# Journal of Visualized Experiments

## Self-administration of drugs in mouse models of feeding and obesity

--Manuscript Draft--

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| **Article Type:** | Invited Methods Collection - Author Produced Video |
| **Manuscript Number:** | JoVE62775R1 |
| **Full Title:** | Self-administration of drugs in mouse models of feeding and obesity |
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| **Additional Information:** | |
| **Question** | **Response** |
| Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations. | La Jolla, CA |
| Please specify the section of the submitted manuscript. | Medicine |
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| Please provide any comments to the journal here. | we have made a video but don't think its quite up to the standard needed |

**TITLE:**

# Self-Administration of Drugs in Mouse Models of Feeding and Obesity

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**SUMMARY:**

The overall goal of this procedure is to describe a method for self-administration of drugs that can be used in mouse models of feeding and obesity.

**ABSTRACT:**

Preclinical studies in mice often rely on invasive protocols, such as injections or oral gavage, to deliver drugs. These stressful routes of administration have significant effects on important metabolic parameters including food intake and body weight. Although an attractive option to circumvent this is to compound the drug in rodent food or dissolve it in water, these approaches also have limitations as they are affected by drug stability at room temperature for extended periods of time, the drug's solubility in water, and that the dosing is highly dependent on timing of food or water intake. The constant availability of the drug also limits translational relevance on how drugs are administered to patients. To overcome these limitations, drugs can be mixed with highly palatable food, such as peanut butter, allowing mice to self-administer compounds. Mice reliably and reproducibly consume the drug/peanut butter pellet in a short time frame. This approach facilitates a delivery approach with minimal stress compared with an injection or gavage. This protocol demonstrates the approach of drug preparation, animal acclimatization to placebo delivery, and drug delivery. The implications of this approach are discussed in studies related to timing of drug administration and the circadian rhythm.

**INTRODUCTION:**

The goal of this method is to deliver drugs in mice via a non-invasive, minimally stressful procedure. Preclinical studies in mice often rely on stressful, invasive routes of drug administration that can have significant impacts on metabolic parameters. For example, repetitive daily oral gavage can significantly decrease caloric intake and weight gain in mice[1]. In addition, oral gavage can be technically challenging and has the potential to cause injuries. As an alternative, mice can self-administer compounds that are mixed in their food or dissolved in their

drinking water [2]. However, this approach has a major limitation, which is, it relies on the natural circadian timing of food or water intake. Furthermore, drug stability or solubility in water can be major issues when chronically delivered in this way. To overcome these limitations, drugs can be mixed with highly palatable foods, such as cookie dough [3], jelly [4,5] or peanut butter [6] to encourage self-administration in mice at a specified time. This approach has the advantage of facilitating drug delivery with minimal stress compared with an injection or daily gavage[1]. This procedure can be adapted to deliver a wide variety of drugs to mice. This protocol demonstrates the process of drug preparation, training, followed by drug delivery in highly palatable food. As an example, this method is used to administer the antipsychotic drug risperidone to C57BL6 female mice. Risperidone is well known to have potent hyperphagic and weight gain effects in patients [7] that is well modelled in rodents [6,8]. This system of administration facilitates a highly translational model that could be used to study a wide variety of drugs and their effects on pathways regulating food intake and body weight [9].

**PROTOCOL:**

All procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, San Diego.

**1. Making the drug-peanut butter pellet**

1.1. Calculate the amount of drug needed to make the desired dose of drug in a pellet of peanut butter and scale to the size of the batch required for an experiment. Importantly, pellets can be kept at -80 °C, depending on drug stability.

1.2. Pulverize the drug tablets using a mortar and pestle.

1.3. Weigh the calculated amount peanut butter required by placing it in a weigh boat on a tared scale.

1.4. Place the peanut butter over a beaker of warm water until melted.

1.5. Add the required amount of pulverized drug into the melted peanut and mix thoroughly.

1.6. Allow the drug-peanut butter mixture to cool so that it can be easily placed into the rubber molds.

1.7. Place the peanut butter-drug mix into a mold. The one used here is a rubber corticosterone pellet mold and creates approximately ~100 mg peanut butter pellets.

1.8. Repeat these steps with peanut butter alone to make placebo pellets.

1.9. Freeze the mold in -80 °C to allow the peanut butter to harden until use.

## 2. Mouse setup

2.1. Singly house mice in standard mouse cages. Line with highly absorbent paper bedding and enrichment, including paper towels and housing dome. This paper bedding facilitates accurate food intake measurements by allowing quantification of spilled food from feces and bedding.

2.2. Provide ad libitum food and water and allow the mice to acclimate to the housing for approximately 3 days.

## 3. Training to self-dosing of drug-peanut butter

3.1. Plan and select the optimum time of the day for the drug administration.

3.2. Fast the mice for 24 hours.

3.3. Take the mold out the freezer, let the rubber mold soften so that the pellets can be easily extruded out of the mold. All training can be completed using placebo control pellets.

3.4. Place a placebo control peanut butter pellet on the wall of the cage approximately 1.5 inches from the base. On the first day, it may take approximately 1 hour for the mouse to consume the peanut butter pellet due to novelty.

3.5. After the training session provide ad libitum access to food and water.

3.6. On the following day, place the peanut butter pellet on the wall of the cage in the same location for further training on non-fasted mice.

3.7. Repeat the training in fed mice for approximately 3 days. The time taken to consume the peanut butter will be less than 30 minutes by the third day of training.

## 4. Experiment

4.1. Randomize mice to treatment groups based on body weight so the groups have the same average body weight before treatment.

4.2. Plan to administer the peanut butter pills (treatment or placebo) to the mice at the same time they were trained to receive the peanut butter pellets.

4.3. Weigh the food and mouse and record the values.

4.4. Ensure that the peanut butter pills (treatment or placebo) are placed at the same location in the cage as established during training.

4.5. Continue the dosing procedure daily for the duration of the experiment.

## REPRESENTATIVE RESULTS:

In the example presented here, peanut butter was used to deliver risperidone to mice daily for 14 days.

This study shows the chronic delivery of risperidone via this method facilitates highly reproducible increase in food intake and body weight compared with control (**Figure 1a,b**). In addition, this delivery method results in highly consistent data compared with alternative, more stressful delivery approaches such as intraperitoneal injections (**Figure 1c,d**).

**Figure 1. The effect of drug delivery methods on food intake and weight gain in mice.** C57BL6 female mice were treated with risperidone (3 mg/kg) in a peanut butter pellet or placebo control pellet daily at 8 AM for 14 days. Mice treated with risperidone in peanut butter had significantly higher daily food intake (**a**) and gained significantly more weight (**b**) compared with control treatment. Furthermore, intraperitoneal delivery of risperidone (3 mg/kg) did not have such robust effects on food intake (**c**) or weight gain (**d**) compared to self-delivery in peanut butter. Data is expressed as mean ± SEM and was analyzed by student t-test.

## DISCUSSION:

When conducting this protocol, it is important to be consistent with the accuracy of the measurements of food intake and body weight and the timing of drug administration throughout the study. While this self-administration method requires a significant training phase, this is particularly important to acclimate the mice to the novelty of the peanut butter and ensure mice consume the drug at the time given. Once established, it also offers great experimental flexibility and can be modified and adapted to deliver drugs at multiple times per day at various doses. This technique works best when mice are consuming normal chow as their main source of nutrition. When mice are fed highly palatable high fat, high sugar diets, this can make the training phase more challenging as some mice are less motivated to consume the peanut butter under these conditions and can be a limitation of this technique.

Compared with existing methods such as injections or oral gavage, this method of self-delivery of drugs causes minimal stress and results in robust and consistent data related to drug-induced effects on metabolic phenotypes including food intake and body weight. Future applications of this technique include studies into of timing of drug delivery on food intake and weight gain in the context of circadian rhythms and metabolic health[10].

## ACKNOWLEDGMENTS:

This work was supported by the National Institutes of Health grant R01DK117872 awarded to OO and the Larry L Hillblom Foundation Fellowship awarded to RCZ.

## DISCLOSURES:

The authors have no conflicts of interest to declare.

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Figure

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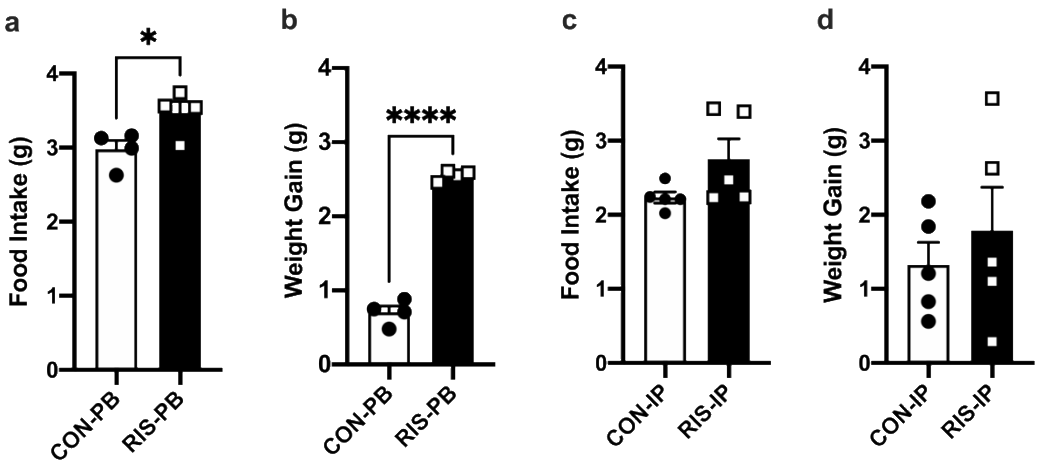

Table of Materials

Click here to access/download;Table of Materials;table materials.xlsx

| Name of Material/ Equipment | Company | Catalog Number | Comments/Description |
|---|---|---|---|
| C57B6/J mice | Jackson Labs, Sacramento, CA, USA | 664 | |
| corticosterone pellet mold | Ted Pella Inc, Redding, CA, USA | 106A | |
| Mouse igloo | VWR, Visalia, CA, USA | 89067-850 | cage enrichment |
| peanut butter | Jif Peanut Butter, Orrville, OH, USA | | Creamy peanut butter |
| pestle and mortar | VWR, Visalia, CA, USA | 470148-960 | |
| risperidone | Patriot Pharmaceuticals, Horsham, PA, USA | 50458-593-50 | |
| rodent chow | LabDiet, St. Louis, MO, USA | 5001 | |
| weigh boat | VWR, Visalia, CA, USA | 10803-148 | |
| weighing scale | Mettler Toledo, Greifensee, Switzerland | MS104TS | |
| Wypall paper X60 | Kimberly-Clark, Corinth, MS, USA | 34865-05 | absorbent paper bedding |

We wish to thank the editors and reviewers for the comments and have addressed them point by point below:

**Editorial comments:**

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. ***We have proofread the manuscript***

2. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.
For example: Ted Pella Inc, etc.

***Removed ® from Jiff description***

3. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).
***The text has been modified and the word "we" removed.***

4. Please revise the Introduction to include all of the following:
a) A clear statement of the overall goal of this method
b) The rationale behind the development and/or use of this technique
c) The advantages over alternative techniques with applicable references to previous studies
d) A description of the context of the technique in the wider body of literature
e) Information to help readers to determine whether the method is appropriate for their application

***Edited as suggested***

5. Please include a one-line space between each protocol step and then highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

***highlighted***

6. Please remove the embedded figure(s) from the manuscript. All figures should be uploaded separately to your Editorial Manager account. Each figure must be accompanied by a title and a description after the Representative Results of the manuscript text.
***removed***

7. Please remove the embedded Table from the manuscript. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Each table must

be accompanied by a title and a description after the Representative Results of the manuscript text.
***removed***
8. Please include at least one paragraph of text to explain the Representative Results in the context of the technique you have described, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. Data from both successful and sub-optimal experiments can be included.
***modified***

9. Please include a title and a description of each figure and/or table. All figures and/or tables showing data must include measurement definitions, scale bars, and error bars (if applicable). Please include all the Figure Legends together at the end of the Representative Results in the manuscript text.
***Description has been added and included at the end of representative results.***

10. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique
***This has now been amended***

11. Please do not use the &-sign or the word "and" when listing authors in the references. Authors should be listed as last name author 1, initials author 1, last name author 2, initials author 2, etc. Title case and italicize journal titles and book titles. Do not use any abbreviations. Article titles should start with a capital letter and end with a period and should appear exactly as they were published in the original work, without any abbreviations or truncations.
***The Jove output style was used in Endnote and has been edited to modify these points***

12. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials. Sort the table in alphabetical order.
***Removed these symbols***

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**Reviewers' comments:**

**Reviewer #1:**

Manuscript Summary:

The proposed mouse self-administration approach is an attractive alternative to i.p. injection,

gavaging, or administration via the diet. Some of the steps of the procedure come across as laborious and could be semi-automated. If obvious approaches did not work, it would be helpful to disseminate that information with the research community (see minor comments).

Major Concerns:
None

Minor Concerns:
Each pellet is made separately by mixing test compound with "a small amount of peanut butter". For large cohorts of mice, the pellet production is quite laborious. Please address: 1) specify the amount of peanut butter for each dosage. 2) If the test compound is not available in tablets, the test compound would have to be weighed for each dosage. Did the authors consider making a bulk mixture of test compound and peanut butter and dispense dosages into the molds by weight or volume, as one would do for compounding suppositories?

*1.1* ***Drug/peanut butter pellets are made in batches and this has now been clarified. "Calculate the amount of drug needed to make the desired dose of drug in a pellet of peanut butter and scale to the size of the batch required for an experiment. Importantly, pellets can be kept at -80, depending on drug stability."***

My other concern relates to the training procedure: The use of a training cage makes the experiment complicated when using large cohorts of mice. Please address: Did the authors try doing the training in the habitual cages? If that did not work, please address problems in the discussion. As most mouse studies start with a few days of acclimation of mice after arrival from the vendor, can the training be performed during this period? Can training be performed with placebo pellets in the group of mice that will receive the test compound pellets after acclimation? Was there any spillage of the pellets? If so, how much?

***We have now modified this description so the training can be done in the home cage.*** The training is done with placebo pellets. This has now been clarified in the manuscript. "All training can be completed using placebo control pellets."

**Reviewer #2:**
Manuscript Summary:
In this manuscript the authors try to find a different approach to delivery drugs to mice with minimal stress in comparison to more invasive protocols such as intraperitoneal injection. To perform this the authors, mix risperidone with highly palatable food and give it in small pellets of peanut butter to mice, which are then able to consume the drug/peanut butter pellet in a short time frame. The final aim of the procedure presented by the authors is to describe a method for self-administration of drugs that can be used in mouse models of feeding and obesity.

Major Concerns:

Minor Concerns: In my opinion, this video-recorded protocol is well done. The English format is appropriate. The protocol is easy to follow and to repeat.