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olf413 Gene Controls Taste Recognition, Preference and Feeding Activity in *Drosophila melanogaster*

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Authors' contributions

This work was carried out in collaboration between both authors. Author BVS conceived and designed the study. Author RR carried out the experiment and did data analysis. Authors BVS and RR prepared the manuscript and are involved in critically reviewing and revising the analysis and the interpretations. Both the authors have read and approved the final manuscript.

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ABSTRACT

Recognition and responsiveness to food taste becomes a crucial event in foraging and feeding behaviour of an organism. Adjusting the feeding behaviour through a sophisticated and robust taste system is critical to fulfil their nutritional needs and facilitate its survival in environment. Palatability of food sources depends on the sensory and motor cues provided by the brain, in co-ordination with the other body systems to enable decisive feeding. *Drosophila melanogaster* is an apt model organism to decipher these behavioural paradigms. Octopamine a neurotransmitter, is required in regulation of feeding behavioural responses. *olf413*, a paralogue of *TβH*, is a gene predicted for its involvement in octopamine biosynthesis. The biological function of this gene is yet to be unravelled. Here we propose this gene function in taste recognition, food preference and feeding activity. We test the *olf413* loss of function mutants for food preference between two fruit extracts using CAFE

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and horizontal box methods. In our study we have used *olf413* gene disruption strain, $olf413^{MI02014}$ homozygous and in transheterozygous condition with another allele isolated in our lab, $olf413^{SG1.1}$. The results show that *olf413* mutants display a severe phenotype in feeding behaviour and there is an allele specific phenotypic distinction between the two strains. Thus implying that *olf413* gene function is required for taste recognition, starvation driven initiation and execution of feeding behaviour of the flies.

Keywords: olf413; Drosophila melanogaster; TβH; food preference; starvation driven feeding response; CAFE; taste response.

1. INTRODUCTION

Adaptive foraging behaviour is critically important for the survival of an organism. Appropriate feeding response is a co-ordinated outcome of physiological conditions necessitating food intake, taste sensing response to decide the palatability and significance of the food, followed by the initiation and execution of feeding behaviour. It involves a complex neuronal networking between the taste sensory neurons, processing and decision making centres in the brain along with the effector motor neurons. The genetics underpinning this complex behaviour can be better understood in *Drosophila melanogaster* model system with its highly potential and feasible genetic manipulative tools. *olf413* is a gene annotated as CG12673 in genome of *Drosophila melanogaster* [1]. Its molecular function is predicted as an enzyme in octopamine biosynthesis pathway owing to the signature domains in the encoded protein [2]. The biological function and mutant phenotypes for this gene have not been demonstrated so far. Here we use a new P-Gal4 insertion allele $olf413^{SG1.1}$ generated in our lab (in press) and the gene disruption strain $olf413^{MI02014}$ generated by Lee et al., (2018) to demonstrate for the first time, the function of *olf413* in regulating the feeding behaviour of *Drosophila melanogaster*. *olf413* function operates at two different levels of feeding behaviour. 1) Taste sensing and food preference decisions 2) Starvation driven feeding activity.

2. MATERIALS AND METHODS

2.1 *Drosophila* Stocks

Drosophila stocks were cultured on standard wheat cream agar media in a 12hr light/ 12hr dark cycle at 22^0C with 60% relative humidity. The following *Drosophila melanogaster* stocks were used: Oregon-K (Drosophila Stock Centre, University of Mysore, Mysuru), w^{1118} (NCBS, Bengaluru), SG1.1/*TM3Sb* (From our lab), Gene disruption strain $olf413^{MI02014}$/*TM3Sb* (#77717, Bloomington Drosophila Stock Centre) [3].

2.2 Loss of Function Mutants for Analysis

The $olf413^{MI02014}$-*GAL4* homozygous survivors were selected from the stock $olf413^{MI02014}$/*TM3Sb*, age matched and used as gene disruption strain for the experiments. $olf413^{SG1.1}$ / *TM3Sb* virgin females were crossed with $olf413^{MI02014}$/*TM3Sb* males to obtain transheterozygotes in F1. The transheterozygous $olf413^{SG1.1}$ / $olf413^{MI02014}$ survivors from F1 were selected for the assay. Oregon-K and w^{1118} stocks were used as controls.

2.3 The Capillary Feeder Assay (CAFE)

We followed the procedure as given by Diegelmann et al. [4] for CAFE assay, with slight modifications. The capillary feeder assay tube is a glass vial of length (7.3cm) and width (3.3cm). A sponge stopper with 4 holes to the size of the 200µl pipette tip were made. The 200µl pipette tip edge was cut in such a manner that the capillary tube could just pass through but not fall off. To provide an extra support for the capillary tube to hold on to the pipette tip, we introduce another 10µl pipette tip with end cut placed on top of the 200µl tip. The capillary tube was introduced running through both these pipette tips.

3 days old flies (for all the control and mutant groups) were collected by pupal isolation to avoid the effect of anaesthetic ether on the behavioural paradigm. The flies were placed in a vial with a moist blotting paper at the base of the vial and kept for starvation for 5 hours. Apple and tomato were ground separately with clean pestle and motor, filtered to remove the debris to obtain the fruit extracts. The brilliant blue food dye was mixed with apple juice whereas the red food dye was mixed with tomato juice. Two capillary tubes were filled with the blue coloured solution and another two capillary tubes were filled with red

coloured solution and were placed in alternating positions. 10 flies were subjected for analysis at a time. The assay was conducted for 100 flies as 10x10 trials for each group. The starved flies were introduced into the experimental vial and sealed with the sponge stopper along with the capillary tubes carefully making sure that the flies do not escape. The flies were allowed to feed on the given choice of fruit extracts in the capillary tubes for 30 minutes. The experiments were carried out at 22^0C. The flies were carefully anesthetized and the colour of the abdomen were observed under a stereo microscope and recorded. The flies having blue abdominal colour were considered as flies which had fed on apple juice whereas the flies having red abdominal colour were flies which had preferred tomato juice. Flies having a shade of mixed brown colour in their abdominal region were the flies which had fed on both apple and tomato juice indiscriminately. The flies which do not have any colour in their abdominal region were the flies which had not fed on any fruit juice even after the starvation period.

2.4 Feeding Assay with Horizontal Box

This apparatus is a horizontal box of 15.8cm in length, 14.5cm in width and with a height of 7.2 cm. The fruit extracts were kept at the four corners of the box in the centrifuge tube caps. The lid of the box was replaced by a sponge stopper with a hole in the centre for introducing the flies into the box during the experiment. The procedure in selecting and screening of the flies for the assay is as similar as mentioned for CAFE assay procedure. Since the area provided was large, 50 flies were subjected for analysis each time. A total of 250 flies were assayed as 50 x 5 trials for each group. Before adding the flies, the fruit extracts were added to its containers inside the box. The starved flies were then introduced into the box through the space created in the sponge stopper lid carefully, making sure that the flies do not escape. The flies are allowed to feed on the given choice of fruit extract in the containers for 45 minutes. The flies were carefully anesthetized and the colour of the abdomen were observed under a stereo microscope and recorded.

2.5 Statistical Analysis

The SPSS program, version 22, was used throughout all of the statistical analysis. The mean and standard error of the mean are displayed in the graphs of the food preferences. To analyze the differences between more than two groups, a one-way analysis of variance (ANOVA) was performed, and the control group and the mutant group were analyzed using Tukey's post-hoc honestly significant difference test. The following degrees of statistical significance were found: *P 0.05; **P 0.01; and ***P 0.001.

3. RESULTS

olf413$^{SG1.1}$ has a P-Gal4 insertion at 1.9 kb upstream of *olf413* transcription site. *olf413*^{MI02014} has a gene disruption T2A-Gal4 insertion in the second intron of olf413 after translation site. It also carries a stop codon immediately after Gal4 coding sequence, hence generates a truncated protein thus forming a null/severe loss of function allele of *olf413*. *Drosophila melanogaster* wild type adult flies have a strong preference for apple juice as food against tomato, given a choice [5,6].

We have used *olf413*^{MI02014} homozygous and *olf413*$^{SG1.1}$/*olf413*^{MI02014} transheterozygous survivor flies to assess 1) their ability to discriminate the two fruit extracts and show appropriate preference between apple and tomato 2) their feeding activity post starvation. We analysed the males and females separately for different parameters. As there was no significant difference observed between sexes, we have presented here the data from analysis of male flies. In the capillary feeder assay (CAFE) the flies have to surpass 2 physiological stages to feed onto the fruit extract provided in the capillary tube, 1) the flies have to climb up the wall of the glass vial and hold onto the tube and feed from the capillary tube end 2) taste, and decide their preference. The result of capillary feeder assay is presented in Figs. 1A-H.

Oregon-K flies prefer apple over tomato fruit extract (53% vs 17% respectively) when given a choice between the two fruit extracts. A very few number of flies fed on both the fruits or do not feed on any of the fruit extracts (Fig. 1A). *w*^{1118} flies have a problem in discriminating between the two fruit extracts as the percentage of flies preferring apple and tomato extract does not show significant difference (42% vs 30% respectively). We observe that only a small percentage of flies are found to be not feeding (Fig. 1B). *olf413*^{MI02014} homozygous survivors show a maintained preference choice between apple and tomato fruit extract feeding as these

flies show a higher percentage of preference towards apple than tomato extract (48% vs 10% respectively) which resembles the Oregon-K flies preference ratios. Mixed fruit extract feeding was significantly less in these flies. There was a statistically significant increase in the number of flies which do not feed on any fruit juice even after 5 hr of starvation (Fig. 1C). Upon comparison these results with that of w^{1118} (its respective control) we can say that this behaviour observed in $olf413^{MI02014}$ flies is not due to the presence of *white* mutation, but solely due to the loss of function of *olf413* gene. In the transheterozygous $olf413^{SG1.1}/olf413^{MI02014}$ survivor flies, we observe that there was a defect in taste response and in discriminating between the two fruit extracts. They did not show selective preference for apple (28% apple and 20% tomato). We find that a statistically significant number of flies (40%) in this transheterozygous condition which do not feed on any of the fruit juice (Fig. 1D).

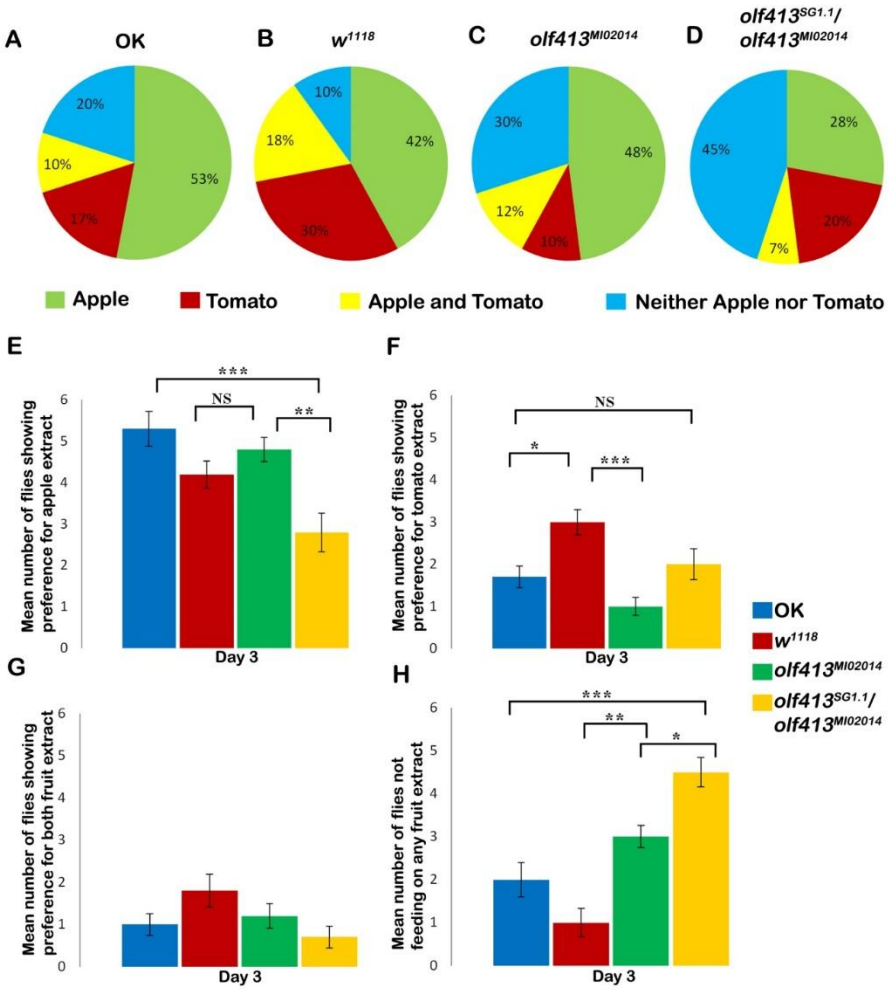

Fig. 1. Loss of function mutant $olf413^{MI02014}$ and transheterozygote $olf413^{SG1.1}/olf413^{MI02014}$ adult flies showing food preference defects in capillary feeder assay

*(A-D) Represent the pie chart of control and mutant strains showing food preference given a choice between apple and tomato fruit extracts. (E) Represents a bar graph with mean number of flies preferring apple fruit extract over tomato extract. (F) Represents a bar graph with mean number of flies preferring tomato extract over apple extract. (G) Represents the bar graph with mean number of flies preferring both apple and tomato fruit extracts indiscriminately given a choice between them. (H) Represents the bar graph with mean number of flies which do not feed on any fruit extract even after 5hr of starvation. Oregon-K serves as a control for $olf413^{SG1.1}/olf413^{MI02014}$ transheterozygotes, whereas w^{1118} serves as a control for homozygous $olf413^{MI02014}$ mutant strains. The error bars represent the Standard Error of the Mean (SEM). Asterisks indicates significant difference with *P < 0.05; **P < 0.01; ***P < 0.001*

In *olf413*$^{SG1.1}$/*olf413*^{MI02014} we find that there is a strong disability in discriminating between the two given choice of fruit extracts (Figs. 1 E-G). They exhibit a highly significant difference (P ≤ 0.001) in the number of flies which do not feed on any fruit extract after the 5hr of starvation in comparison with their control Oregon-K flies (Fig. 1H). *w*^{1118} flies also display a defect in discriminating between the two fruit extracts provided (Figs. 1 E-G). *olf413*^{MI02014} homozygous flies do not have any problem in discriminating between apple and tomato fruit extracts (Fig. 1E-G). They show a highly significant (P ≤ 0.001) proportion of flies which do not feed on any fruit extract after starvation as compared to its control *w*^{1118} flies (Fig. 1H).

olf413$^{SG1.1}$ and *olf413*^{MI02014} flies have certain level of motor disability (our data unpublished). Surprisingly high number of flies did not eat either of the two fruit extract despite 5 hours of starvation prior to testing. This lack of motivation driven feeding activity raised a question, if these flies had problem to reach out to the food source placed at a height in capillary tubing and hence could not feed. In order to test this possibility we conducted a second experiment for food preference in a box where the fruit extract were presented in a small container placed at the bottom of the box. The flies in this case did not have to climb or hold onto the capillary tubing in order to feed on the fruit extract (details in materials and methods). The results are presented in Figs. 2A-H.

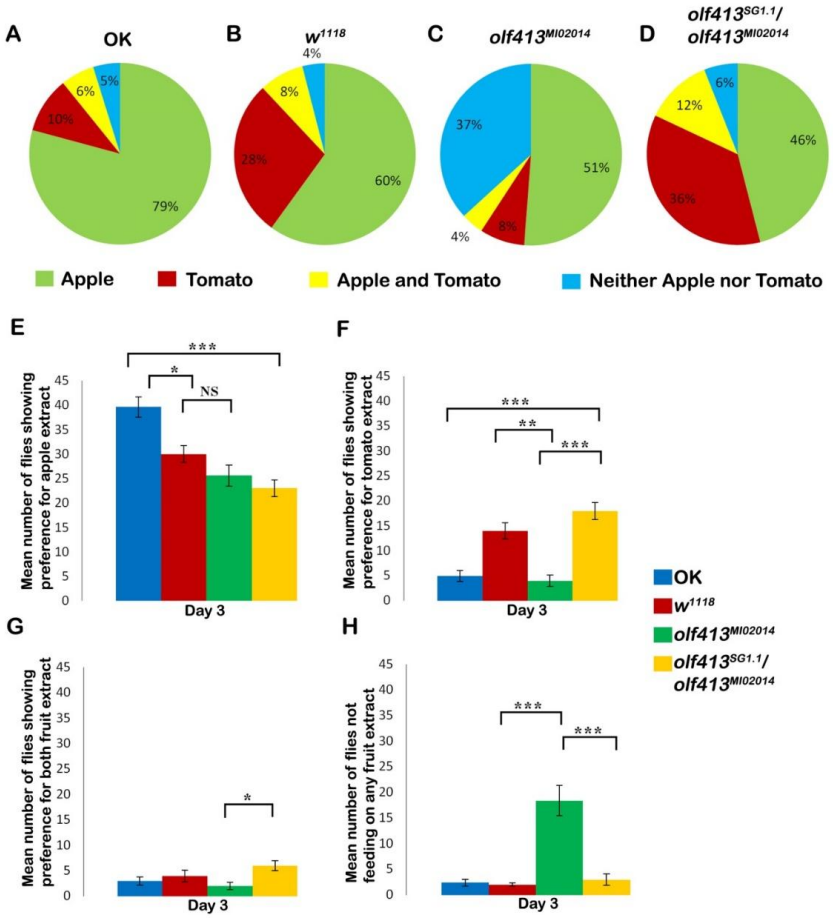

Fig. 2. Loss of function mutant *olf413*^{MI02014} and transheterozygote *olf413*$^{SG1.1}$/*olf413*^{MI02014} adult flies showing food preference defects in horizontal box paradigm

(A-D) Represents the pie chart of control and mutant strains showing food preference given a choice between apple and tomato fruit extracts. (E) Represents a bar graph with mean number of flies preferring apple fruit extract over tomato extract. (F) Represents a bar graph with mean number of flies preferring tomato extract over apple extract. (G) Represents the bar graph with mean number of flies preferring both apple and tomato fruit extracts indiscriminately given a choice between them. (H) Represents the bar graph with mean number of flies which do not feed on any fruit extract even after 5hr of starvation. Oregon-K serves as a control for olf413$^{SG1.1}$/*olf413*^{MI02014} *transheterozygotes, whereas w*^{1118} *serves as a control for homozygous olf413*^{MI02014} *mutant strains. The error bars represent the Standard Error of the Mean (SEM). Asterisks indicates significant difference with *P < 0.05; **P < 0.01; ***P < 0.001*

The results of Oregon-K and w^{1118} flies using horizontal box were very much similar to that of the results obtained for these flies in CAFE (Figs. 2A, B). $olf413^{MI02014}$ homozygous survivors showed a preference between apple and tomato fruit extract feeding with a significantly high percentage of preference towards apple than for tomato extract (51% vs 8%) which resembles the Oregon-K flies preference rates. The percentage of flies preferring both apple and tomato extracts was also less in these flies. Striking observation was that, there was a statistically significant increase in the number of flies (37%) which do not feed on any fruit juice even after the starvation time window (Fig. 2C). The transheterozygous $olf413^{SG1.1}/olf413^{MI02014}$ survivor flies, showed a defect in discriminating between the apple and tomato fruit extracts (46% apple vs 36% tomato). A small percentage of flies (12%) showed mixed feeding (Fig. 2D).

$olf413^{MI02014}$ homozygous survivors show a highly significant impairment with not able to feed on the fruit extracts as compared to its respective control w^{1118} ($P \leq 0.001$) post starvation (Fig. 2H). These flies maintained significantly high preference ($P \leq 0.01$) choice for apple against tomato fruit extract (Fig. 2E-G). When compared with $olf413^{SG1.1}/olf413^{MI02014}$ transhetrozygotes, these flies also show a highly significant difference ($P \leq 0.001$) in the behaviour 'not able to feed' onto the provided fruit extract (Fig. 2H). $olf413^{SG1.1}/olf413^{MI02014}$ transheterozygous flies do exhibit a strong defect in discriminating between the fruit extracts (Figs. 2E-G).

4. DISCUSSION AND CONCLUSION

Molecular function of *olf413* gene is predicted to be in octopamine synthesis [2]. The copper type II ion binding, tyrosine- beta-hydroxylase like and dopamine -beta-hydroxylase domains predicted in the encoded protein [7] serve as a basis for its proposed function as an enzyme paralogous to Tyramine β hydroxylase (TβH) [8] which has a function in octopamine synthesis [9]. As octopamine is known to be a neurotransmitter, neuromodulator and neurohormone [10,11] involved in various biological activities including appetitive foraging, motor activity and taste response mediated behaviours [12-15], we have studied for the first time, the new annotated gene *olf413* for its role in these behaviours of *Drosophila*. The two loss of function P-Gal4 insertion alleles, $olf413^{SG1.1}$ and $olf413^{MI02014}$ were studied for food preference and feeding activity. Apple juice and tomato extracts, the most and the least preferred fruit extracts respectively by the wild type *Drosophila melanogaster* were used for testing the taste preference ability of the *olf413* mutants. The capillary feeder assay showed that, $olf413^{MI02014}$ homozygous flies did not have deficits in taste discrimination, as their preference was very high for apple juice against tomato, similar to that seen in Oregon-K control flies. Contrary to this, the transheterozygotes $olf413^{SG1.1}/olf413^{MI02014}$ exhibit a severe disability in taste discrimination. Equal number of flies ate tomato/ both fruits as that of the flies which ate apple extract. They fail to show the normal taste response preference for apple.

Intriguing observation was that both the mutant strains showed very high number of flies (30%-45%) which did not feed on any fruit extract, despite the 5 hour of prior starvation which they were subjected to. Our earlier experiments have shown that *olf413* mutant flies had climbing deficits (unpublished). It is possible that, the flies failed to feed because they primarily had a problem in feeding activity, or they were not able to climb up to the capillary tubes containing fruit juice (materials and methods). The results from the second set of experiments, where the food preference assay was carried out in a box with fruit extracts presented at the bottom, were able to draw a discretion between the two possibilities, for not feeding. In the box set up, we found that, about 94% of the $olf413^{SG1.1}/olf413^{MI02014}$ were able to feed but had strong taste response deficits, as they were not selective upon apple against tomato. In case of $olf413^{MI02014}$ homozygous flies, interestingly we found that, they continued with their normal discriminating preference to apple against tomato. The most intriguing observation here was that significantly very high number (37%) of flies did not feed on any fruit extract, though there was no climbing required. They had intrinsic defect in performing feeding activity. Earlier studies carried out using TβH and Tdc2 (tyrosine decarboxylase) loss of function flies have shown that the octopamine is required in ventral paired median octopamine neurons of suboesophageal zone of *Drosophila* brain to promote feeding initiation in starved flies. They fail to exhibit proboscis extension response to sucrose despite several hours of starvation [16-18]. Our study reveals important findings: a) *olf413* function is required for normal feeding behaviour of *Drosophila melanogaster*, b) there is clear allele specific phenotypic distinction between $olf413^{SG1.1}$ and $olf413^{MI02014}$. $olf413^{SG1.1}$

delineates the functional requirement of the gene for taste recognition and food preference responses, as well as the climbing ability. *olf413*^{MI02014} interestingly separates out the requirement of *olf413* function for starvation driven initiation and execution of feeding activity of the fly. Thus our study for the first time demonstrates the *in vivo* biological requirement of *olf413* gene function for multiple attributes of foraging behaviour of *Drosophila melanogaster* adult flies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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