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Minibox: Custom solo or semi-group housing chambers for long term housing of rats with miniscopes ☆,☆☆

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Minibox –Solo and Semi-Group Housing Chamber

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ABSTRACT

In this detailed procedure, we include open-source methodologies using 'solidworks' designs for creating solo or semi-group housing units for rats wearing miniscopes for long periods of time. Builds are optimized to preserve rat health and prevent hardware destruction. We include all prices and suggestions for purchasing strategies to reduce overall build-costs.

- Chambers are optimized for long-term housing to protect rats wearing delicate headstages (e.g., miniscopes).
- Designed to be low-cost, efficient supplement to operant chambers and provides numerous benefits to long-term miniscope imaging. The housing chambers can be augmented by installing cameras, commutators, or different types of floor grids depending on experimental conditions.
- The chambers can also be secured to one another to create "rat-duplexes", allowing experimenters to control the degree of social isolation.

Specifications table

Subject area:	Neuroscience
More specific subject area:	Behavioral Neuroscience
Name of your method:	Minibox –Solo and Semi-Group Housing Chamber
Name and reference of original method:	A modular, cost-effective, versatile, open-source operant box solution for long-term miniscope imaging, 3D tracking, and deep learning behavioral analysis
Resource availability:	https://github.com/NJBeacher/NJBeacher.github.io

☆ **Related research article:** Nicholas J. Beacher, Jessica Y. Kuo, Miranda Targum, Michael Wang, Kayden A. Washington, Giovanna Barbera, Da-Ting Lin, A modular, cost-effective, versatile, open-source operant box solution for long-term miniscope imaging, 3D tracking, and deep learning behavioral analysis, MethodsX, Volume 12, 2024, 102,721, ISSN 2215–0161, https://doi.org/10.1016/j.mex.2024.102721.
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Background

This procedure is an extension of our previous procedure [1] that outlines the methods for building an operant chamber suitable for long-term miniscope imaging, 3D tracking, and deep learning behavioral analysis. Using similar materials, this procedure outlines the methods for building modifiable housing units that can be used for single or semi-group housing rats wearing delicate headstages such as miniscopes. These boxes may be used in tandem with our previously designed operant chambers. We have included detailed instructions and a companion video for the full construction of the housing chamber (see https://youtu.be/6Bcyd6ozczc?si=NjTjTA2aJjH8V2p3) and additional instructions for semi-group housing rats in duplex style connected units (https://youtu.be/B8BvHccKWk4?si=5ZQX67a10WSZVPno**)**. All solidworks designs are organized in the tables below and are found here: https://github.com/NJBeacher/NJBeacher.github.io.

This updated box is designed for housing rats wearing miniscopes. This version allows for a simple alternative method for housing animals that continues to reduce infection and protect miniature microscopes (miniscopes) from damage by components of standard rodent home cages. Housing chamber height and wall opacity can be adjusted to accommodate various experimental demands. The modifiable nature of the chamber also permits neural [2] and video data collection by attaching cameras [1] and commutators [2]. We outline a further modification by extending a single unit into a duplex unit which share walls. These adaptations enable experimental designs to apply varying degrees of social interaction across the shared duplex wall, with the option to continue extending units as desired. We have included detailed instructions and a companion video for this modified extension as well (see https://youtu.be/B8BvHccKWk4?si=5ZQX67a10WSZVPno).

These housing chambers are smaller and cost-efficient than the previously designed operant chambers. Similar to our previous designs, our costs are designed to scale with construction of 10 boxes (see Tables 1-6 for detailed price outlines). For example, constructing a single solo house ($879.05) or duplex unit ($712.40) vs. constructing 10 solo units (average is $419.05 per box) or duplexes ($242.40 per box). This reduction in cost is primarily a result of sharing acrylic from a single large order that scales as opposed to smaller individual orders.

Method details

Constructing the open-source housing chamber

Please see the attached instructional video (see https://youtu.be/6Bcyd6ozczc?si=NjTjTA2aJjH8V2p3) for a detailed walkthrough of each step. Costs of rails, acrylic, and components are detailed in Tables 1-3.

1. Constructing the side walls (see 0:12 https://youtu.be/6Bcyd6ozczc?si=NjTjTA2aJjH8V2p3)
   a. Measure 3″ from the bottom end of rails 1A and 1B

Table 1
McMaster rails necessary for each housing chamber.

Label	Description	Solid Works Part	Rail Length (in)	Quantity / Box	Part ID	Cost / Inch	Total Cost / Box
1A, 1B, 1C, 1D	Square, 4-Slot	1A_1D_16in47065T801_T-Slotted Framing rail	16	4	47065T807	0.77	49.28
2A, 2B	Square, 3-Slot	2A-2B_9_5in6575N217_T-Slotted Framing Rail	9.5	2	6575N217	0.54	10.26
3A, 3B, 3C	Square, 3-Slot	3A_3B_3C_11_5in6575N217_T-Slotted Framing Rail	11.5	3	6575N217	0.54	18.63
4A, 4B, 4C	Square, 4-Slot	4A_4C_11_5in47065T801_T-Slotted Framing rail	11.5	3	47065T807	0.77	26.57
5A, 5B	Square, 2-Slot	5A_5B_11_25in47065T846_T-Slotted Framing	11.25	2	47065T846	0.50	11.25
6A	Round, 2-Slot	6_11_5in6575N218_T-Slotted Framing Rail	11.5	1	6575N218	0.48	5.52

Dimension information and pricing regarding the rails used for the housing chamber. SolidWorks designs were modified based off designs available in the links for each Part ID. All designs for rails are included: https://github.com/NJBeacher/NJBeacher.github.io The total cost does not scale with additional boxes like the acrylic cost scales. We estimate a single box costs 121.51 for these rails.

Table 2
Acrylic panels necessary for each housing chamber.

Label	Solid Works Part	Width (in)	Length (in)	Thickness (in)	Quantity /Box
pA	pA	10	12.5	0.1875	2
pB	pB	12	13	0.1875	1
pC	pC	9.75	12	0.1875	1
pD	pD	9.875	12	0.1875	1
pE	pE	2	10	0.1875	5

Information regarding the acrylic dimensions used for the housing chamber. Larger orders of acrylic are suggested as this scales with cost as they will typically be cut from a single acrylic sheet. Therefore, each part being ordered should be adjusted for each website to maximize the total pieces acquired from a single ‘cut’. For a single box our cost is estimated at $500 (approximately $100 per part per order), however, we estimate this cost to be $500 for 5 boxes, or $600 for 10 boxes. For example, part pA should get 2 orders due to requiring 2 parts/box while part pE only requires 1 order of 33 units for the same price due to the smaller size. This practice is done by maximizing order quantities in our practice using ACME-plastics.

Table 3
Additional hardware components for each housing chamber.

Name	Description	Manufacturer	Quantity/ Box	Part ID	Cost/ Part	Cost/ Box
Rail-to-Rail Hinge	Housing chamber door hinge	McMaster-Carr	2	47065T347	19.39	38.78
Pull Handle	Door handles for main/social	McMaster-Carr	1	47065T595	12.84	12.84
Antislip Cover**	Interspaces acrylic floors/gasket. Optional if using Med Associates flooring	McMaster-Carr	1	47065T362	19.01	19.01
Panel Gasket *	Used as gasket to keep acrylic panels firmly in place.	McMaster-Carr	1	7437N11	8.21	8.21
Corner Concealed Bracket	Attach rails (internally)	McMaster-Carr	4	5537T315	3.08	12.32
Corner Surface Bracket	Attaches rails (externally)	McMaster-Carr	14	47065T267	11.60	162.40
Deadbolt	Locks housing chamber	Reliabilt	1	3,728,814	3.98	3.98

Information regarding the additional hardware components used for the housing chamber. We estimate this cost to be 257.54 for 1 box and a small savings of ~20.00 per box due to using gasket across all 10 boxes.

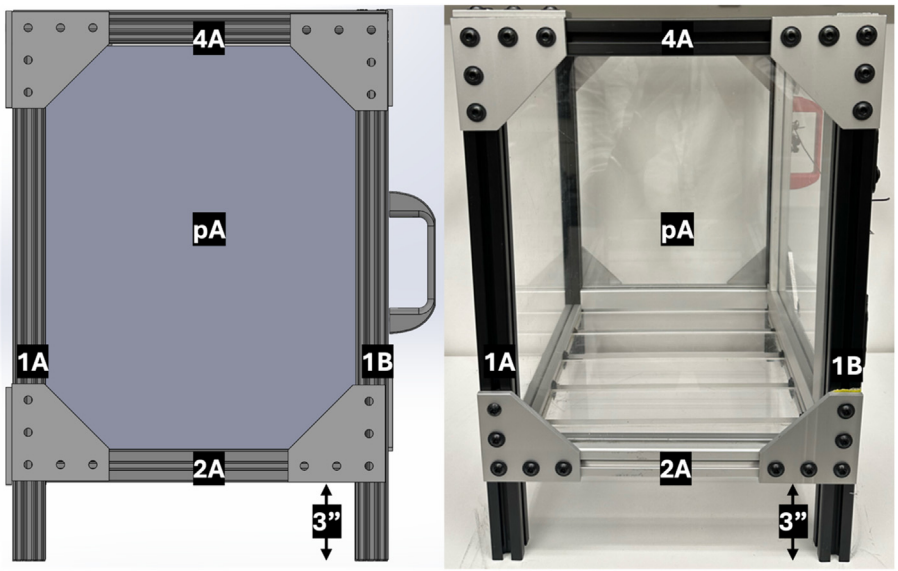

Fig. 1. Left side of the housing chamber.

b. Orient rail 2A such that the smooth face will be facing the interior of the housing chamber.
c. Using two surface brackets, secure rail 2A to rails 1A and 1B at the 3″ mark (Fig. 1).
d. Repeat steps 1a-1c to secure rail 2B to rails 1C and 1D (Fig. 2).
e. Measure 2.5″ from the bottom of rails 1B and 1C.
f. Orient rail 3A such that the smooth face is towards the top of the box.
g. Using two corner concealed brackets, secure rail 3A to rails 1B and 1C at the 2.5″ mark (Fig. 3).
h. Repeat steps 1e-1 g to secure rail 3B to rails 1A and 1D, however, orient rail 3B such that the smooth face is towards the back of the box (Fig. 4**A**).
i. Slide plexiglass pA between rails 1A and 1B and another pA between rails 1C and 1D.
j. Slide plexiglass pB between rails 1A and 1D.
k. Secure a deadbolt with locking grooves facing downwards onto the front face of rail 1C.

2. **Securing the chamber roof (see 4:01** https://youtu.be/6Bcyd6ozczc?si=NjTjTA2aJjH8V2p3)
   a. Orient rails 4A, 4B, and 4C, such that 4B is perpendicular to and between 4A and 4C. These are the roof framing rails.
   b. Attach two surface brackets onto the top face of rail 4B at either end and use these brackets to secure rails 4A and 4C (Fig. 4**B**).
   c. Attach four additional surface brackets onto rails 4A and 4C on the outer face of the rails such that these brackets will secure onto rails 1A, 1B, 1C, and 1D.
   d. Secure the roof framing rails onto rails 1A, 1B, 1C, and 1D.
   e. Slide plexiglass pC such that it sits in the grooves of rails 4A, 4B, and 4C (Fig. 4**B**).
3. **Constructing the chamber door (see 5:47** https://youtu.be/6Bcyd6ozczc?si=NjTjTA2aJjH8V2p3)
   a. Orient rail 5A such that the two smooth faces are towards the bottom and the inside of the box.
   b. Secure two surface brackets to either end of the outer face of rail 5A (Fig. 3; Left).

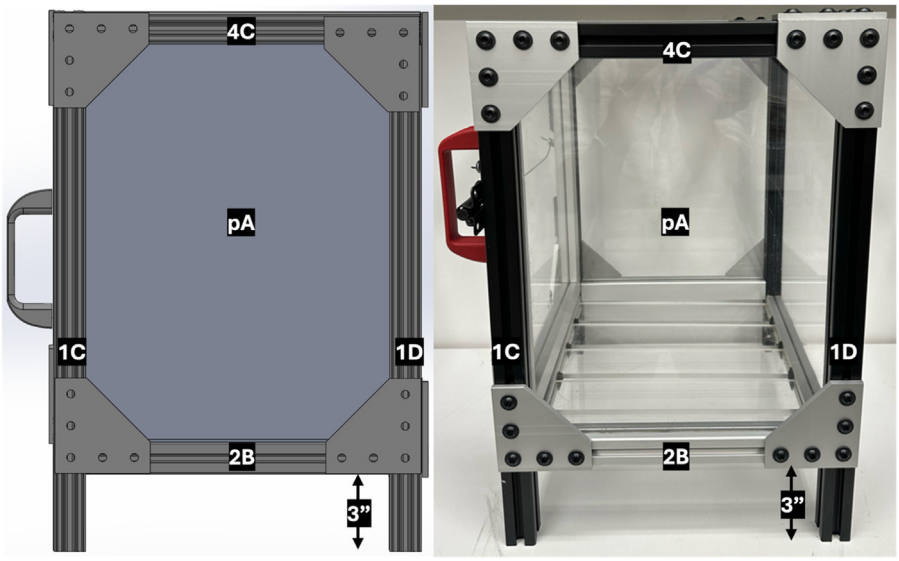

Fig. 2. Right **side of the housing chamber.**

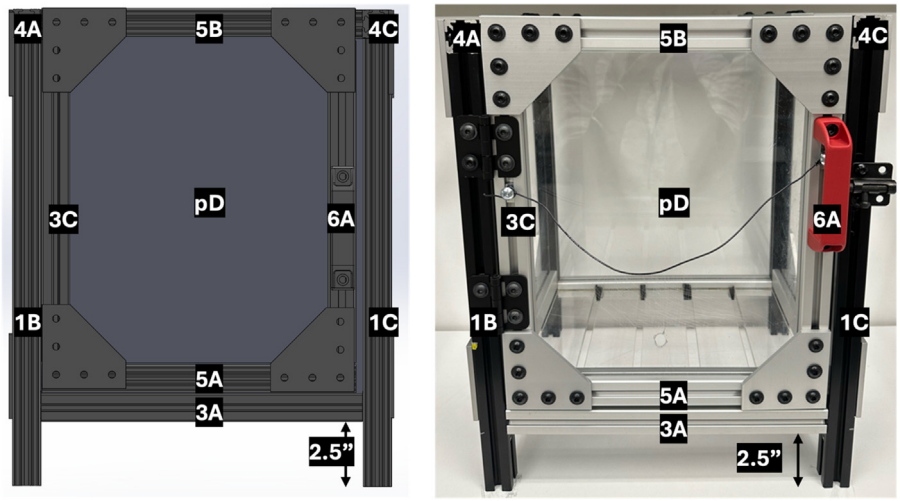

Fig. 3. Front face of the housing chamber.

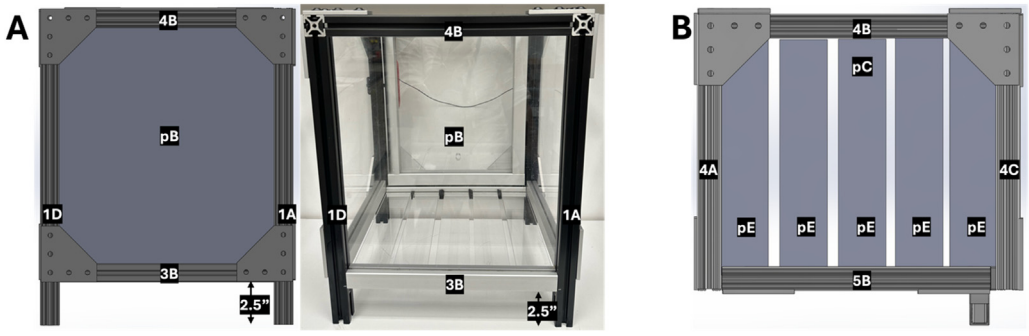

Fig. 4. Back and top-down faces of the housing chamber. **(A)** Back face of the housing chamber. **(B)** Top-down face of the housing chamber. Note that plexiglass pane pC is the roof of the housing chamber, and the 5 plexiglass panes pE are used as a flooring alternative to MedPC floor grids. However, these can still incorporate the med-associates style floor if necessary (see instruction in [1]).

Table 4
McMaster rails necessary for each rat duplex extension.

Label	Description	Solid Works Part	Rail Length (in)	Quantity / Duplex Extension	Part ID	Cost / Inch	Total Cost / Box
1E, 1F	Square, 4-Slot	1A_1D_16in47065T801_T-Slotted Framing rail	16	2	47065T807	0.77	24.64
2C	Square, 3-Slot	2A-2B_9_5in6575N217_T-Slotted Framing Rail	9.5	1	6575N217	0.54	5.13
3D, 3E, 3F	Square, 3-Slot	3A_3B_3C_11_5in6575N217_T-Slotted Framing Rail	11.5	3	6575N217	0.54	18.63
4D, 4E	Square, 4-Slot	4A_4C_11_5in47065T801_T-Slotted Framing rail	11.5	2	47065T807	0.77	17.71
5C, 5D	Square, 2-Slot	5A_5B_11_25in47065T846_T-Slotted Framing	11.25	2	47065T846	0.50	11.25
6B	Round, 2-Slot	6_11_5in6575N218_T-Slotted Framing Rail	11.5	1	6575N218	0.48	5.52

Dimension information and pricing regarding the additional rails used for a single housing extension. SolidWorks designs were modified based off designs available in the links for each Part ID. All designs for rails are included: https://github.com/NJBeacher/NJBeacher.github.io**.** We estimate a single box costs 82.88 for these rails and we estimate no price savings for 10 boxes.

Table 5
Acrylic panels necessary for each duplex extension.

Label	Solid Works Part	Width (in)	Length (in)	Thickness (in)	Quantity / Box
pA	pA	10	12.5	0.1875	1
pB	pB	12	13	0.1875	1
pC	pC	9.75	12	0.1875	1
pD	pD	9.875	12	0.1875	1
pE	pE	2	10	0.1875	5

Information regarding the acrylic used for the housing extension. We anticipate a cost of 500 per box and the same price for 10 boxes due to shared sections of part pA between boxes.

c. Attach rail 6A to the right side of rail 5A using the surface bracket. Ensure that rail 6 is oriented such that one slotted face is towards the outside of the box, and the other allows for an acrylic wall to slide into the door frame.
d. Attach rail 3C to 5A using the left surface bracket such that the flat face of rail 3C is towards the inside of the operant chamber.
e. Orient hinge joints to face outwards and secure the right halves of two hinges onto the outer face of rail 3C.
f. Secure a door handle onto the outer face of rail 6A at a comfortable height.
g. Insert plexiglass pD into the door frame between rails 5A, 6A, and 3C.
h. Orient rail 5B such that the smooth faces are towards the inside and the top of the chamber.
i. Attach two surface brackets to the outer face of rail 5B
j. Using the surface brackets, secure rail 5B to rails 6A and 3C.
k. Slide the door up from the bottom of rail 1B Tighten the hinges to rail 1B
l. Adjust the height of the deadbolt on rail 1C if necessary **(**Fig. 3**; Right)**.

4. Inserting the plexiglass flooring (see 9:01 https://youtu.be/6Bcyd6ozczc?si=NjTjTA2aJjH8V2p3)
   a. Insert 5 pieces of plexiglass pE into the interior slotted faces of rails 3A and 3B Either rail may be lowered to allow for the plexiglass to slide into position (Fig. 4B).
   b. Secure plexiglass pieces using thick rubber gaskets.

Extending a single unit into duplex units

It is possible to easily modify a single housing unit into a duplex unit. Although not directly shown in these videos, these boxes are designed to expand it into multiplexes along each common shared wall. Please see the attached instructional video (see https://youtu.be/B8BvHccKWk4?si=5ZQX67a10WSZVPno) for a detailed walkthrough of each step. Costs of rails, acrylic, and components are detailed in Tables 4-6.

1. Detaching the Right Wall of the Housing Unit (see 0:10 https://youtu.be/B8BvHccKWk4?si=5ZQX67a10WSZVPno)
   a. Detach the door by the hinges to allow for easier modification.
   b. Remove plexiglass roof pC and loosen the corner surface bracket that secures roof rails 4B and 4C.
   c. Loosen the corner concealed brackets that connect rail 3A to 1C, and rail 3B to rail 1D. Leave these brackets with rails 1C and 1D.
   d. Pull the right wall of the housing chamber away.
2. **Constructing the Housing Extension (see 0:59** https://youtu.be/B8BvHccKWk4?si=5ZQX67a10WSZVPno**)**
   a. Measure 3″ from the bottom end of rails 1E and 1F **(**Fig. 5**B and E)**.
   b. Orient rail 2C such that the smooth face will be facing the interior of the housing extension.
   c. Using two corner brackets, secure rail 2C to rails 1E and 1F at the 3″ mark on the underside of the wall.
   d. Install plexiglass pA into the central grooves of rails 1E, 1F, and 2C.

Table 6
Additional hardware components for each duplex extension.

Name	Description	Manufacturer	Quantity/ Box	Part ID	Cost/ Part	Cost/ Box
Rail-to-Rail Hinge	Housing Chamber door hinges	McMaster-Carr	2	47065T347	19.39	38.78
Pull Handle	Door handle	McMaster-Carr	1	47065T595	12.84	12.84
Antislip Cover**	Interspaces acrylic floors/gasket. Optional if using Med Associates flooring	McMaster-Carr	1	47065T362	19.01	19.01
Panel Gasket *	Used as gasket to keep acrylic panels firmly in place.	McMaster-Carr	1	7437N11	8.21	8.21
Corner Concealed Bracket	Attach rails (internally)	McMaster-Carr	4	5537T315	3.08	12.32
Tee Surface Bracket	Attach rails to join units	McMaster-Carr	1	47065T278	12.10	12.10
Deadbolt and bracket	Locks door	Reliabilt	1	3,728,814	3.98	3.98
Corner Bracket	Attach lower rail of shared wall	McMaster-Carr	2	47065T236	7.92	15.84
Drop-in Fastener	Attaches deadbolt bracket	McMaster-Carr	2	47065T003 (pack of 4)	5.44	5.44

Information regarding the additional hardware components used for the housing extension. We anticipate a cost of 129.52 per box due to shared walls and structural space with other housing units. Additional boxes estimate a cost savings of ~20 per box due to reusing gasket between units.

e. Attach two corner concealed brackets to the bottom face of rail 4D and reverse the remaining single tightening screws on both corner concealed bracket.
f. Secure the corner concealed brackets on rail 4D to the top of rails 1E and 1F.
g. Attach a tee surface bracket onto the top face of rail 4D such that the center screws are along rail 4D and the bracket is pointed forward **(**Fig. 5**F).**
h. Measure 2.5″ from the bottom end of rails 1E and 1F **(**Fig. 5**C and D).**
i. Orient rail 3D such that the smooth face is towards the top of the box. Attach rail 3D to rail 1E at this mark using a corner concealed bracket.
j. Orient rail 3E such that the smooth face is towards the back of the box, and attach rail 3E to rail 1F at this mark using a concealed bracket.
k. Attach rail 4E to the tee surface bracket on the top face of rail 4D.

3. **Attaching the Housing Extension (see 4:04** https://youtu.be/B8BvHccKWk4?si=5ZQX67a10WSZVPno**)**
   a. Insert plexiglass panel pB.
   b. Secure corner concealed brackets onto rail 1E and 1F opposite of rails 3D and 3E.
   c. Attach rail 3A to 1E and 3B to 1F at the 2.5″ mark, and the tee surface bracket on 4D to rail 4B
   d. Tighten all brackets.
   e. Secure the corner concealed brackets on 1C and 1D onto rails 3D and 3E respectively. Attach such that the bottom face of rails 3D and 3E are at the 2.5″ mark. Attach the corner surface bracket on 4C to 4E.
   f. Tighten all brackets.
4. **Constructing and Attaching the Doors (see 6:04** https://youtu.be/B8BvHccKWk4?si=5ZQX67a10WSZVPno**)**
   a. Construct a new housing chamber door as previously detailed in step 3 of “Constructing the open-source housing chamber.”
   b. Add a deadbolt lock onto the front face of rail 6B, within the door handle.
   c. Attach the new housing chamber door onto rail 1B This replacement is necessary as the original placement of the deadbolt will impinge on the free movement of housing extension doors.
   d. Adapt a deadbolt lock using two drop-in fasteners.
   e. Attach the original door by sliding the topmost hinge onto rail 1E, followed by the modified deadbolt bracket, and the remaining hinge **(**Fig. 5**A).**
   f. Align the deadbolt on rail 6B and deadbolt bracket on rail 1E such that the door can lock. Secure each hinge and deadbolt.
   g. Confirm deadbolts will lock each side of the extension.
   h. Insert plexiglass roof panels pC, and assemble custom plexiglass flooring as detailed previously in step 4 of “Constructing the open-source housing chamber” **(**Fig. 5**F)**

Method validation

Overall benefits of the housing chamber

When used in conjunction with the open-source operant chambers, these modifiable housing chambers provide several benefits to long-term miniscope imaging. In this way, the original designed operant chamber [1] can be used for both housing and recording, while this chamber can be used to house additional rats overnight and share the common chamber during recording sessions for optimal data collection procedures. By fitting water bottles and establishing a 24/7 light cycle using MedPC LED modules, these chambers provide a dedicated housing option for rat subjects that maintains the degree of cleanliness and safety provided by the operant chamber. By using the same metal rails and acrylic components as the operant chamber, these housing chambers are similarly easy to clean and represent a similar home environment. These materials also avoid the less sanitary bedding and sawdust of traditional shoebox cages, thereby reducing infection risk and allowing for miniscope equipment to remain affixed for the entirety of the

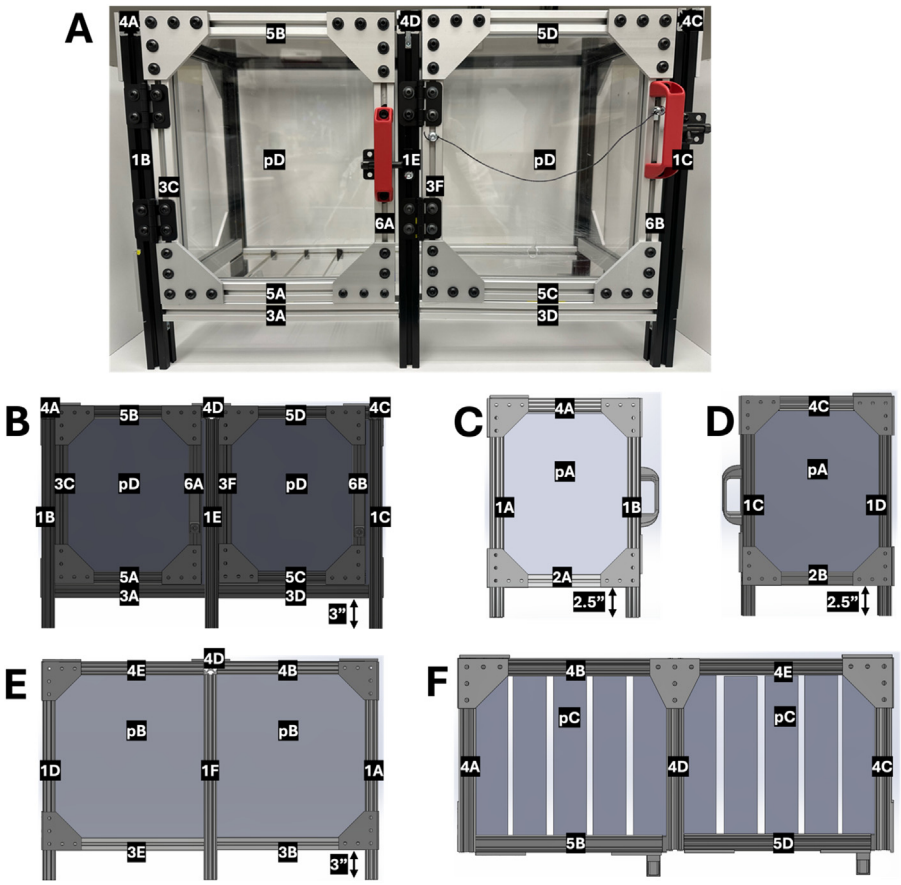

Fig. 5. Composite of the completed duplex housing unit. **(A)** Photo of the front face of the completed duplex housing chamber. **(B)** Model of the front face of the duplex housing chamber. **(C)** Model of the left face of the duplex housing chamber. **(D)** Model of the right face of the duplex housing chamber. **(E)** Model of the back face of the duplex housing chamber. **(F)** Model of the top face of the duplex housing chamber. Note that plexiglass pane pC is the roof of the housing chamber, and the 10 plexiglass panes pE (see Fig. 4) are used as a flooring alternative to MedPC floor grids.

experiment. These housing chambers maintain the high quality long-term miniscope imaging made possible by the operant chamber. As the equipment is permanently affixed on rat subjects, the housing chambers reduces rat exposure to isoflurane and protect subject health [3].

These housing chambers also reduce the cost of recording from multiple animals in an experiment. For example, while it is possible to house four animals, each in their dedicated operant chamber, it would be more cost-efficient to construct one operant chamber and three housing chambers, rotating animals between locations as necessary. As detailed in Tables 1-3, three housing chambers with one operant chamber reduces the cost of materials by more than $1000 compared to four operant chambers, prior to factoring in recording equipment and MedPC components. In summary, housing chambers used in combination with operant chambers reduce costs and materials, continue to protect equipment such as miniscopes and headcaps, and maintain animal welfare by avoiding excess isoflurane exposure [3].

Potential experiments for isolated animals

These housing chambers can also be used to modulate and control specific variables in an experimental condition. Cameras may be mounted using ThorLabs Optical Posts as previously described for the operant chamber [1]. Similar camera settings may also be applied. Using clear plexiglass, the housing chamber allows for omnidirectional observation of the subject. For example, experiments that examine social isolation as a variable would benefit from periodic video recordings, both during the day and overnight. Behavioral comparisons may be made over the course of this example longitudinal study.

Our design for the housing chamber does not include MedAssociates accessories such as levers, but our custom operant chambers have instructions for accommodating commercial materials and cameras (see: [1]). If neural recording in the housing chambers is desired, modifications can be made to adjust the height of the chamber and fit a commutator [2]. This housing chamber can also accommodate a MedAssociates floor grid in place of acrylic panels in the same method as the operant chamber design [1]. This is vital should experimental conditions require interventions such as a planned delivered shock paradigm [4].

The addition of these parts enables behavioral experiments in the chambers such as open field [5], One such longitudinal, open-field experiment could assess overnight alcohol drinking behaviors in rats with variable genotype knockouts. In this experiment, subjects have access to separate bottles of ethanol and water. Alcohol consumption would be monitored by intake volume, and persistent 24/7 recordings would allow for comparison of behavior analyzed using deep learning methodologies [6-9]. Within these modes of responding, different behaviors may arise such as the amount of alcohol consumed in a sitting, time between sittings, persistence around a bottle or lack thereof [10]. These variables would be quantifiable by analysis of the video recordings and identification of distinct behavioral microstates [7].

Potential experiments for semi-housed animals

Finally, the housing chambers allow for a unique experimental condition by securing units to each other in a duplex or multiplex arrangement. In this layout, it is possible to control for various factors and degrees of social interaction and isolation the rat experiences. For example, opaque or clear acrylic allows for adjustable visual stimulus by a social neighbor, and drilling holes into the shared plexiglass wall may allow for more direct and constant social interaction, from sniffing to whisking. Furthermore, complete isolation for rats [11] and humans [12-14] increases stress. Stress can impact reward processing in the limbic region and other regions of the brain [15,16]. Rats are typically group housed, or they are housed individually next to each other on shelves [17]. However, in certain experimental paradigms, isolation from other rats is necessary to prevent damage to key pieces of hardware, such as a miniscopes [1,18]. Our lab has previously identified rats exert more effort for familiar social partners while socially isolated [19] and this effect can now be further explored using this duplex style technique in rats wearing miniscopes.

Our new housing chamber design includes options for semi-group housing in which rats can share walls with a dedicated social partner. Social interaction has been found to be highly rewarding in both male and female rats, and motivation for social interactions changes as function of isolation and partner familiarity [19]. It is critical to account for these behavioral trends when designing experiments, especially when dealing with aspects of social reward [20,21], drug reward [19,21], and choosing between social or drug reward [22-25].

Certain experiments utilize social isolation in rats as a variable because of human relevance [26]. In humans, social isolation increases stress [12-14], drug use [27], and other issues [28]. Our custom housing chamber enables a minimalist version of our previous operant chamber to efficiently utilize resources between rats. We also include the option for semi-grouped rats in the duplex options. This way, researchers can take full advantage of all these newly created contextual tools at their disposal. Our housing chamber can be used to explore a spectrum of social isolation manipulations. Miniscope imaging, can be used to explore neural processing [2,18,29-33] in synchrony with behavioral posture changes identified using deep learning procedures[7] during reintroduction of the social partner [6,19,31], Ultimately, the modifiable housing chambers are cost-efficient and suitable for numerous housing and experimental purposes.

Limitations

Not applicable.

Ethics statements

All experiments were conducted in accordance with the guidelines of Institutional Animal Care and Use.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Nicholas J. Beacher: Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Michael W. Wang:** Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing, Visualization. **Matthew C. Broomer:** Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing. **Jessica Y. Kuo:** Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing, Visualization. **Da-Ting Lin:** Methodology, Validation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Resources, Funding acquisition.

Data availability

All data is shared in the GitHub links embedded in the paper.

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