 2011).

In addition, many mammalian species display sex differences in social play of juveniles. Generally, male rats are more likely to initiate and engage in play-fighting behaviors than females, whereas females are more likely to withdraw from a play initiation (Meaney et al., 1985). Although mice do not generally display 'rough and tumble play,' other measures to assess social reciprocity and play include nose-to-nose sniffing, anogenital sniffing, following, crawling over/under, and social grooming (Crawley, 2007). Unlike the mating/courtship behavioral differences seen in adult animals, these social behaviors are seen pre-pubertally, *i.e.*, before sexual maturity. Any disparities seen in behavior at this stage are more likely due to the perinatal environment, sex chromosomes or other prenatal effects than to differences in gonadal hormones circulating at the time of the behavioral trial.

In order to effectively model behavioral disorders using animals of both sexes, it is important to assess baseline sex-related behavioral differences, especially when studying disorders for which sex-related behavioral effects are expected, such as autism. The sex bias of autism has a male to female ratio of 4:1 and even higher in cases without dysmorphic features and Asperger syndrome (Moldin & Rubenstein, 2006). Recent research has stimulated the generation of animal models to better elucidate the role of specific genes and

environmental influences in the pathogenesis of autism (Oddi, Crusio, D'Amato, & Pietropaolo, 2013).

Current neuroscience literature supports a variety of mouse behavioral assays designed to maximize relevance to social deficits specific to autism (Silverman, Yang, Lord, & Crawley, 2010). The results from previous studies using mouse models of autism have underscored the importance of including age and sex as relevant factors (Oddi et al., 2013). The strains chosen for this study were inbred (C57BL/6) and outbred (CFW and CF1) mice, commonly used as general multipurpose models. On postnatal days 27–33, we investigated the effect of sex on distinct behaviors. Using a series of running wheel assays developed to evaluate autism-like behaviors (Karvat & Kimchi, 2012), we measured the animals' ability to gain and maintain a routine, assessed repetitive behavior, evaluated cognitive rigidity and examined social interactions. This behavioral array was chosen because of its relevance to the core symptoms of autism and in preparation for future work assessing autism-like behaviors following developmental insults. Examining the animals before puberty should reduce behavioral variations due to circulating sex hormones. By assessing three mouse strains, we attempted to find information that applies more generally to the species, rather than to only one strain. Most importantly, these assays provide needed insight into pre-pubertal sex-related differences in behavior.

2. Materials and Methods

2.1 Animals

The animals used in this study were 3 strains of mice: C57BL/6 (n=16; 8 males and 8 females), CFW (n=24; 12 males and 12 females), and CF1 (n=20; 10 males and 10 females). The mice were received from the vendor (C57BL/6, Jackson Laboratory; CFW and CF1, Charles River) at approximately 3 weeks of age (P20–P21). Initial body weights were taken on the day of arrival, which was P20 for C57BL/6 and P21 for CFW and CF1. All animals were maintained in our animal facility on a 12:12 light/dark cycle and were provided with TekLad 2016 rodent chow and tap water *ad lib*. The C57BL/6 mice remained on their previous light schedule (lights off 6pm), while the CFW and CF1 mice underwent a phase shift to correspond with the researchers' schedule. On day one lights went off at 6pm. On day 2 the mice underwent a 3 hour phase shift, with lights off at 3pm. The CFW mice underwent another 3 hour shift on day 3 resulting in lights off at 12pm (note that daylight savings time started immediately after this shift so that the lights actually went off at 1pm during trials), whereas the CF1 mice underwent a 2 hour shift on day 3 resulting in lights off at 1pm. The light schedule on subsequent days was maintained. Within each strain, two mice of each sex were housed separately to be used for social testing purposes only; in CFW and CF1 strains these mice were also shipped separately. After a week-long acclimation period, the experimental animals were tested with a series of wheel-running assays (see section 2.2). Euthanasia was performed following a standard protocol of intracardial perfusion with 4% paraformaldehyde; final body weights, testis and uterine weights were taken at this time to assess growth and sexual maturity. The C57BL/6 mice of both sexes were perfused in two groups on postnatal days 34 and 35. All CFW and CF1 females were perfused on postnatal day 33, while all males were perfused on postnatal day 34. Animal care provided was in

accordance with the Guide for the Care and Use of Laboratory Animals (Guide for the Care and Use of Laboratory Animals, 2011) and procedures were approved by our Institutional Animal Care and Use Committee. Note: The trials for each strain were completed independently so that only one strain of mice was housed and tested at a time.

2.2 Test Procedure

Animals were tested during the dark phase of the light cycle to ensure they were in their most active period when tested. Video was collected using a camera with night-vision capability under red lights. The wheel-running assays used a plexiglass cage (34.5 cm × 23 cm × 19 cm, covered) lined with corncob bedding and fitted with a standard plastic mouse running wheel (14 cm diameter) that either freely turned or was jammed by a metal pin. To remove olfactory cues, the layer of bedding was changed and the entire cage was cleaned with 70% ethanol between trials. We followed the protocol of Karvat & Kimchi (2012) to assess wheel running behaviors. Modifications were made to assess sex differences in behavior, as specified subsequently. *Stage 1: gaining and maintaining a routine.* For the C57BL/6 strain each mouse was placed in the cage for 20 minutes and permitted to run on the wheel freely for 4 consecutive days; the trial time was reduced to 15 minutes and period reduced to 3 consecutive days for CFW and CF1 strains, as analysis of C57BL/6 data revealed this timing should be sufficient for routine wheel running to be established. *Stage 2: repetitive behavior.* Each mouse was recorded for 15 minutes in the cage with the wheel jammed on days 5–6 (for C57BL/6) or days 4–5 (CFW and CF1). For each sex, time spent grooming and burrowing in the bedding on days the wheel was free (1–4 or 1–3) and on days the wheel was jammed (5–6 or 4–5) was assessed. *Stage 3: cognitive rigidity.* Cognitive rigidity is defined as the inability to forgo a habit/routine. Time spent interacting with the wheel when it was jammed was measured and evaluated for sex-related differences in cognitive rigidity. *Stage 4: social interaction.* On day 7 (C57BL/6) or day 6 (CFW and CF1) each study mouse spent 10 minutes in the cage with the jammed wheel and an age and sex-matched novel mouse. The novel mouse was placed into the cage first, followed by the study mouse to allow the observer to identify the mice and score behaviors accordingly. We tested for social interaction by assessing the time spent by the study mouse engaging in social investigation with a novel mouse. Contact and following behaviors were assessed for differences in social behavior between the sexes.

2.3 Scoring

Behavior scores were recorded using the Noldus Observer software version 4.1. For all days, the following items were measured: latency to begin wheel running; directionality and duration of wheel running; interaction with wheel without movement (*i.e.*, sitting on wheel), and duration of burrowing, grooming, and interaction with the cage's water nozzle. For durations, percentage of total trial was used instead of time, because the total time differed among strains. Burrowing was scored when pieces of the bedding were intentionally moved by the mouse. On the days the wheel was jammed, "wheel running" was scored as interaction of the mouse with the wheel that would have made the wheel move if it were free. Time the mouse spent on top of the jammed wheel was excluded from the wheel interaction score. On the final day, interaction of the study mouse and a novel, age and sex-matched mouse provided information on sociability. Any physical engagement between the

mice, whether initiated by the study mouse or by the novel mouse, was scored as social interaction, divided into contact and following behaviors.

2.4 Statistics

Running wheel assays (*i.e.*, latency and duration of wheel running and stereotypical behaviors) were analyzed for sex, day and sex $\times$ day interactions in SAS (9.3, SAS Institute Inc.) using a mixed linear model with the restricted/residual maximum likelihood (REML) estimation method. The Kenward-Roger approach was used to approximate inference about fixed effects to estimate denominator degrees of freedom in tests for fixed effects. Day and sex $\times$ day were specified as random effects. Data were transformed as appropriate to reach normality: for C57BL/6 data the square root was taken for the duration of wheel running and interaction with the water nozzle, and for the latency to wheel run; for CFW the duration of wheel running data was squared, the square root was taken for duration grooming data and the natural log was taken for duration of interaction with the water nozzle; for CF1 the duration of wheel running data was squared, while the square root was taken for latency to wheel run. To adjust for the number of simultaneous tests to assess the effect of day, the sequential Bonferroni test was used (Rice, 1988). The latency and duration of contact and following behaviors of the study mouse with a novel mouse were analyzed by t-test with sex as an independent variable. Strain differences in wheel running duration was analyzed by one-way ANOVA with pairwise multiple comparisons made using the Holm-Sidak method. Because we found no significant sex differences within each strain, this analysis combined data for all mice of a given strain.

3. Results

In all 3 strains (C57BL/6, CFW and CF1), pre-pubertal mice follow patterns of wheel running behavior characteristic of normal adult mice, with some differences among strains; however, there are not significant differences in wheel running behaviors between the sexes at this age.

3.1 Latency of Behavior

In a typical animal gaining a routine, latency to run should decrease following the initial introduction to the novel wheel. The mixed model analysis for latency to begin wheel running revealed a significant effect of day for analyses of days 1–6 for C57BL/6 ($p < 0.0001$) and days 1–5 for CFW ($p < 0.0001$) and CF1 ($p = 0.0002$) (Fig 1, A–C). For days the wheel was free, latency to run was significantly less on day 2 compared to day 1 across all strains ($p < 0.0001$), displaying appropriate routine gaining. Cognitive rigidity is evaluated by the inability to forgo a routine. Latency to run increased on the second day the wheel was jammed as compared to the first ($p < 0.001$ for all strains), suggesting that the mice in our study remembered the wheel was jammed the previous day and could adjust their routine. We found no significant difference in latency to run on the wheel between males and females across days for C57BL/6, CFW, or CF1 ($p = 0.3304$, $p = 0.9399$, $p = 0.5988$, respectively). The observer noted that C57BL/6 males were more inclined to explore the cage on day one and appeared to initiate wheel running later than C57BL/6 females. The mean latency to run on the wheel for females was 159.8 ± 11.2 seconds and for males was

238.8 $\pm$ 40.0 seconds; however, the latency data across all days failed to reach statistical significance for sex ($p=0.3304$) and there was no significant sex $\times$ day interaction ($p=0.4062$). Social behavior was demonstrated by the latency of the study mouse to initiate contact or begin following the novel mouse. For all strains, t-tests indicated there was no difference between sexes in latency to contact or follow the novel mouse (Fig 2, A–B) ($p=0.134$ to $p=0.942$).

3.2 Duration of Behavior

The percentage of trial duration spent wheel running was also analyzed using a mixed effects model in SAS (Fig 1, D–F). Duration analysis again revealed a significant day effect during days 1–6 for C57BL/6 ($p<0.0001$) and days 1–5 for CFW ($p<0.0001$) and CF1 ($p<0.0001$). Total duration of wheel running increased across days the wheel was free; however, interaction with the wheel dropped for days when the wheel was jammed. In a comparison of wheel running duration on day 1 (first day free) vs. day 4/3 (last day free) and also day 5/4 (first day jammed) vs. day 6/5 (second day jammed), all p-values reached significance ($p<0.0003$ for all comparisons) using the Bonferroni adjustment, except CFW day 1 vs. day 3. This follows the expected pattern of behavior for animals lacking rigidity to change habits, *i.e.*, a normal mouse should decrease its interaction time with the wheel on the second day that the wheel is jammed, which was apparent on day 6/5. We found no significant difference in percentage duration of wheel running between the sexes across days for C57BL/6, CFW, or CF1 ($p=0.6146$, $p=0.1674$, $p=0.4881$, respectively). However, there were strain differences in percentage duration of wheel running for the last day the wheel was free ($p<0.001$), first day the wheel was jammed ($p=0.001$), and second day the wheel was jammed ($p<0.001$) (Table 1). Total duration of contact and following behaviors with the novel mouse were examined from the social interaction test (Fig 2, C–D). The t-tests indicated no significant differences between sexes in contact or following behaviors of the study mouse with a novel mouse ($p=0.114$ to $p=0.866$).

We also evaluated the total duration percentage of stereotypical behaviors, such as burrowing, grooming, and interaction with the water nozzle. The ability to freely run on a wheel reduces stereotypical behaviors, *e.g.*, burrowing in bedding (Karvat & Kimchi, 2012). Therefore, a typical animal should spend more time performing stereotypical behaviors when the wheel is jammed vs. free. We compared these behaviors for the last day the wheel was free (day 4/3) vs. the first day it was jammed (day 5/4), and found that grooming and water nozzle interaction were displayed more often when the wheel was jammed ($p<0.0003$ for all strains). The inability to run on a wheel increased these stereotypical behaviors in both sexes. A day effect was noted for grooming behaviors in both CFW and CF1 strains (CFW $p<0.0001$, CF1 $p=0.0042$) (Fig 3, E–F). Statistical analysis of grooming in C57BL/6 mice was hindered by its infrequency in this strain, yet these animals displayed the same pattern of behavior as seen in other strains (Fig 3, D). A day effect of interaction with the water nozzle was displayed across all days ($p<0.0001$ for all strains) (Fig 3, G–I). Burrowing was exceedingly rare while the wheel was free and represented a small percentage of time spent on days the wheel was jammed (Fig 3, A–C; Fig 4). A sex effect was seen for water nozzle interaction in CF1 mice across all days, in that males were more likely to interact with the nozzle than females ($p=0.0263$), but not in any other strain ($p=0.3143$ and

$p=0.5966$). In addition, brief mutual grooming was displayed in both sexes during the social interaction trial. Both sexes displayed similar patterns of behavior in that they were more likely to interact with the wheel when it was free and performed other behaviors when it was jammed. See Figure 4 for a representation of total duration of all behaviors by sex and across days. Three days are shown for each sex from all strains: the last day the wheel was free (left column) and the subsequent two days when the wheel was jammed. This figure clearly displays our main findings: within each strain there was a lack of sex differences in behavior; however, strain differences in wheel running behavior were apparent (analysis in Table 1).

3.3 Morphology

Mouse morphology data, including body and gonad/uterine weight, can be found in Fig 5. The weights of both CFW and CF1 mice matched expected growth curves (Charles River Laboratories International Inc., 2014a, 2014b); C57BL/6 mice were above the expected weight at 3 weeks, but matched published growth curve weights by 5 weeks (The Jackson Laboratory, 2014).

4. Discussion

Wheel running is widely studied in rodents (Novak, 2012), as it appears to be a highly rewarding activity (Werme et al., 2002). Karvat and Kimchi (2012) used this instinctive wheel running propensity in mice to develop a behavioral test relevant to autism-like behaviors. It assesses key features including rigidity to change habits, repetitive stereotypical behaviors and impaired social interactions. We used this technique to evaluate sex differences in these behaviors in pre-pubertal mice. Overall, we found no significant sex differences in wheel running behaviors in 3 strains of pre-pubertal mice.

In adult rats, assessments of wheel running have repeatedly shown higher levels of activity in females (Beatty, 1992), and also shown that wheel running activity is variable along the estrous cycle (Kent, Hurd, & Satinoff, 1991). Some studies of general activity and wheel running in adult mice have reported sex differences (Broida & Svare, 1984; Garland et al., 2011; Swallow et al., 1998), while others have not (Lamberty & Gower, 1988). Sex steroids do appear to influence wheel running in adult C57BL/6 mice, in which females exhibit greater activity (*i.e.*, distance and duration) than males at baseline; gonadectomy significantly reduced levels of wheel running (distance, duration, and velocity) in both males and females, which was at least partially recovered upon steroid replacement (Bowen et al., 2012). In addition, control Hsd:ICR mice showed sex differences in wheel running measures, including minutes per day and revolutions per day, and sex differences approached significance in average velocity (Garland et al., 2011). However, another study using C57BL/6 found no effect of estrous cycle on general activity in the open field assay (Meziane et al, 2007). With no previous data on pre-pubertal wheel running in mice, we explored the possibility of sex differences, which could result from differences in neural structure/circuitry of the sexes established perinatally. The similarity in wheel running behavior provides further evidence that wheel running behaviors may be linked to circulating sex steroids and differences only emerge after hormone levels increase following puberty. On the other hand, it is also plausible that a pre-pubertal sex difference in this

behavior exists, but the sensitivity of the behavioral test may be inadequate to detect a difference between males and females (Kelly, Ostrowski, & Wilson, 1999). It is worth noting that two of the strains underwent photoperiod phase shifting in the week before testing; however, this did not have noticeable effects on behavior in this assay, which was done in the dark phase for all strains.

We also found a lack of variability in social behaviors between the sexes. There were no significant sex differences in contact or following behaviors of the study mice with the novel mice. While it should be noted the study and novel mice used in the C57BL/6 strain were shipped in the same container prior to being separated for 13 days before the social interaction test, the study and novel mice in the other strains were shipped and housed separately to maintain novelty. Similar results were found across all strains for both contact and following behaviors. Social grooming, self-grooming, nose-to-nose sniffing, ano-genital sniffing, following and exploratory activity were exhibited by both sexes.

Another factor that was inherent to our study is the timing of puberty. Since the mice were not reared onsite, they were received following weaning around 3 weeks of age. Mice were given a week-long acclimation period, followed by the 6–7 day wheel-running assay. Perfusions were then performed between postnatal days 33 and 35. Puberty is the transitional period prior to adulthood that terminates with the production of mature gametes and the start of reproductive activity (Mayer et al., 2010). Sexual maturity is usually reached in females with the onset of estrus between 40–51 days, depending on weight and other environmental conditions (Pinter, 2007). Uterine weight and morphology are standard measures of estrogen action in immature female rodents (Evans, 1941). The timing of puberty in males is suggested to begin around day 40 (Albert, 1984). Considering the age of the animals, we can be relatively confident that our study was conducted before either sex reached sexual maturity. The variability in uterus weights suggests that some females might have been further along in puberty than others (Fig 5, C). Puberty occurs over time with the onset of neuroendocrine events that stimulate the pulsatile release of GnRH, which activates the pituitary/gonadal axis and directs gonadal steroid hormone production and the maturation of gametes (Colledge, Mei, & d'Anglemont de Tassigny, 2010; Mayer et al., 2010). Considering that increased gonadal hormone activity begins prior to sexual maturity at the conclusion of puberty, we cannot be certain that all animals were tested before the initiation of increased gonadotropic activity. However, with behavioral trials completed prior to P33 the influence of circulating gonadal hormones should have been minimal.

Both sexes showed characteristic wheel running behavior, spending the majority of each trial interacting with the wheel when it was free and more time performing other activities (*e.g.*, stereotypical behaviors, general locomotion) when it was jammed. Given that sex differences were not detected in this assay, the results show that baseline pre-pubertal sex-related differences are not strong enough to influence behavior in running-wheel studies. Furthermore, the inclusion of three strains provides evidence that these results apply more generally to the species, rather than to only one strain. We found differences among strains, including duration of time spent wheel running (or attempting to run) when it is both free and jammed. Interestingly, sex differences in wheel running have been found in adult mice related to two of our strains (C57BL/6: Bowen et al, 1992; CFW and Hsd:ICR both descend

from same Swiss-Webster stock: Swallow, Carter, & Garland, 1998), but we found no sex differences within any of our tested strains before sexual maturity. We conclude that sex-related behavioral differences can be discounted when designing future experiments to measure the effects of developmental insults on wheel running behavior in juvenile mice. Therefore, if sex differences in the running-wheel assay emerge after a developmental insult, one can make inferences regarding relative susceptibility of males vs. females to that treatment.

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Fig. 1. Wheel running behavior. (A–C) Latency of mice to run on wheel (mean sec $\pm$ SEM). A) C57BL/6, B) CFW, C) CF1; (D–F) Duration of wheel running (mean % total trial $\pm$ SEM). D) C57BL/6, E) CFW, F) CF1. * = first day the wheel was jammed. When the wheel is jammed, “wheel running” is defined as any activity that would make the wheel move if it were free.

Fig. 2. Social behaviors. (A–B) Latency of social interaction with novel mouse (mean sec $\pm$ SEM), analyzed for sex differences within strains. A) contact, C57BL/6, $p=0.215$; CFW, $p=0.134$; CF1, $p=0.447$. B) follow, C57BL/6, $p=0.765$; CFW, $p=0.942$; CF1, $p=0.595$. (C–D) duration of social interaction with novel mouse (mean % total trial $\pm$ SEM). C) contact, C57BL/6, $p=0.548$; CFW, $p=0.114$; CF1, $p=0.495$. D) follow, C57BL/6, $p=0.817$; CFW, $p=0.770$; CF1, $p=0.866$.

Fig. 3. Duration of stereotypical behaviors (mean % $\pm$ SEM). (A–C) burrowing: A) C57BL/6, B) CFW, C) CF1; (D–F) grooming: D) C57BL/6, E) CFW, F) CF1; (G–I) water nozzle interaction: G) C57BL/6, H) CFW, I) CF1. * = the first day the wheel was jammed.

Fig. 4. Duration (mean %) of all behaviors by sex and day. Note: WhInt, running or sitting on wheel; H2ONozzle, interact with water nozzle; Other, includes cage exploration and climbing on top of jammed wheel.

Fig. 5. Mouse morphology. A) Female initial and final body weight (g) by strain (mean $\pm$ SEM), B) Male initial and final body weight (g) by strain (mean $\pm$ SEM), C) Scatter plot of uterine weights (g), D) Scatter plot of paired testes weights (g). Note: Uterine weights were not collected for C57BL/6.

Table 1

Strain differences in duration of wheel running (mean % + SEM) on last day free (Last Free), first day jammed (Jammed 1), and second day jammed (Jammed 2).

Strain	Last Free	Jammed 1	Jammed 2
C57BL/6	69.9 ± 2.96 ^a	16.6 ± 0.73 ^b	4.9 ± 0.48 ^a
CFW	55.0 ± 1.86 ^b	13.6 ± 0.63 ^a	2.3 ± 0.52 ^b
CF1	69.0 ± 2.71 ^a	12.1 ± 0.84 ^a	7.7 ± 0.50 ^c

^{abc} Differing superscripts in a given column indicate statistically significant difference ($p < 0.05$) across strains in % duration wheel running (or attempt to run in wheel when jammed) for that column. Males and females did not differ within strains for this measure (Fig 1, D–F), so are both included in this analysis.