optoPAD, a closed-loop optogenetics system to study the circuit basis of feeding behaviors

José-Maria Moreira et al
Figure 1. The optoPAD system. (A) Concept for the use of closed-loop capacitance measurement of feeding with optogenetic manipulation of neurons in behaving flies. The interaction of the fly with the food source triggers the activation of the LED. (B) Overview of the components of the optoPAD, the Figure 1 continued on next page
Figure 1 continued

flyPAD arena and the high-power RGBA LEDs. (C) Algorithm for real-time detection of food interactions. Extracted food interaction bouts (activity bouts) are shaded in gray. (D) Schematic overview of the optoPAD experimental dataflow.

DOI: https://doi.org/10.7554/eLife.43924.002
probability of feeding burst onset

time after activity bout onset [s]
Figure 1—figure supplement 1. Quantification of the probability of the occurrence of a feeding burst onset depending on the time after activity bout onset. Bins are 0.1 s wide and probabilities are plotted for two different genetic background controls feeding on yeast, on an optoPAD channel triggering light activation.

DOI: https://doi.org/10.7554/eLife.43924.003
Figure 2. Increasing light intensity affects the feeding behavior of flies expressing different optogenetic effectors.

(A) Irradiance of all four LEDs increases linearly with increasing voltage (for red and amber above 2 V, for green and blue above 2.5 V). The average value of the three measurements is shown and error bars indicate standard error of mean.

(B) Difference in total number of sips on the stimulated (ON) and unstimulated (OFF) food patches of the same arena for 24 hr starved male flies expressing CsChrimson under the control of Gr5a-GAL4, and

Figure 2 continued on next page
Figure 2 continued

corresponding genetic controls. Both food sources contained 5 mM sucrose solution in 1% agarose. (C) Difference in total number of sips on the stimulated (ON) and unstimulated (OFF) food patches of the same arena for 3 days yeast-deprived, mated female flies expressing GtACR1 under the control of 57 F03-GAL4, which labels taste peg GRNs, and corresponding genetic control. For genotypes, see Materials and methods and key resources table. Both food sources contained 10% yeast solution in 1% agarose. The numbers below the graphs indicate the number of flies tested in each condition. ***p<0.001, **p<0.01, *p<0.05, ns non-significance. Boxes represent median with upper/lower quartiles; groups compared by Wilcoxon rank-sum test, followed by Bonferroni multiple comparison test when more than two groups were compared.

DOI: https://doi.org/10.7554/eLife.43924.006
Figure 2—figure supplement 1. Mean number of sips per feeding bursts during open-loop stimulation (1 s ON, 2 s OFF) measured on both food patches of the same arena for 3 days yeast-deprived, mated female flies expressing GtACR1 under the control of 57 F03-GAL4 or 67E03-GAL4, which both label taste peg GRNs, and corresponding genetic control (Steck et al., 2018). Both food sources contained 10% yeast solution in 1% agarose. The numbers below the graphs indicate the number of flies tested in each condition. ****p<0.0001. Boxes represent median with upper/lower quartiles; groups compared by Wilcoxon rank-sum test, followed by Bonferroni multiple comparison test for two comparisons.

DOI: https://doi.org/10.7554/eLife.43924.007
Figure 2—figure supplement 2. Difference in total number of sips on the stimulated (ON) and unstimulated (OFF) food patches of the same arena for 3 days yeast-deprived, mated female flies expressing CsChrimson under the control of SS02299-GAL4, and corresponding genetic controls. All genotypes have been supplemented either with (+) or without (-) 5 ml of 400 mM all-trans retinal for 6 days. Both food sources contained 10% yeast solution in 1% agarose. The numbers below the graphs indicate the number of flies tested in each condition. ***p<0.001, **p<0.01, *p<0.05, ns non-significance. Boxes represent median with upper/lower quartiles; groups compared by Wilcoxon rank-sum test, followed by Bonferroni multiple comparison test when more than two groups were compared.

DOI: https://doi.org/10.7554/eLife.43924.009
Figure 3. Creating virtual taste realities for Drosophila using the optoPAD. (A–D) Total number of sips from the unstimulated (LED OFF) and the light-stimulated (LED ON) food patches of the same arena by flies expressing CsChrimson (A and C) or GtACR1 (B and D), under the control of Gr64f-GAL4 (A and B) or Gr66a-GAL4 (C and D) (left side of the panels). Difference in total number of sips on the stimulated (ON) and unstimulated (OFF) food patches for flies expressing CsChrimson or GtACR1 (A and C), under the control of Gr64f-GAL4 (A and B) or Gr66a-GAL4 (C and D), and corresponding genetic controls (right side of the panels). All flies were 24 hr starved male flies (for genotypes, see Materials and methods and key resources table). The food substrate is indicated in each panel. The numbers below the graphs indicate the number of flies tested in each condition. ***p<0.001, **p<0.01, *p<0.05. Boxes represent median with upper/lower quartiles; groups compared by Kruskal-Wallis test, followed by Dunn’s multiple comparison test.

DOI: https://doi.org/10.7554/eLife.43924.016
Figure 4. The optoPAD allows for complex dynamic closed-loop experimental designs. In all experiments, 5–7 days old female Gr66a-GAL4 > CsChrimson flies were used. (A) Schematic overview of the dynamic virtual taste reality experiment: every five minutes the contingency of the
activity bout initiation

Moreira et al. eLife 2019;8:e43924. DOI: https://doi.org/10.7554/eLife.43924
11 of 13
Figure 4 continued

experiment is reversed (in each experimental block the fly’s interaction with a different channel triggered light stimulation). (B) Number of sips from channel 1 (upper half of the plot) and channel 2 (lower half of the plot) across time in the changing virtual taste reality setting described in A. Columns and lines represent mean and the standard error of the mean, respectively. The trials leading to LED activation are shaded in red. (C) Onset of light stimulation (red box) can be freely set to occur at different times after the initiation of an interaction with food (delay of 1.5, 3 and 6 s). The lower part of the diagram represents a representative capacitance trace with the onset of food contact marked with an arrow. (D) Duration of activity bouts in flies exposed to light after different delays relative to the initiation of food interactions and corresponding controls (experimental design described in C). Plotted are the duration of activity bouts for the stimulated flies (light) and for the same number of trials that were longer than 1.5, 3 and 6 s (from left to right) performed by the ‘no light’ control flies. (E) Schematic of the experimental design in which light activation was set to happen in a probabilistic manner. (F) Duration of activity bouts of the catch trials. Plotted are the duration of activity bouts for the stimulated flies (light) and for a selection of 10% of all the trials that were longer than 1.5, 3 and 6 s (from left to right) performed by the ‘no light’ control flies. (G) Cumulative feeding for the four different groups of the experiment described in (E). Line represents the mean and the shading the standard error of the mean. Dotted line indicates the 1100 sips threshold used to calibrate the data for the internal state of the animal. (H) Duration of activity bouts of the catch-trials for sip-calibrated flies (trials performed until the flies had reached a total of 1100 sips). Plotted are the duration of activity bouts for the stimulated flies (light) and for a selection of 10% of all the trials that were longer than 1.5, 3 and 6 s (from left to right) performed by the ‘no light’ control flies. For genotypes, see Materials and methods and key resources table. ***p<0.001, **p<0.01, *p<0.05, ns non-significance. The numbers below the graphs in D, F and H indicate the number of flies tested in each condition. In D, F, and H, boxes represent median with upper/lower quartiles. In D, F and H, groups were compared by Kruskal-Wallis test, followed by Dunn’s multiple comparison test. In B, the total number of sips for all bins in each channel during each period of 5 min was compared by Wilcoxon rank-sum test.

DOI: https://doi.org/10.7554/eLife.43924.021
Figure 4—figure supplement 1. Activity bouts of individual flies in a dynamic experimental protocol experiment. (A) Raster plot of the data shown in Figure 4B. Every line corresponds to a single fly undergoing the dynamic experimental protocol. Gray marks activity bouts with the food source not triggering light stimulation and red marks activity bouts triggering light activation and hence likely activation of bitter neurons. Dashed lines symbolize the time point at which the channel triggering light activation was changed. (B) Summed time flies are interacting with the food in a 20 s wide bin for non-light triggering channel (gray) and light triggering channel (red). Data in the bin are normalized by the total number of flies in the assay.
DOI: https://doi.org/10.7554/eLife.43924.002