Cologne, 11th September 2016

Dear Dr. Nguyen,

We resubmit our revised version of JoVE55024R1 “The CApillary FEeder assay measures food intake in *Drosophila melanogaster*” for publication. We appreciated the constructive criticisms of the Associate Editor and the reviewers and addressed each of their concerns as outlined below.

We incorporated a new figure and supporting data into the manuscript. Both are addressing how the variability of the assay due to evaporation can be reduced, a concern two of the reviewers had. We believe that we have substantially revised and improved the manuscript and hope that is now ready to be turned into a JoVE video.

Sincerely yours,

Soeren Diegelmann

**Editor comments:**

1. Formatting:

-Do all authors have the same affiliation?  
*We provided an updated version.*

-Please use the American standard for punctuation, with periods used for commas instead of commas, throughout the manuscript and in all figures and tables (including the materials table).

*We updated all tables and figures with the American standard using periods instead of commas.*

2. Grammar:

Line 115: “stereo foam plug”

*We replaced the word “stereo foam plug” with the “sponge bung”.*

Line 198: “to keep huminity constant inside the assay” - humidity  
*We corrected the misspelling.*

3. Branding:

5.6 – “Statistica”  
*We replaced the term by “statistical software”.*

**Reviewer comments:**

**Reviewer #1**

Major Comments

1) Since larval crowding during development can significantly affect adult body size (which correlates with absolute feeding), the authors might highlight the importance of careful development. That said, aren't the conditions used by the authors (35 females per vial with 3 days of egg-laying) quite crowded?

*We agree with the reviewer that the development conditions play a significant role for adult body size. We set up the crosses for all genotypes tested at the same time and rear the animals under the same conditions (2.1). The vials for “breeding” the flies differ from the one for the “CAFE assay” by an over 2.5 time increase of volume. We clearly indicated in the retyped manuscript the dimensions of the “breeding” and “CAFE assay” vials. We included both vials also in the table of specific materials. We use the bigger vials to provide more space and food sources to avoid problems with overpopulation as the reviewer pointed out.*

2) There's really no good scientific reason yet to normalize feeding by body weight. People just do it. The tone of the text could be adjusted to make this a suggestion, but there's no reason to indicate that it should be part of a definitive protocol. Hence, the entire third experiment (food demand between sexes and normalizing with body weight) doesn't seem scientifically relevant at this point. I'd recommend less "scientific" discussion and more focus on the technical protocol instead.

*We reformulated the manuscript and stating that we suggest normalizing the consumption to the body weight. Normalization is essential if e.g. control and mutants differ significant in their body weight, otherwise low intake could be misinterpreted. We also rephrased the interpretation of the result in figure 3 and focus more on the technical protocol of the CAFE assay.*

3) If flies consume 0.2-0.6 uL of food per day (which weighs approximately 0.2-0.6 mg), how much does this consumption contribute to the fly body weight measurements? Does it add significant variability to the results?

*We agree with the reviewer that the intake of food lead to an increase in body weight, but at the same time the excretion system of the fly is lowering the weight potentially. Body weight therefore is a dynamic value, especially for long term experiment. We measure the body weight of the flies before the experiment and look for potential significant differences. The influence of consumption on body weight is therefore not addressed in the assay. We include this point in the discussion as one aspect to consider.*

4) As mentioned above, there are many different iterations of the assay using different sized enclosures, different foods, different capillary sizes, etc. Highlighting some of the possibilities, or at least why the authors think their protocol should be the definitive one, or is better than others, might be valuable.

*As the reviewer pointed out there are numerous variation of the CAFE assay setup. Especially the lid range from simple sponge vial stopper with openings to placing the capillaries to plastic lids like in our setup, as the reviewer pointed out. We ourselves performed experiments using the sponge variation. We experienced that for example the secure* [*positioning*](https://dict.leo.org/ende/index_de.html#/search=positioning&searchLoc=0&resultOrder=basic&multiwordShowSingle=on&pos=0) *of the capillaries during the handling and the experiment period can be an issue. The usage of a custom-made lid avoids this drawback and we gained more reproducibility. It allowed us to perform the assay successfully during student practical courses.*

*We added a note at point 1.2 to highlight other possibilities for the lid and retyped the manuscript at specific point to guide the reader to alternatives.*

5) One of the advantages of the CAFE assay is that supplements to the food, such as dyes, are unnecessary. These supplements can (and often do) affect feeding behavior. The food colorants that the authors use aren't necessary to visualizing the meniscus and shouldn't be the gold standard for the assay.

*We agree that food dyes are not a necessity for the assay. Nevertheless the hard to see decline of the meniscus without this supplement makes data collection time consuming, which could be challenging if multiple assays are performed in parallel. We see a weak avoidance of food containing the dye. We included a note at point 3.1 to show that problem and to make clear that addition of food dyes is not mandatory for the assay. We also highlighted that if two dyes are used the supplemented dyes should be randomized.*

6) Humidity within and outside of the CAFE chamber is important for minimizing evaporation and improving signal-to-noise. Applying water to the stereo foam plug (step 4.5) doesn't seem like it would work well. Not only is that a very limited amount of water that would evaporate quickly, but isn't that the only source of fresh air for the flies? For long-term experiments, I would suggest using agar in the vial itself as an alternative source of humidity and water, instead of the filter paper and water drop that probably dries out.

*Indeed the humidity is a critical point for the assay. We included new additional devices we use to minimize the evaporation and to lower the signal to noisy ratio. This includes water filled vials inside the plastic box and box which can be placed above the ready to use CAFE assay vials to create an own humidity environment. We added the information of how much water is supplied to the central sponge bung daily in a long term experiment. The sponge bung is not the only source for fresh air for the vials. The six opening which can hold the capillaries can lead air through. The capillary is secured by the second pipette tip but there are still gaps for air exchange, but too small to let flies escape out of the vial.*

7) Do the authors observe any fly deaths in their setup? If so, how are these handled? Any good set up should be usable for many days with young flies without mortality.

*During our 3 h experiment we hardly see any death flies. Occasionally one fly dies during the fasting period before the experiment. In this situation we still perform the experiment and divide the consumption by for example 19 flies. For the long term assays we observe the survival of the flies every day. Depending on the food source provided we see 1-3 dead flies at the end of day 4. We always use the numbers of flies at the beginning of a 24 h period to estimate the consumption per fly in a vial. We included a new point (4.6) and Note to make sure people notice death flies and to calculate the consumption accordingly.*

8) There is no discussion of signal-to-noise. Namely, what percent of the "feeding" should background (evaporation) represent to be a good data set? Looking at Figure 3, each of the values can be multipled by the number of flies per chamber (20) to get the total consumption. One can then compare this to the evaporation presented in Table 2. On the lowest sucrose concentrations, feeding (0.2 uL) is far outweighed by evaporation (2-2.5 uL). Even on the higher concenrations (0.1 and 1M sucrose), evaporation represents 20-80% of the measurement! That's awful, and I believe for the publications that mention it, evaporation is best if it's less than 10% of the data. Lower would obviously be better. At these high levels of evaporation, one must also consider that the effective nutrient concentration in the capillaries has changed by quite a bit. There's zero evaporation with 2M sucrose, but this is not a commonly used food concentration.

*We are thankful to the reviewer for this comment. By reviewing the data inside table 2 we noticed that the data shown are representing loss in mm not µl. The conversion of the values was not performed. The values shown were not from the actual experiment shown in figure 3. When the right data and the conversion were performed we saw the evaporation for the lower concentrations is around 28 %, the value for evaporation at 0.1 M solution, taking the females data set into account is around 15 %. All other comparisons show evaporation less than 10 %. We updated the table for the evaporation control and include data from a two choice assay performed in two ways. One time without further humidity approaches and one time in parallel with extra water filled vials and using a cover for the plastic box during the experiment (plus applying water to the central sponge bung every 24 h (new Figure 2 and new Table2).*

9) Given that the assay has been used very successfully without custom parts, a "definitive" protocol that uses a custom-made lid seems like a poor choice. The lid and O-ring certainly look nice, but the authors should justify any significant advantages or, otherwise, highlight alternatives. I've seen some labs use a standard stopper that likely results in high evaporation. I've also seen the use of rubber septa.

*We rephrased the usage of the lid and pointed out possible alternatives. We included a note (see 1.2) addressing alternative ways and advantages of our system. We show in the new figure 2 and table 2 that additional devices to control humidity lower the evaporation significantly and therefore help to perform the assay more reproducible.*

**Reviewer #2**

Minor Comments

Line 47: The authors mentioned already in the abstract that their report demonstrates using the assay with single or multiple flies, tho there are no data presented or modifications shown to successfully measure drinking of one fly in their setup.

*We took out the term “single fly” as the reviewer mentioned correctly that we didn´t show data for a single fly. Our setup is indeed not useful for single flies and should be used with minimum of 8 flies.*

Line 66: at the beginning of 21st century. just state the year.

*We retyped the sentence.*

Line67: Capillary drinking assay was described much earlier than by Ja et al. Dethier's Hungry fly from 1976 is a well known example. Benzer first published it in Drosophila and gave it the name.

*We put the establishment of the CAFE assay in Drosophila in the right context.*

Line 68: 1. meniscus decline also indicates evaporation. 2. "missing" is not the best word; it's not missing, it was consumed or evaporated. and it indicates food uptake too. Please, reformulate the sentence.

*We reformulated the sentence and avoided the usage of the word “missing” and pointed out the influence of evaporation.*

Line 83: should be "measuring food response", not consumption. Is there any reason to bring a new term - PE, instead of the widely used term - PER? Explanation or citation would be welcome. How would they measure consumption with either?

*The more widely used term “PER” is used in the whole manuscript. The term “response” is used.*

Line 83-85: these two sentences are exclusive of each other. with only the later being correct. PER doesn't measure uptake.

*We retyped the sentence, see above*.

Line 88: this is a very vague technical description of these two assays. Moreover, both of these assays measure not only PER/PE but also amount of time spent drinking which can be used to estimate volume.

*We aware of the short description of the techniques, we believe that more detailed explanation is beyond the focus of our manuscript. The reader is guided to excellent references about those new developed techniques. We include an additional sentence pointing out the change to estimate food intake.*

Line 89: Do authors understand under PE assay proboscis extension AND the following drinking?

*We mean both possibilities.*

Line 101: This sentence should be part of abstract instead of the one mentioning single fly experiment.

*We retyped the abstract and took out the term “single fly”.*

Line 116: the conical openings do not fit the capillaries. They fit pipette tips.

*We retyped the sentence pointing out the presumable usage of the pipette tips to hold the capillaries in the assay.*

Line 117: there is no supplementary figure in the version I review. Cross section of the lid would be helpful.

*We supplied a cross section within the technical drawing; visual information inside the media file should help the viewer to understand the usage of tips and capillaries*.

Line 119: better description of capillaries needed. ID/OD, cut/polished, inner filament Y/N?

*We provided more information for the capillaries within the comments at the table for specific materials.*

Line 133: this is confusing. are flies allowed to lay eggs for 3 days in each vial or only in the first one, then 1 day in the second and two in the last one?

*We reformulated the point to give a more clear time line. We also included when adult flies should be discarded.*

Line 138: dry or wet weight?

*We included the term “wet”.*

Line 149: Are capillaries refilled or discarded and always new fresh capillaries are used?

*We included the handling of used capillaries after measurement of the meniscus (see also point 5.1).*

Line 156: is there a difference between pre-starved and starved? It should be 'fasted' instead of 'starved' anyway.

*We agree with the reviewer and used the term “fasted” instead.*

Line 163: The stock solution is calculated wrong. 102.6g of sucrose should be FILLED to 100mL, not add to 100mL water.

*We corrected the calculation of the solution.*

Line 164: explaining serial dilution seems rather unnecessary. Moreover, the described method introduces potentially larger error than classical serial dilution.

*From our experiences with students in the lab this explanation works fine and we therefore would like to keep it. We included the % w/v of the stock solution.*

Line 168: should describe what the reason to do that is. What c of EtOH is being made?

*We reformulated the point to state clearly the usage of the solution. We included the c for EtOH*.

Line 173: not sure if 1 or 10 capillaries in a bundle is inserted. I assume it will be obvious on the video.

*We rewrote the sentence to make clear that multiple capillaries are filled simultaneously. This point should be clear seeing the media file.*

Line 179: Quantifying evaporation in empty vials is misleading; the evaporation is higher in empty vials than in vials with flies. Using high c of bitter solution in one capillary with flies inside gives much more accurate values. Is wet filter paper always placed in the CAFÉ vials? If so, it should be mentioned.

*We rephrased the usage of the filter paper and included a new figure showing the reduction in evaporation.*

Line 183: I'm not sure what the authors mean with ".. placing the BACK of the pipette tip NEXT to…".

*We rephrased the sentence. This point should be clear seeing the media file*.

Line 187: Should not all ends be at the same height in all vials? Would be nice to specify which height it is/should be.

*We exchanged the term “one” by all and stated at which height we normally let the capillaries end.*

Line 188: This sentence suggests that filter paper is always in the tube. Is it? Is it wet? How is it kept wet in long running assays? Is it exchanged/wetted for days running assays?

*The filter paper is indeed always in the prepared vial. It´s wet (see point 2.6). Fresh water can be applied via the sponge bung in the central opening of the lid (see point 4.5, which we retyped for gaining more clarity regarding the water supply).*

Line 195: What's the point of the humidity controlled incubator? Or do the authors mean in case the assay is not in the incubator? In any case, the humidity inside of the tube is influenced by number of flies and the amount of drinking; and the following excretion.

*We reduced the variability of our assay placing the prepared vials within a climate controlled chamber described in point 4.5.*

Line 197: This sentence indicates that wet filter paper is not inside of the tube. Overall, the humidity control is not well specified and humidity (or lack of it) has a huge effect on drinking. It should be clearer and consistent.

*We retyped point 4.5 stating more clearly that the bottom filter paper stays inside the vial and fresh water is applied via the centrally placed sponge bung.*

Line 201: The note should be just the second sentence. The first sentence should be part of the protocol. And the variability is probably influenced more by humidity than temperature (within the room temperature range).

*We eliminated the first sentence and pointed out the role of humidity for the assay*.

Line 207: A care should be taken to note if the liquid is reaching the bottom of the capillary AND the markEND at the same time. Otherwise, the liquid probably shifted and was not reachable by the fly.

*We included the problem if the liquid was not accessible to the flies within point 4.7.*

Line 215: Authors probably mean "dissection" or "stereo" microscope.

*We replaced “binocular” with “dissection microscope”.*

Line 220: This sentence should read: "To transfer data directly to a spreadsheet, use USB connected caliper (Figure 1E)." Normal digital caliper will not connect to computer

*We retyped the sentence according to the reviewers’ suggestion.*

Line 226: The formula doesn't need "x 1uL" as 14.6mm is 1uL

*We eliminated “x 1ul”*

Line 228: As mentioned above, this is not very precise method. But depends on humidity control.

*We included new evaporation data, figure and table showing the effect on evaporation.*

Line 236: as mentioned earlier. is the calculation to dry weight or wet? If wet, is it gradually adjusted as flies drink in the assay? With higher concentration of sucrose and starved flies, the uptake can be very significant.

*We included the term “wet weight” (point 2.3). The weight is measured before the experiment to see if significant differences exist. The influence of consumption on body weight during the experiment is not addressed with this assay.*

Line 239: Is there any reason to use Statistica over another statistical program?

*We deleted the branding and used the term “statistical software”.*

Line 251: rather sex difference than body size as will be mentioned later

*We included this interpretation in the discussion section*.

Line 262: Is it similar or the same food choice?

*We used the term “similar” because we used a 23 % EtOH source instead of 15 % EtOH in the first experiment.*

Line 264: It's just "Sucrose", not "Suc-EtOH"

*We followed the reviewers’ suggestion.*

Line 272: This experiment is very inconclusive as it is not clear whether it is the effect of weight or sex. The flies of the same sex and of different weight should be used if the weight effect is being studied.

*We included the interpretation of sex differences in the ability to perform the assay in the discussion section.*

Line 274: statement is not reflected in the described experiment? Why is it mentioned then?

*Mated female prefer protein-rich diets over sucrose containing solution. Therefore the reduction in sucrose intake (0.1-2M) in our 3 h experiment could reflect a prolonged search in females for their “right” food and less time spent in drinking the supplied food.*

Line 276: So mated females AND males were used

*We added the term “mated male and female”.*

Line 280: First, it is said that flies didn't feed "…appreciably on the lower concentrations…" but then big conclusion is drawn from those data?

*We eliminated the term “appreciably” as it appears indeed too strong in the context.*

Line 291: wet filter paper is mentioned here but not in the protocol. Still, how is it kept wet over days lasting experiments?

*We retyped the protocol, stating now how the humidity device is kept wet during the period of the experiment.*

Line 292: Are the food dyes always used or is it just for display in the figure? If the former, should be mentioned to randomize used dyes as they cause different aversive response.  
*In a two choice assay the use of the food dyes is randomized to avoid any bias because of aversive responses to the dye. We included this information within point as a note in point 3.1.*

Line 295: A cross section of the lid would be helpful.

*A cross section of the lid is provided as a technical drawing in a supplementary figure.*

Line 298: In the picture, it seems like the liquid is too far for the fly to reach and the fly is only touching the capillary rim.

*The fly in the picture reach the liquid which is at the end of the capillary. You can see actually that the fly is drinking because of the red staining inside the proboscis. This point will be also possible to illustrate in the media file showing a drinking Drosophila in close up.*

Line 303: Should be ..."sucrose (suc)" and "...ethanol (EtOH).." Actually, the figure doesn't show any "Suc", so why to use it in the legend?

*We followed the reviewers’ suggestion.*

Line 304: Be consistent with description of solutions. "solution containing 15% EtOH" vs "EtOh containg sucrose solution". I assume both are sucrose with EtOH but the description is unclear.

*We retyped the legend for figure 2 and focused on consistency of naming the solutions.*

Line 312/315: Is it 27 males or 27x20 males? Same for females

*We included the term “xx trials with xx (fe)male flies each”.*

Line 318: mg per fly are shown in Table 1 but ug/fly are mentioned in the legend. Use of ug and mg is inconsistent few more times throughout the MS

*We eliminated this confusion and used mg throughout the new manuscript version.*

Line 334: again, not Suc-EtOH; just Suc

*We eliminated “-EtOH”.*

Line 340: Is really intake of total sucrose calculated and shown or is it just the sum of all the solutions? I suspect it's the later.

*We retyped the legend.*

Line 350: As mentioned before, it was not invented decade ago; just published for use in D.m.

*We eliminated the half sentence*.

Line 357: The "pre-strvation" is rather confusing term. As opposed to "post-starvation'? The correct term should be "fasting" as we don't really know how and if the fly is actually starving when placed on water only.

*We followed the use of the term “fasting” in the revised manuscript*.

Line 361/363: Other versions of CAFE assay were described (Masek & Scott, 2010) that addressed this caveat and should be mentioned.

*We included the citation.*

Line 366: there is not such thing as "standard D food vial". Size or volume should be specified.

*We changed the terms and use CAFE assay vial and breeding vials in the manuscript. More information is given in the table of specific materials*.

Line 371: Pool et al 2014 can be cited here.

*We included this citation.*

Line 381: Spill also depends on viscosity of the liquid (EtOH for example) and should be mentioned.

*We included the problem of viscosity (point 4.3), which is stated.*

Line 385: Food accessibility should not be mentioned as pitfall but rather as a part of the main protocol. And it needs to be mention what this means in detail as it is a common problem. (moving the liquid away from the end of the capillary due to evaporation, moving of the assay or drinking)

*We included the food accessibility in point 4.9 and guide the reader to discard the data if food was not available to the flies. We didn`t notice moving liquid from the end of the capillaries due to evaporation in our system.*

Line 391: Vertical or horizontal position in MAFA?

*The eating position of the fly in the MAFA assay is mentioned in the sentence.*

Line 402: CAFÉ can be used for measuring robust drinking in as little as 3 mins.

*We retyped the sentence.*

Line 402: It's mentioned several times in the MS but proboscis extension is not a feeding assay.

*We eliminated the PER assay here as it´s not a feeding assay.*

Line 409: Yet, even in this MS, it is mostly not.

*We suggest doing so.*

Line 413: "…metabolic AND taste research…"

*We included the term “taste”.*

Line 480: missing tab

*We included the tab.*

Fig 1 Cross section of the capillary/pipette tip arrangement is needed. C) The food is too far in the capillary for the fly to reach it or bad lighting. D) the two pictures (placement/brightness/shadows are too different

*Figure1. We included a technical drawing in a supplementary figure, which shows a cross section. The media file will help to understand the arrangement of pipette tips and capillaries.*

*The food is reached by the fly in figure C, which can be seen in the red staining inside the proboscis. The media file can be used to visualize a drinking fly from the capillary end*.

*D) We adjusted the brightness and contrast of the picture and its orientation. The staining of the abdomen with red or blue food color is seen within the picture. The same placement/ shadow would not add any new information.*

Fig 3 x-axis should be "Sucrose dilutions"; not a gradient. Why is w1118 sometimes shown in the figures and why it's in the tables when it is mentioned at the beginning that ALL flies shown are w1118. Seems unnecessary. And inconsistent.

*We followed the suggestion of the reviewers for the x-axis and eliminated the genotype inside the figure. We keep the genotypes inside the figure legends and table (legend) as this helps the reader if the data is viewed without reading the manuscript first.*

All tables must fit on one page. I see no reason for them to be so spread. They are very confusing the way they are now. And some, like the 3rd page of Table 5 is completely out of place.

*We arranged all tables to fit on one page to eliminate a source of confusion if printed out.* *We simplified the table 1 by only showing the bodyweight per fly in mg.*

Table 1 keep the naming consistent "/male vs (male)". It's not necessary to put "total weight" and "per fly" if # of flies is always 100. It's just moving decimal point.

*We consistently use the term “(male)” or “(female)” in the revised version of the tables.*

Table 2 The variability is rather large. Often around 2 x difference in different vials. What is the explanation?

*We found two mistakes in table 2. The values shown were in mm and not converted to µL and not from the experiment shown in table 5 and figure 3. We corrected our mistake including the µL values for evaporation on three days. This also reduced the variability.*

Table 3 The variability of the results is really large. 3.36vs0.14 or 2.67vs2.05 in the same day?

Table 4 Again so much variability. -0.26 to 0.8 under the same conditions?

That's a lot of variability. Maybe showing preference of two different concentrations of sucrose would better illustrate that CAFÉ assay can be very precise and highly reproducible assay. It would also show an experiment more relevant for other researchers besides those in EtOH field (where the authors are).

*We believe the reviewer confused the two variants of solutions used in this experiment. 3.36 (Suc+EtOH) vs 0.14 (Suc). We therefore color labelled the different columns and also changed the order (to follow Figure 3A). The STDEV and STERROR for the solutions show a normal value and a listed under each column. The differences among the solutions tested are statistical significant. We agree that showing results of data of preferences between two sucrose concentrations would result in less variation. In our tutorial we intend to show that the CAFE assay can be successfully used with different solutions and show the effect of food supplements. The experiment was already shown in the first publication of the assay in the fly and seem to us like a perfect representative result example.*

Table 5 More ## after decimal point need to be shown for the low c of sucrose. It's meaningless as it is now

*We included the third visual decimal point in all tables to avoid the rounded zero value.*

**Reviewer #3**

Major Comments

I have one general comment about the manuscript: I would like to see a clearer handling of the general discussion concerning how to measure Drosophila feeding. …………... This could be inserted in the introduction (perhaps at the start of the second paragraph) and once this has been established, the authors have the freedom to move past the discussion and describe the CAFÉ assay in isolation.

*We rephrased the introduction and pointed out possible other assays in Drosophila. We wrote more clearly for which scientific question which assay would be an appropriate one. We believe that in combination with the discussion section the viewer should be able to choose an assay for their specific scientific question*.

Minor comments

Line 236: I am not entirely sure why the authors suggest to normalise intake to body mass. Without any knowledge about nutrient absorption this seems unnecessary and could even lead to over-interpretation of the data.

*We reformulated the manuscript and stating that we suggest normalizing the consumption to the body weight. Normalization is essential if e.g. control and mutants differ significant in their body weight, otherwise low intake could be misinterpreted.*

Line 261-266: there is also the possibility that males and females differ in their proficiency at feeding from the capillaries and therefore that the comparisons below reflect this difference.

*We included the possibility of a difference in the ability to drink from the assay between male and females in the text.*

Line 384: tling of solid particles is important and it is worth noting that this has lead researchers to use water soluble yeast extract in some assays that use capillary feeders. However, this is an incomplete source of nutrition for flies and can impose fitness costs. I feel this is an important point to make to further clarify the scope of uses for this assay.

*We included the possibility and the potential downfall of the usage of water soluble yeast extract within the text.*

**Reviewer #4**

Line 62: 1. On line 62, there is a statement that few researchers in mammalian systems have examined the circuitry of eating disorders. This is too strong a statement, in fact there is a large community of mammalian researchers examining the circuitry of eating disorders studying systems and circuitries of the hypothalamus and gut, involving several signaling systems including ghrelin, NPY, and serotonin, to name a few.

*We rephrased the sentence and highlighted the numerous efforts made in the mammalian field, including three new references.*

Line 76: Change the text "already after" to "after only"

*We followed the reviewers suggestion and replaced “already after” with “after only”.*

Line 109, 124: The term "assay" is used to describe the final equipment setup, a more appropriate term such as "prepared assay vials" should be used.

*We defined the use of the term “assay” more clearly and rephrase when vials are mentioned.*

Line 115: What is a "stereo foam plug"?

*Word replaced with “sponge bung”.*

Section 2.3: line 136: Consider combining this with section 2.4, and emphasizing at the beginning of this section that it is important to measure the weight of the flies prior to feeding, and that this number (ug/fly) can be used to adjust for the total volume of food to be used in a given experiment to load the capillaries.

*We combined the points 2.3 and 2.4 but leave the sub-points in place as they are important to setup the assay correctly for a 3 h or long-term experiment.*

Section 3.1: Please include the % sucrose (w/v) final values, as many researchers use % sucrose instead of molar concentrations.

*We included the % sucrose (w/v) for the stock solution, for clarity and readability we would like to not include the % value for each diluted working solution.*

Section 4.5: line 194: The use of a humidification device within the assay tube is mentioned as being an option. This particular reviewer, however, has found that the addition of a humidification device to CAFÉ assay tubes appears to be necessary to prevent increased evaporation and confounded consumption measurements. This is especially important when the assays will be performed in a non-humidity controlled environment at 25°. I would advise adding stronger wording encouraging the use of a humidifying device in an assay under most conditions. That being said, the presence of such a device (foam plug, filter paper, etc. soaked with water) will introduce a confound into the system and flies will drink from it, therefore a mesh barrier is necessary to prevent flies from doing this.

*We retyped the note for point 4.5 and highlighted the use of humidifying devices. The usage of a mesh barrier surrounding this water sources seems desirable but we haven´t used such a barrier in our assay yet.*

Line 247: replace the word "a" with "the"

*Word replaced*

In Figure 1: it would be helpful to also show what the underside of the lids in 1A and 1B look like with the assembled tips and tubes present.

*Figure 1: We included a technical drawing of the customized lid, including a cross section drawing in the supplementary figures. The assembled lid with tips and tubes should be shown in the media file; we believe that this way allows the viewer to understand the concept of the lid much easier as an additional picture of the underside of the lid.*

General comments: How often and with what do you clean the lids with? Do you reuse your vials or capillary tubes?

*We include additional sentences at point 4.7 and 5.1 showing the washing and the handling of the capillaries after the experiment. We include the soap which we use for cleaning in the table of specific materials.*