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Increased Juvenile and Adult Body Weights in BALB/cByJ Mice Reared in a Communal Nest

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Both wild and laboratory mice and rats preferentially rear their young in communal nests and indiscriminately nurse any of the young within the nest. In this study, BALBc/ByJ mice reared under communal nesting (CN) conditions (3 dams and their litters sharing a common nest) were compared with BALBc/ByJ mice raised in single (one dam with her litter) nests (SN) in body weight from birth into adulthood; food and water intake and body composition were compared between adult mice. Compared with SN female mice, female CN mice (measured only until weaning) exhibited significantly higher body weights at postnatal days 11 and 25. Male CN mice were significantly heavier than were male SN mice at postnatal day 25 and at 20, 26, and 30 wk of age. There were no differences between adult male mice from CN and SN groups in 48-h food and water intake or body composition (total lean:total fat ratio; measured by quantitative MRI). In conclusion, BALB/cByJ mice reared under communal nesting conditions showed more robust juvenile growth rates than did mice raised with a single dam and litter per cage. In addition, body weights of male CN mice remained higher than male SN mice into adulthood.

Abbreviations: CN, communal nesting; PND, postnatal day; SN, single nesting.

Communal nesting, a form of alloparenting in which 2 or more lactating female conspecifics rear their young within a common nest while sharing parental duties, occurs in many social species. In some species, communally nesting females also nurse offspring other than their own. Several rodent species including mice (*Mus musculus*) and rats (*Rattus norvegicus*) preferentially rear their young in communal nests and indiscriminately nurse any of the young within the nest.[4,6,10] In mice, communal nesting (CN) occurs ubiquitously in wild and laboratory populations when provided the opportunity.[1,3,11] In a 1995 study of a seminatural population of mice, 90% of dams nested communally.[6]

Most of the published literature on CN in mice attempts to explain the evolutionary and functional significance of communal nesting in wild mouse populations.[3-5] However, the drive to nest communally persists in domesticated mice despite the absence of obvious survival challenges in climate-controlled and predator-free laboratories. Relatively few studies[8,13] have explored whether the offspring of laboratory mice reared in CN exhibit important differences in physiology as juveniles or adults compared with mice reared in a single nest (SN; one dam and her litter in the cage).

Some laboratory studies have found that pups raised in CN (2 or more lactating mouse dams with their litters within a single cage) have greater preweaning growth rates[10] and heavier body weights at weaning[13] than do pups raised in a single nest. Other studies have found no effect of CN compared with SN on weaning weights in mice.[6,8] None of the published studies followed mice beyond weaning to determine whether weight differences were present in adults.

In the current study, BALBc/ByJ mice reared under CN or SN were compared in regard to body weight (from birth into adulthood), food and water intake, and body composition as adults.

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Materials and Methods

Animal care and use. Animal procedures were approved by the Pennsylvania State University (PSU) Institutional Animal Care and Use Committee and performed in AAALAC-accredited facilities.

Male and female BALB/cByJ mice were purchased at 6 wk of age from The Jackson Laboratory (Bar Harbor, ME) to be used for breeding in an unrelated experimental study. On arrival at our facility, male mice were housed individually and female mice were housed 3 to 4 per cage in animal rooms maintained at 18 to 24 °C and 30% to 70% relative humidity under a 12:12-h light:dark cycle in polypropylene cages (27 cm × 15 cm × 13 cm) with corncob bedding (Bed-o'Cobs, 1/4-in. , The Andersons, Maumee, OH) and stainless steel wire lids. Food (Labdiet 5001, PMI, St Louis, MO) and water were provided ad libitum. Dirty cages and bedding were cleaned weekly. The SPF health status of mice in this facility was monitored by sentinel surveillance. Mice were tested routinely and remained negative for common mouse parasites and pathogens, including ectoparasites, pinworms, pathogenic enteric protozoa, mouse hepatitis virus, Sendai virus, Theiler murine encephalomyelitis virus, *Mycoplasma pulmonis*, mouse parvovirus, murine minute virus, enzootic diarrhea of infant mice virus, pneumonia virus of mice, reovirus 3, ectromelia virus, and lymphocytic choriomeningitis virus throughout the study period.

At 8 wk of age, 1 male and 2 or 3 female mice were placed together in a single cage for breeding. An excess number of female mice were bred to allow for the delivery of sufficient numbers of litters of appropriate birth date, size, and composition to create CN and SN cages for the study. Male mice were removed after 10 d. Female mice remained together until visibly gravid, at which point each dam was housed individually until after parturition. Nesting material (Nestlets, Ancare, Bellmore, NY) was available in all breeding and experimental cages throughout the study period.

Neonatal manipulation. On the day of birth, the cage containing the dam and litter were removed from the cage rack to a table within the animal room. Each dam was placed in a clean cage while the pups in the litter were sexed and weighed. For SN cages, all the pups from a single litter and nesting material from the original cage then were placed in the clean cage with the dam and returned to the cage rack. SN litters consisted of 4 to 6 pups of both sexes (pups were culled or added by fostering if needed to bring the litter total to 4 to 6) with a mean dam:pup ratio of 1:5.

For CN litters, a similar procedure was followed, with the exception that 3 dams with viable litters of at least 3 pups were placed together in a clean cage. Pups were sexed and weighed, and then nesting material from each of the 3 litters plus, if needed, additional fostered pups from other litters were combined to make a total of 14 to 19 pups of both sexes (average dam:pup ratio, 1:5.2) per CN cage. All pups included in a single communal nesting cage had been born within a 24- to 48-h time span. Pups from different litters were placed at different areas of the communal nesting cage. The 3 dams were placed in the communal nesting cage, and the cage was returned to the cage rack.

The following day, pup numbers in SN and CN litters were counted again. A total of 3 pups (2 from CN litter 1 and 1 from SN litter 1) died overnight; 4 additional (same age) pups were fostered into CN 1 at this time. Pups then were left undisturbed except for routine husbandry procedures until postnatal day (PND) 11 to 12, at which point all pups were weighed, and the litters and dams in each CN and SN cage were transferred to a larger cage (44.5 cm × 24 cm × 15 cm) of similar composition and stainless steel wire lid with corncob bedding. Nest material from each cage was transferred with the pups.

SN pups were weighed and weaned on PND 25. In the CN cages, all pups in the cage were weighed and weaned on PND 25 of the youngest litter in the cage.

Experimental animals. Pairs of male weanlings were chosen randomly from each cage (that is, CN or SN) and housed together undisturbed until approximately 6 mo of age in polypropylene cages (27 cm × 15 cm × 13 cm) with corncob bedding (Bed-o'Cobs, 1/4-in., The Andersons) and stainless steel wire lids. Food (Labdiet 5001, PMI) and water were provided ad libitum. Pairs were chosen from multiple cages of each nesting condition in an attempt to limit disproportionate contribution of individual litters to CN and SN means. Excess weanling mice were used for other research studies or euthanized.

The SN experimental group consisted of a total of 14 male mice originating from 7 SN cages. One or 2 male pups from each of the SN litters were kept as experimental animals, with the exception of a single SN litter that contributed 4 male mice. The CN experimental group consisted of a total of 14 male mice originating from 5 CN cages: 2 male pups were kept from each of 3 CN cages and 4 male mice from each of 2 CN cages, for a total of 14 pups.

At approximately 26 wk of age, each experimental mouse was transferred to individual housing (under the same cage conditions as described previously) for food and water intake measurements. The mice remained in individual housing until euthanasia 25 d after transfer to individual housing.

Body weight measurements. Pups in each SN and CN cage were weighed within 24 h of birth (PND 1), at PND 11, and at PND 25 (weaning weight). Preliminary sexing of pups was attempted at birth, with definitive sex determination at PND 11. To ensure that pup ages were the same, only weight data obtained from CN litters 1, 2, and 4 were used to make statistical comparisons to SN pup weight for PND 1, PND 11, and PND 25. Pups in each of these 3 CN litters were born within a 24-h time span. CN litters 3 and 5 included pups born within 48 h of each other. Adult SN and CN male mice were weighed at 137 to 141 d (20 wk), 181 to 186 d (26 wk), and 206 to 210 d (30 wk) of age.

Body composition analysis. Body composition measurements were made on 30-wk-old mice in vivo by using a quantitative MR method (EchoMRI 100; Echo Medical Systems, Houston, TX). Prior to each quantitative MR run, the system was calibrated by using a known standard. To obtain measurements, each mouse was placed into a plastic cylinder (inside diameter, 4.7 cm), which then was inserted into the MR instrument. The scan time for each mouse was 80 to 90 s, after which they were returned to their home cages. The output information was expressed as lean tissue mass and fat mass in grams.

Food and water intake measurement. Food intake in 26-wk-old mice was estimated by weighing chow pellets before and after a 48-h period. Cages were inspected for spillage and uneaten food at the beginning and end of the procedure. Water intake was measured from 25-mL graduated tubes (0.2-mL graduations) from readings obtained at the beginning and end of the same time period.

Statistics. Data were entered into a spreadsheet (Excel, Microsoft, Redmond, WA), and differences between CN and SN groups were examined by Student *t* test (independent groups) or, where repeated measures were involved, ANOVA (one between-groups factor and one repeated measures factor) was conducted. These analyses were carried out within Excel or SPSS (version 18, IBM, New York, NY). The alpha level was set at a P value of less than 0.05 (2-tailed).

Results

Body weight and pup mortality. There was no statistically significant difference in body weight at birth between CN and SN groups or between male and female pups. The 3 CN dams consistently combined their pups into a common nest area within 15 to 20 min of placement into the communal cage. Statistically significant ($P < 0.05$) differences in body weight between female CN and SN mice were evident by PND 11 and in both male and female CN mice at weaning (PND 25). Female CN mice weighed more than did SN female mice at both PND 11 and 25, and male CN mice weighed more than did SN male mice at weaning (Table 1). There was no statistical interaction between sex and nesting condition (CN or SN) at either PND 11 or at weaning. In addition, 94% (73 of 78) of pups raised in CN survived to weaning, whereas 98% (44 of 45) of pups raised in SN survived to weaning. These proportions were not statistically different (95% confidence level, Z = 0.659).

Male CN mice had significantly ($P < 0.05$) higher body weights than did SN mice at all time points measured after weaning (Table 2). Body weight measurements were taken at the same time for all adult experimental mice. Mouse ages at the time of adult body weight measurements varied by a range of 5 d due to the variance in birth dates between litters. Adult mice were weighed at 20 wk (137 to 141 d) of age, 26 wk (181 to 186 d); and 30 wk (206 to 210 d). After the 26-wk measurement, mice were transferred to individual housing for estimation of food and water intake. All mice lost weight (average of 5% for both CN and SN groups) after this transfer. However, CN mice remained heavier than SN mice when measured at 30 wk of age.

Body composition and food and water intake. There were no differences in 48-h food and water intakes at 26 wk of age; data were corrected for body weight. At 30 wk of age, CN mice had significantly ($P < 0.05$) greater lean mass than did SN mice, but the lean:fat ratio did not differ between the 2 groups (Table 2).

Table 1. Body weight (mean ± 1 SD) of BALB/cByJ mice at birth, PND 11, and weaning

		Body weight (g) at					
		Birth	*n*	PND 11	*n*	Weaning	*n*
Communal nesting	Female	1.62 ± 0.26	31	7.23 ± 0.74[a]	35	11.76 ± 1.13[b]	35
	Male	1.53 ± 0.23	18	7.16 ± 0.64	12	12.26 ± 0.51[b]	12
	Total	1.59 ± 0.25	49	7.21 ± 0.71	47	11.89 ± 1.03[b]	47
Single nesting	Female	1.60 ± 0.20	22	6.80 ± 0.72	26	10.20 ± 1.31	26
	Male	1.49 ± 0.21	23	6.77 ± 0.66	18	10.87 ± 0.86	18
	Total	1.54 ± 0.21	45	6.79 ± 0.69	44	10.47 ± 1.18	44

Significant difference ([a]$P < 0.05$; [b]$P < 0.0001$) between CN and SN mice.

Table 2. Body weight (g; mean ± 1 SD) and body composition of adult male BALB/cByJ mice (*n* = 14)

			at 30 wk		
	Body weight at 20 wk	Body weight at 26 wk	Body weight	% Fat	% Lean
Communal nesting	30.72 ± 1.77[a]	33.39 ± 1.38[b]	31.72 ± 1.31[b]	13.04	78.13
Single nesting	29.09 ± 1.85	31.34 ± 2.18	29.76 ± 1.66	12.36	78.71

Significant difference ([a]$P < 0.05$; [b]$P < 0.0001$) between CN and SN mice.

Discussion

Mouse communal nests consist of 2 or more female mice rearing their young within the same nest space, and both wild and laboratory mice will nest communally when given the opportunity.[1,3,10] Parental duties, including nursing, are shared by all the mothers within the nest. Mouse pups raised in a CN, therefore, are influenced by multiple mothers with varying maternal physiology and behavior and by a large number of both sibling and nonsibling cohorts of often varying ages.

In laboratory settings, mouse growth rate and body weight at weaning tends to be inversely proportional to litter size.[2] Nonetheless, some studies[10,13] examining the effects of CN rearing on mouse growth rates have reported increased growth rates and weaning weights in mice raised in large litters under CN conditions compared with mice raised in SN litters. Typically in those previous studies, a uniform dam:pup ratio was maintained for both CN and SN conditions (by strictly limiting litter sizes).[10,13] However, other studies in which either the litter size was not reported or not controlled have not found significant differences in juvenile pup weight between CN and SN litters.[6,8] None of the previously published studies differentiated between growth rates in male and female mice, determined whether weight disparities between CN and SN persisted into adulthood, or evaluated the body composition variations that underlay these differences.

For the purposes of the current study, mean dam:pup ratios for both CN and SN groups were similar (1:5 for SN compared with 1:5.2 for CN), but litter sizes were unequal and varied within a predetermined range for each group. In our study, female BALB/cByJ pups reared in a communal nest (CN condition) weighed more at PND 11 and weaning than did female SN mice. Male BALB/cByJ pups reared in a CN weighed more at weaning than did SN mice and maintained this difference throughout adulthood until euthanasia at 30 wk of age. The increased body weight of CN male mice appeared to reflect comparable increases in both lean and fat compartments, according to body composition comparisons conducted by using MRI at 30 wk of age. Consistent with this finding, there was no difference in lean:fat ratio between CN and SN groups. Metabolic function appeared comparable in CN and SN groups, because food and water intakes did not differ between them once differences in body weight were taken into consideration.

Both CN and SN mice lost weight after transfer from pair to individual housing at 28 wk of age. Both groups lost an average of 5% of body weight in the 2 wk between the start of individual housing to reweighing at 30 wk of age. In mice, a change from group to individual housing may be stressful and results in a variety of physiologic and behavioral changes including loss of body weight, altered immune function, and decreased resistance to tumor growth.[9,12] In the current study, both CN and SN male BALB/cByJ mice responded equivalently with regard to body weight changes after this alteration in home cage environment.

Various hypotheses have been proposed to explain why mice and other species rear their young in communal nests. Some authors have suggested that CN behavior evolved as a social adaptation, allowing successful adult cohabitation in environments with either limited or unevenly distributed resources and not as a means to directly benefit offspring growth or survival.[3,5] As an example, man-made structures such as barns and granaries provide ample food and nesting sites for mice but within a limited physical space. Wild house mice inhabiting these environments can reach extremely high population densities[1] and form communal nests more frequently than do field dwelling mice,[3] whose populations tend to be of much lower density, presumably because resources in the field are dispersed more broadly.

However, this hypothesis does not explain the tendency for captive mice with unlimited food and water to prefer communal nesting, unless space is the primary limiting factor. If so, supplying more nesting space should result in decreased CN. This outcome does not appear to occur, as one study[6] found that wild-derived female mice living in captivity predominantly chose to raise their litters in communal nests even when unused nest boxes were available. In our current study, BALB/cByJ CN mice initially were housed in standard-size mouse cages with 3 dams and their litters per cage. All mice were moved to larger cages when the litters were 11 to 12 d old, but the dams continued, without exception, to maintain all the pups in a single nest.

Another hypothesis suggests that CN may occur in mice because it reduces pup mortality and as a result, improves reproductive success and evolutionary fitness in genetically related individuals.[4] Previous studies found that related female mice preferentially nest together and wean greater numbers of offspring in their lifetime than unrelated females who nest together.[4,7] Another found that female house mice living under seminatural conditions tended to establish communal nests with MHC-similar partners, [7] which could indicate a preference for genetic relatedness.

Nonetheless, genetic relatedness does not appear to be the only factor driving female mice to combine their nests with other dams'. Familiarity with another female mouse, regardless of relatedness, also improves the probability of reproductive success,[4] but unrelated and previously unfamiliar laboratory mice will rear litters communally and have been shown to wean more offspring over a 6-mo period compared with SN females.[4] In our study, all breeding female mice were of the same inbred strain and therefore MHC haplotype but were not necessarily familiar with each other prior to placement in a CN cage. There was no significant difference between CN and SN experimental groups in the proportion of pups surviving to weaning in the current study, but we calculated pup survival for only one litter per breeding female mouse. Calculations of lifetime reproductive success rates might better reflect possible advantages in pup survival due to CN.

A key component of CN in mice that could enhance pup survival is the occurrence of communal nursing. Female mice voluntarily nurse any pup within the CN. Lactation requires the expenditure of large amounts of energy; therefore nonoffspring nursing is costly and could potentially disadvantage a mouse's own offspring if the quantity or quality of milk provided to them decreased.[5] However, the potential benefits from nonoffspring nursing as it occurs during CN may outweigh the disadvantages. Pup growth rate and survival within CN could be enhanced if the increased number of nursing pups within the nest resulted in increased milk production by each mother or if the quality of available milk or the amount of nursing time each pup experienced was increased due to the presence of multiple dams.

In our study, with similar dam:pup ratios for CN and SN litters, there was no difference in survival between CN and SN, but CN pups were heavier than were SN pups as early as PND 11 and continued to weigh more than SN mice throughout adult life. A previous study[13] that raised both C57BL/6J and A/J pups under CN conditions found that CN pups were heavier than SN pups at weaning on PND 21. Another study using BALB/c mice[11] found that CN pups spent more time nursing than did SN pups until PND 14 and weighed more than SN pups at weaning. This difference occurred even though CN dams had less mammary development while nursing equivalent pup numbers. In addition, the caloric content of milk did not differ between CN and SN mothers, and individual CN mothers spent less total time nursing than did SN mothers.[11]

Increased thermoregulatory stability due to the presence of larger numbers of pups and dams potentially could enhance pup growth rate and survival within a CN nest. A previous study[11] partially tested this hypothesis by housing a virgin female mouse with a dam and her litter. The virgin female mouse assisted the dam in nest building, hovering over and retrieving of pups, but did not lactate. The presence of the virgin female mouse did not affect the growth rate of the pups in regard to body weight at PND 9. In fact, the PND 19 body weights of pups housed with their dam and a virgin female mouse tended to be decreased when compared with those of a single dam and an equivalent size litter.[11]

The presence of greater numbers of pups huddled together in the CN perhaps contributes to improved thermoregulatory stability in a CN litter. However, if that idea were true, SN litters with greater numbers of pups would be expected to show increased growth rate and survival. However, in a previous study,[11] PND 9 and 19 body weights of SN litters consisting of 14 pups and 1 dam were lower than those of SN litters of 7 pups and 1 dam, even though mammary gland development appeared to be greater in the dam raising 14 pups.

In summary, although speculation abounds, the functional and evolutionary significance of communal nesting in mice and other species has not been explained definitively. The current study demonstrated that male and female BALB/cByJ mice reared under communal nesting conditions showed more robust juvenile growth rates than did mice raised with a single dam and litter per cage. In addition, body weights of male CN mice remained higher than those of male SN mice into adulthood. Higher adult body weights in male CN mice were not due to increased caloric consumption as adults, and percentages of fat and lean in total body composition between CN and SN mice were not different. Whether these results are true for other inbred strains remains to be seen. Future studies likely should further examine the differential effects of variations in preweaning social environment on the physiology and behavior of mice and how genetic background influences such effects.

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