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Establishing New Isosexual Pairs in Adult Male Guinea Pigs (*Cavia porcellus*) to Facilitate Social Housing

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Guinea pigs (*Cavia porcellus*) are a commonly used species in biomedical research. As social creatures, compatible guinea pigs should be housed together unless scientific objectives or veterinary care require otherwise. Extensive literature suggests that adult male guinea pigs are highly aggressive in the presence of females, but data are lacking regarding the compatibility of cohoused adult males in the absence of females. Most studies that use adult males do not report housing densities. We used serial wound scoring and observations of behavior to determine whether unfamiliar adult male guinea pigs will develop stable, prosocial isosexual pairs. Wound scoring was performed before and 24 h after pairing. Serial behavioral observations assessed affiliative and agonistic behaviors at 0.5, 2, 24, and 48 h after pairing. Wound scoring and behavioral observations continued weekly for 1 mo and monthly thereafter. Wound scores were significantly higher at 24 h after pairing as compared with baseline and all other time points. Wounding was rare after week 2, indicating reduced aggression. Furthermore, affiliative behaviors significantly increased over time while agonistic behaviors were rare. Together, these data suggest that unfamiliar adult male guinea pigs establish stable prosocial pairs after an acclimation period. As was done in the present study, providing ample space, separate shelters for each animal, and the absence of female guinea pigs will likely facilitate successful pairing. We recommend consideration of a social housing program for adult male guinea pigs to provide companionship and enrich their housing environment.

Abbreviation and Acronym: GP, guinea pig(s)

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Introduction

Domesticated guinea pigs (GP; *Cavia porcellus*) have been used for over 200 y in biomedical research and continue to be valuable models of human disease; this use can be attributed to their gentle temperament, commercial availability, and extensive historical uses in research.[59] GPs are used as models in many disciplines including cardiology, immunology, infectious disease, nutrition, otology, and pharmacology.[59] The outbred Dunkin Hartley GP is an established and common model of progressive, chronic, and adult-onset diseases, including osteoarthritis, Alzheimer disease, tuberculosis, and asthma.[1,64] Many of these conditions have strong sex predilections, with more pronounced phenotypes in adult males.[1,64] Models that require the use of adult male GPs pose difficulties in balancing scientific objectives and providing species-appropriate social housing.

GPs are social creatures that thrive in groups.[46] They tend to eat communally, lay together, and interact frequently with members of their group.[3,9] The *Guide for the Care and Use of Laboratory Animals* recommends cohousing social species like GPs unless a scientific exemption, medical reason, or individual incompatibility exists.[27,46] A social environment improves animal welfare by promoting species-specific behaviors and provides social support during stress that can be inherent to some studies. This benefit is well described across a variety of species, including GPs and other rodents.[35,45,48,50,54,57] In the wild, *Cavia aperea*, one of the most widely studied *Cavia* species, typically live in communities consisting of one male and several females.[2,50] Once offspring reach sexual maturity, which occurs at about 2 mo of age in females and 3 mo of age in males, the males leave the group.[46,50] Some *Cavia* species show variations in this social structure that include the presence of a smaller sexually mature male in a restricted range of the primary social group and roaming males waiting at a group's periphery until the existing dominant male dies or can be overthrown.[2] Although previously thought to descend from *Cavia aperea*, domesticated GPs are most closely related to *Cavia tschudii*; the natural social structure of this *C. tschudii* has not been described but may mirror one of the many systems described in other *Cavia* species.[61] Both sexes of GP can be housed in iso- or heterosexual groups before sexual maturity.[6,32,40,46,58] Existing literature suggests isosexual housing of adult male GPs is associated with significant aggression and may not be possible in a research setting, particularly if the male is reared with or cohoused in proximity to females.[36,51,52,59] The creation of de novo adult male social pairs in a biomedical research setting has not yet been systematically assessed. Furthermore, published studies that use adult male GPs generally do not report housing

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details. These factors, along with personal communications between the authors and the vendors who recommend individual housing of retired male breeders, may discourage attempts to pair-house sexually mature males.

The goal of this study was to assess the compatibility of 24 previously unfamiliar adult male Dunkin Hartley GPs who were placed in new social pairs after arrival at our facility. These GPs were concurrently enrolled in a long-term approved primary study. Given their social nature and enrollment in a long-term study, isosexual pair housing was attempted and evaluated to promote species-specific behaviors and animal welfare. To evaluate the stability of isosexual pairs, we performed serial wound scoring and observations to assess fighting, affiliative, and agonistic behaviors.

Materials and Methods

Literature review. To assess the prevalence of social housing in adult male GPs, we conducted a systematic literature review. First, we performed a PubMed search using the following search criteria: ("guinea pigs"[Majr] OR "guinea pig*"[ti] OR guineapig*[ti] OR "cavia porcellus"[ti] OR "c porcellus"[ti] OR "cavia aperea"[ti] OR "domestic cavies"[ti]) NOT ("Systematic Review"[Publication Type] OR "Meta-Analysis"[Publication Type] OR "Guideline"[Publication Type] OR "Editorial"[Publication Type] OR "Comment"[Publication Type]). Results were filtered to include articles in English and those published in the last 5 y (date range 5/17/2018 to 5/17/2023), yielding 1,121 articles for further analysis. Results were exported in PubMed format and uploaded to Covidence for a 2-step review process: screening and final review. Covidence (Veritas Health Innovation, Melbourne, Australia, www.covidence.org) is a web-based collaboration software platform that streamlines the production of systematic and other literature reviews. For screening, articles were excluded that did not use live GPs (examples include in vitro and ex vivo work, computational studies, and published protocols or methodologies), were not primary research (i.e., corrections, review articles, and case reports), involved client-owned or wild populations of GPs, could not be obtained, or involved only short-term terminal procedures or tissue harvest. Two hundred and ninety-five articles were excluded during the screening process. For the final review, methodology sections were assessed for descriptions of GP sex, age, and housing density. Articles that described group housing of adult male GPs were included. Adult male GPs were defined as at least 3 mo of age or weighing at least 600 g at the study start.[46,50] The systematic literature review is summarized in Figure 1 according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and adapted from the autopopulated schematic from Covidence.

Animals and husbandry. Five- to six-month-old male, intact Dunkin Hartley GPs (n = 24) were purchased from an AAALAC-accredited commercial vendor (Elm Hill Labs, Chelmsford, MA) and housed in an AAALAC-accredited facility in accordance with the Animal Welfare Act, PHS Policy on Humane Care and Use of Laboratory Animals, and *Guide for Care and Use of Laboratory Animals*.[8,14,27] Standard housing practices at the vendor included individual housing of adult male GPs whenever they were not actively participating in breeding (personal communication). The vendor routinely establishes breeding harems (1 male with 4 or 5 females) when GPs reach

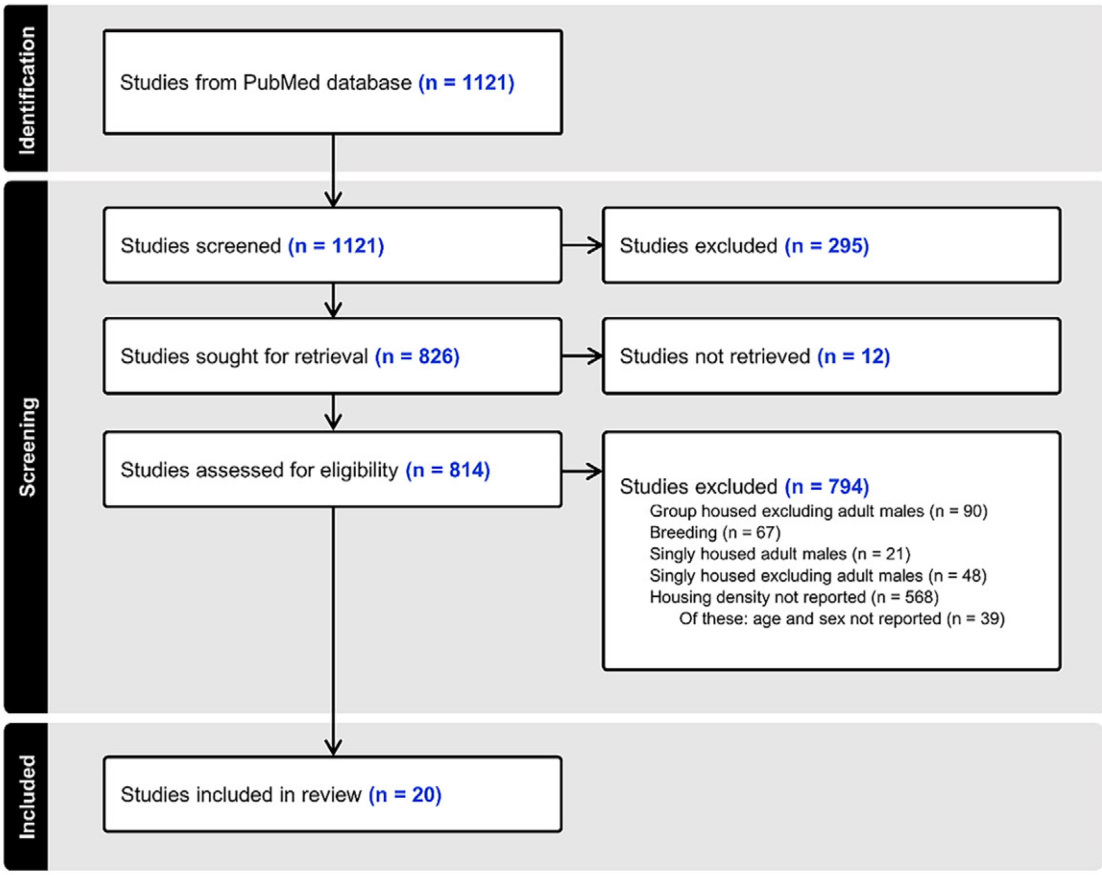

Figure 1. Schematic of literature review process.

5 wk of age. None of the males we procured had been housed together at the vendor after reaching sexual maturity. The exact breeding history or genetic relationship of the GPs included in our study was not known. Because adult males had been housed singly or with breeding females at the vendor, the ones used in our study were deemed to be unfamiliar; however, we cannot rule out the possibility that some of the males were littermates and therefore had lived together with their dam before weaning (approximately 4 mo before the present study). GPs were negative for Sendai virus, *reovirus 3*, lymphocytic choriomeningitis virus, pneumonia virus of mice, *Encephalitozoon cuniculi*, *Salmonella*, *Bordetella bronchiseptica*, *Streptococcus* species, *Streptococcus pneumoniae*, and common endoparasites and ectoparasites based on vendor report.

All GPs were assigned to a primary research protocol and were concurrently enrolled in the social housing study described herein. The primary research protocol focused on assessing osteoarthritis as the GPs aged; this condition occurs naturally in Dunkin Hartley GPs.[38] All animal use had been approved by the Medical University of South Carolina IACUC. The sample size ($n = 24$) was based on that of the primary study, which was powered to meet study objectives; on arrival to our facility, all GPs were paired with a conspecific for inclusion in this secondary study assessing social compatibility. Therefore, the sample size for the present social housing study was not determined via power analysis. After arrival to our facilities, GP were paired as described below. They then received a 7-d acclimation period before beginning the primary study.

All GPs were housed in standard plastic GP drawer-style enclosures (24-in. × 32-in. × 10-in., Allentown) with suspended floors. Enclosures included 2 shelters (either plastic hut or plastic igloo) and one enrichment manipulandum that was rotated weekly (nylon bone, wooden block, or small ball). GPs had access to timothy hay (Kaytee), Certified GP Diet #5026 (LabDiet), and reverse osmosis filtered water ad libitum. A rotation of supplemental vegetables was provided approximately 5 times per week. Sanitization schedules were as follows: enclosures every other week, accessories weekly, and cage pans with noncontact paper chip bedding 3 times per week. Macroenviroment parameters were as follows: 12:12 light:dark cycle (on at 0600 and off at 1800), temperatures between 68 and 79 °F (20 to 26 °C), and 30% to 70% humidity.

Experiment housing. Standard shipping crates that were used for animal transport consisted of a solid, opaque, thick cardboard partition separating 2 GPs per crate. Crates allowed olfactory and auditory cues but prevented visual or physical contact. The GPs were shipped directly from the vendor to our facility. When the GPs arrived from the vendor, they were examined by an American College of Laboratory Animal Medicine-certified veterinarian for preexisting conditions or wounds before pair housing. After this examination, GPs were paired with the conspecific from the same shipping crate.

Wounds scoring. GPs were assessed individually without sedation for the presence of wounds. Initial assessment occurred immediately before pair housing (i.e., at receipt). Subsequent wound scoring occurred 24 h and at 1, 2, 3, and 4 wk after pair housing and monthly thereafter until they reached endpoints related to their primary study. Wounds were counted, and their individual location and severity were recorded at each time point. Wound locations were recorded as mouth, pinna, remaining face (all areas of the face other than mouth or pinna), dorsum, ventrum, genitalia, forelimbs, or hindlimbs. Wound severity was scored according to the following criteria: 0, no wounds; 1, 'punctate' superficial wounds not penetrating the dermis; 2, 'punctate' wounds penetrating the dermis; or 3, lacerations (Figure 2). Each individual wound on a given animal received a severity score of 0 to 3. Severity scores corresponding to each wound were then summed for a single animal, yielding the total wound score. A total of 9 wound examinations were performed on each guinea pig during the 4-mo study. All identified wounds healed by secondary intention without the need for systemic antibiotics or primary closure.

Behavioral observations. Behavioral observations were conducted to assess for affiliative and agonistic behaviors in the pairs. One of 2 trained observers stood approximately 1 to 2 m from the front of the enclosures and observed the pair for 10 min for the presence or absence of the target behaviors.[5,11] Behavioral observations were conducted for 10-min periods at 0.5, 2, 24, and 48 h and at 1, 2, 3, and 4 wk after pair housing and monthly thereafter until they reached endpoints related to their primary, concurrent study. All observations were made in the morning, except for 0.5 and 2 h after pairing, as that timing depended on the time at which the GPs were delivered to the facility. Observers recorded displayed affiliative behaviors

A

Wound Severity Score	
Score	Criteria
1	Superficial punctate wound
2	Punctate wound penetrating the dermis
3	Laceration

B

C

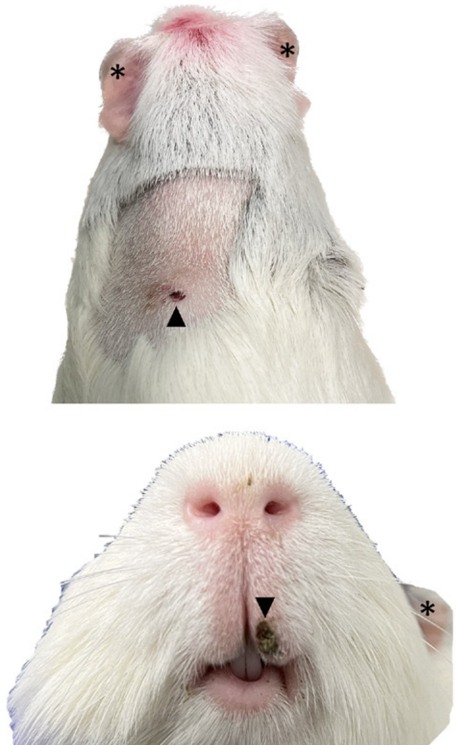

Figure 2. Wound severity scoring. (A) Criteria for assigning wound severity scores. Severity of each individual wound was scored based on these criteria and summed to yield a wound score for each animal. Images of wound severity scores from representative wounds, including (B) a superficial, punctate wound not penetrating the dermis (severity score 1) and (C) a punctate wound penetrating the dermis (severity score 2). Ears are marked with an asterisk (*) for orientation.

Table 1. Ethogram for recording behavioral observations

Category	Behavior	Operational definition
Affiliative	Proximity	Present within the same quadrant of the enclosure
	Social contact	Physically contacting one another, including the heads, limbs, or sides of the animals touching
	Allogrooming	Grooming a conspecific with their mouth or tongue
	Shared shelter	Physically present within the same shelter
	Eating together	Simultaneously eating from the same feeder or hay ration
Agonistic or dominant	Chasing	One animal displacing the location of another by chasing
	Chattering	Low pitch, rapid noise made by repetitive contact of the teeth
	Biting	Wounding or attempting to wound the skin of a conspecific with the mouth
	Mounting	Placing one or more forelimbs on the back of a conspecific

To receive a score of '1,' affiliative behaviors must have been present for more than 1 s (i.e., passing by one another while in motion did not reach the threshold for proximity or social contact).

(proximity, social contact, allogrooming, sharing a shelter, and eating together) and/or displayed dominance or agonistic behaviors (chasing, chattering, biting, and mounting) as described in Table 1. The presence or absence (i.e., zero or one) of each behavior was recorded per pair; the frequency, duration, and initiator of the behavior were not noted. Each behavior observed during a given session received a score of '1.' To receive a score of '1,' affiliative behaviors must have been present for more than 1 s (i.e., passing by one another while in motion was not scored as proximity or social contact). Observed affiliative behaviors were summed, yielding a cumulative affiliative score for each pair. Observed agonistic or dominant behaviors were summed, yielding a cumulative aggression score for each pair. Affiliative and agonistic behaviors included in the ethogram have been previously described in GPs;[4,9,36,40,49,53] however, our scoring system does not include all such behaviors described for GP. A total of 11 observation sessions occurred during the 4-mo study.

Sample size attrition. One pair of GPs were removed from this study and singly housed approximately 1 h after pairing due to incompatibility. A wound score of greater than 10, the presence of lacerations (a wound score of 3; Figure 2A), or prolonged agonistic behaviors were used as objective measures of incompatibility. One animal in the incompatible pair had a wound score of 11 at 1 h after pairing; this score included a single laceration (one wound with a severity score of 3). This pair was used only to determine the frequency of successful pairing within the cohort. Due to the limited data collected from this pair (receipt and 0.5 h after pairing), their data were excluded from all other analyses. Additional pairs were removed at various time points if at least one animal in the pair reached endpoints related to their primary, concurrent study. The number of pairs present at each time point after cohousing was as follows: 11 pairs from 0.5 h to 3 wk; 10 pairs at 4 wk; 7 pairs at 2 mo; and 6 pairs at months 3 and 4.

Statistical analysis. Data were analyzed using Prism 9.4.1 (GraphPad Software, San Diego, CA) and SAS v9.4 (SAS Institute, Cary, NC). Generalized linear mixed-effects models that assumed an underlying Poisson distribution for the analyses of behavioral counts over time were used to account for clustering of outcomes within pairs and within animals. Time was treated as a continuous variable in all analyses, and random effects were used for animal and pair identifiers. The random animal effects helped account for within-animal clustering of repeated measurements over time, while the random pair effects helped account for within-pair clustering. Generalized linear mixed-effects models use all available data, and inferences based on them are valid if data are 'missing completely at random,' which is the situation in our study because the absence of data points was due to the scheduled endpoints associated with the primary study rather than to any observed factors such as their behavior or degree of wounding.[26] Due to nonlinearity in analyzing wound scores over time, 2 separate models were constructed: one based on 0.5 to 24 h, and one based on 24 h to 4 mo. All hypothesis testing was 2-sided ($\alpha = 0.05$), and Bonferroni adjustments were used to correct for multiple comparisons.

Results

Literature review of social housing in adult male GPs. The PubMed search using the terms mentioned above yielded 1,121 articles. Of these, 295 were excluded during the screening process because they met exclusion criteria and 12 were not accessible. Eight hundred and fourteen articles passed the screening phase and went on to the final review. Only 20 articles (2.5%) described group housing of adult male GPs (Table 2).[4,7,13,15,16,18,19,22-24,31,34,39-42,56,60,65,66] Eight of these had obtained the GP from an onsite breeding program, 7 obtained GP from a commercial vendor, one received GP from another institution, and 4 did not report the source of the GP. In one study, male GPs were castrated after arrival from the commercial vendor and prior to group housing.[27] Twenty-one publications (3%) reported that male GPs were housed individually. The vast majority of articles (n = 568, 70%) did not report housing density; 39 of these also omitted age and sex. Sixty-seven (8%) publications involved breeding (either mating or receipt of time pregnant dams that were subsequently nursing offspring), and 90 (11%) reported group housing of females of any age and juvenile males. Finally, 48 (6%) publications described individual housing of juvenile GP and adult females or did not provide the sex (n = 5) or age (n = 1) of singly housed GP (Figure 3).

Observed affiliative behaviors. We observed 159 affiliative behaviors among the paired GPs during the 11 observation sessions (Figure 4A). The most common affiliative behaviors observed were proximity (78 total events) and social contact (60 total events; Figure 4A). The pairs showed wide variation in the number of observed affiliative behaviors. However, despite this variation, the number of prosocial events observed increased significantly ($t = 2.47$, $P = 0.02$) over time (Figure 4B) based on a generalized linear mixed-effects model.

Observed aggressive behaviors. Agonistic or dominant behaviors, jointly referred to hereafter as aggressive behaviors, were rare, with 12 total events observed among the pairs across the 4-mo study (Figure 5A). Prevalence of these behaviors did not correlate with the duration of pair housing. The initiator of the aggressive behavior was not recorded. Of the behaviors observed, mounting (n = 4), chasing (n = 3), and chattering (n = 3) were the most common (Figure 5A). Among the 11 pairs, 6 were never observed to show any agonistic behaviors. Three

Table 2. Studies that documented group housing in adult male guinea pigs

No. of adult males	Age	Weight (g)	Source of animals	Housing density	Strain	Reference
25	N/R	500–700	Commercial vendor	2	N/R	4
4[a]	Approximately 2 y	N/R	N/R	2	Dunkin Hartley	7
31	N/R	151–900	Onsite breeding colony	N/R	13/N	13
48[b]	7–20 wk	350–1,000	Onsite breeding colony	Up to 8	Dunkin Hartley	15
30[b]	10–12 wk	435–849	Onsite breeding colony	2–4	Dunkin Hartley	16
24	4–12 mo	N/R	N/R	2–3	Rosetta, Angora	18
15	N/R	600–1,200	Commercial vendor	1–2	N/R	19
41	16 wk	N/R	Commercial vendor	5–12	Dunkin Hartley	22
11	2–9 mo	430–950	Onsite breeding colony	N/R	N/R	23
17	N/R	400–1,200	Onsite breeding colony	N/R	N/R	24
24	3 mo	N/R	N/R	2	Dunkin Hartley	31
20	3–12 mo	N/R	Commercial vendor	N/R	Dunkin Hartley	34
18	14–22 mo	N/R	Onsite breeding colony	9	N/R	39
30	N/R	867–922	Onsite breeding colony	10	N/R	40
30	6–24 mo	N/R	Onsite breeding colony	10	N/R	41
26	6 mo	896–1,071	Commercial vendor	2–3	Dunkin Hartley	42
14[a,b,c]	12–24 mo	832–1,132	Commercial vendor	N/R	Dunkin Hartley	56
3	7–11 wk	503–525	Commercial vendor	N/R	Dunkin Hartley	60
96	6 mo	550–650	Another institution	4	Dunkin Hartley	65
2	N/R	400–750	N/R	N/R	N/R	66

N/R, not reported.
[a]Retired breeders.
[b]Includes unspecified number of males and females.
[c]All males were castrated.

pairs showed aggression at 0.5 h, with 2 of these pairs also showing aggression during follow-up observations. Two pairs showed no aggression at 0.5 h but did show at least one instance of aggression during follow-up. During each observation session, 70% or more of the pairs displayed no agonistic behaviors (agonistic score of 0; Figure 5B). At any given observation session, no more than 2 agonistic behaviors were observed within a pair, resulting in a maximum observed agonistic score of 2. Furthermore, an agonistic score of 2 was only observed within the first 2 h after pairing. Thereafter, pairs either displayed no or only one agonistic behavior during observation sessions, indicating that the frequency and severity of agnostic behaviors decreased over time (Figure 5B).

Wound scores and severity. All wounds were counted and scored for individual GPs. A total of 72 wounds were observed during 9 scoring sessions, with the greatest number of wounds being present at 24 h after pairing (n = 43) (Figure 6A). Fewer wounds were noted 1 wk after pairing and were rarely seen thereafter (Figure 6A). Wounds were most commonly present on the mouth (n = 23), dorsum (n = 25), and the remainder of the face (excluding the mouth and pinna) (n = 14; Figure 6A). No wounds were present on the genitalia at any time point, and wounds were rare in other locations. Eight of the 11 pairs (73%) presented with wounds at 24 h after pairing. The remaining 3 pairs (27%) showed no wounds at any time. Severity scores for all wounds seen on a given animal were summed at each time point, yielding a total wound score. The total wound scores were significantly higher at 24 h after pairing as compared with wound scores obtained immediately before pairing (t = 3.45, P = 0.002; Figure 6B). Wound scores then slowly tapered off over time, reaching significantly (t = 7.21, $P < 0.0001$) lower levels at all subsequent time points compared with the 24-h score (Figure 6B). Means differences from the 24-h score were 2.1, 2.4, 2.5, 2.6, 2.6, 2.6, and 2.6, respectively,

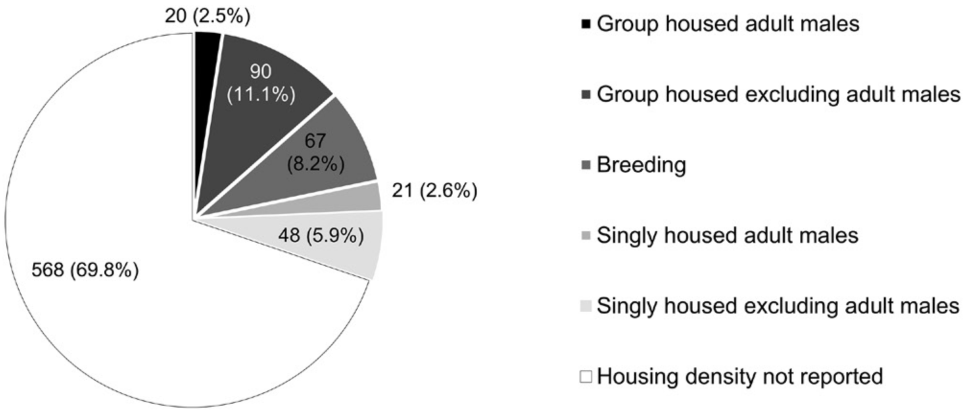

Figure 3. Literature review results assessing prevalence of group housing of adult male guinea pigs in research settings.

A

Number of Pairs Displaying Affiliative Behaviors						
Time After Pairing	Proximity	Social Contact	Shared Shelter	Allogrooming	Eating Together	Number of Pairs
0.5 hr	6	5	0	0	0	11
2 hr	7	6	0	0	0	11
24 hr	5	3	2	0	0	11
48 hr	9	6	2	0	0	11
Week 1	9	9	1	1	0	11
Week 2	9	6	0	0	0	11
Week 3	10	6	3	0	2	11
Week 4	6	6	3	0	1	10
Month 2	7	6	0	1	1	7
Month 3	5	3	0	0	1	6
Month 4	5	4	0	0	3	6

B

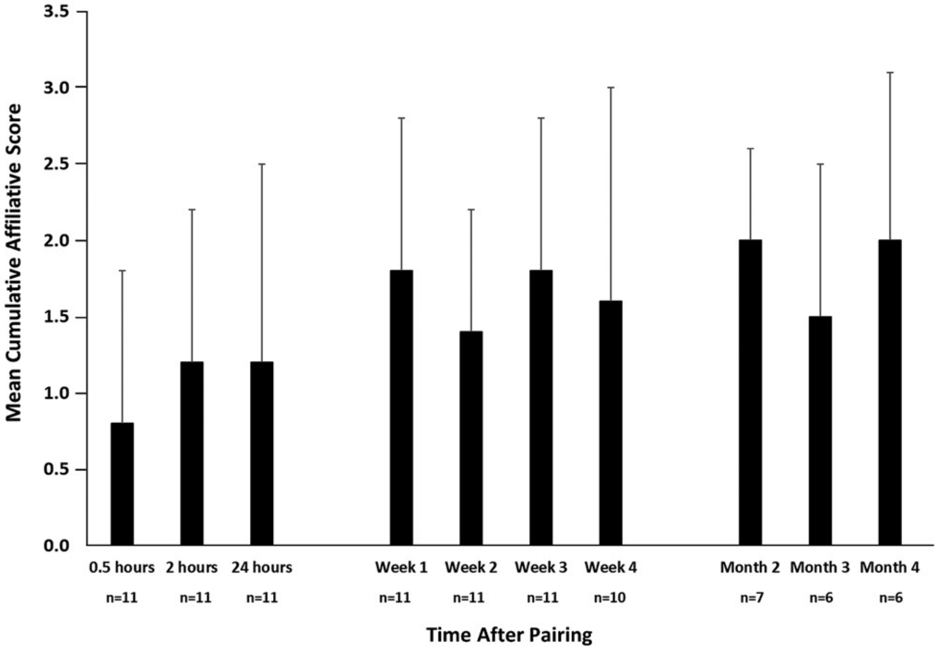

Figure 4. Observed affiliative behaviors within pairs. (A) The number of pairs displaying affiliative behaviors at each observation session. The final column indicates the number of pairs observed at each time point. Otherwise, rows represent a time point and columns represent behaviors assessed in the ethogram. (B) Mean cumulative affiliative scores at each time point after pairing. Bars represent standard deviation.

for weeks 1, 2, 3, and 4 and for months 2, 3, and 4. Of the 72 wounds observed, 83% ($n = 60$) received a wound score of one, indicating that the wounds were typically minor and superficial despite conspecific fighting (Figure 6C). Twelve wounds rated a severity of 2 (17%; Figure 6C). One pair of GP was deemed to be socially incompatible approximately 1 h after pairing due to a cumulative score of 11 in one GP, including a single wound with a severity of 3 and frequent agonistic behaviors. None of the other GPs had a wound severity score of 3 throughout the study (Figure 6C). The incompatible pair was used only to determine the frequency of successful pairing within the cohort (11 of 12 pairs, 92% success rate). Due to the limited data collected from this pair (receipt and 0.5 h after pairing), their data were excluded from all other analyses.

Discussion

This study demonstrates the successful establishment of new, compatible social pairs in 11 of 12 pairs (92%) of adult male Dunkin Hartley GPs. The need for a systematic evaluation of compatibility in previously unfamiliar adult male GPs is indicated by 1) the paucity of peer-reviewed studies reporting social housing in adult male GPs (2.5% of the studies assessed); 2) the large number of reviewed publications that omit details of GP housing density, age, and/or sex (69.8% of studies); 3) existing literature, including the *Laboratory Animal Medicine* textbook, indicating that social housing of adult male GPs may be unfeasible in a research setting; and 4) vendor recommendation to singly house adult males (personal communication).[36,51,52,59] The failure to report subject and housing parameters persists despite increasing pressure to improve rigor and reproducibility in animal research and the widespread endorsement of reporting standards by journals and funding agencies.[43] The ARRIVE Guidelines, which have been endorsed by over 1,000 journals, include a comprehensive checklist of experimental and methodological parameters that should be reported in publications.[43] Because these guidelines were first

A

Number of Pairs Displaying Dominant or Agonistic Behaviors					
Time After Pairing	Chasing	Chattering	Biting	Mounting	Number of Pairs
0.5 hours	2	2	0	0	12
2 hours	1	1	0	1	11
24 hours	0	0	0	0	11
48 hours	0	0	0	0	11
Week 1	0	0	0	1	11
Week 2	0	0	0	0	11
Week 3	0	0	0	0	11
Week 4	0	0	1	0	10
Month 2	0	0	1	1	7
Month 3	0	0	0	0	6
Month 4	0	0	0	1	6

B

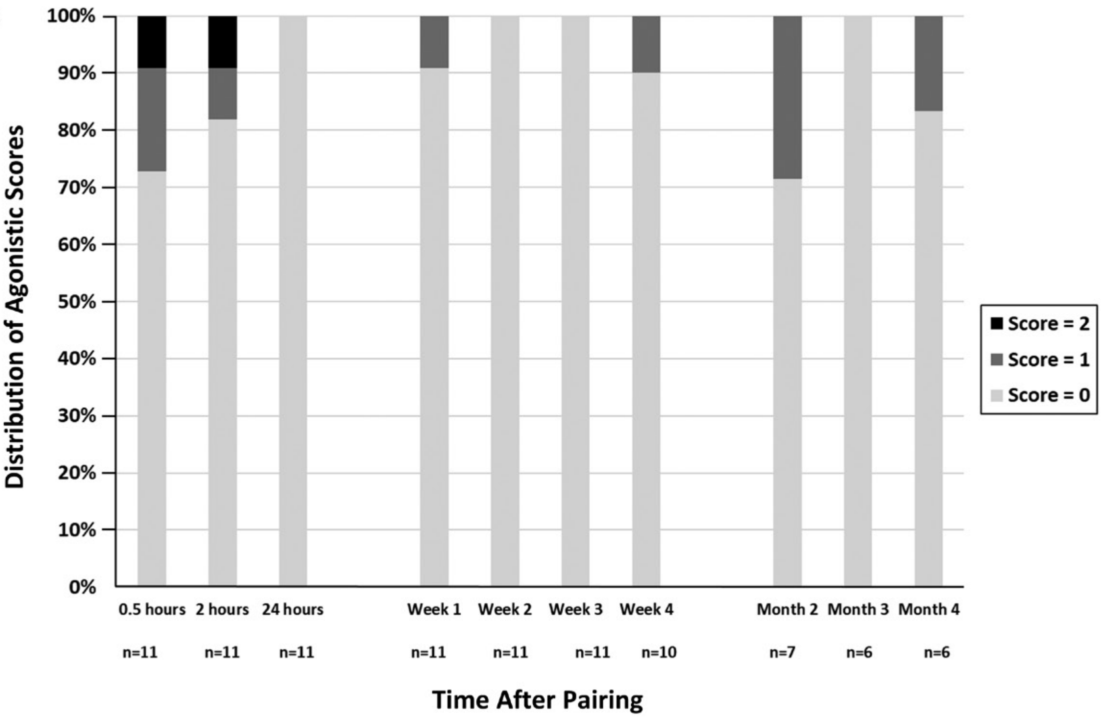

Figure 5. Observed dominant or agonistic behaviors within pairs. (A) The number of pairs showing dominant or agonistic behaviors at each observation session. The final column indicates the number of pairs observed at each time point. Otherwise, rows represent a time point and columns represent behaviors assessed in ethogram. (B) Distribution of agonistic scores based on the percentage of pairs at each observation session that received a score of 0, 1, or 2.

published in 2010, we reviewed publications from the last 5 y, assuming that older studies would be more likely to lack information on housing density, age, and/or sex. Although animal age and sex are included in the 'essential 10' reporting components and housing and husbandry details are recommended in the ARRIVE Guidelines, 568 of the studies we assessed did not report at least one of these parameters, and 39 of these failed to report all 3.[43] Only 20 of the studies we reviewed reported social housing in adult males.[4,7,13,15,16,18,19,22-24,31,34,39-42,56,60,65,66] Eight of these had onsite breeding programs, which can facilitate the establishment of isosexual groups in juvenile males or littermates before their use as adults.[13,15,16,23,24,39-41] In addition, one study that obtained male GPs from a commercial vendor castrated them before creating social groups;[56] hormonal alterations associated with castration may improve the creation of isosexual groups and be a viable option when scientific objectives do not require reproductively intact GP. Familiarity with cohoused conspecifics before puberty, including genetic relation or age at introduction, was not described in any of the publications, making the present study the first to report the de novo creation of stable isosexual pairs in adult male GP and to systematically evaluate conspecific interactions for prosocial and aggressive behaviors.

In the present study, we achieved social housing in stable pairs despite their history of individual or harem (1 male: multiple females) housing at the commercial vendor. Upon arrival at

A

Number of Lesions and Locations									
Time After Pairing	Mouth	Pinna	Remaining Face	Dorsum	Ventrum	Genitalia	Forelimbs	Hindlimbs	Number of Animals
Receipt	0	1	0	1	0	0	0	0	22
24 hours	17	0	8	13	2	0	2	1	22
Week 1	3	0	6	8	0	0	1	0	22
Week 2	0	0	0	2	0	0	0	2	22
Week 3	0	0	0	0	0	0	0	0	22
Week 4	1	0	0	1	0	0	0	1	20
Month 2	2	0	0	0	0	0	0	0	14
Month 3	0	0	0	0	0	0	0	0	12
Month 4	0	0	0	0	0	0	0	0	12

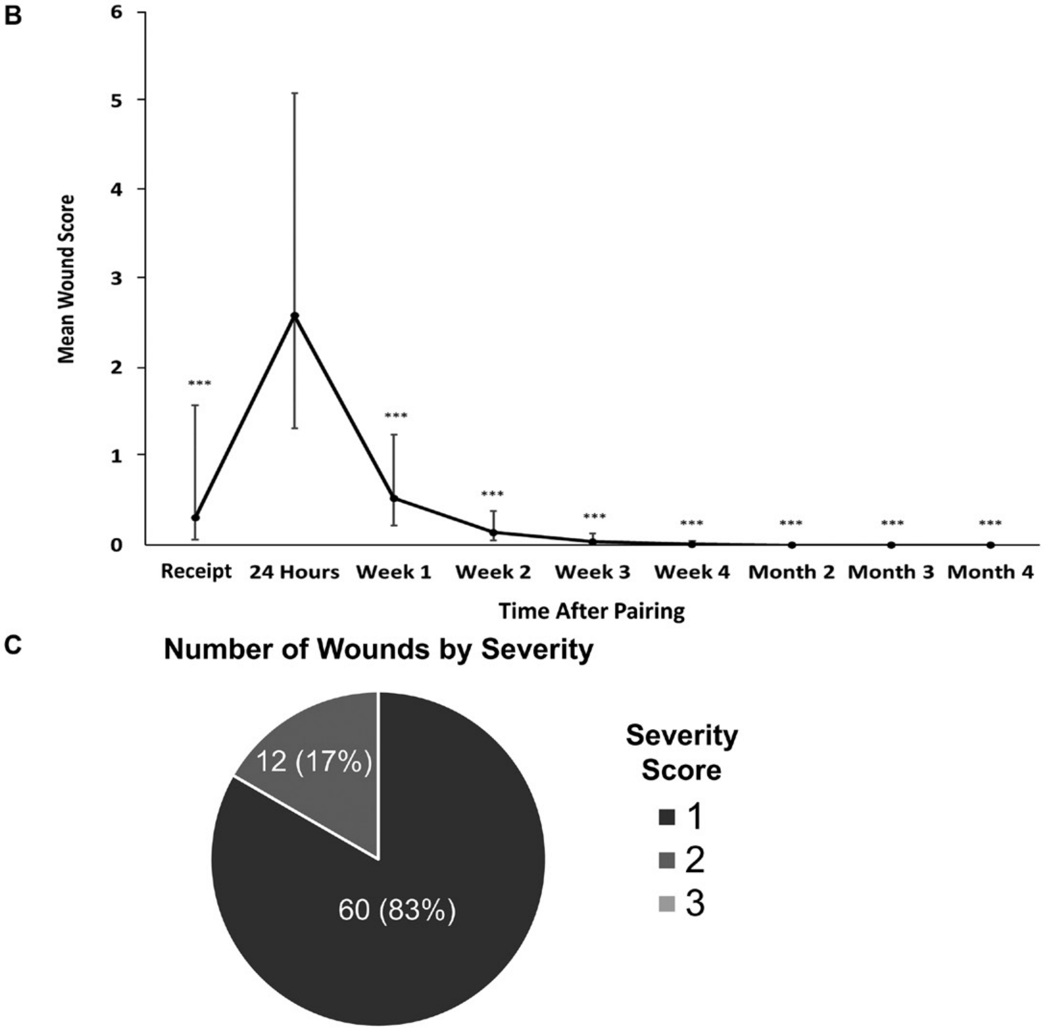

Figure 6. Wound characteristics. Wounds were assessed in each individual animal. (A) Number of distinctly observed lesions and their locations at each time point. 'Receipt' represents lesions present on arrival before pair housing. The final column indicates the number of animals evaluated at each time point. Otherwise, rows represent a time point and columns represent a given anatomic location. (B) Mean wound score at each time point. Bars represent standard deviation. *P* values were determined by using generalized linear mixed effects models to account for within-animal and within-pair clustering, with Bonferroni adjustment for multiple comparisons. ***$P < 0.0001$, as compared with the score at 24 h after pairing. (C) Severity of all wounds observed during the study.

our facility, GPs were immediately paired with the conspecific that shared the same primary shipping container. The shipping containers had been partitioned to prevent physical or visual contact between GP but allowed the perception of auditory and olfactory cues. A shared stressful event can facilitate social bonding in other species, leading us to pair GP that arrived in the same primary shipping container.[21,29,63] When selecting which unfamiliar animals to pair, alternative approaches that can be considered and may be equally or more successful in predicting compatibility include, but are not limited to, temperament, body weight, and duration of prior individual housing. Future studies could also assess the compatibility of adult male GPs without shipping to a new facility. We assessed compatibility after pairing by serial scoring of wounds and observing for affiliative, agonistic, and dominant behaviors. Wound scoring data indicated that most of the newly paired GPs fought, resulting in wounding. Despite the prevalence of wounding, lesions were small and superficial, with 83% and 17% of wounds receiving severity scores of one and 2, respectively (Figure 6C). Facial (54%) and dorsum (34%) wounds were the most common; no genital wounds were observed (Figure 6A). This finding contrasts the predilection sites of wounding in male mice, with dorsum and genital wounding being common and facial wounds being rare.[12,33] The GP's face and dorsum should be inspected thoroughly when examining them for fight wounds.

In addition to wound severity and distribution, our peak total wound score occurred at 24 h after pairing, with a steady decline in scores thereafter. When wounding occurred during the first 24 h after pairing, one GP of the pair had a higher total wound score. This suggests an emerging dominance hierarchy as alpha male GPs have previously been shown to bite more often than nonalpha males.[50] The pattern of the same GP having a lower wound score was generally true throughout the study but was not always the case. We did not identify the initiator of agonistic or affiliative behaviors in the present study; therefore, we inferred the presence of an alpha male based on the wound scores at 24 h after pairing. This inference may not fully describe the social dynamic within the pair. Furthermore, 3 pairs had no wounds throughout the study, preventing any assumption about social dominance. Future studies could evaluate other parameters to more fully describe the social hierarchy formed between adult male GPs. After acclimation and presumed establishment of social hierarchies, wounding rarely occurred; wound scores were significantly higher at 24 h after pairing than at all other time points assessed (Figure 6B). The subsequent decreased propensity for wounding indicates the stability of pair social dynamics. Stable social hierarchies result in predictable behaviors and social interactions.[50,51] The predictability of interactions is important to ensuring animal welfare because social incompatibility can lead to stress, injury, or death.[27] Furthermore, bonding between conspecifics, indicating compatibility, has been shown to attenuate the stress response in GPs, as indicated by diminished production of cortisol after exposure to unfamiliar conspecifics or environments.[51] Together, these data suggest that transient stress likely occurs as social rank is established and stable bonds are formed. The benefits of long-term social housing, indicated by a reduction in wounding and an increase in affiliative behaviors, likely outweigh the initial stress. Nonetheless, increased monitoring of newly paired adult male GPs is warranted, particularly during the first 24 h after pairing.

Although the presence of wounds confirmed fighting between pairs, we rarely observed aggressive behaviors, particularly after 2 h of cohabitation. This was true despite the more frequent observations made during the first 48 h after introduction, which allowed the assessment of evolving pair dynamics and aligned with existing recommendations.[27] The agonistic or dominant behaviors included in our ethogram are well described in GPs, but our ethogram did not include all possible aggressive behaviors.[9,36,49,53,54] Other displays of dominance or aggression include scent marking, vocalization other than teeth chattering, snorting, teeth display with an open mouth, standing on rear legs, and barbering.[36,49,53,54] Future studies could monitor additional aggressive behaviors to further characterize group dynamics. Many factors likely contributed to the paucity of observed aggressive behaviors. For example, ear nibbling has been described in crowded or stressful environments, but ear wounds were rare in our study.[46] We used best practices and previously published recommendations to mitigate aggression when pairing unfamiliar adult males, although we did not test the efficacy of such measures. These measures included providing enclosures with ample floor space (24-in. × 32-in. × 10-in.), a separate hut for each GP as a visual barrier and retreat, 2 water bottles and separate feeders for pelleted diet and hay to reduce resource guarding, and increased monitoring during the introductory phase.[3,59] Future studies could evaluate the value of these and other factors for promoting the successful pairing of unfamiliar adult male GPs. Our facility housed only male GPs during the time of this study. Studies of adult males reared in isolation or social settings have shown that males are extremely aggressive in the presence of females.[51-53] The presence of females in the same room as socially housed adult males could result in severe wounding and prolonged distress. Enclosure size, enclosure complexity, and proximity to females should be considered when socially housing adult male GPs.

Affiliative behaviors were frequently observed in this study, including social contact, hut sharing, and eating together, with the observed affiliative behaviors significantly increasing over time. These behaviors are consistent with previously described prosocial behaviors in GPs.[9,40,59] While existing literature suggests that boars may not be socially compatible, we achieved successful stable pairs as indicated by infrequent agonistic behaviors, frequent affiliative behaviors, and low total wound scores in 11 of the 12 pairs. Although fighting has been well described among adult males, a wound-scoring system has yet to be published for GPs. Our wound scoring system was adapted from a published system used in nonhuman primates.[17] Our observed peak in wound scores at 24 h after pairing correlated with the more frequently observed agnostic and less frequently observed affiliative behaviors at early time points, suggesting this wound scoring system is a good proxy for fighting. Only one pair was deemed to be incompatible, requiring separation due to fighting and wound severity approximately 1 h after pairing. This pair had been introduced prematurely during transit due to a divider failure, allowing unsupervised, physical contact between the 2 GPs before arrival at our facility. At the initial examination, both GPs had minor superficial wounds (wounds score of 6 and 1), presumably due to fighting during transit. Approximately 1 h after pairing, one animal sustained additional wounds, yielding a score of 11, including the only wound with a severity score of 3. The incompatible pair was used to determine the frequency of successful pairing in the 12 pairs (92%), but the pair was excluded from all other data analyses. This approach to data analysis was used in at least one other study investigating the success of social housing in nonhuman primates.[30] In the present study, we used a wound score of greater than 10, the presence of lacerations (Figure 2A), or prolonged agonistic behaviors as objective measures for incompatibility. Establishing such parameters should involve input from the IACUC, veterinarians, and researchers, as the parameters we used may not be appropriate for all GPs, facilities, or studies. Staff education is also critical to establishing expected amounts of aggression, reporting procedures, and parameters for intervention. Facilities should also have a plan for managing GPs that are deemed to be incompatible after pairing. This plan could include a slower return for pairing the same 2 GPs, creating new pairs to assess compatibility with other conspecifics, or providing a veterinary justification for permanent individual housing after documented incompatibility. Because our GPs were involved in an ongoing concurrent study, a limited number of GPs were available, and we were concerned about the individual propensity to fight, we did not reintroduce the 2 GPs that were incompatible in the present study. Further studies are needed to determine if reintroduction or the creation of new pairs would yield stable social relationships if pairs are deemed incompatible at first introduction. The premature introduction of these 2 GPs during transit may have contributed to their unsuccessful pairing, although other unassessed factors, including temperament and housing density immediately before shipping, may also have contributed. Direct contact with unfamiliar adult male GPs should be avoided during transit. Inadvertent introduction during transport may hinder successful pairing at the receiving facility.

While this study indicates that boars can be housed in stable social pairs, facilities should consider the animal's overall welfare and study objectives when socially housing previously unfamiliar males. We inferred social compatibility in the present study based on the frequency of affiliative behaviors, rarity of agonistic behaviors, and decreased wounding after an introductory period. Field studies assessing a variety of *Cavia* species indicate several possible social schemes, including harems with single males, multimale-multifemale groups, and roaming adult males existing at the periphery of harem territories.[2,50] Additional work is needed to determine the social or solitary housing preferences of domesticated adult male GPs in captivity. While we rarely saw agonistic behaviors in the present study, a live individual made the behavioral observations; this may have limited the ability to capture a wider range of aggressive and/or affiliative behaviors. Video recording was not feasible in the present study due to equipment design and enclosure complexity that impeded visualization of the entire enclosure. Observer presence has been shown to affect a variety of animal behaviors, including pain expression, prosocial or agonistic behaviors, self-directed behaviors, stereotypic behaviors, and activity levels.[10,20,25,28,37,44,47,55] Despite the presence of an observer, compatibility of unfamiliar conspecifics has been successfully assessed in other species.[11,30] Video recordings could eliminate observer effects. Our finding of a high incidence of prosocial behaviors and comparatively rare occurrence of aggressive behaviors suggests pair compatibility; however, further analyses of behavior frequency, time spent performing each behavior, and various group sizes are an important future direction. Likewise, future studies could measure hormone levels, including cortisol, testosterone, norepinephrine, and oxytocin, to further characterize physiologic indicators of stress and social compatibility when creating new social groups.[3,11,36,40,54,62] We did not have a complete housing and breeding history of the GPs included in this study, including duration of single housing as compared with breeding or number of litters sired. We also did not know the genetic relationship between males; therefore, some of the GPs may have been littermates, living together with their dam before weaning (approximately 4 mo before the study started). These and other aspects of their social history could affect pairing success. Future studies could compare the compatibility of adult males with known histories, including use as a breeder and housing densities. Future studies could also assess the social compatibility of different GPs to identify strain differences that could affect pairing success.

Our data demonstrate the compatibility of pair-housed adult male Dunkin Hartley GPs. Because wounding, and therefore agonistic behaviors, occur more frequently during the first 24 h after pairing, increased monitoring is warranted during this time. Investigators should consider study objectives and duration, pairing-related stress, and overall animal welfare because social housing may not be appropriate in all situations, particularly if female GPs are present. With sufficient space and resources, unfamiliar adult male GPs can form stable social pairs in a research setting.

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