Journal of the American Association for Laboratory Animal Science
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Vol 62, No 1
January 2022
Pages 38–47

Preference of Escaped Mice for Live Capture or Glue Traps and Relevance to Pest Control Programs

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Insects are potential disease vectors for research animals. Therefore, implementing an effective pest control program is an essential component of any animal care and use program. The *Guide for the Care and Use of Laboratory Animals* emphasizes the humane use of traps; however, insect traps commonly use glue that can entrap escaped research mice, leading to their potential distress and injury. This situation is challenging for research facilities attempting to identify insect populations. In an effort to improve pest control in animal facilities, we sought to characterize the behavioral interactions of mice with common vermin traps. Three experiments using different combinations of traps (glue trap, live mouse trap with a clear viewing window, and live mouse trap with a red-tinted viewing window) were used in multiple behavioral testing arenas to address these questions. Experiments 1 and 2 were performed in a small arena, and Experiment 3 was performed in a simulated mouse housing room. Dependent measures included exploration of the test environment, grooming behavior, time spent near each trap, and latency to capture. Results indicate that mice were captured significantly more quickly by live traps than by glue traps, and were far more likely to enter a live trap as compared with a glue trap. Mice did not appear to differentiate between clear or red-tinted window live traps. Taken together, the results indicate that deploying both a live trap and a glue trap will allow humane capture of escaped mice yet will also capture insects in the same environment.

DOI: 10.30802/AALAS-JAALAS-22-000073

Introduction

Pests are potential vectors of disease for both research animals and personnel, and establishing a pest control program is essential for animal research facilities.[9,14] Prevention is the foundation of a successful pest management program, and quick identification of pest species in a research vivarium is key to preventing an infestation.[19] Identification of vermin is typically aided by using pest control traps that are likely to capture the vermin that are most likely to inhabit a given geographic area. Humane capture of feral or wild mice is best accomplished with the use of live traps, and many facilities use live mouse traps with a viewing window to facilitate the daily inspection of traps.[1,14] Live traps alone, however, are ineffective in capturing insect pests due to large openings that allow insects to freely enter and exit the trap.

Commercial pest control services commonly use small, foldable glue traps to capture, monitor the effectiveness of the current insect pest program and to identify captured insects. For example, during an outbreak of wild mice in our facility, glue traps helped us to detect the presence of *Ornithonyssus bacoti* (tropical rate mite) in different locations.[4] However, the strong adhesives in these insect traps could also capture escaped and wild mice in the facilities, thus create a welfare concern. The *Guide* states, "if traps are used, methods should be humane."[14] In addition, AAALAC International FAQ on Frequency of Monitoring Rodent Traps indicates that "alternatives to 'sticky/adhesive' live board traps should be used for mice to avoid unnecessary animal distress."[1] Unfortunately, the elimination of adhesive-based pest control traps limits options for identifying insect pests.

To reduce accidental capture of mice in glue traps, adhesive pest control traps could be placed on walls or doors, thereby keeping the glue trap off the floor and minimizing welfare risk. When we tested this option at the University of Chicago (unpublished data), fewer insects were captured in wall-mounted glue traps as compared with glue traps on the floor. For example, in a room with a booklice (*Liposcelis* spp.) infestation, 2 booklice were captured in a wall-mounted glue trap as compared with 21 that were captured in the nearby glue trap on the floor at 18 days after traps were placed. This difference could reduce the identification of invasive insect species, especially those that are thigmotactic.[8,20] Other approaches to restricting mouse access to glue traps involve encasing glue traps in barriers that permit insect, but not mouse, entry. Unfortunately, such modifications can also limit the identification of some pests. For example, the American cockroach (*Periplaneta americana*), which is prevalent in most major cities, may grow to the length of a mouse (up to 53 mm),[16] and may not be able to enter such modified traps.

When animal care staff are notified of an escaped mouse, temporary room-level adjustments can be made to increase the chances of humane capture such as adding more live traps, baiting existing live traps, and removing potentially inhumane traps. To avoid inhumane capture of escaped mice, one option is to use humane live traps on the floor and glue traps on the walls to avoid inhumane capture. However, this practice makes 2 assumptions: (1) that the escaped mouse will enter and become captured by a live trap, and (2) that an escaped mouse will be ensnared in a glue trap if it were on the floor. Currently, we are unaware of empirical evidence in support of these assumptions.

In this study we monitored mice in the presence of different pest traps under conditions that simulated an escaped mouse in a colony room. We investigated whether mice behave differently in the presence of the different types of traps, presented either alone or in combination. Specifically, we were interested

Submitted: 24 Jul 2022. Revision requested: 24 Aug 2022. Accepted: 21 Oct 2022.
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in whether escaped mice exhibit a preference for one type of trap over another. One report on wild house mice in various building-types found that wild mice were caught more often in live traps than glue traps.[5] However, the significance and applicability of findings in that report are not clear with regard to escaped research mice due to lack of sufficient methodological details and the lack of peer review. In addition, behavioral research has indicated wild mice and research mice have very different responses to the same tests.[13] On our campus during a recent wild mouse infestation, glue traps anecdotally seemed more effective than live traps when both trap types were present. However, the absence of systematic records prevents confirmation of this potential difference. Nonetheless, based on this information, we predicted that research mice would be caught more often and more quickly on glue traps as compared with live traps. Finally, we explored methods for improving capture rates with live traps. Mice are less sensitive to red light (> 600 nm) as compared with humans and with other colors in the visible light spectrum.[12,23] In addition, they are generally less anxious in darker spaces.[6] Therefore we predicted that the addition of red-tinted filters to the viewing windows of live traps would make them less aversive to escaped mice as compared with live traps with the standard clear viewing window. To evaluate these predictions, mice were tested in a series of arenas that contained different combinations of trap types. Specifically, we tested the hypotheses that; 1) escaped research mice would be caught more often in glue traps than in live traps and, 2) the addition of red tint would increase capture rates in live traps.

Materials and Methods

Husbandry and animal care. The Animal Care Program at the University of Chicago is AAALAC-accredited, and all animal work was approved by the University's IACUC. Mice were housed in the UChicago ARC facilities RRID: SCR_021806. They were group housed in solid-bottom polysulfone individually ventilated cages (Allentown Jag 75 micro-Barrier, Allentown, NJ), with 1/4-in. corncob bedding (Teklad 7097, Envigo, Indianapolis, IN) with shredded paper for enrichment (Bed-r'Nest [The Andersons, Maumee, OH, USA]), an irradiated diet (Teklad 2918, Envigo, Indianapolis, IN) and acidified tap water ad libitum. All cages, bedding, and enrichment were autoclaved prior to use. Cages were changed every 14 d in a Class II Type A2 biosafety cabinet (NuAire, Plymouth, MN). Housing rooms were maintained on a 12:12-h light:dark cycle (lights on at 0600 h, lights off at 1800 h) with humidity ranging from 30% to 70%. Temperature ranged from 20 to 24 °C (68 to 76 °F). Light illuminance on the experimental room floor ranged from 169 to 212 lx via 48-in. fluorescent bulbs (Sylvania FO32/835/ECO, color temperature: 3500 Kelvin, Wilmington, MA) with one bulb per ballast throughout the room ceiling, which held 8 ballasts. Both the housing room and the experimental room had no supplemental light, including red light, during the dark phase except during rare occurrences of researchers entering the housing room at night. In these instances, red light would have been used. The door portholes were equipped with a shade that is kept shut while not in use and the window contains red tint.

Mice were checked daily by the animal care staff to assure good health and appropriate food, water, and cage conditions. The following agents were excluded as verified by PCR on samples obtained from exhaust air dust as previously described:[17] Sendai virus, pneumonia virus of mice mouse hepatitis virus, mouse parvoviruses, reovirus, epizootic diarrhea of infant mice, mouse encephalomyelitis virus, ectromelia virus, lymphocytic choriomeningitis virus, murine adenovirus 1 and 2, murine cytomegalovirus, hantavirus, lactate dehydrogenase-elevating virus, *Filobacterium rodentium*, *Mycoplasma pulmonis*, *Salmonella* spp., *Citrobacter rodentium*, *Clostridium piliforme*, *Streptobacillus moniliformis*, *Corynebacterium kutscheri*, and endo- and ectoparasites such as *Giardia muris*, *Myobia musculi*, *Myocoptes musculinus*, *Radfordia affinis*, *Syphacia* spp., and *Aspicularis tetraptera*. Mouse norovirus, *Rodentibacter pneumtropicus, R. heylii*, and *Helicobacter* spp. were endemic in the vivaria. All procedures and housing were in compliance with the *Guide for the Care and Use of Laboratory Animals*.[14]

Experimental Design

Methods and procedures common to all experiments. Female and male wild-type C57BL/6 mice, 22 to 34 d of age, were used for all experiments for a total of 122 mice; mice were obtained from inhouse breeding colonies. Mice were group-housed, up to five mice per cage, until the afternoon of their behavioral trial, at which time a mouse was transferred to a cardboard holding box for transport to the behavioral testing arena. Immediately before the behavioral test, mice were weighed, and their sex was confirmed via visual inspection of the anogenital region. Trials were conducted between the times of 1600 h and 2000 h in Experiments 1 and 2 to match the interval of the day during which high levels of research activity are occurring in mouse housing rooms, with mice being removed from and returned to cages or transported. Spanning the dusk photocycle transition also allowed us to observe behavior that would presumably be performed by an escaped mouse around the beginning of its circadian active phase if the mouse was not captured before the dark phase. The time at which the experiment took place was shifted in Experiment 3 because we had found that the dark phase did not increase capture of mice in Experiments 1 and 2. Running the experiment in the light also facilitated hand-capturing of mice. Each mouse was used in only one trial with no crossover between experiments. Mice were euthanized with carbon dioxide at a flow rate of 30 to 70% chamber volume per minute after the conclusion of the behavioral test.

Behavioral tests. To better mimic a real-life setting in which the space is novel, mice were not given an opportunity to explore or habituate to the arena prior to behavioral testing. All behavioral tests began by placing a mouse into the arena by the investigator, who then immediately left the room. Behavioral tests were monitored from an adjacent, acoustically isolated room via a camera that was mounted inside the testing room outside of and above the wall of the arena and linked via closed-circuit digital video (Camera/router: D-link, DCS-5020L, Irvine, CA).

Each behavioral test ended after a predetermined interval or after a mouse became captured in a trap. Mice that were captured in a trap were immediately removed from the testing arena and euthanized. After each behavioral trial, the walls of the enclosure were sprayed with and live traps were doused in 70% isopropyl alcohol, followed by rinsing in tap water. Live traps were then partially dried with paper towels and allowed to air-dry. Glue traps were discarded after each trial. Video recordings were stored for later scoring offline. All scoring was performed by the same investigator (JS). Ethogram descriptions and definitions used in behavioral scoring are described in Figure 1. Recorded video was carefully analyzed with frequent use of pause, rewind, and slow-motion playback to verify accurate timing and action based on Figure 1 definitions. Experiments 1 and 2 ended after a maximum of 3 h or after trap capture, whichever came first. Experiment 3 ended after a maximum of 6 h or trap capture, whichever came first.

Behavioral parameter	Description
Trap investigation (d, f)	Subject is less than a body length (measured from tip of nose to base of tail) away from the trap and is engaged with the trap demonstrated by having its head directed towards the trap, sitting or grooming next to the trap, or climbing and walking on the trap
Climb investigation (d, f)	Any behavior taking place while the mouse had all four feet on top of a trap
Door investigation (d, f)	Trap investigation at an opening of a trap
Head enters trap (f)	At least one eye is fully nonvisible or half of both the eyes are nonvisible from entering the head into the trap
Body enters trap (f)	Greater than 50% of the body (measured from tip of nose to base of tail) has entered into the trap. This is consistent with a capture if the body enters a glue trap, and can be down without a capture in a live trap
Grooming (d, f, l)	Cleaning of face, or any licking or scratching, of the body for greater or equal to two seconds
Sleeping (d, f, l)	Lack of movement (eyes closed if visible) in a crouched position for greater than 20 seconds
Target box exploration (d, f)	Greater than 50% of the body (measured from tip of nose to base of tail) is inside of a target box
Recoil	A sudden draw back with head and/or body associated with trap exploration, followed by grooming or ambulation away from trap

d = duration, f = frequency

Figure 1. Description of parameters and behaviors assessed in Experiments 1 and 2 of this study.

Experiment 1: Interactions with a single trap. (n = 45 mice; 22 females, 23 males). This experiment aimed to characterize general features of mouse behavior in the presence of traps. To evaluate mouse behavioral responses toward traps, 3 different traps were tested within the arena (Figure 2): (A) a glue trap containing synthetic peanut butter scent (Mouse and Insect Glue Board #72TC, Catchmaster, Bayonne, NJ, USA; "Glue"), (B) a mouse live trap with a clear window (Victor Tin Cat #M308, Woodstream, Lancaster, PA, USA; "Clear"), (C) a mouse live trap with a red-tinted window (Colored Light Filter, McMaster-Carr, Santa Fe Springs, CA, USA; "Red"). The red filter allows less than 10% light transmission between 420 and 600 nm of the visible spectrum and is the same wavelength filter used on door porthole windows in the rodent housing rooms. The investigator randomly chose mice and experiments or trap types in a manner that alternated trap types and equally allocated the number of total mice per trap group and numbers of males and females per trap group. The type of trap tested varied from trial to trial so that the order of mice chosen from each cage for each trial would not correlate to the trap type order.

The behavioral testing arena was constructed of moisture resistant high-density polyethylene (HDPE) plastic and measured 1.22 × 0.6 × 0.6 m (4 × 2 × 2 ft). It was placed on the floor of an animal housing room that was dedicated to behavioral

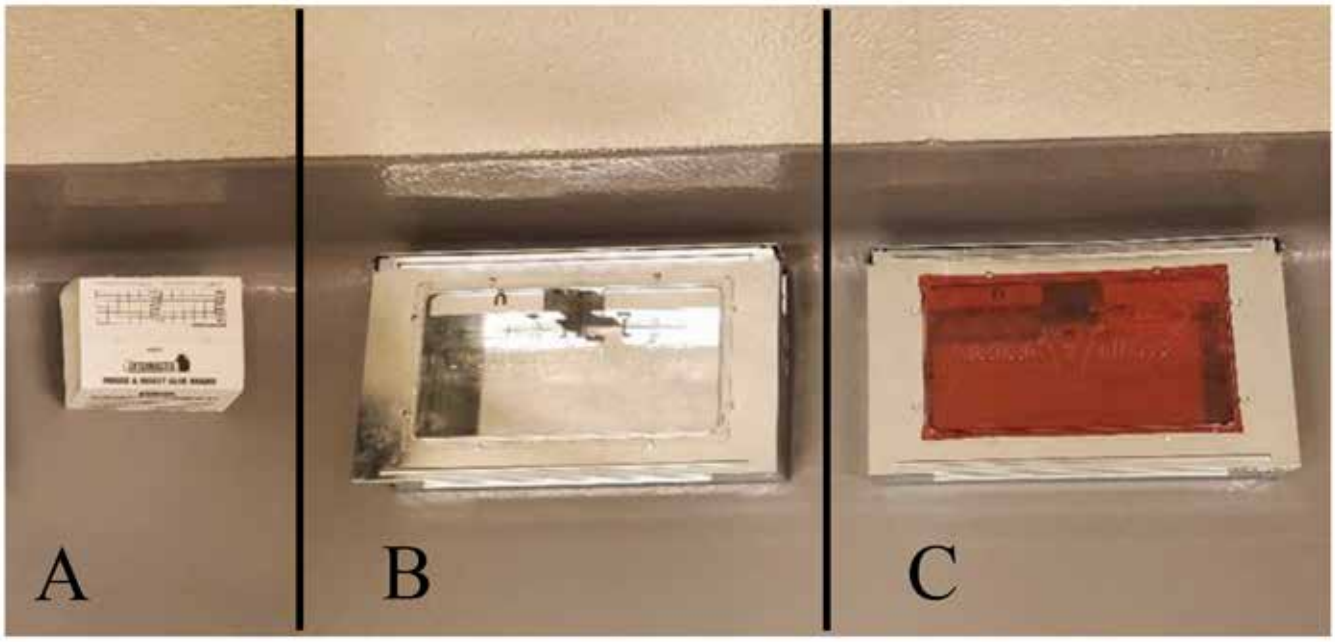

Figure 2. Traps tested in experiments. (A) the glue trap, (B) the live trap with a clear viewing window, and (C) the live trap with a red-tinted viewing window. All 3 trap types s were assessed in Experiments 1 and 2, and only A and B in Experiment 3.

testing for the entire study interval. Lines on the floor delineated a start zone at one end of the arena (30 × 60 cm) and a target zone (33 × 60 cm) at the opposite end of the arena, along the long axis. Prior to each test trial, a single trap was placed in the target zone, centered against the back wall of the zone (Figure 3A).

The test began when a mouse was removed from the transport cage by its tail and placed in the start zone against the middle of the front wall. The test ended when either: (1) the mouse was captured, or (2) 3 h had elapsed without the mouse being captured. Key behavioral parameters measured for Experiment 1 included latency to approach a trap and time until capture (see Figure 1 for a complete ethogram of all behavioral measures scored from videos). One male mouse was excluded from the Red group in Experiment 1 because its capture time was unclear (it hovered in the entryway of the trap for an extended period of time, and whether it moved through the trap door could not be determined).

Experiment 2: Trap preference in open field. (n = 49 mice; 25 females, 24 males). This experiment tested mouse preference for trap type. The arena contained two different traps, each situated in the middle of the shorter walls at opposite ends of the arena's long axis, as in Experiment 1 (Figure 3B). Experiment 2 was conducted in the same testing room and arena, and at the same times of day, as Experiment 1. A pilot study conducted prior to the start of the study indicated that mice tended to prefer the end of the enclosure furthest from the room's door; the arena was reoriented such that both ends of the arena were equidistant from the door.

Each end of the arena contained a target zone (30 × 60 cm), with a trap against the middle of the opposite far walls (Figure 3B). In each trial, one of the 3 trap types (Glue, Clear, Red) was placed in a target zone at each end of the arena. Thus, each trial permitted evaluation of the preference for one trap type over another. Combinations of traps evaluated against one another were: Glue compared with Clear, Glue compared with Red, Clear compared with Red, and Glue compared with Glue. Trap combinations were randomly selected using a random number generator, and placement locations (left compared with right) were alternated from trial-to-trial.

A mouse was released into the arena by gently sliding it out of a clean plastic opaque cup (Solo Plastic Party Cup- 18 oz, Dart Container Corporation, Mason, MI) onto the floor at the center of the arena equidistant from each trap and against a wall. The opaque cup prevented mice from seeing the traps or arena prior to trial onset. Key behavioral parameters measured for Experiment 2 included investigation time for each trap type and latency to capture (see Figure 1 for a complete ethogram of all behavioral measures scored from videos).

Experiment 3: Trap preference in typical room setting. (n = 28 mice; 11 females, 17 males). This experiment assessed whether trap preferences characterized in Experiment 2 extrapolated into a more naturalistic environment, specifically one that closely simulated conditions that an escaped mouse would encounter in an animal housing room in the vivarium.

Venue. A vacant room in the vivarium (area: 16.1 m^2) was outfitted with animal care and husbandry equipment in a manner that simulated an occupied rodent housing room (Figure 4).

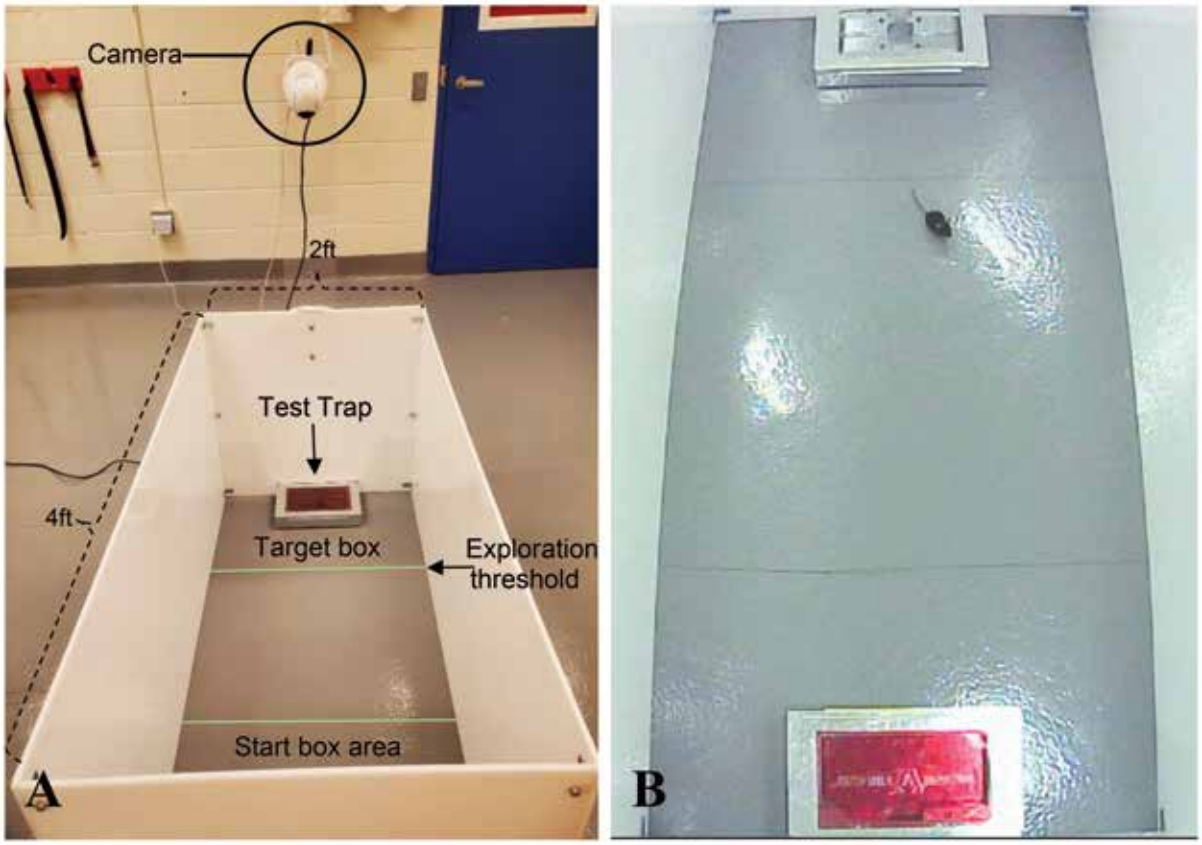

Figure 3. Images of experimental arena used in Experiments 1 and 2. (A) A view from the testing room with the camera over one end of the enclosure, reflecting conditions in Experiment 1 in which a single trap was placed on the floor in the target box. Individual mice were placed in the start box. (B) A representative frame of the camera's field of view for Experiment 2 of a mouse exploring the enclosure. Two traps were used, one placed at each end of the arena within target zones indicated by lines. In Experiment 2, mice were released in the middle of the arena, against a wall equidistant from each trap.

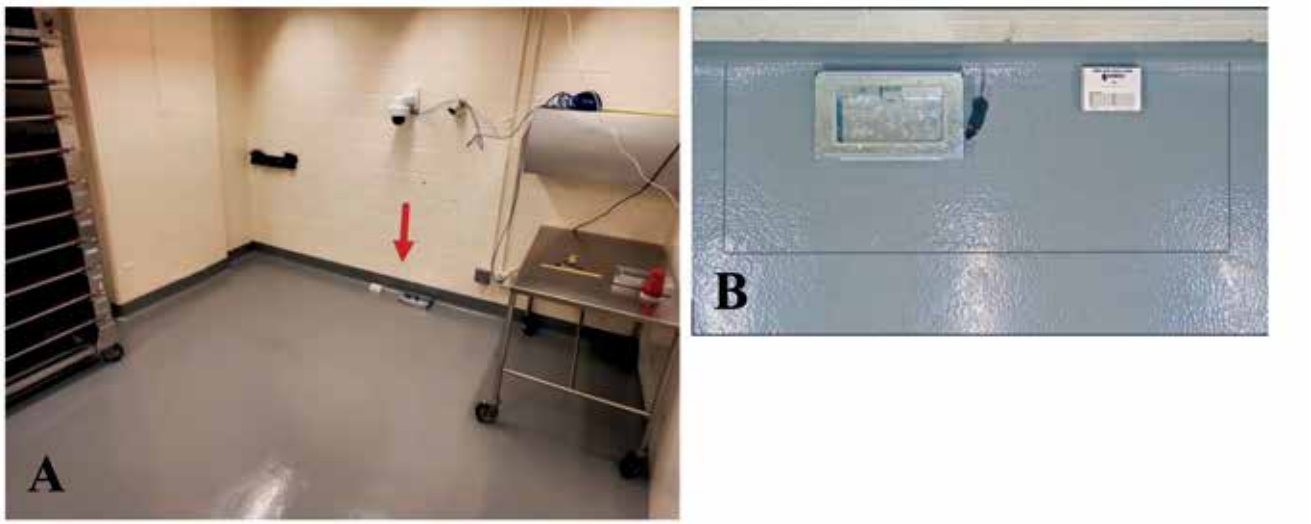

Figure 4. Image of the test room in Experiment 3. (A) A Glue trap and a Live trap can be seen against the far wall (red arrow). The 4 ft high-density polyethylene (HDPE) wall is visible on the right of the image. (B) A representative frame of the camera (main camera, located above red arrow) field of view for Experiment 3 of a mouse exploring the enclosure.

Equipment included an IVC rack (Thoren Caging Systems, Hazelton, PA), a class II biosafety cabinet (SG403 – Class II Type A/B3, The Baker Company, Sanford, ME), and a metal table (121.9 × 76.2 × 86.4 cm^3) (Figure 4A). No animals or cages were present on the IVC rack during the study interval. The room was also outfitted with a tall (1.2 m) plastic (high-density polyethylene; HDPE) wall creating a 3.7 × 4.4 m^2 barrier to prevent mice from reaching the room door during the test. A target zone (92 × 33 cm^2) was marked on the floor against the wall and one Clear and one Glue were placed adjacent to the wall at the center of the target zone (Figure 4B). The left/right positioning of the traps was alternated from trial-to-trial. A camera (Axis M5525-E PTZ Network Camera, Axis, Lund, Sweden) was fastened to the wall over the target box, and 2 additional cameras (DCS-5020L, D-Link, Irvine, CA) were positioned on another wall and on the ceiling; this array permitted recording of mouse activity in the entire room.

Each trial began when a mouse was released from a clean plastic opaque cup (as in Experiment 2) onto the floor beneath the biosafety cabinet. The trial concluded either when a mouse was captured in one of the 2 traps, or after 6 h (360 min) had transpired, whichever came first. All tests were performed between 0830 h and 2000 h.

The testing room was sanitized between trials as follows: the floor was mopped with a quatracide solution (Labsan 256 CPQ, Sanitation Strategies, Holt, MI). The bottom metal lining and wheels of the IVC rack were also cleaned with a mop and quatracide after each trial. At approximately the midpoint of the study, we realized that mice frequently hid behind a vertical beam on the bottom metal lining of the IVC rack and did not explore the room. In an attempt to discourage this behavior, the undercarriage of the rack was cleaned thoroughly after all subsequent trials as follows: a scrub brush was used to apply GP 100 (Sanitation Strategies, Holt, MI) to rid the rack of mouse urine and feces that were not otherwise eliminated by the previous room sanitization practices. The undercarriage/lining was then cleaned with tap water and a sponge, followed by the normal quatracide mopping. The key behavioral parameters measured for Experiment 3 were latency to capture and trap type for capture.

Statistical methods. Statistical analysis was performed using Stata 17 by the University's Biostatistics Laboratory and Research Computing Group (StataCorp LLC, College Station, TX). Statistical significance was defined as $P < 0.05$. Data were summarized using percentages for categorical variables and median values for continuous measures. The percentage of mice that were trapped was compared across groups using the Fisher exact test. Time to first approach was compared across groups using the Kruskal–Wallis test. Time until trapped was compared across groups using the log-rank test, and the median time was estimated using the Kaplan–Meier method. Mice that were never trapped were censored at the end of the experiment. Pairwise comparisons were only performed if the overall comparison was statistically significant. Time until capture was censored for all experiments. For this reason, medians were used instead of average total times.

Results

Experiment 1: Interactions with a single trap. All of the mice in the Clear and Red groups were captured, and 9 of 14 mice were captured in the Glue group (Figure 5A). Capturing a research mouse took significantly longer with the Glue trap as compared with either the Clear or Red trap (Figure 6). The total capture time did not differ significantly between the Clear group and the Red group (Figure 6). The differences in time until capture were large between the Glue and the Live trap groups; no significant differences were detected between any of the groups in the time it took for mice to first approach each trap (Table 1, Figure 7). Females and males did not differ significantly in capture time (Table 2).

The substantial variations in the time to capture between groups complicated comparison of other behaviors such as grooming, sleeping, and trap investigation. Mouse behavior changed over the 180 min, with mice displaying much more activity and movement toward the beginning of the trial, with more prolonged grooming and sleeping after about 75 min into the experiment. Even the use of proportions of time spent doing certain activities would not be an accurate comparison because capture total time ranged from less than a minute to 180 min.

Experiment 2: Trap preference in open field. Mice in an arena with at least one live trap were more likely to be captured and were captured more quickly than were mice in the arena with only Glue traps (Figure 8, Table 2). Similar to the total time until capture for the Red and Clear groups in Experiment 1, the total

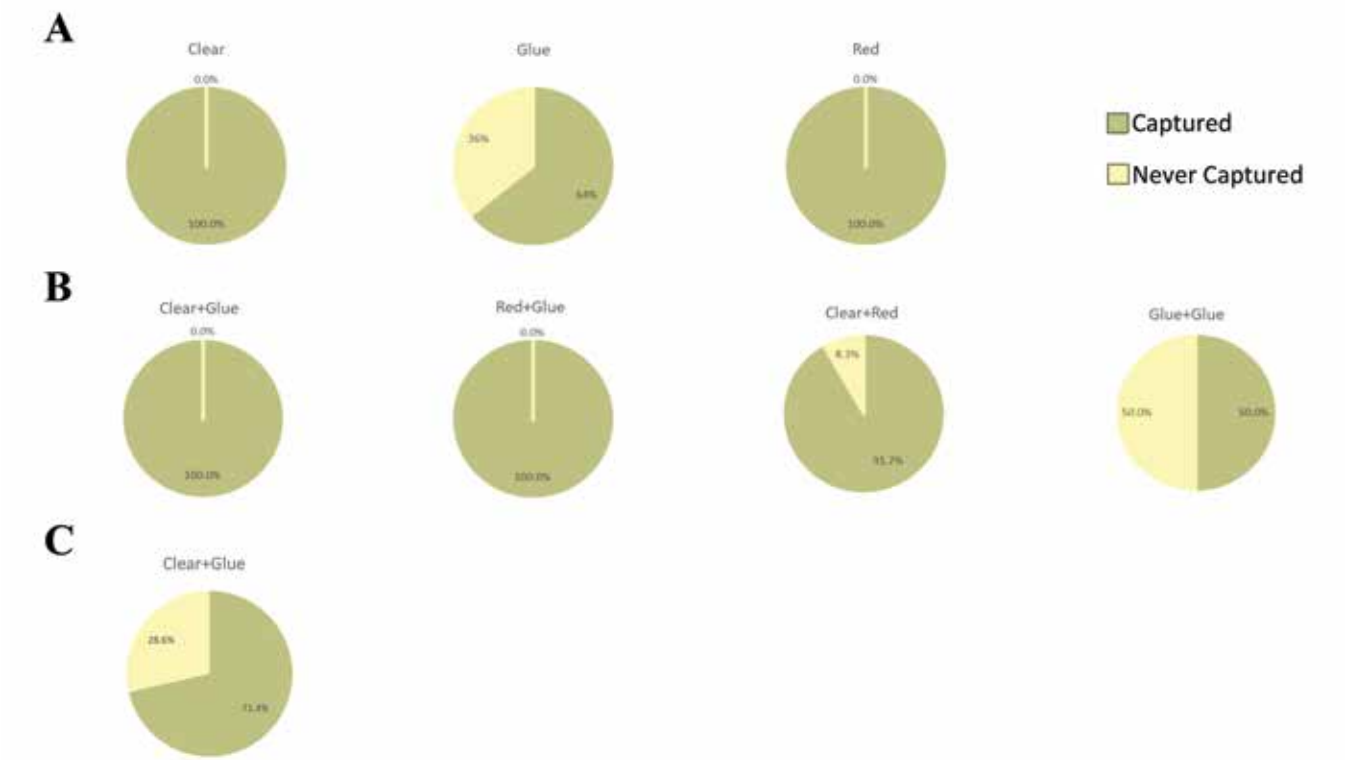

Figure 5. Percentage of mice captured per group for Experiments 1 (A), 2 (B), and 3 (C). Mice in Experiment 1 were placed into an arena for a maximum of 3 h with either a Clear, Glue or Red trap. Mice in Experiment 2 were given the same maximum time and chose between 2 traps in the same arena (pairing of 2 traps that were either Clear, Glue, or Red). In Experiment 3 mice chose between Clear and Glue traps while in a mouse housing room for a maximum time of 6 h. Groups that had a live trap type (Clear or Red) available had fewer mice that never become captured compared with groups with only a Glue trap.

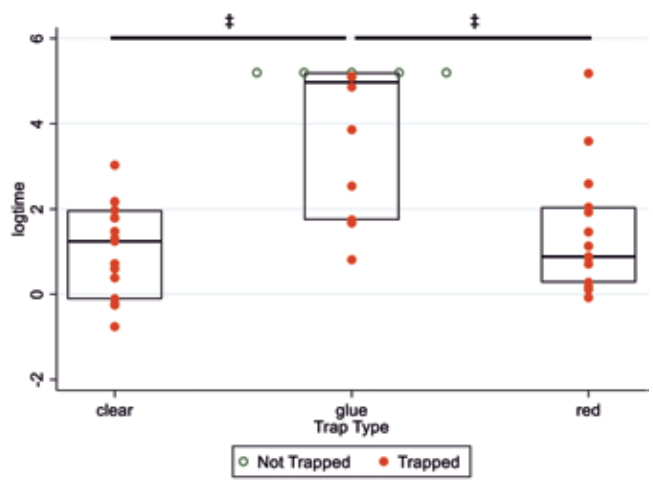

Figure 6. Box plot of the range and median log values for total time until capture in Experiment 1 for each trap type. The plot ignores censoring and assumes the maximum time for mice that were not trapped by 180 min (underestimates true time to being trapped). ‡ = $P < 0.001$, * = $P < 0.05$, all other pairwise comparisons did not have a significant difference.

time until capture were not different between the Clear+Glue and Red+Glue groups (Figure 8). In addition, the Clear+Red group was not different from the Clear+Glue and Red+Glue groups in the total time until capture. However, the total capture time for the Glue+Glue group was significantly different from those of the other 3 experimental groups. Of the 8 mice that were never captured, 7 were in the Glue+Glue group and 1 was in the Clear+Red group. All mice in the Clear+Glue and the Red+Glue

Table 1. Median time (min.) until first approach for each trap type in Experiment 1. The P value for all groups was >0.05, indicating there was no significant difference in the time it took for mice to first approach each trap type.

	Trap Type			
	Clear	Glue	Red	P value[a]
N	15	14	15	
Median (min.)	0.53	0.33	0.38	0.43

[a]Kruskal-Wallis test was used to obtain P value

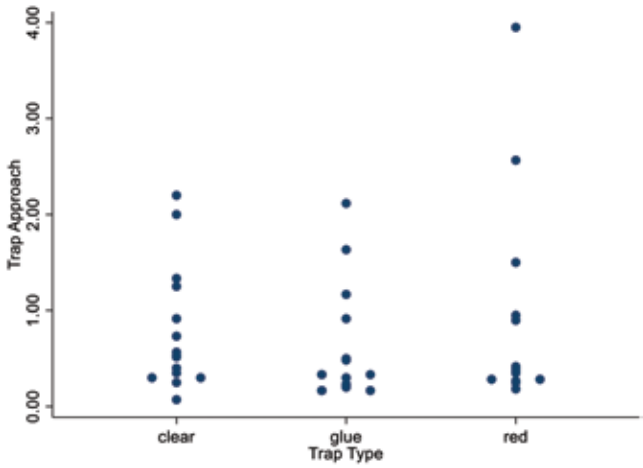

Figure 7. Dot plot of individual mice for the latency to approach each trap type in minutes for Experiment 1.

Table 2. Comparison of median time (minutes) until capture of females and males inthe 3 trap types in Experiments 1, 2, and 3.

	Overall	Female	Male	*P* value[a]
Experiment 1				
N	44	22	22	
Clear (min.)	3.5	1.8	4.4	0.26
Glue (min.)	144.7	180[b]	128.5	0.33
Red (min.)	2.6	2.8	2.6	0.69
Experiment 2				
N	49	25	24	
Live+Glue (min.)	2.8	1.5	4.5	0.71
Clear+Red (min.)	4.2	8.3	0.7	0.08
Glue+Glue (min.)	131.2	180[b]	14.2	0.17
Experiment 3				
N	28	11	17	
Live+Glue (min.)	30.7	43.9	19.2	0.78

[a]Log-rank tests were used to obtain *P* values.
[b]Underestimate of true value due to censoring

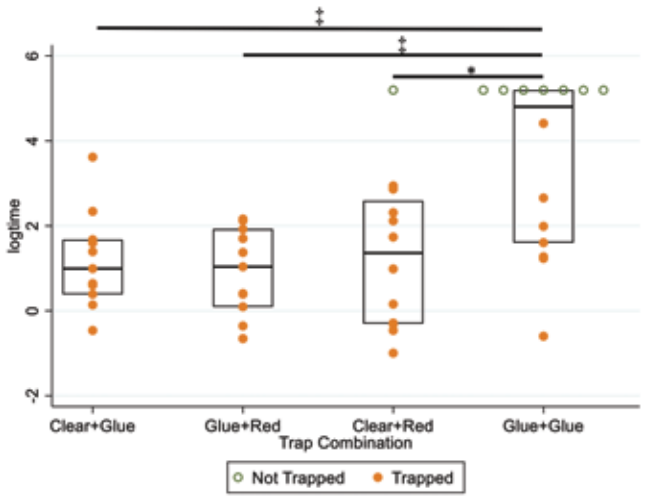

Figure 8. Box plot of the range and median log values for time until capture for Experiment 2 for each trap group. The plot ignores censoring and assumes the maximum time for mice that were not trapped by 180 min (underestimates true time to being trapped). ‡ = $P < 0.001$, * = $P < 0.05$, all other pairwise comparisons did not have a significant difference.

were captured (Figure 5B). Because the total times until capture, the percentages of mice captured, and the trap type preference were not significantly different between the live trap groups in Experiments 1 and 2 (Figure 9A), we concluded that mice had no preference for the red tint. Thus, the Clear+Glue and the Red+Glue groups were combined and called "Live+Glue" for further statistical analysis, and the Clear and Red traps were collectively called "Live" traps.

When in an arena with a Glue trap and a Live trap, mice were captured in the Live trap 91% of the time (Figure 9B). Of the 23 mice in these tests, 17 visited both traps at least once before being captured, while 6 mice were captured in the first trap they approached, including the only 2 mice that were captured in the Glue trap (Figure 9C). The 17 mice that investigated both live and glue traps before being captured were more likely to approach the Glue trap first (13 of 17), and all of the mice that first approached the Glue trap were later captured in a Live trap (data not shown). Mice in the Live+Glue trial spent most of their time near the Live trap or in the Live trap target box as compared with the Glue target box or the center area of the arena (Figure 9D). Total time until capture did not differ by sex for any of the trap groups.

Experiment 3: Trap preference in typical room setting. In the mouse housing room, 71% of the 28 mice were captured in either a Clear live trap or a Glue trap within the allotted 360 min (Figure 5C). Of the mice that were captured, 75% (15 of 20) were caught in the Live trap (Figure 9E). The time to capture did not differ significantly based on sex (Table 2).

Discussion

Designing a pest control program for vivaria is essential, yet approaches for pest control are limited by humane considerations in the animal research environment. Traps are used primarily to identify pests and to inform the pest control program of which pests are present so that they can construct an appropriate management plan. Traps can be used in close proximity to the research animals without causing potential negative side effects associated with other pest control methods, such as pesticides.[2] The best types of traps for capture of insect pests and wild mice have not yet been identified empirically, but both types are an integral component of a pest control program. The appropriate use of Glue traps to identify and monitor insect activity must be balanced with the welfare concerns that these traps pose for rodents. Our study analyzed mouse behavior toward Live rodent traps and Glue traps to better inform their use in vivaria.

Behavioral assessment of mice toward 2 types of Live traps and a Glue trap was performed to determine which trap type was most effective at capturing research mice, and which trap captures the most mice, indicating preference. To investigate this, mice were tested in 2 open fields that contained the different types of traps. In general, mice prefer to move to a protected space[18] and all traps in this study provided some level of shelter. The Glue trap was least effective at capturing mice in terms of frequency of capture and length of time until capture compared with either of the Live traps. The difference between the time until capture between the Glue trap only groups and the Live trap groups would have been even greater if mice had been allotted a capture time of infinity (in other words, if mice with longer capture times were uncensored) because all mice in the Clear and Red groups were captured in less than 180 min, while 5 of 14 mice in the Glue group were never captured (Figure 5A).

In an effort to enhance the standard commercial live mouse trap with a viewing window, we added a red tint over the trap's window. We expected that the mice would look into the trap and consider it as a dark place to seek refuge, given that mice have a low capacity to see light in the red spectrum[12,23] although humans can see through the tint to inspect the mice. However, Experiments 1 and 2 showed that mice were indifferent to the added red tint; mice were captured at the same rate in live traps with clear or red-tinted windows (Figure 6). When the Clear trap and the Red trap were tested together at opposite ends of the arena, trapping results were almost equal (Clear = 5, Red = 6, Figure 9A). We also found no difference between Live trap types when each was paired with a Glue trap with regard to either the type that captured the mouse or the total time it took to capture the mouse (Figure 8). Therefore, clear and red Live traps were equally effective in capturing mice. One possible explanation for this lack of difference may be that the red tint on the live trap did not provide as much darkness for the mice as was once thought, given that recent literature has found that rodents do indeed have physiologic responses to red light.[7,21] Another explanation may simply be that the level of darkness had no effect on the mouse's behavior in this setting.

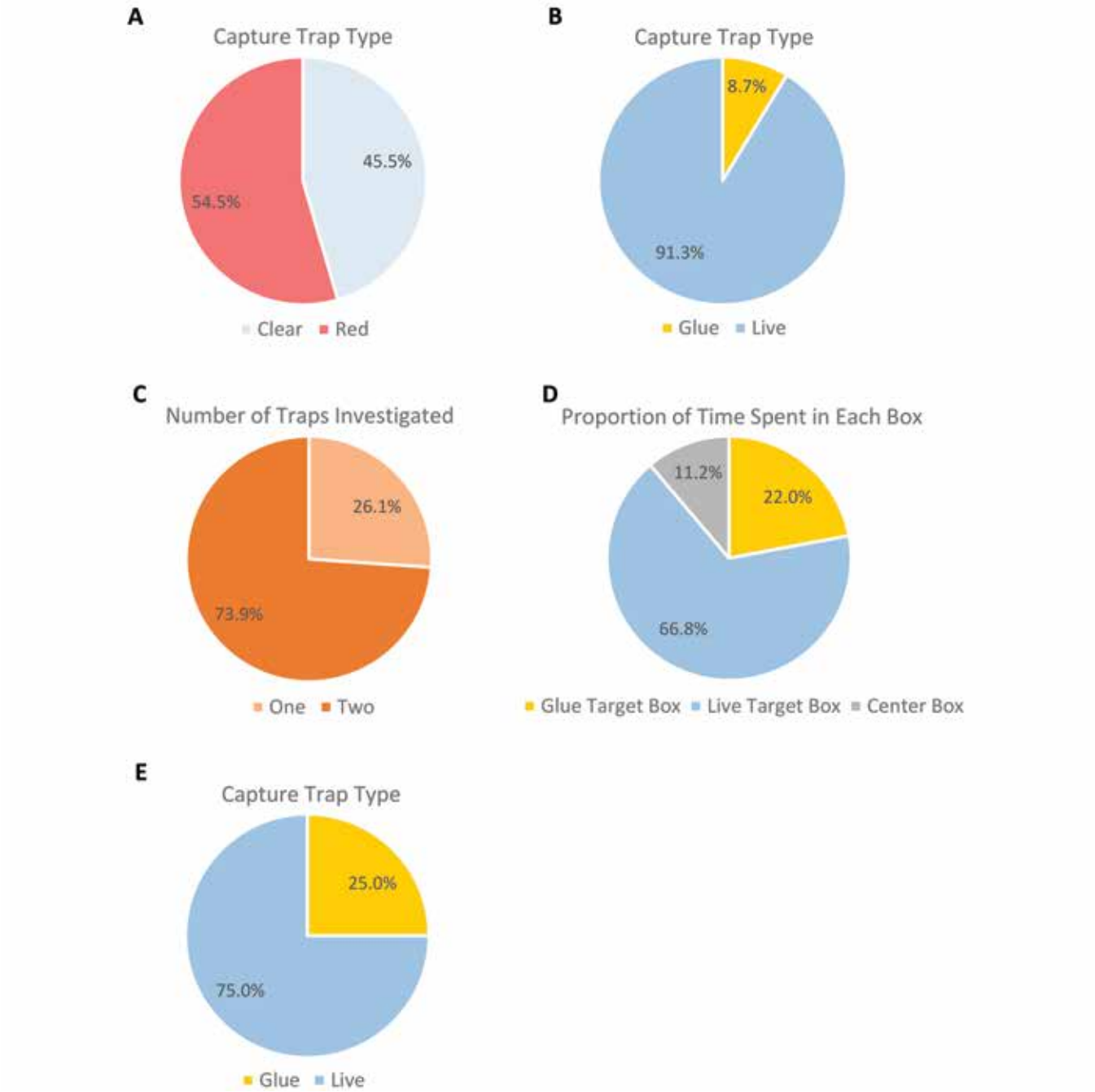

Figure 9. Trap preferences of lab mice in Experiments 2 and 3. (A) Percentage of mice captured per trap in the Clear+Red group from Experiment 2 ($P > 0.05$ by Fisher exact test). Mice from the Clear+Glue and the Glue+Red group were combined and collectively called "Live+Glue," because we found no difference in capture time between the Clear and Red group from Experiment 1, and no difference between the Clear+Glue and the Glue+Red group in Experiment 2. (B) Percentage of mice captured per trap in the Live+Glue for Experiment 2 ($P < 0.05$ by Fisher exact test). (C) Of the 23 mice in the Live+Glue group, 17 investigated both traps in the arena. (D) Average total time allocation of mouse activity in regions of the arena in Experiment 2 Live+Glue. (E) Percentage of mice captured per trap of all mice captured in Experiment 3 ($P < 0.05$ by Fisher exact test).

Adding a Live trap to an arena that contained a Glue trap significantly increased capture (Figure 5B, 8). Mice in groups with at least one Live trap in the arena were captured with the same frequencies and total times until capture. Adding a second Glue trap to the arena did not result in faster capture, as the capture times were not significantly different between the Experiment 1 Glue group and the Experiment 2 Glue+Glue group (102 min and 98 min, respectively).

In the experiments evaluating mice exposed to 2 traps at a time, approaching one trap can be viewed as an escape to this space or the avoidance of another. These escape or avoidance behaviors are confounded by the innate drive of mice to explore.[18] Anxiety can be defined as the conflict between a drive to approach a potentially threatening stimulus and the drive to avoid this same stimulus.[11] We assume that mice in this study experienced this conflict because they frequently investigated and then fled from all 3 types of traps; moreover, open field tests are often used to assess anxiety.[26] Mice approached all 3 trap types with the same, and fairly quick, latency (Table 1). Thus, the traps were apparently not different in their approachability and

were not an object of avoidance for mice when visualized from a distance. Only after investigating the traps did the mice show avoidance of the Glue trap, as they spent 3 times as long near the Live trap in the Live+Glue group in Experiment 2 (Figure 9D). We could not compare other anxiety-related behaviors, such as grooming and sleeping, in a way that would provide useful information because the way mice spent their time in the arenas changed from more exploratory in the beginning to more stationary behaviors as time went on. Mice in the Glue only groups were largely the only mice to remain in the arena long enough to show this shift in behavior, given that mice in Live trap groups were captured during the exploratory phase. These behaviors were not removed from Figure 1 in order to inform readers that these anxiety-related behaviors had been considered and measured. Future studies could include a category of grooming for a duration of less than 2 seconds. In our study, the video was not close enough to the mouse to allow us to determine with certainty that movements observed were grooming rather than other short movements of the head or limbs.

Mice spent more time near an object or at the ends of the arena, rather than away from an object or in center of the floor (Figure 9D). Even though mice were much more likely to be captured in a Live trap, they did not necessarily evade the Glue trap, as some mice would repeatedly climb on and even sleep on top of the traps. Mice seemed to avoid the inside of the Glue traps, however, with higher proportions of mice not being captured in Glue only groups (Figure 5A, B). Also, some mice were captured without purposefully entering the trap, as some slipped off the trap while jumping from the top of the trap toward the top of the arena. These mice appeared to land off balance on the edge of the trap and then slip onto the glue interior of the trap. Others would get their tail stuck while facing away from the trap. The data showed that 29% of mice captured by the Glue trap in Experiments 1 and 2 did not appear to have intentionally entered the trap. Mice that seemingly were trapped accidently on the Glue were still considered to be captured in this study because that could occur in a real-life situation unless the mice could immediately free themselves from the target box in which they were stuck.

Mice appeared to avoid the inside of the Glue traps. One of the most interesting behaviors we saw was an apparent recoil of the mouse after inspecting the open sides of a Glue trap. Although recoiling was observed in all experiments, this behavior was difficult to assess for several reasons. The velocity of the recoil, the distance that the head and body moved, and the activity proceeding the recoil varied. Therefore, the behavior could not be accurately quantified. Estimated ranges include the following: Experiment 1: 78% to 86% of Glue and 10% to 17% of Live displayed a recoil behavior, and in Experiment 2: 61% to 83% Live+Glue recoiled, with approximately 87% of the total associated with the Glue trap, and none (0%) associated with the Clear+Red. Two nonexclusive explanations are possible. One was whether the scent of the glue on the trap was repellent to mice, and the other was whether the mice were momentarily getting their vibrissae (whiskers) stuck to the glue. Recoil behavior would more appropriately be interpreted in a blind analysis in which the trap type not being visible in the video. However, a striking amount of research has studied rodent vibrissae and their somatosensory pathway;[3,10,15,22,24,27] this information could suggest mice sense the sticky glue with their whiskers. Future studies could leave the glue cover paper on the folded Glue traps and determine whether mice more readily enter the trap if the glue is covered. However, this would not be a realistic situation.

Our results were consistent with a study[5] on the efficacy of various traps for wild house mice. This study placed traps inside buildings to capture wild rodents and in all experiments, the nonglue traps were more than twice as effective at capturing wild mice.[5] Mouse activity was often detected on sticky traps, and yet the mice were not captured, suggesting that the mice were detecting the glue.[5] Furthermore, mice show curiosity but they also approach novelty cautiously and may stick their heads into traps, which could help to favor the Live traps. On entry-way inspection of Live traps, nothing inhibits the mice as glue would.[5] Mice are known to move their vibrissae in a focused direction toward an object or location of interest.[22] In a study that evaluated the extent to which mice used vibrissae for object localization, the data should that mice can locate objects at a correct position 86% of the time when their heads are fixed and only their whiskers could move, yet their detection of the location of the object fell to chance rates (51%) after their whiskers were trimmed.[22] Vibrissae help mice to locate objects, but they also allow discrimination of textures of objects.[3,10,15,24,27] Vibrissae have even been described as being comparable to the use of fingertips by primates to discern textures of objects.[3] Part of this texture discrimination is explained in *slip-stick theory* that describes whiskers moving across various coarse textures; this happens at different rates depending on the coarseness of the texture.[15,25] Several studies have examined the use of vibrissae for texture discrimination via in rodents, but none have evaluated sticky textures.[3,10,15,24,25,27]

Evidence supporting mouse avoidance of Glue traps was accrued in Experiment 2, which tested 2 trap types simultaneously (Figure 9C). The majority of mice visited both traps and investigated the Glue trap first. However, mice were not typically trapped by the first trap they encountered. All mice that approached both traps and also went to the Glue trap first were captured in a Live trap. One may question whether investigating a Glue trap first increases chances of being captured in a Live trap. Data collected in this study indicates that the simple answer would be "no" because mice in a Live+Glue group were captured after about the same total time as Clear or Red in Experiment 1 and Clear+Red in Experiment 2 (Figure 6 and 8). One would expect that if mice had been captured more quickly in the Live+Glue as compared with the Clear+Red, the median total time would be higher in the Clear+Red group, but these values were not significantly different. Future studies could evaluate whether mice try to escape from the Glue traps.

In the study performed in a housing room, the Glue and Live trap covered a much smaller surface area of the floor as compared with the first 2 experiments. In addition, mice had a greater area to explore and more places to hide in this larger space. Mice hid on the base of the IVC rack above the floor and behind one of 2 vertical columns in a large majority of the trials. Some mice stayed in these locations for the entire 6 h period, but some would eventually leave that location to explore the room for a short time before returning to the hiding spot. At one point in the study, consecutive mice hid behind the same column for substantial amounts of time. Closer inspection showed thar urine staining was present in a hole on the base of the rack that led to the wheel. The cleaning protocol was then changed to eliminate urine residue that may have been attracting mice to this spot. In subsequent trials, the locations in which mice spent most of their time varied and were more similar in distribution to those of the first several trials. Overall, the results from Experiments 1 and 2 translated fairly well to Experiment 3 in terms of the rate of capture in a Live trap (Figure 9E). A potential refinement to reduce the capture of mice in Glue traps

even more would be to position the Glue trap between 2 Live traps; however, testing that idea was beyond the scope of this study. Another consideration is whether the presence of caged mice in the room results in different trap capture rates and trap preferences, as scent cues could change the exploratory behavior of an escaped mouse behavior.

Integrated pest management is challenging in vivaria due to limited availability of methods that do not interfere with research or cause animal welfare concerns. A major aspect of a pest control program is the surveillance of unwanted pests, which informs programs of the types of pests present in areas of greatest concern. Although use of traps does not adversely affect mice used in research, they are not ideal because of welfare concerns. Specifically, Glue traps are highly effective at identifying insect pests, but are also a major hazard to escaped mice that could potentially become stuck on them. If the facility is notified of an escaped mouse, Glue traps may be removed from the floor and more Live traps be added. This is the practice we have adopted at our institution. Data from this study showed that research mice are more likely to enter a Live trap over a Glue trap. Therefore, if a facility is not informed of an escaped mouse and uses Glue traps on the floors, deploying both a Live trap and a Glue trap together provides an effective way to both identify insects and humanely capture mice.

Acknowledgments

We thank The University of Chicago Laboratory Animal Medicine Residency Training Program for funding this project. We also thank Keith Fournier for constructing the arena and research room for this study and Joe Mecyssine for lending his camera information technology skills, without which this project would not have been possible. In addition, we thank the University of Chicago's Biostatistics Laboratory and Research Group.

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