Journal of Insect Physiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

The adult foraging assay (AFA) detects strain and food-deprivation effects in feeding-related traits of *Drosophila melanogaster*

Bryon N. Hughson[a,1], Ina Anreiter[a,b,1], Nicholas L. Jackson Chornenki[a], Keith R. Murphy[c,d,e], William W. Ja[d,e], Robert Huber[f,g], Marla B. Sokolowski[a,b,*]

[a] Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada
[b] Child and Brain Development Program, Canadian Institute for Advanced Research (CIFAR), 180 Dundas St. West, Suite 1400, Toronto, Ontario M5G 1Z8, Canada
[c] Program in Integrative Biology and Neuroscience, Florida Atlantic University, Jupiter, FL 33458, USA
[d] Department of Neuroscience, The Scripps Research Institute, 130 Scripps Way 3B3, Jupiter, FL 33458, USA
[e] Center on Aging, The Scripps Research Institute, 130 Scripps Way 3B3, Jupiter, FL 33458, USA
[f] JP Scott Center for Neuroscience, Mind & Behavior, Biological Sciences, Bowling Green State University, Bowling Green, OH 43614, USA
[g] Radcliffe Institute for Advanced Study, Harvard University, 10 Garden Street, Cambridge, MA 02138, USA

ARTICLE INFO

Keywords:
Drosophila melanogaster
Adult foraging assay
Food deprivation
Food search
Ingestion
Thigmotaxis

ABSTRACT

We introduce a high-resolution adult foraging assay (AFA) that relates pre- and post-ingestive walking behavior to individual instances of food consumption. We explore the utility of the AFA by taking advantage of established rover and sitter strains known to differ in a number of feeding-related traits. The AFA allows us to effectively distinguish locomotor behavior in Fed and Food-Deprived (FD) rover and sitter foragers. We found that rovers exhibit more exploratory behavior into the center of an arena containing sucrose drops compared to sitters who hug the edges of the arena and exhibit thigmotaxic behavior. Rovers also discover and ingest more sucrose drops than sitters. Sitters become more exploratory with increasing durations of food deprivation and the number of ingestion events also increases progressively with prolonged fasting for both strains. AFA results are matched by strain differences in sucrose responsiveness, starvation resistance, and lipid levels, suggesting that under the same feeding condition, rovers are more motivated to forage than sitters. These findings demonstrate the AFA's ability to effectively discriminate movement and food ingestion patterns of different strains and feeding treatments.

1. Introduction

Animals meet their daily energetic demands by searching for and consuming food (Stephens et al., 2007). Energy needs are more easily satisfied when food is abundant, and negative perturbations in metabolic set points stimulate increased locomotion and food search (Corrales-Carvajal et al., 2016; Dethier, 1976). In the fruit fly (*Drosophila melanogaster*) foraging behavior combines the detection of olfactory and gustatory cues through contact chemo-sensation (Dahanukar et al., 2005; Dethier, 1976). Gustatory receptor neurons, located in tarsi and labella, allow a fly to detect attractive nutrients and aversive toxins (e.g. by-products of decomposition); this information is used to make decisions on whether to ingest a specific food item (Itskov and Ribeiro, 2013; Ling et al., 2014). In the wild, flies face heterogeneity in both the quantity and quality of nutrients as they encounter fallen fruit in orchards (Reaume and Sokolowski, 2006). Due to the complexity of natural habitats, *Drosophila* researchers use controlled laboratory environments to characterize foraging behavior. Food consumption, or its likelihood, is quantified using a variety of techniques (Deshpande et al., 2014; Itskov and Ribeiro, 2013) such as measuring the volume ingested from a capillary tube over time in the Capillary Feeder (CAFE) assay (Ja et al., 2007) or by counting the number of proboscis extensions when fly tarsi are stimulated by food as in the Proboscis Extension Response (PER) assay (Scheiner et al. 2004; Shiraiwa and Carlson, 2007). Alternatively, *post hoc* analyses measure the quantity of coloured, fluorescent, or radiolabeled food ingested (e.g. Allen et al., 2017; Carvalho et al., 2005; Tanimura et al., 1982; Thompson et al., 1991). Further approaches to measuring ingestion include the flyPAD (fly proboscis and activity detector) and FLIC (fly liquid food interaction counter), which rely on changes in capacitance or resistance across sensors to measure the physical interaction of an individual fly with food (Itskov et al., 2014); Ro et al., 2014). Automated systems for measuring actual

* Corresponding author.
E-mail address: marla.sokolowski@utoronto.ca (M.B. Sokolowski).
[1] Co-first authors.

http://dx.doi.org/10.1016/j.jinsphys.2017.08.011
Received 15 March 2017; Received in revised form 23 August 2017; Accepted 26 August 2017

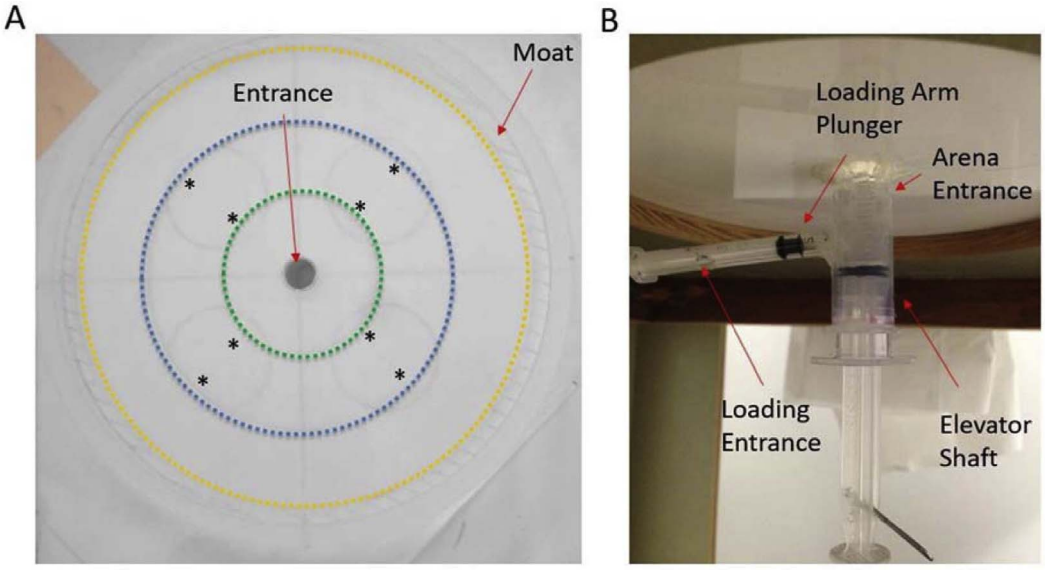

Fig. 1. The adult foraging assay (AFA) for examining locomotion and feeding in adult flies. (A) The AFA testing arena. The entrance is shown in the open position at the center of the arena, and the space for the moat is noted at the edge. The arena is 90 mm in radius from the center to the inner edge of the moat. * Denotes the location of the sucrose drops. The dashed circumference lines mark the inner (green), middle (blue) and outer (yellow) zones. The movable opening in the floor attached to the loading arm is shown open in this figure but, once closed, the entire surface of the arena is uniformly white, except for the sucrose drops that were dyed blue to increase visibility. (B) Fly loading arm underneath the arena. The loading arm consists of a 1 mL horizontal syringe, with a hole large enough for a single fly to enter, leading into a 5 mL vertical syringe, placed within a 10 mL vertical syringe. The front ends of both syringes were cut, with the end of the horizontal 1 mL syringe leading into a hole at the bottom of the vertical 5 mL and 10 mL syringes, and the open end of the 5 mL syringe leading into the arena. Moving the outer 10 mL syringe results of displacement of the holes in the two vertical syringes, closing the entrance into the shaft and preventing the fly from moving backwards. The loading arm is shown in the closed position, when open the plunger is pulled back past the loading entrance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

consumption with high resolution, the Expresso and ARC (Activity Recording CAFE) have also been described (Yapici et al., 2016, Murphy et al., 2016). While these assays provide excellent measurement of food intake, they do not provide any information about how the animals find food in a large open space.

Food search is a more complex behavior than simply food intake and its study in the laboratory requires artificial foraging environments (Bell et al., 1985; Corrales-Carvajal et al., 2016; Pereira and Sokolowski, 1993; Tanimura et al., 1982; Toshima et al., 2014). The characterization of walking behavior during foraging requires precise and reliable tracking of a fly's movement. Measures such as distance traveled and speed in addition to food intake allow us to examine specific pre- and post-ingestion exploratory patterns that may influence successful food search. It is important to distinguish pre- and post-ingestion exploratory behaviors since previous research has shown that they are influenced by metabolism and differ from each other (Bell et al., 1985; Nagle and Bell, 1987; Burns et al., 2012). When flies find a food patch they perform either local search or ranging behaviour (Bell et al., 1985, Kim and Dickinson, 2017). In local search, flies remain close to the food source and often circle around it (Kim and Dickinson, 2017). In ranging, flies move further from the food source and their movement patterns are in straighter paths. Hungry flies perform more intensive search behavior than well-fed flies (Bell, 1990).

Here, we introduce a novel cost-effective design for an adult foraging assay (AFA) that permits quantification of pre- and post-ingestive behavior as well as the number of times a fly feeds on a food source. The AFA is flexible with respect to the configuration of drops of sucrose or other food sources and is designed to minimize handling stress by allowing the fly to enter the chamber on its own accord. This entry method eliminates the potential confounds of handling effects that arise from aspirating or tapping a fly into a test arena (Barron, 2000; Burns et al., 2012; Trannoy et al., 2015). The AFA arena is surrounded with a water moat to prevent flies from walking onto the arena wall and lid. This obviates the need for a slippery fluon coating of arena surfaces (Dierick and Greenspan, 2006), a method that may lead to excessive grooming in test subjects. We assess the ability of this assay to characterize variation in foraging behavior by using rover (for^R) and sitter (for^s) strains of the *foraging* (*for*) gene of *D. melanogaster* (Burns et al., 2012; de Belle et al., 1989; Kaun et al., 2007; Osborne et al., 1997; Sokolowski, 1980). Rovers and sitters have been shown to differ in feeding behavior both as larva (Sokolowski, 1980) and adult flies (Pereira and Sokolowski, 1993; Kent et al. 2009; Burns et al., 2012). Our results show that the AFA provides an enhanced method for the phenotypic characterization of adult rover and sitter foraging behavior and their plastic responses to food deprivation, and that these differences correlate with several metabolic traits.

2. Materials and Methods

2.1. Fly strains and handling

The Rover (for^R) and sitter (for^s) strains used in these experiments have isogenized for^R 2nd chromosomes, originally described and called B15 in Bauer and Sokolowski (1985), or for^s, 2nd chromosomes, originally described and called E2E3 in Sokolowski (1980); and share isogenized X and 3rd chromosomes from the rover B15 strain (25). Rover and sitter strains were re-isogenized in 2013 as described in Allen et al. (2017), and share the same newly isogenized X and 3rd chromosome. Flies were maintained at 25 °C, in a 12:12-h light/dark cycle at 60% relative humidity with lights on at 0800 h. Flies were reared on a standard yeast-sugar-agar medium (Anreiter et al., 2016). Virgin female flies were collected immediately after eclosion and placed into vials of n = 20 containing 10 ml of standard food. For proof of principle of the AFA, we chose to test virgin females to avoid confounding effects of oviposition site selection. Approximately 24 h after collection, flies had their wings clipped under brief anesthesia with CO_2. We found that wing clipping did not abolish strain differences in food-search behavior, but prevented flies from jumping onto the arena lid. Un-clipped Fed flies would often jump onto the arena lid, which prevented consistent filming and resulted in too many mistrials. Flies were transferred to