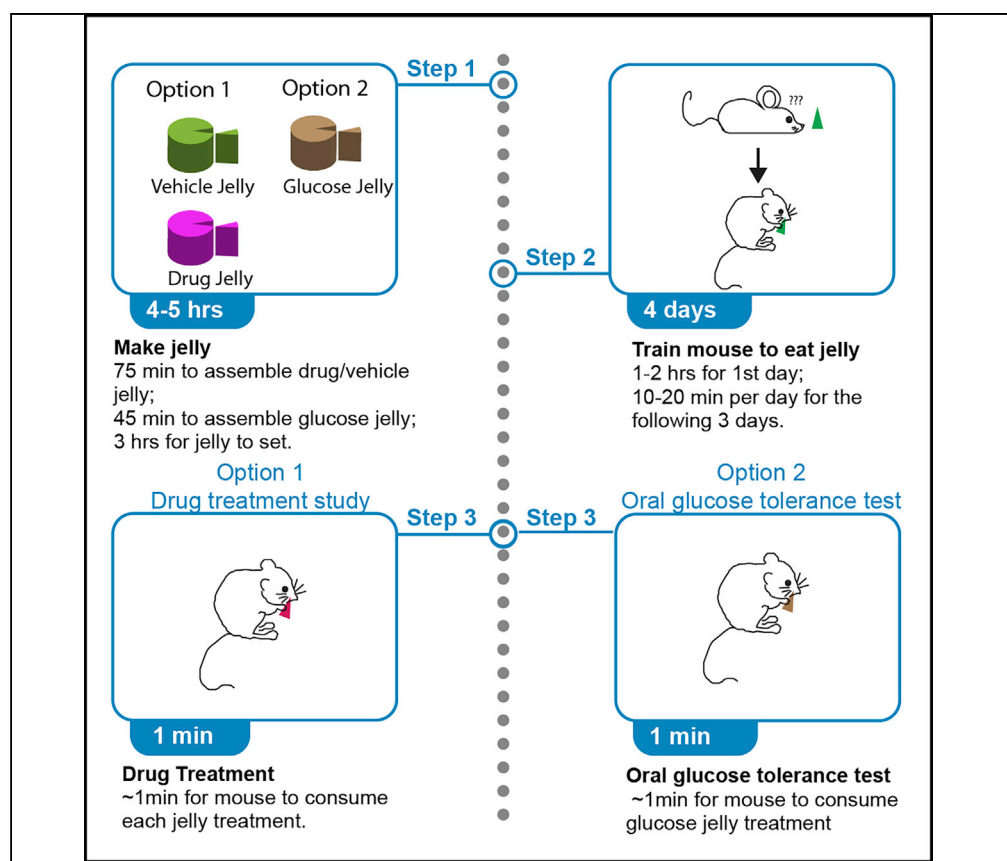


Protocol

Method for voluntary oral administration of drugs in mice



Substances are commonly administered orally in mouse experiments. Here, I describe how to voluntarily administer substances orally in a time- and dose-controlled manner to laboratory mice. This minimizes injury potential and stress often associated with the commonly used intragastric gavage technique. Here, the drug is incorporated into artificially sweetened and flavored jelly and given to mice previously trained to eat the jelly. This can be used for acute and chronic oral drug treatment or for oral glucose tolerance tests.

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HIGHLIGHTS

Voluntary oral administration of drug to mice minimizing stress

Allows for time-controlled drug dosing

Can be used for oral glucose tolerance test in mice

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Protocol

Method for voluntary oral administration of drugs in mice

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SUMMARY

Substances are commonly administered orally in mouse experiments. Here, I describe how to voluntarily administer substances orally in a time- and dose-controlled manner to laboratory mice. This minimizes injury potential and stress often associated with the commonly used intragastric gavage technique. Here, the drug is incorporated into artificially sweetened and flavored jelly and given to mice previously trained to eat the jelly. This can be used for acute and chronic oral drug treatment or for oral glucose tolerance tests.

For complete details on the use and execution of this protocol, please refer to Zhang et al. (2010) and Cox et al. (2010).

BEFORE YOU BEGIN

Option 1: make drug and vehicle jellies (for drug treatment study)

⌚ Timing: 15 min + 30 min + 15 min + 5 min + 10 min + 3 h

1. 2% sucralose solution (wt/vol in H₂O)

⌚ Timing: 15 min

- For 100 mL of 2% sucralose solution, dissolve 2 g of sucralose powder in 100 mL of H₂O

Note: We used a sucrose-derived non-caloric sweetener, sucralose, to avoid introducing additional calorie into the jelly. Moreover, unlike sucrose, sucralose does not stimulate insulin or incretin hormone release, or alters gastric emptying (Fujita et al., 2009, Ma et al., 2009), making it suitable for masking drugs in studies investigating effects on glucose homeostasis.

⏸ **Pause point:** Can be aliquoted, for example an aliquot of 45 mL in a 50 mL plastic tube and stored at −20°C for up to 3 months.

2. 8% gelatin stock (wt/vol in 2% sucralose solution)

⌚ Timing: ~30 min

- For 50 mL gelatin stock, weigh out 4 g of gelatin powder and transfer into a 100 mL glass bottle with a stirrer bar.
- Place the glass bottle on stirring hot plate and start stirring.
- Add 50 mL of 2% sucralose solution into the bottle with constant stirring.



d. Heat up to 55–60°C with bottle cap loosely screwed on, till solution becomes clear.

Caution: Hot solution

▮ **Pause point:** Can be stored at –20°C for up to 3 months. When using, warm up on heating block at 55–60°C till solution becomes clear again. Start stirring when the stock becomes liquid. I recommend freezing the gelatin stock solution in aliquots to avoid repeated freeze-thaw cycles and thus maintaining a consistent quality of the gelatin stock for different batches of jellies.

⌚ **Timing:** ~30 min

3. Drug solution

⌚ **Timing:** 15 min

a. Dissolve or suspend the respective drug in 2% sucralose solution with other ingredients(s) if required by the drug reconstitution instruction. For example, if 0.1% Tween 80 is required, prepare the drug in sucralose solution with 0.1% Tween 80.

Note: For the amount of drug to be added in, please see **Note** under step 5d.

4. Vehicle solution

⌚ **Timing:** 5 min

a. This is the solution without adding the drug. As for the above example described in step 3, use sucralose solution with 0.1% Tween 80 as vehicle solution.

5. Make the drug jelly

⌚ **Timing:** 10 min + 3 h

a. Use a 24-well flat-bottom tissue culture plate as the jelly mold. One jelly refers to the jelly formed in one well.

Alternatives: Round or V-bottom tissue culture plates could be used as an alternative. Also culture plates with 96 or 384 wells are possible but I found the flat-bottom 24 tissue culture plate allows for easier scooping out of the jelly.

b. For 1 drug jelly, add 450 µL of drug solution into one well, and then add 1,300 µL of gelatin stock into the well.

⚠ **CRITICAL:** Gelatin stock solution does not need to be very hot, however should be clear and runny to ensure proper mixing with the drug solution.

Note: If the drug is heat-sensitive, let the gelatin stock solution to cool down at about 22°C (room temperature in most laboratories) before adding into the drug solution. Make sure the gelatin stock remains clear and runny to ensure proper mixing the drug solution.

c. Add 150 µL of flavoring essence imitation (see [Key resources table](#)). Mix thoroughly with spatula

⚠ **CRITICAL:** Ensure thorough mixing.

Note: This step adds flavoring to the jelly to further enhance the palatability. The volume of flavoring chosen here works quite well in the current studies. However, it can be altered according to the strength of the flavoring used, mice preference, or study suitability. If an

increase in volume is considered, keep in mind that this will increase the volume of the jelly and thus the time mice would use to consume the piece of treatment jelly (see **Note** under step 5d).

d. Cover with the plate lid and let the jelly to set at 4°C for at least 3 h.

Pause point: The jelly can be stored at 4°C for several days without losing its flavor, however the drug stability in the jelly needs to be taken into consideration. The maximum period stored at 4°C for jellies used in the current studies was 3 days. Sealing the jelly plate with parafilm will prevent water loss from the jelly and thus maintain jelly texture and palatability.

Alternatives: While we have not tried this, an alternative to storing jellies at 4°C would be storing them at –20°C. This would prolong the storage period and thus allow for big batch of jellies being made to avoid between-batch variations. If this option is chosen, make sure the jellies are completely set before transferring them to –20°C. Additionally, the drug stability in jelly at –20°C needs to be considered.

Note: To ensure the consumption of the entire piece of jelly by the mouse in a single attempt so that the drug achieves a peak concentration in the circulation, the volume of the jelly is an important factor to consider. The finished jelly has a total volume of 1.9 cm³ (450 + 1,300 + 150 = 1,900 µL) (Figures 1A and 1B). This cylindrical block will then be cut into 8 equal pieces with a scalpel and one of these pieces with an approximate volume of 0.24 cm³, will be given to each mouse (Figure 1C). This volume of jelly can be consumed by a mouse (of 22–32 g body weight of both genders in current studies) in a single attempt that takes less than 1 min (Methods Videos S1 and S2). Please see suggestions under [Troubleshooting](#) for young mice that need jelly of smaller volume.

Note: Drug dosing: Since 1/8th of 1 jelly will be given to one mouse per treatment, each jelly needs to contain the amount of drug sufficient for 8 mice and dissolved in 450 µL of drug solution. For example, we used 10 mg/kg dosage for each Rimonabant treatment, average body weight of the mice we used was 30 g. Thus, we made the jelly with each jelly containing 2.4 mg of Rimonabant: 10 mg/kg × 30 g × 8 mice = 2.4 mg resulting in a drug concentration of 5.33 mg/mL (2.4 mg/0.45 mL).

Alternatives: An alternative to cutting one jelly into 8 equal pieces is to pipette 240 µL (i.e., 0.24 cm³) of the jelly mixture (containing the drug solution, gelatin stock and flavoring) onto a parafilm, and let these individual jelly droplets to set at 4°C. Each jelly droplet is equivalent to 1/8th of jelly, thus can be given to mouse for treatment without cutting. While this is possible and more convenient, one caution is that pipetting hot jelly mixture can yield inaccurate volumes, thus producing jelly droplets containing vary amount of drug depending on the deviations from targeted volume.

Alternatives: Another alternative is to prepare smaller jellies to have each jelly contain the amount of drug appropriate for 1 treatment. If this option is chosen, a smaller mold would be required. I suggest using a shallow mold for easy scooping the smaller individual jellies. In addition, please see **Notes:** drug dosing under step 5d (this step) to adjust the volumes of each jelly components. It is worth to note that while this option enhances the consistency of drug quantity in each drug jelly, it does not enhance drug dosing precision. To achieve precise drug dosing, the amount of jelly given to individual mouse needs to be weighed out individually based on the total drug contained in the jelly and the body weight of the mouse. If this level of precision is required for drug treatment, please follow steps for making and giving jelly for oral glucose tolerance test (Option 2).

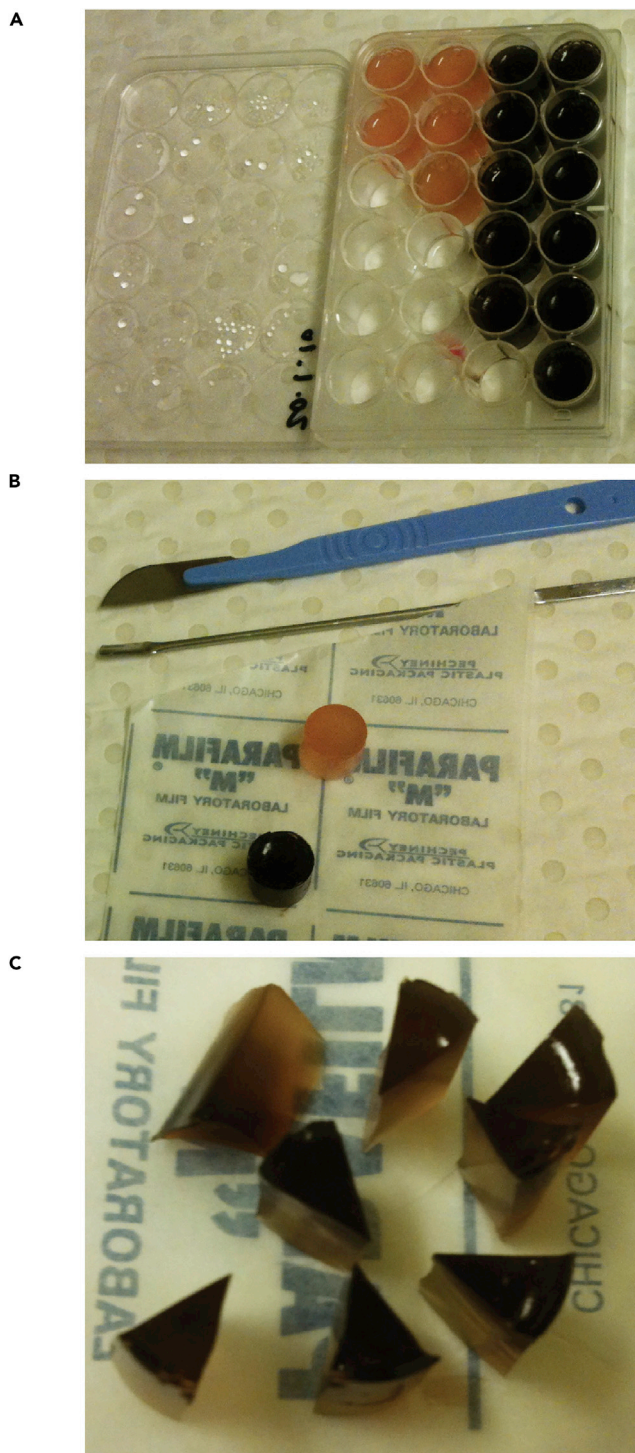


Figure 1. Representative images of the jelly

(A) Jelly in the well of a 24-well flat-bottom tissue culture plate. Pink and brown jellies are flavored with strawberry and chocolate flavoring essence, respectively.

(B) Jelly that has been taken out of the well using the micro spatula.

(C) One jelly shown in (B) has been cut into 8 equal pieces using a scalpel.

6. Make Vehicle jelly

⌚ **Timing:** 10 min + 3 h

- a. For 1 vehicle jelly, follow above steps outlined for drug jelly, but substitute the 450 μ L of drug solution with the vehicle solution.

Option 2: make glucose jelly (for oral glucose tolerance test)

⌚ **Timing:** 15 min + 30 min + 3 h

This voluntary oral administration method can also be used for an oral glucose tolerance test in mice. Below is how to prepare the glucose jelly.

7. 75% glucose solution (wt/vol in H₂O)

⌚ **Timing:** 15 min

- a. Dissolve 0.9 g of glucose in 1.2 mL of H₂O in a small glass vial with lid loosely screwed on a stirring heating plate (~ 55°C). This amount is for 1 glucose jelly.

Note: I recommend to prepare this glucose solution for individual glucose jelly in individual glass vial since glucose tolerance test requires precise glucose dosing and the calculation of glucose dosage is based on the total amount of glucose in one jelly.

8. 14% gelatin solution (wt/vol in H₂O)

⌚ **Timing:** 30 min

- a. Dissolve 0.7 g of gelatin in 5 mL H₂O on stirring heating plate as described for making gelatin stock.

⏸ **Pause point:** Can be stored at –20°C for up to 3 months. Before use, warm up on heating block at 55°C–60°C till solution becomes clear again. Start stirring when the stock becomes liquid.

9. Make glucose jelly

⌚ **Timing:** 3 h

- a. For 1 glucose jelly, transfer the entire glucose solution from 1 glass vial to 1 well of the 24-well tissue culture plate.
- b. Add 650 μ L of gelatin solution into the well.
- c. Add 150 μ L of flavoring essence imitation (See [Key resources table](#)).
- d. Mix thoroughly with spatula.
- e. Cover the plate with the lid and leave at 4°C for at least 3 h to allow proper setting of the jelly.

⚠ CRITICAL: Gelatin solution need to be warmed up and be clear and runny to ensure thorough mixing.

Note: Glucose jelly dosage for oral glucose tolerance test:

Each glucose jelly contains 0.9 g of glucose. Scoop out the jelly from the well with micro spatula and weigh the jelly on a fine balance. Calculate the amount of jelly for each mouse according to its body weight. For example, for 3 g/kg oral glucose tolerance test, if the whole jelly weighs 2.4 g, a 30 g mouse needs to have 0.24 g of glucose jelly: $3 \text{ g/kg} \times 0.03 \text{ kg BW} / (0.9 \text{ g glucose} / 2.4 \text{ g jelly}) = 0.24 \text{ g}$.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Sucralose	Sigma-Aldrich	Cat #69293
Gelatin	Davis Gelatine, manufactured by GELITA NZ Ltd., distributed by GELITA Australia Pty. Ltd	n/a
Flavoring essence imitation	QUEEN Flavouring Essence Imitation Strawberry, Queen Fine Foods Pty. Ltd. QLD, Australia	n/a
Glucose	Sigma-Aldrich	Cat #G7021
Experimental models: organisms/strains		
Mice	Researcher	n/a
Other		
24-well flat-bottom cell culture plate with lid	Costar	Cat #3524
Small disposable weighing tray	SARSTEDT	Cat # 71.9923.211 PVC, 35 × 35 mm

Alternatives: The above listed gelatin and flavoring essence imitation can be replaced with other brands or equivalent products.

STEP-BY-STEP METHOD DETAILS

Step 1: Jelly-dosing training

⌚ **Timing:** 4 days, 1–2 h for 1st day, 10–20 min per day for following 3 days

Since mice exert innate avoidance to novel food (Kronenberger and Medioni, 1985), a training period is necessary for mice to overcome neophobia and eat the jelly. The training involves fasting the singly housed mouse overnight followed by vehicle jelly (that does not contain a drug) presentation and 3-day follow-up jelly presentation without food restriction at the same time of the day.

1. Make the vehicle jelly
2. Fast singly housed mice over night

Note: We typically remove the food from hopper at 5 pm.

3. Next morning between 8:30 to 9:00 am, scoop out vehicle jelly from the well with micro spatula and cut into equal 8 pieces (Figures 1B and 1C).
4. Open mouse cage lid and place 1 piece of jelly on the cage floor or a small disposable weighing tray (see [key resources table](#)), then put the lid back on. Wait till the mouse finishes the jelly then refeed the mouse.

⚠ **CRITICAL:** Leave the mouse as un-disturbed as possible.

Note: Typically it takes about 15–30 min for the mice to eat the jelly for the first time. See suggestions under [Troubleshooting](#) for mice that have difficulties in overcoming neophobia or accepting the jelly.

5. Give 1 piece of vehicle jelly to each mouse in the morning of the following 3 days without fasting them the night before.

Note: If the drug will be given at a particular time of the day rather than in the morning, place the piece of vehicle jelly on the cage floor at that time instead of the morning. If the drug will be given multiple times of the day, give the piece of vehicle jelly to mice at those times.

▮▮ **Pause point:** In our experience, once being trained, mice maintain the interest to the jelly even after jelly has been absent for a few weeks (3 weeks are the longest break occurred in current studies).

Step 2: Option 1: drug treatment study

⌚ **Timing:** few minutes per treatment, duration depends on study requirement

Give mice oral drug treatment according to study protocol.

6. Make vehicle and drug jellies.
7. Give 1/8th of vehicle or drug jelly to mice in the control or treatment group, respectively.

Note: The timing, frequency, and length of treatment depend on the study protocol. For each treatment, it may take 10–20 min depending on the number of mice under study. For 12 mice for example, it typically takes about 10 min per treatment.

Step 2: Option 2: oral glucose tolerance test

⌚ **Timing:** 10–20 min for glucose jelly dosing depending on number of mice under test + oral glucose tolerance test duration

8. Make glucose jelly the day before the test day.
9. On the day of oral glucose test day, calculate the amount of glucose jelly each mouse needs to receive based on its body weight.
10. Weigh out the amount of glucose jelly required for each mouse and give it to the mouse.

⚠ **CRITICAL:** The 0 time point in the oral glucose tolerance test is when the mouse finishes the entire piece of glucose jelly in a single attempt that takes about 1 min after jelly presentation.

EXPECTED OUTCOMES

After 2–4 days of training, the majority (over 95%) of our mice started to eat the jelly within 1 min after the presentation of the jelly in their cage and finished the entire piece of jelly in a single attempt. In our experience, after the initial 2–4 days of training period to overcome neophobia, mice maintain their interest to consume jelly even when the jelly is re-introduced after 2–3 weeks of absence. Thus this method can be used for studies when drug is only given periodically. It is worth noting that while out of the scope of this protocol, a pre-evaluation of drug metabolism and pharmacokinetics may be warranted for each drug administered.

This method can also be used to deliver glucose to mice for an oral glucose tolerance test. Mice consume the glucose jelly in the same manner as consuming the vehicle jelly during the training session. The effectiveness of this method to orally deliver glucose is evidenced by the sharp rise in serum glucose and insulin concentrations after consuming the glucose jelly (glucose dose 3 g/kg) with the peak glucose and insulin concentrations in serum achieved at 30 min and 5 min, respectively, after the completion of glucose jelly consumption (Cox et al., 2010).

LIMITATIONS

This voluntary jelly-dosing method include a training period of approximately 2–4 days to ensure consistent and reliable drug delivery. This lag may be suboptimal if a study involves dosing an animal only once. However, once the mice have been trained, they maintain their acquaintance and interest to the jelly even after a jelly-free period (3-weeks is the longest we have used). Thus the 2–4 days of training period could be built in at the pre-study stage if the training period is relative lengthy for a short drug treatment during the study.

To ensure the accurate drug dosing and avoid injury caused by fighting over the jelly (particularly for male mice), the mice need to be individually housed during time of jelly presentation. This may present logistic challenge for studies that do not permit single housing and thus have to treat each mouse individually during jelly treatment. For this I have some suggestions under [Troubleshooting](#).

One key for this method to be successful is that the drug is sufficiently palatable in flavored and sweetened vehicle. This may be challenging for drugs that have low solubility or strong unpleasant taste. I have included several potential solutions under [Troubleshooting](#). However, for drugs that precipitate out at low temperature, i.e., at 4°C during the jelly setting period, this method would not be suitable. Furthermore, although we have not encountered this in any of current studies, mice may develop conditioned taste aversion and withdraw from voluntary jelly consumption if the drug elicits aversive side-effects. Thus, this voluntary dosing method would not be suitable for drugs that cause malaise.

TROUBLESHOOTING

Problem

Drug has limited solubility and precipitates in drug solution.

Potential solution

Incorporate the amount of drug for 6 mice rather than 8 mice into 1 jelly, then give 1/6th the jelly to each mouse. Mice generally finish the entire piece of jelly in a single attempt; however, completion of the jelly consumption may take a little longer time.

Problem

Drug has its own diluent with high viscosity and not readily mixes with water.

Potential solution

The drug can be masked into a flavored and sweetened paste using the instructed viscous vehicle instead of gelatin and give to the mice that have been trained to lick the paste from a small tray. The training protocol is essentially the same as that used for jelly, except a small tray containing 200 - 250 µL of flavored and sweetened paste rather than a piece of jelly being presented to mice. Mice will lick off the tray and finish this portion also in about 1 min as has been successfully applied in our study ([Cox et al., 2010](#)).

Problem

During the jelly training, mouse does not eat jelly within 30 min after jelly presentation.

Potential solution

Reasons for this problem could be that the mouse is disturbed by lab noise, or more neophobic than other mice. I suggest leaving the jelly in the cage while keeping the mouse fasted for another 0.5–2 h and monitor every 15 min. Refeed the mouse if the mouse eats the jelly within this period. If the mouse still does not eat the jelly, leave the jelly in the cage and refeed the mouse to avoid prolonged fasting. Monitor the mouse during the day. In current studies, the majority of mice that did not eat the jelly at fasting condition and had to be refeed to avoid prolonged fasting ate the jelly by the end of the day.

Problem

Mouse does not finish the entire piece of vehicle jelly in a single attempt.

Potential solution

This is likely due to the size of the jelly piece being too big for the mouse, e.g., young mice.

Give 1/10th of the jelly to the mouse. In this case, the amount of drug in the drug jelly to be used in later “treatment” stage needs to be re-calculated accordingly as explained in the note under: [Before you begin](#)/Option 1/step 5d.

Problem

Mouse eats vehicle jelly but not the drug jelly.

Potential solution

This may happen when the drug has strong adverse smell or taste. Potential solution is to increase the amount of flavoring essence in jelly, or use a different flavoring essence. If possible, reduce the drug dosage.

Problem

Mouse eats the drug jelly initially, then stops eating it.

Potential solution

The drug may have aversive side-effects and cause conditional taste aversion. Using a different flavoring essence may resume jelly consumption temporarily. Reduce the drug dosage if possible to reduce potential side-effects.

Problem

The study does not permit single housing mice that would allow for convenient jelly dosing for individual mouse.

Potential solution

If the cage is sufficiently big, dividers (we made it from autoclaved cardboard) can be placed inside the cage during the jelly treatment time to separate the mice to individual compartment. Place one piece of jelly per compartment and remove the dividers after all mice finished their jelly. This worked well for us in the setting of 2 mice per cage (cage size: 34.5 cm (L) × 11.5 cm (W) × 13.5 cm (H)). Using dividers that fit inside the cage and thus allow for the closure of cage lid helps since an open cage creates distractions and stress for mice.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Lei Zhang, l.zhang@garvan.org.au

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate/analyze datasets/code.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xpro.2021.100330>.

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AUTHOR CONTRIBUTIONS

L.Z. developed the method, designed and conducted the experiments, and wrote the article.

DECLARATION OF INTERESTS

The author declares no competing interests.

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