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Effects of Pair Housing on Patency of Jugular Catheters in Rats (*Rattus norvegicus*)

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Chronic vascular access devices are widely used in a variety of species for repeated blood sampling or substance administration. Jugular catheters are commonly used for studying addiction-related behaviors in rats. Rats with catheters have historically been individually housed for the duration of the study to prevent cage mates from damaging the catheter. The 2 goals of this study were to determine 1) the effects of pair housing on catheter patency and 2) the effects of pair housing on catheter patency of rats in a study of opioid self-administration and cue-induced reinstatement of opioid-seeking behavior. The latter study also represented an opportunity for experimental refinement as it evaluated the temporary use of a barrier that allowed for pair-housed rats to be physically separated. Male Heterogeneous Stock (HS; *n* = 24) and Sprague–Dawley (SD; *n* = 121) rats were allocated to either single- or pair-housed condition. To assess the effect of social housing on catheter patency, rats (HS, *n* = 24; SD, *n* = 36) were monitored in their assigned housing condition for one month, with scheduled evaluation of catheter patency and structural damage. To examine the effect of social housing on catheter patency during a study of opioid self-administration and cue-induced reinstatement of opioid-seeking behavior, rats (SD, *n* = 85) were monitored in their assigned housing condition with similar routine patency evaluations. Catheter patency rates between single- and pair-housed rats were not statistically different in the first experiment, and pair-housed animals were successfully maintained on an infusion study in the second experiment. The use of a barrier between pair-housed rats after surgery allowed continued social contact with no observed adverse effects. These results suggest that, pair housing is a viable option for rats with chronic vascular implants, and may improve their wellbeing by allowing them to display species-typical social behaviors.

Abbreviations: CI, Confidence Interval**;** HS, Heterogeneous Stock; SD, Sprague-Dawley; OR, Odds Ratio

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The widespread support of species-appropriate social housing in biomedical research has increased in the recent years and, as has the number of publications that can be found citing social housing as a key variable within their investigations.[13,28,29,31] Regulations state that appropriate social interactions are essential to the wellbeing of laboratory animals, and that single housing of social species should be the exception.[12] Further guidelines state that a disrupted or restricted social environment can negatively affect animals of all ages and may lead to maladaptive behaviors such as self-injury and stereotypies.[11]

The benefits and behavioral effects of social housing in rodents, and rats in particular, have been reported in previous literature and provide an important foundation for research into the feasibility of socially housing animals used in behavioral research.[2,5,27,32] Rats are a naturally social species,[27] and social housing in the laboratory does not impede critical behaviors such as feeding.[5] Moreover, the beneficial effects of social housing include reduced anxiety-like behavior[2] and attenuated motivation to self-administer rewarding substances such as sucrose and cocaine.[32]

Some situations still require that rats be single-housed in the laboratory. Such situations include health concerns, social incompatibility, and specific research paradigms. Findings from previous literature indicate that single housing, if not properly accounted for, may significantly affect the interpretation of results.[1,7,9,10,16,17,19,28] Single housing can be stressful[17] and can alter gut microbiotia,[7] both of which may induce anxiety-[1] and depressive-like behavior.[16] These conditions may manifest as weight gain,[28] poor grooming, inactivity, learned helplessness[20] and social avoidance.[19] Social isolation also reduces normal behavioral repertoires, such as ultrasonic vocalization,[10] and increases consumption of rewarding substances such as sucrose and cocaine.[9]

Although social isolation may introduce an experimental variable and adversely affect research outcomes, a limitation of pair housing in the research setting is concern that a cage mate may damage an implanted device. One example of this is the study of addiction-related behaviors using self-administration paradigms. In these cases, rats with indwelling jugular catheters self-administer controlled substances over an extended period of time. Rats participating in these studies are often individually housed in order to prevent damage to the catheter, which must remain patent for the duration of the experiment. Limited information is available about the incidence of cage mate-related catheter complications and the methods used to socially house rats with chronic vascular implants.

The development and refinement of appropriate practices to socially house rats with chronic implants are important factors in optimizing animal wellbeing and successful research outcomes. The principles of replacement, reduction, and refinement (3Rs) were developed several decades ago to promote the humane use

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of animals in research.[22,26] With this in mind, we studied whether social housing was a feasible experimental refinement for rats participating in an opioid self-administration and reinstatement study. Rats used for this investigation were already part of our laboratory's rat colony and not needed for experiments for meeting the laboratory's primary research goals. The 2 goals of the current study were to determine the effects of social housing on catheter patency (Experiment 1) and on catheter patency of rats participating in an opioid self-administration and cue-induced reinstatement study (Experiment 2). Opportunities for refinement were identified throughout the study, one of which was an additional aim for Experiment 2. We examined the use of a 'buddy barrier' for pair-housed rats participating in the opioid self-administration study. For this study, we hypothesized that the long-term patency rates of catheters in individually housed and pair-housed rats would not be different.

Materials and Methods

Animals and Housing. All activities described (Figure 1) were approved by the University of Michigan IACUC. Rats were housed in an AAALAC-accredited facility (University of Michigan, Ann Arbor, MI). Male Heterogeneous Stock (NMcwiWFsm:HS, referred to as HS) rats (n = 24) and Sprague–Dawley (Crl:SD and NTac:SD, referred to as SD) rats (n = 121) were used in this experiment. HS rats, which have been used for fine genetic mapping of complex traits, including drug abuse,[24] were obtained from a breeding colony maintained by Dr. Leah Solberg Woods at Wake Forest School of Medicine. SD rats were obtained from Charles River Laboratories (Barrier R04, Raleigh, NC and Barrier C72, Kingston, Canada) and Taconic (Barrier IBU16, Germantown, NY). Rats were pair-housed on arrival in static cages (Allentown, Allentown, NJ) with corncob bedding (Bed-o'Cobs 1/8", The Andersons Lab Bedding, Maumee, OH) that was changed weekly by animal care staff. Rats had access to food (5L0D PicoLab Laboratory Rodent Diet, LabDiet, St Louis, MO) and water ad libitum. The room was maintained on a 12:12-h light:dark cycle with a temperature of 22.2 °C, and humidity between 30% and 70%. Rat colonies at the institution were monitored semiannually for GDVII, H-1, KRV, LCMV, MAV, MPUL, NS-1, PVM, REO, RPV, RVM, SDAV/RCV, Sendai virus, pinworms, and fur mites.

Catheter Construction. *Plastic*. The base of the catheter was made from a 200 uL plastic pipette tip (P1 Technologies, Roanoke, VA) that was cut approximately 8 to 10 mm from the end with the widest opening. The long end of the guide cannula (C313G-5UP/SPC, 5 mm pedestal height; P1 Technologies, Roanoke, VA) was curved to be almost perpendicular to the pedestal. The cannula was then inserted through the top of the pipette tip base and fed through an opening at the bottom of the pipette tip (Figure 2 A). Silastic tubing (P1 Technologies, Roanoke, VA) was cut to 10 cm in length and beveled at one end. A silicone bead was placed around the silastic tubing approximately 3.0 cm from the beveled end. The silicone bead was allowed to dry overnight. Shrink tubing (P1 Technologies, Roanoke, VA) was cut to 4 mm length and the unbeveled end of the silastic tubing was run through the shrink tubing. The silastic tubing was then attached to long end of the cannula to the point where the tubing was flush with the pipette base. The shrink tubing was then secured by heat around the silastic tubing and metal cannula to secure and protect the silastic tubing's connection to the cannula. A dummy-cannula (C313DC/CAC/SPC, P1 Technologies, Roanoke, VA) was cut to 4 mm from the base of the pedestal and screwed on to the top of the cannula so that the dummy-cannula was flush with the top of the pipette base. A small piece of Parafilm (Sigma, St Louis, MO) was then wrapped around the area where the dummy-cannula and pipette base met to prevent the sealing of this junction. To secure the cannula inside the pipette base, the base of the catheters was then filled with dental cement (Ortho-Jet, Lang Dental Manufacturing Company, Wheeling, IL). As the cement was drying, a piece of oval-shaped mesh (P1 Technologies, Roanoke, VA) was placed on the pipette base and secured with an additional layer of dental cement. The mesh was approximately 1.5 cm wide × 2.0 cm long and affixed to the catheter base so that the cannula was angled approximately 45 degrees from the long-axis of the mesh. Once the cement was dry, the Parafilm was removed and a sanding stone was used to smooth down any sharp edges at the bottom of the catheter created by the dental cement. Each catheter was flushed after construction with sterile water to confirm that it was intact and sterilized via a previously established and institutionally approved method using ethanol and orthophthaladehyde solution (Cidex, OPA, Advanced Sterilization Products, Irvine, CA).

Metal. To prevent a rat from damaging a cage mate's catheter, we constructed a different set of catheters with metal external components. Construction followed the same steps as described above. However, the plastic base was replaced with an 8 mm (range 7 to 9 mm) tall metal base cut from 304 stainless steel tubing (Grainger, Lake Forest, IL) and the dummy-cannulas were fitted with a metal collar cut from the same tubing as the base.

Surgery. All rats underwent surgery to place an indwelling catheter into the jugular vein, as adapted from methods reported by others.[15] University of Michigan's *Guidelines on the Performance of Surgery in Rodents* were followed, which included use of aseptic surgical technique. All catheters were made inhouse as described above and surgeries were performed by 2 trained experienced surgeons. Animals were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection. The nonsteroidal antiinflammatory analgesic carprofen (5 mg/kg) was injected subcutaneously prior to surgery and 24 h after surgery to control postoperative pain and inflammation. A dorsal incision was made between the scapulae, and the mesh backport was implanted subcutaneously. The attached catheter was tunneled subcutaneously to the ventral cervical region, where the tubing was inserted into the jugular vein and anchored into the vessel with synthetic, nonabsorbable suture (Braunamid, Jorgensen Labs, Loveland, CO). After completion of the surgery, rats were given a subcutaneous injection of saline (3 mL) to mitigate any adverse effects of blood loss during vascular surgery. Rats received daily intravenous infusions of heparin (100 units/mL, 0.05 mL) and gentamicin sulfate (1mg/mL, 0.05 mL) to maintain catheter patency and decrease the risk of infection. After surgery, toenails were trimmed weekly for each rat to decrease the risk of tissue trauma from scratching.

Catheter Patency and Structural Integrity Evaluation. Catheter patency was evaluated in a standardized manner every 7 to 10 d during Experiment 1 (3 time points) and every 12 to 15 d during Experiment 2 (2 time points). The testing consisted of an intravenous infusion of methohexital sodium diluted in sterile saline (10mg/mL; 0.1 mL) into the catheter through the dorsal access port. If ataxia was not observed within 10 s of the infusion, the catheter was deemed nonpatent, and the animal was removed from the study. After the final patency assessment, rats were euthanized with CO_2 and a necropsy performed on animals with nonpatent catheters to evaluate catheter position within the vessel.

Recognizing that catheter patency was also related to the structural integrity of the dorsal access port to which the catheter

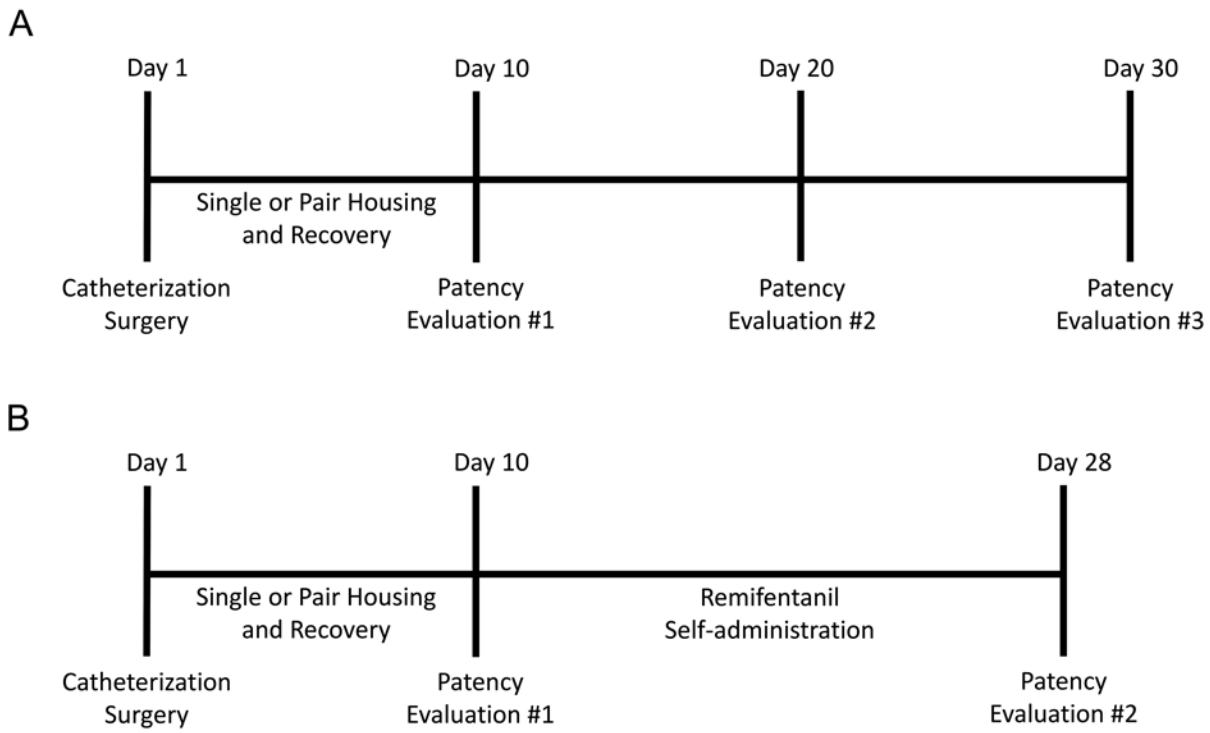

Figure 1. (A) Experimental methods and timeline for Experiment 1. A total of 24 Heterogeneous Stock (HS) rats (paired $n = 16$; single $n = 8$) and 36 Sprague–Dawley (SD) rats (paired $n = 24$; single $n = 12$) were used in this experiment. (B) Experimental methods and timeline for Experiment 2. A total of 85 SD rats (paired $n = 48$; single $n = 37$) were used in this experiment.

was attached, we visually evaluated external components of the catheter system daily for signs of damage. Signs included 1) loss of dummy cannulas, 2) dummy cannula damaged from cage mate gnawing, and 3) compromised threaded pedestal around the guide cannula. Displaced dummy cannulas were replaced as needed. Structural integrity was scored using a fixed-interval 0 to 1 sampling method; a score of 0 indicated no signs of structural damage, and a score of 1 indicated that at least one of the above listed signs was observed.

Experiment 1: Evaluation of Housing Condition, Stock, and Catheter Type on Catheter Patency. A total of 60 male rats were used in this experiment (HS, $n = 24$; SD, $n = 36$; weight, 300 to 500 g). Rats (HS, $n = 16$; SD, $n = 24$) were randomly chosen to be pair-housed in standard static cages after catheter implantation surgery. All HS rats had plastic catheters implanted and SD rats had both plastic ($n = 18$) and metal ($n = 18$) catheters implanted. Rats were paired randomly after surgery; thus, the cage mate after surgery may or may not have been the same cage mate as prior to surgery. Both rats in the pair had catheters. Feeding after surgery continued as the standard husbandry protocol of ad libitum access to food and water. Rats remained in their home cages for the duration of the experiment. Catheter patency and structural integrity evaluations were performed as outlined above.

Statistical Analysis. The following packages were used for statistical analyses: R version 3.4.3 (CRAN) and GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA). For Experiment 1, we performed a Log-rank (Mantel–Cox) test to compare differences in the percentage of catheters remaining patent over time between 2 groups: single- compared with pair-housed rats. The effect of housing condition, rat stock, catheter material, and clinical intervention on catheter patency rate were statistically computed using Fisher exact tests. The effect of housing condition on catheter damage was also evaluated using Fisher exact test. Group sizes were not calculated in advance, as this study began within a single laboratory using rats that were not required for the laboratory's primary aims; therefore, a limited number of animals were available. The interaction between housing condition and rat stock was evaluated using a χ^2 test. A Cox proportional hazards model that included housing condition, rat stock, and catheter material was used to determine what combinatorial effect they had on overall catheter patency. A P value less than 0.05 was considered statistically significant for all tests.

Experiment 2: Evaluation of Housing Condition on Catheter Patency in Rats Participating in an Opioid Self-Administration and Opioid-Seeking Study. A total of 85 Sprague–Dawley rats (weight, 225 to 275 g) participating in an opioid self-administration study were used in Experiment 2. All rats were implanted with jugular catheters as described above and, on recovery, were housed singly ($n = 37$) or in pairs ($n = 48$). Both rats in the pair had catheters. Single-housed animals had plastic catheters implanted and pair-housed animals had metal catheters implanted. Rats were paired randomly after surgery; therefore, the cage mates may or may not have been cage mates prior to surgery. Pair-housed rats were separated from full physical contact with each other for 5 d, which corresponded to the first half of the recovery period, by use of a 'buddy barrier' (described below). After the barrier was removed, rats remained pair-housed and completed their recovery period. Catheter patency was assessed immediately before and after opioid self-administration. Rats with nonpatent catheters were removed from the study. Three pair-housed rats were removed from study for either non-catheter related health concerns or failure to acquire opioid self-administration.

Buddy Barrier. Construction of the buddy barriers was similar to methods described by others.[5] Perforated stainless-steel sheets (1.651 mm/16 Ga, Metal Supermarkets, Fort Wayne, IN)

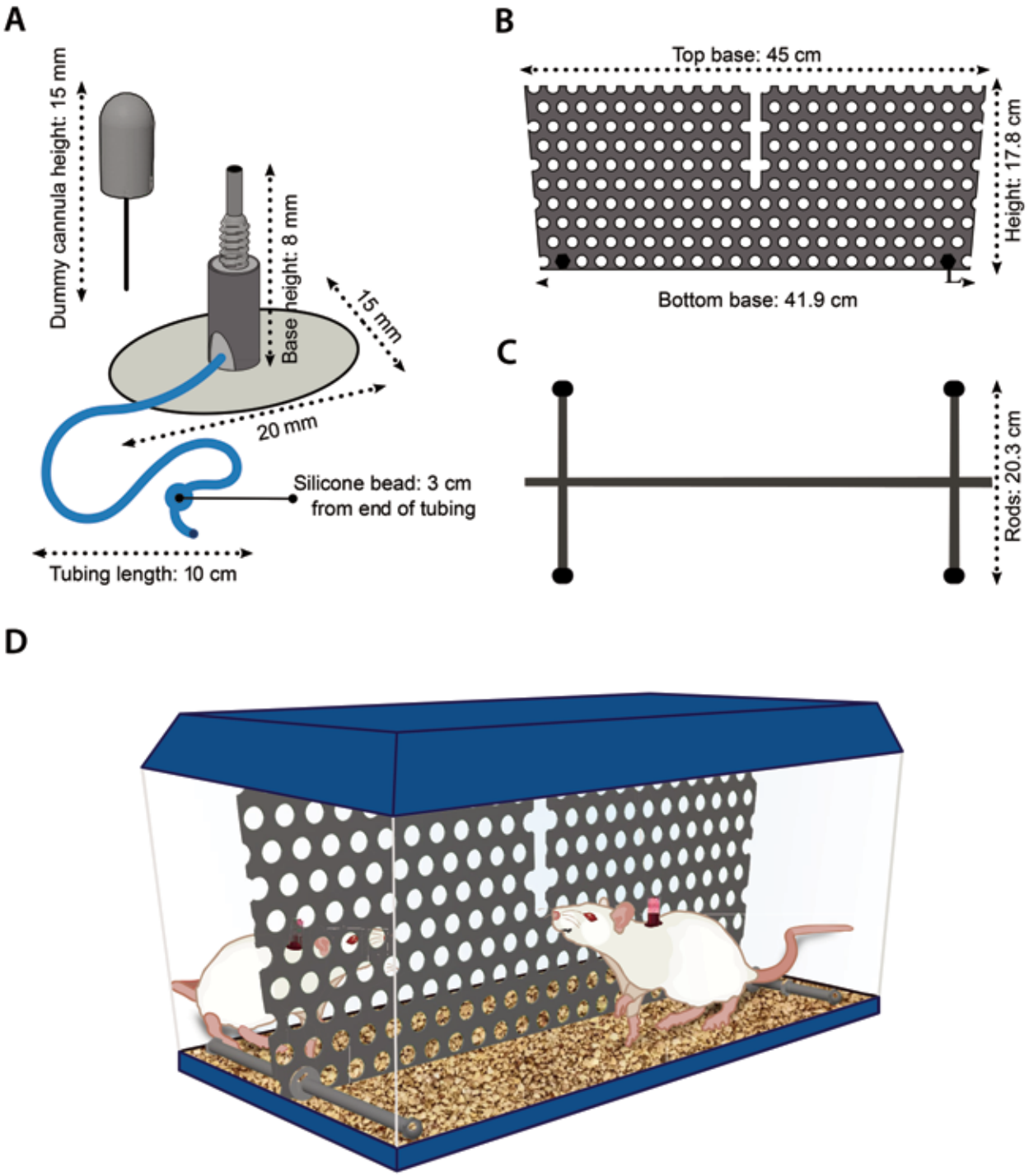

Figure 2. (A) Design of the jugular vein catheter. (B) Design of the 'buddy barrier", side view. (C) Design of the "buddy barrier", aerial view. (D) Schematic of the 'buddy barrier' in a housing cage with catheterized rats.

were cut to fit inside our standard, nonventilated, rodent cage (PC10196HT, Allentown, Allentown, NJ). Perforations had a 12.7 mm diameter with 17.5 mm centers and were staggered in parallel diagonal rows. An off-center channel was cut to accommodate the wire feeder (Figure 2 B). To support the barrier, 316 stainless steel threaded rods, with a diameter of 7.9 mm (McMaster-Carr, Elmhurst, IL), were cut into 8 in. sections and secured by stainless steel fender washers and hex nuts through a bottom perforation on both sides - of the barrier. Two rods were used to stabilize the barrier, one placed at either end. Stainless steel cap nuts were attached to the ends of the rods (McMaster-Carr, Elmhurst, IL) (Figure 2 C). All materials were approved for use by the IACUC and husbandry and veterinary staff at the University of Michigan. As the sheet ran through the wire-top cage lid, food and water were provided to each rat separately. The placement of the barrier resulted in reduced cage space for each rat; however, it continued to meet AAALAC guidelines. These temporary barriers were used for 5 consecutive days and allowed for visual, olfactory, auditory, and limited tactile contact between cage mates (Figure 2 D).

Opioid Self-Administration Paradigm. Rats with patent catheters underwent 15 consecutive days of remifentanil

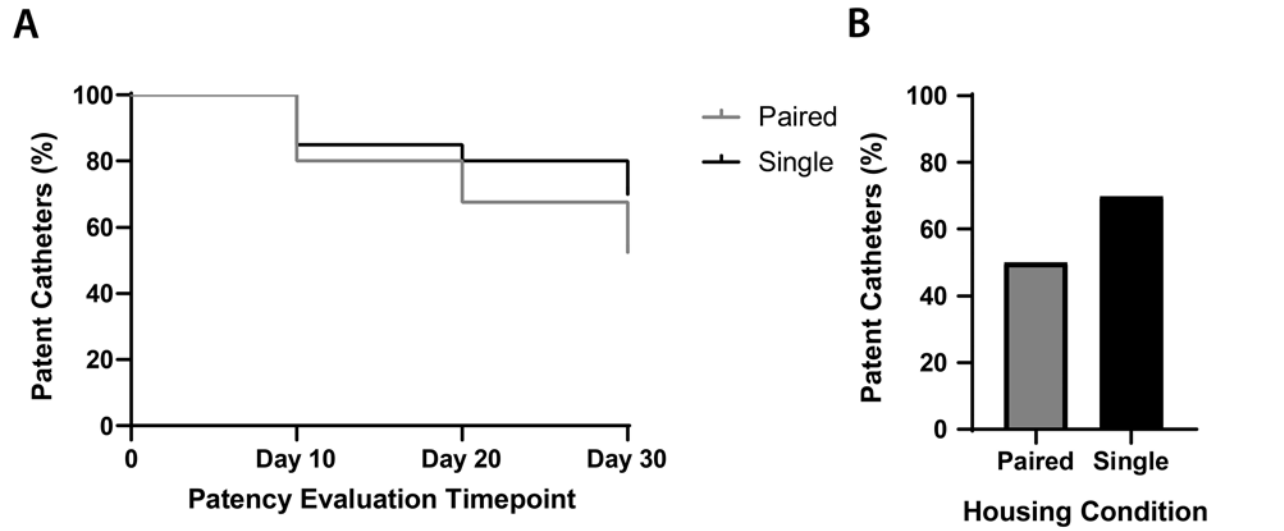

Figure 3. (A) Effect of social housing on catheter patency rate over time. There were no significant differences between the catheter patency rate curves for single- and pair-housed rats (P = 0.2384; Mantel-Cox test)). Patency checks were performed every 7 to 10 d; days on the x-axis are approximate. (B) Effect of social housing on catheter patency rate at the end of the study. Catheter patency rates for single- and pair-housed rats at the end of the study were not significantly different (P = 0.1739; Fisher exact test).

self-administration. Self-administration occurred in standard behavioral testing chambers (20.5 cm × 24.1 cm × 29.2 cm; MED Associates, St Albans, VT) located in sound-attenuating boxes. Rats were tethered via their external access port to a syringe pump containing the remifentanil for the duration of the session; one self-administration session took place each day, lasting for no more than 3 h. After the last self-administration session, a final catheter patency evaluation was performed.

Results

Experiment 1: Effect of social housing on long-term catheter patency. Overall, the pattern of catheter loss over the course of the study was not significantly different between single-housed rats and pair-housed rats (Figure 3 A, P = 0.2384). In addition, the percentage of catheters that were patent at the end of the study did not differ significantly between rats that were single-housed (70%) or housed in pairs (50%; Figure 3 B, Odds Ratio = 2.3333, 95% CI = 0.7793 to 7.829, P = 0.1739).

Effect of stock on long-term catheter patency. When evaluating catheter patency across stocks, regardless of housing condition, HS rats had a significantly higher patency rate than SD rats, 79% compared with 42% respectively (Figure 4, Odds Ratio = 5.320, 95% CI = 1.714 to 15.62, P = 0.0072). We found a significant interaction between housing condition and rat stock on catheter patency (Figure 4, P = 0.0124), indicating that the difference in patency between single- and pair-housed rats was greater in HS rats, as compared with SD rats. However, when evaluating the effect of housing condition on catheter patency for each stock separately, no significant differences were found between single- (100%) and pair-housed (69%) HS rats (Figure 4, OR = 0, 95% CI = 0.000 to 1.405, P = 0.1304) and single- (50%) and pair-housed (38%) SD rats (Figure 4, OR = 0.6000, 95% CI = 0.1602 to 2.198, P = 0.4991).

Effect of catheter material on catheter patency and structural integrity. To decrease the risk of damage to the external catheter components by a cage mate, we used metal catheters in a subset of SD rats. Observers could not be blind when performing structural evaluations of the different catheters, as metal and plastic catheters were easily distinguishable. Catheter type (plastic or metal) had no significant effect on overall patency rate (OR = 0.7955, 95% CI = 0.2257 to 3.194, P = greater than 0.9999). Given these findings, we created a model to investigate the association between housing condition, rat stock, and catheter type on overall patency rates. Rat stock was found to be the only significant predictor (P = 0.0137). Catheter damage showed a significant effect of housing condition (Figure 5 A, OR = infinity, 95% CI = 1.148 to infinity, P = 0.0431), as pair-housed rats had significantly higher levels of structural damage to the external catheter components than did single-housed rats (20% compared with 0%), presumably from cage mate gnawing.

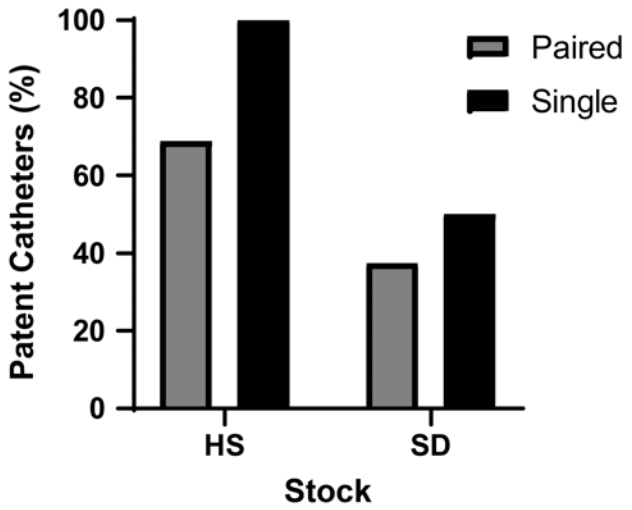

Figure 4. Effect of rat stock on catheter patency rate at the end of study. Regardless of housing condition, HS rats had a significantly higher patency rate than SD rats (P = 0.0072; Fisher exact test. A significant interaction was found between stock and housing condition (P = 0.0124; χ^2 test). No significant differences were found between single- and pair-housed HS rats (P = 0.1304) or single- and pair-housed SD rats (P = 0.4991; Fisher exact test).

Effect of housing condition on proportion of rats requiring clinical intervention. We also evaluated the reports of clinical intervention between single- and pair-housed rats. The level of veterinary clinical intervention required in pair-housed rats (18%) was not significantly different from that of single-housed

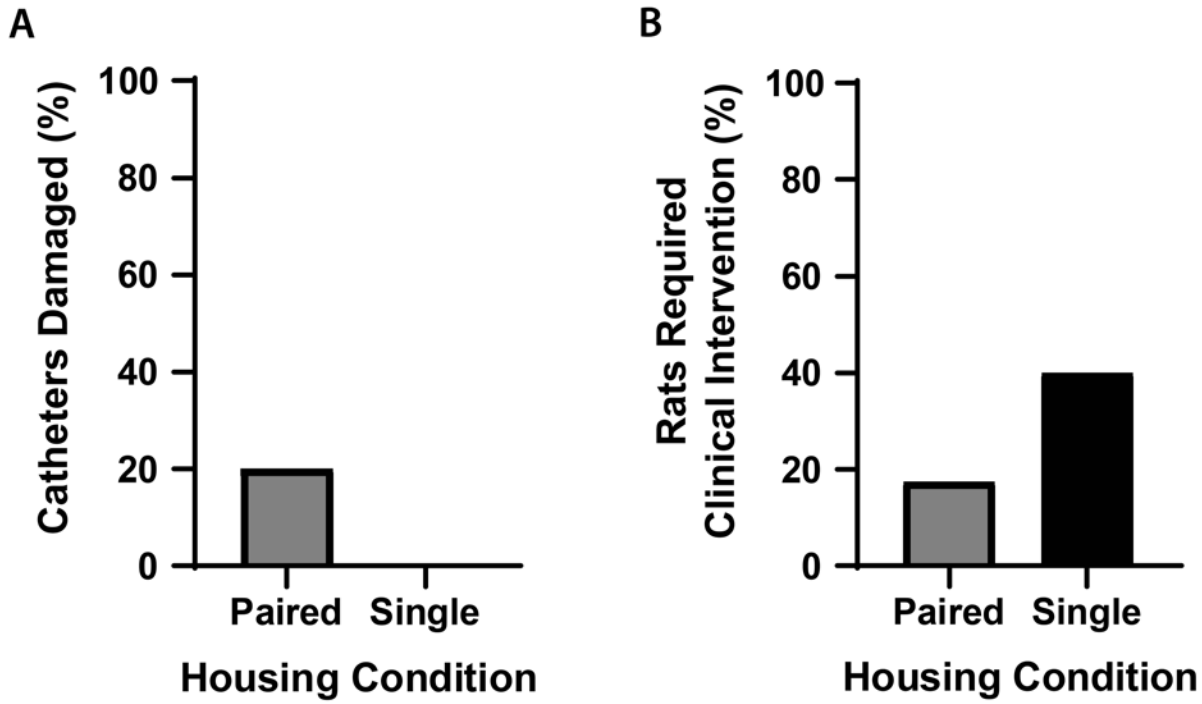

Figure 5. (A) Effect of housing condition on catheter damage. Pair-housed rats had significantly higher levels of catheter damage when compared with rats that were single-housed ($P = 0.0431$; using Fisher exact test). (B) Effect of housing condition on veterinary clinical intervention. Percentage of rats requiring veterinary clinical intervention was not statistically different between single- and pair-housed animals ($P = 0.1113$; Fisher exact test).

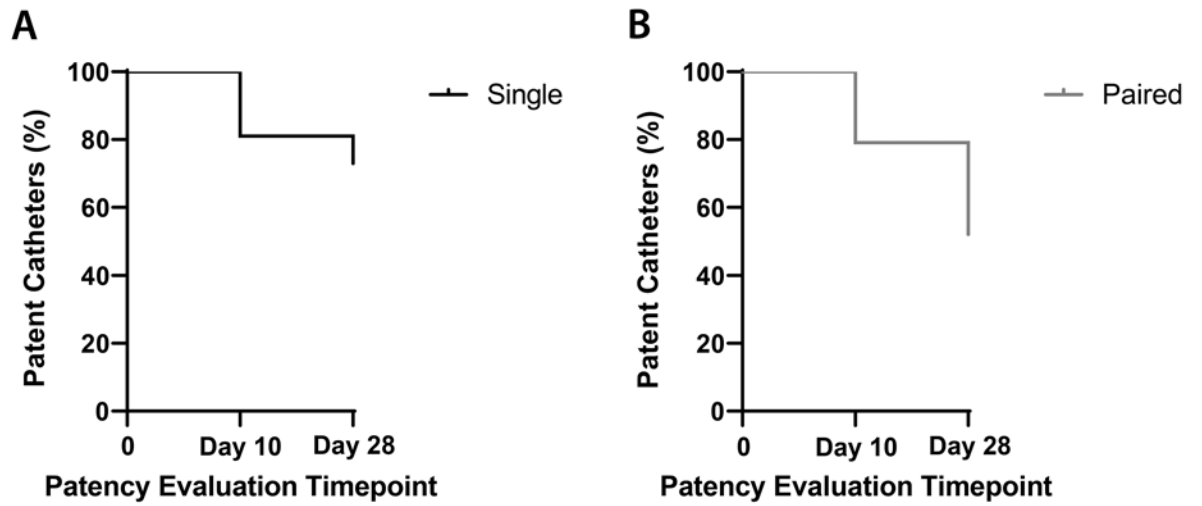

Figure 6. (A) Catheter patency rate over time in single-housed rats on an opioid self-administration and reinstatement study. (B) Catheter patency rate over time in pair-housed rats on an opioid self-administration and reinstatement study.

rats (40%) (Figure 5 B, OR = 0.3182, 95% CI = 0.09331 to 1.004, $P = 0.1113$).

Experiment 2. Effect of social housing on catheter patency of rats participating in an opioid self-administration paradigm. To determine whether social housing would affect our ability to maintain rats in an opioid self-administration study, we evaluated catheter patency of single-housed and pair-housed rats throughout the course of a study. A direct comparison of catheter patency rates between the 2 groups over the duration of the study was not performed, as pair-housed rats were implanted with metal catheters and single-housed with plastic. Nonetheless, at the end of the study, catheters remained patent in 73% of catheters in single-housed rats (Figure 6 A) as compared with 51% of pair-housed rats (Figure 6 B). This lower patency rate of pair-housed rats is consistent with results in Experiment 1.

Discussion

With the increasing efforts to house all social species used in research under socially-appropriate conditions, we studied the effect of social housing on long-term patency and structural integrity of catheters in rats maintained on a long-term intravenous infusion study. Results demonstrated that rats with chronic vascular implants could safely be housed in pairs.

Overall, the catheter patency rates of singly-housed and pair-housed rats were not statistically different. This finding led us to believe that pair housing of rats with jugular catheters would be

feasible if it was compatible with the research design and purpose. When analyzing possible factors affecting patency rates, one of our most robust findings was a difference between rat stocks. HS rats had a significantly higher proportion of catheters remain patent for the duration of the experiment than did SD rats. HS rats are bred to maintain a high level of genetic diversity, thus more closely representing a natural population such as humans.[25] This rat stock may be better able to sustain a chronic implant due to heterosis, in which heterozygosity could allow beneficial or advantageous characteristics or traits to dominate, leading to increased vigor.[4] Thus, based on our results, strain or stock of rat appears to affect the long-term patency of vascular catheters to a greater extent than housing conditions, perhaps due to differences in behavior and/or the foreign body immune response displayed by different rat stocks.

Pair-housed rats exhibited gnawing of the exposed plastic components of the access port of its cage mate. Rat incisors grow continuously, which may, in part, drive their behavior to gnaw on inanimate objects.[6,30] For wild rodents, inanimate items may include sticks and bark, while for laboratory rodents, this may be wooden blocks and nylon bones provided for environmental enrichment.[8] The location of the exposed plastic access port on the dorsum gave the rats an opportunity to display species-typical behavior in the absence of alternative preferred items. Previous work established that a change in implanted material allowed social housing of rats with headstages.[21] Thus, in an attempt to decrease the ability of a cage mate to gnaw on integral external catheter components, such as the plastic threading on the guide cannula, we constructed and tested catheters with metal external components. We found that metal and plastic catheters maintained similar proportions of catheter patency. Given the external damage observed with plastic components, we used metal catheter components for pair-housed animals in Experiment 2 and recommend their use in studies using pair-housed rats.

Because we observed gnawing of the catheters by socially-housed rats, we evaluated the number of cases of veterinary intervention required for pair-housed and single-housed rats. Previous literature has reported that rats will work for access to other rats[18] and even demonstrate prosocial behavior.[3,23] Our pair-housed rats were reported to veterinary staff less frequently than singly-housed rats for scratching at the dorsal backport; this behavior can lead to tissue damage and require an entry into an animal's medical record. A possible explanation for this is that pair-housed rats were less at risk of developing stereotypies or self-injurious behavior when provided with the opportunity to display species-typical social behaviors.

In an effort to refine our practices and improve our science, we evaluated each step in our process and identified opportunities for refinement to the operative and postoperative periods. To maximize the time for healing to occur after surgery and potentially to decrease loss of catheter patency during the immediate postoperative period, a 'buddy barrier' was made and placed into the cage for 5 d immediately after surgery. This corresponded to the first half of the recovery period. The 'buddy barrier' allowed animals to remain in stable pairs with tactile, olfactory, auditory, and visual contact while reducing play behavior that had the potential to disrupt the healing process. The percentage of catheters remaining patent in pair-housed SD rats in Experiment 2 (51%) was higher than the pair-housed catheter patency rate of SD rats recorded in Experiment 1 (38%). Further, no adverse physical or behavioral effects were observed when this barrier was used in the cage. We did not study possible long-term behavioral effects from the acute use of the 'buddy barrier', and this could be an area for future study. We support the use of a separating system for an acute period (up to 5 d) after surgery to facilitate healing and to allow the animals to adapt to the implant. This practice may also help preserve catheter patency of pair-housed rats throughout the study. However, this was not directly assessed in the current study and warrants further investigation.

Our study had several limitations. All rats used in this study had previous exposure to conspecifics, either at the vendor or prior to experimental use at our institution; this may have influenced their behavior. Social housing conditions have been shown to alter behavior across a variety of research paradigms.[2,14,32] In addition, cage mate data for our pair-housed rats was not available, so our data were analyzed assuming that a pair-housed rat's catheter patency outcome was independent of their cage mate's. This may also represent an area for further exploration. In the spirit of the 3Rs[22,26] and to reduce animal numbers, we used rats that were already part of our laboratory's research program and so, were limited in number of animals available and their sex. Studying potential differences due to sex, age, and strain variations in conjunction with larger sample sizes can be done in future studies.

In conclusion, our data support our hypothesis that the catheter patency rate of socially-housed rats is not significantly different from that of single-housed rats. Pair-housed rats were less likely to display self-directed behavior and require veterinary intervention. Acute use of a separating system, such as the 'buddy barrier', requires further exploration, but allows pair housing of physically separate rats. Compared with single housing, pair housing allows social contact and may increase the overall wellbeing of rats under study.

Acknowledgments

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