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Research Article

T-CAFE: A MODIFIED CAFE FOR THE MEASUREMENT OF AMOUNT OF FOOD CONSUMPTION BY A SINGLE FLY

Rambhadur P. Subedi[1]*., Rekha R. Vartak[2] and Purushottam G. Kale[1]

[1]Ramniranjan Jhunjhunwala College, Ghatkopar (W) Mumbai-400086
[2]Homi Bhabha Centre for Science Education-TIFR, Mankhurd, Mumbai- 400088

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ABSTRACT

Accurate measurement of food intake by organism is crucial, especially when working in therapeutic area. Various methods have been developed to address this problem. Food labeled with radioactive tracers, proboscis extension method and capillary feeder (CAFE) assay are some of the popular methods for measurement of food intake. Among these, Capillary feeder and radioactive tracer methods are shown to measure intake amount with greater accuracy. We hereby present a modified version of CAFE method that gives additional advantages of working with single flies and provides an opportunity to measure the amount of excreta produced. Simplicity of assembly of the feeding apparatus and the cost efficiency are some of the other advantages of the method presented here. This study also confirms the higher consumption of food by female as compared to male *Drosophila* adults, as reported using other methods. It also establishes that food consumption in the flies, housed individually, is greater in dark period as compared to the period of light.

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INTRODUCTION

In the experiments involving administration of a substance of interest, mixed with food, to an animal it is essential to measure the amount of food being consumed. While it is fairly easy for most other animal models, it poses a challenge for insect models. Among insects, *Drosophila melanogaster* is the most widely used model system and several methods have been introduced for feeding and measuring the food intake. Food labeled with radioactive tracers (Geer *et al.* 1970, Thompson *et al.* 1991), proboscis extension (Wong *et al.* 2009) and capillary feeder (CAFE) (Deshpande *et al.* 2007) assays are some of the methods developed for feeding and assessing the food intake in *Drosophila* flies. Among these, CAFE and radioisotope labeling have been shown to provide the most consistent results with high sensitivity.

We have designed a modified version of CAFE method (T-CAFE: Tip Capillary Feeder), wherein feeding can be monitored in a single *Drosophila* fly with considerable accuracy.

Most of the reports address to the amount of food consumed by a certain number of flies over certain duration, without considering the variations in the consumption by individual flies. The method presented in this paper can provide greater resolution at single organism level so that the variations due to age, sex and genotypes can be addressed. This method allows designing of experiments wherein variations in individuals as a response to certain treatment is to be studied.

The T-CAFE method also allows the collection of the fecal matter produced by an individual *Drosophila* fly during specific intervals. This can indirectly be used to evaluate the amount of test chemicals being metabolized by the organism.

Another merit of T-CAFE method is that it is non-invasive and exerts no trauma of any kind on the flies. It also allows the microscopic observation of the flies during the experiment and the food consumption can be linked to the proboscis extension, thus, authenticating the present method of analysis of food intake.

MATERIALS AND METHODS

T-CAFE assembly

This apparatus is simple and effectively helps to monitor feeding in *Drosophila*. It can be assembled in any laboratory and does not need sophisticated equipment.

Corresponding author:* **Rambhadur P. Subedi
Ramniranjan Jhunjhunwala College, Ghatkopar (W) Mumbai-400086

Materials used

1. Universal 1ml micropipette tip: Tarson (serving as fly retainer)
2. Sharp cutting blade
3. Standard volume-50µl capacity Capillaries - Banglore genei
4. Cotton plugs: non-absorbent cotton
5. *Drosophila* Liquid media: 5% yeast extract and 5% sucrose
6. Liquid paraffin

The narrower end of universal 1ml micropipette tips are cut, using a blade, such that a standard volume capillary tightly passes through the cut portion without leaving any gap. Through the wider opening of the tip a fly can be transferred using self-made fly transfer aspirator (RamAspire) (Subedi *et al.* 2017). The wider end of this 'fly retainer' can subsequently be closed using cotton plug. A fixed volume of liquid food can now be introduced in the capillary using a micropipette and 5µl of liquid paraffin be introduced at the opposite, non-feeding end of the capillary for minimizing evaporation (Fig 1).

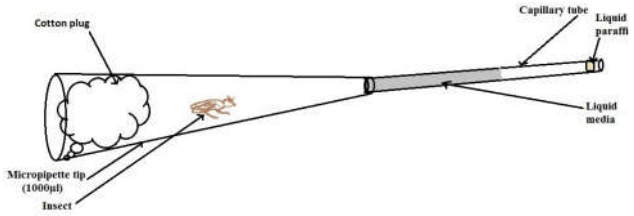

Fig 1 T-CAFE assembly. T-CAFE mainly consists of a 1000µl micropipette tip that acts as a fly retainer and a capillary tube that holds the liquid food for the flies. Liquid paraffin is placed at the end of the capillary tube for minimizing the loss by evaporation.

Drosophila husbandry and experimentation

Drosophila melanogaster (Canton-S strain) flies were initially raised and maintained on Cornmeal-Sucrose-Agar medium at 26^{o}C with light and dark cycle of 12 hours each, fairly coinciding with the natural circadian cycle. One day old, male and female virgin flies were separated and the individuals were transferred to T-CAFE tubes (Fig 2) using self-made fly transfer aspirator (RamAspire). For the measurement of food consumption, 5 sets of 10 assemblies with a male fly each and 10 assemblies with a female fly each were prepared and placed in the cavities made on the polystyrene sheet, as a hold. The entire assembly was placed over a tray containing water to increase humidity and minimize evaporation.

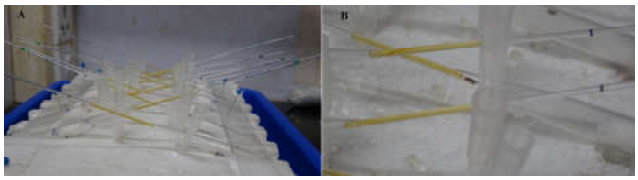

Fig 2 Drosophila feeding with T-CAFE. A) Individual T-CAFE units are arranged on a platform made for holding T-CAFE tubes. B) A single fly can be observed feeding on liquid media with extending its proboscis on T-CAFE capillary.

A volume of 40µl of *Drosophila* liquid feed was introduced into the capillary using a micropipette from the fly retainer end and the level is marked using a permanent marker. From the opposite end 5µl of liquid paraffin was introduced to minimize any change in volume of the feed by evaporation. After every 12 hours, the length emptied due to feeding of the fly was recorded. The initial 12 hour readings were not considered for quantification (period of habituation). Another set of T-CAFE tubes were also assembled without flies (as control), to account for evaporative loss, if any, since the end of the capillary facing the fly retainer has a free access to air. The reduction in the level of fluid between the marking and the fly retainer end in the control and experimental set up were then related to the volume of liquid food consumed by the fly. The data was gathered continuously for 7 days for each T-CAFE assembly. The food consumption was expressed as mean ± standard error µl/day for male and female flies.

Measurement of weight of excreta

After removing flies from the T-CAFE tubes, cotton plug and capillary was disassembled from each unit. The fly retainer part was then weighed in a high precision weighing machine (Citizen scale, model CX220). Subsequently, the fly retainer was washed, dried and weighed again. The differences in the weight represented the weight of excreta.

Statistical analysis

The mean amount of food consumed and the amount of excreta produced by male and female fly was analyzed with standard deviation. To establish the difference in the amount of food consumed and excreta produced by male and female flies and over light and dark period, student's t test was performed using Graphpad prism software package. For all the analysis, α-value was set at 0.05. The mean values were eventually presented with standard error.

RESULTS

Food intake measurements in 1-2 day flies using T-CAFE tubes

Daily average food intake by male and female fly was measured every 12 hours. The female flies showed higher food consumption in comparison to males flies (p-value: 0.0006) (Fig 3). The mean amount of food consumed by male flies was 0.627±0.029µl while the female flies consumed 0.810±0.043µl per day which was about 30% higher than the consumption by male flies.

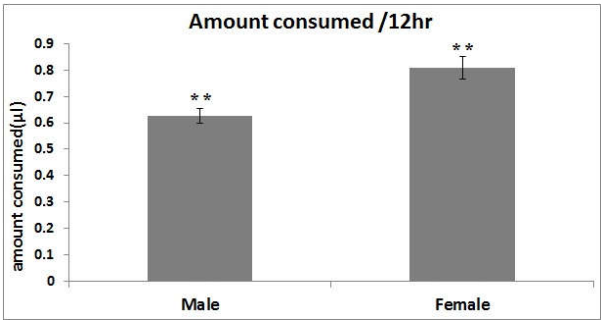

Fig 3 Male Vs. Female food consumption. Female flies consume more food compared to male flies. (** represents p-value <0.01)

Amount of excreta produced by each fly

Using T-CAFE feeding method, it was also possible to measure amount of excreta produced by each fly. The excreta produced were measured on the 7th day of feeding. No significant differences in excreta production was observed between male

and female flies (p-value: 0.6254). The average amount of excreta produced by male flies was 114.314 ±10.668 µg while female produced 110.357 ±11.085 µg of excreta per day (**Fig 4**).

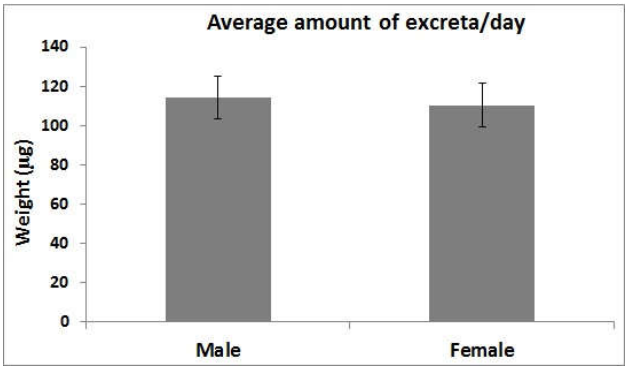

Fig 4 Excreta produced per day by *Drosophila* flies

Higher food consumption of Drosophila flies during dark period

The male as well as female *Drosophila* flies exhibited a significant increase in the amount of food consumed in dark period as compared to the period of light (p-value: <0.0001) (Fig 5). The food consumption during dark period was about 48% higher as compared to under illumination for the male as well as female flies.

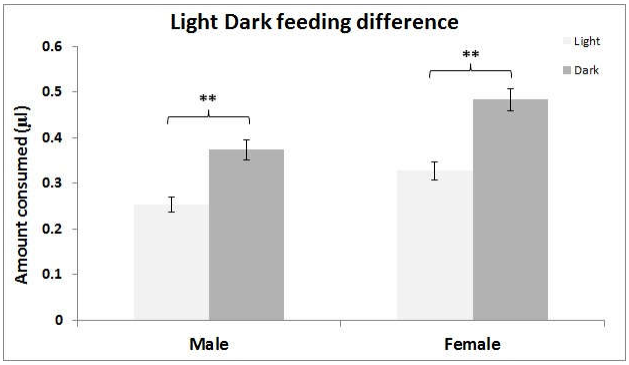

Fig 5 Dark Vs. Light feeding difference**.** Flies exhibit higher amount of feeding in dark duration (night time) compared to under period of illumination. (** represents p-value <0.01)

DISCUSSION

Measurement of food intake is one of the crucial parameters for any model system. Though, this is quite easy for most of the animal models wherein it is possible to inject particular amount of food with or without test substances directly into the digestive tract of an animal, using a gavage (Atcha *et al.* 2010). For insect models, measurement of consumption of food with substance of interest, however poses a challenge because of the presence of pre-oral mouthparts adapted to a specific mode of feeding (Wong *et al.* 2009). The techniques have been developed for the measurement of feeding in insects but most of them involve groups of insect. Moreover, these techniques are either indirect based on the measurement of extension of proboscis (Wong *et al.* 2009) or invasive involving mixing of labeled component/dye with food (Geer *et al.* 1970, Deshpande *et al.* 2014). The method described in this report provides a simple, less cumbersome and low-cost, yet accurate method of measurement of food intake in *Drosophila* model system.

Using this method, we were able to feed *Drosophila* and measure not only the amount of food consumed but also the excreta produced by the individual flies. This can be particularly useful when measuring the amount of a test compound absorbed/metabolized and even the modifications of a test compounds in the GI tract of the model insect, if the excreta is subjected to chemical analysis. The existing methods of excreta measurement vaguely represent the actual amount of excreta produced. Moreover, these methods mostly use chemical or radio tracers that require sophisticated instrumentation (Wayland *et al.* 2014, Apger and Wolfner 2013). The measurement of excreta using T-CAFE method, however, needs improvement in terms of sensitivity. An easier way to achieve this will be to use a weighing machine with better precision. The observed excreta weight may also be an underestimate of actual values as small amount of waste material deposited on cotton plug was not being estimated in the method presented here.

The measurement of the amount of food consumed by T-CAFE method is comparable to the radiotracer method, which gives relatively accurate estimation. Deshpande *et al.* (2014) have reported a greater amount of food consumption when studied by CAFE method than what was established by radiotracer method. This higher value could probably be due to greater dispensing of fluid than can be sucked by the insect using its proboscis. Also, feeding mechanism in the CAFE method is physically challenging due to vertical placement of the capillaries (Ro *et al.* 2014). This drawback has been avoided in T-CAFE method by placing the capillary in near horizontal position. It should be noted that in T-CAFE method, the flies are restricted within a small space. This limits the activity of enclosed flies and subsequently may be lowering the calorific need. We suspect this could be another reason for the lesser amount of food consumed by flies in T-CAFE method.

In agreement with the earlier reports (Carvalho *et al.* 2006) the current study has established that the female *Drosophila* flies consume greater amount of food in comparison to male flies of comparable age (fig 3). This is presumably related to the higher calorific need in egg production (Lints and Soliman 1988). It has been noticed that *Drosophila* flies, both male as well as female, consume higher amount of food during the hours of darkness as compared to the period of illumination. This is contrast to the findings by Xu *et al.* (2008) and Seay and Thummel (2011) who have used similar methods including the CAFE assay. These reports, however, involve either study on groups of flies in contrast to individual flies in the present investigation or were largely invasive in nature. Wong *et al.* (2009) have shown that flies when housed individually led to lesser feeding in comparison to the flies kept as group of 2 or more number. The same lab have also shown that these individual flies exhibit little variation in the amount of feed during different light period. However, this study does not report the amount consumed by these individual flies during dark period.

Overall, T-CAFE technique allows the designing of experiments with individual insects and would help in finding out fly to fly variations in feeding as well as egestion. The variation of individual flies in response to a test compound can also be followed through this technique, a crucial step towards 'personalized' medicines.

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