Video Article

Operant learning of *Drosophila* at the torque meter

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Abstract

For experiments at the torque meter, flies are kept on standard fly medium at 25 C and 60% humidity with a 12hr light/12hr dark regime. A standardized breeding regime assures proper larval density and age-matched cohorts. Cold-anesthetized flies are glued with head and thorax to a triangle-shaped hook the day before the experiment. Attached to the torque meter via a clamp, the fly's intended flight maneuvers are measured as the angular momentum around its vertical body axis. The fly is placed in the center of a cylindrical panorama to accomplish stationary flight. An analog to digital converter card feeds the yaw torque signal into a computer which stores the trace for later analysis. The computer also controls a variety of stimuli which can be brought under the fly's control by closing the feedback loop between these stimuli and the yaw torque trace. Punishment is achieved by applying heat from an adjustable infrared laser.

Protocol

Fly medium

The composition of the fly food is critical for learning (Guo et al., 1996):

- Water 1000 ml
- Cornmeal 180 g
- Soybean 10 g
- Yeast 18.5 g
- Agar 7.5 g
- Molasses 40 g
- Syrup (sugar beet) 40 g
- Nipagin 2.5 g

Every vial is supplied with a dab of fresh, living yeast paste and a piece of filter paper to provide an additional surface for flies and pupae.

Fly breeding and staging

The following procedure is performed every day, leading to precisely staged animals grown at the appropriate density. All newly ecclosed flies since the last procedure on the previous day are collected for breeding and experiments. The oldest vials without any remaining living pupae are discarded. Four day old flies are added to a fresh vial for egg deposition over night. The density of female flies should be approximately 20 for each vial, adjusted for the size of the vial and the strain used. The ideal density is one that is high enough for the fly medium to liquefy during the larval stages and low enough such that all larvae have pupated before the first flies ecclose. The egg-laying flies from the previous day are removed and discarded.

Fly preparation

Flies are kept on standard cornmeal/molasses medium as described above at 25°C and 60% humidity with a 12hr light/12hr dark regime. After briefly immobilizing 24-48h old flies by cold-anaesthesia, the flies are glued (SuperGlue UV glass adhesive, 505127A, Pacer Technology, Cucamonga, Ca., USA) with head and thorax to a triangle-shaped copper hook (diameter 0.05mm) the day before the experiment. The animals are then kept individually overnight in small moist chambers containing a few grains of sucrose.

Apparatus

The core device of the set-up is the torque compensator (torque meter) (Götz, 1964). It measures a fly's angular momentum around its vertical body axis, caused by intended flight manoeuvres. The fly, glued to the hook as described above, is attached to the torque meter via a clamp to accomplish stationary flight in the centre of a cylindrical panorama (arena, diameter 58mm), which is homogeneously illuminated from behind. The light source is a 100W, 12V tungsten-iodine bulb. For green and blue illumination of the arena, the light is passed through monochromatic broad band Kodak Wratten gelatin filters (#47 and #99, respectively). Filters can be exchanged by a fast solenoid within 0.1s. Alternatively, the arena is illuminated with 'daylight' by passing it through a blue-green filter (Rosco "surfblue" No. 5433), or no filter at all. The transmission spectrum of the Rosco blue-green filter used in this study is equivalent to that of a BG18 filter (Schott, Mainz) and constitutes an intermediate between the Kodak blue and green filters (Brembs and Hempel de Ibarra, 2006; Liu et al., 1999). The arena can be rotated around the fly using a computer controlled electric motor. In such a 'flight-simulator' situation, the angular velocity of the arena is proportional to, but directed against the fly's yaw torque (coupling factor $K=-11°/s•10^{-10}Nm$). This enables the fly to stabilize the panorama and to control its angular orientation. This virtual 'flight direction' (i.e., arena position) is recorded continuously via a circular potentiometer (Novotechnik, A4102a306). An analogue to digital converter card (PCL812; Advantech Co.) feeds the arena position and the yaw torque signal into a computer which stores the traces (sampling frequency 20Hz) for later analysis. Punishment is achieved by applying heat from an adjustable infrared laser (825 nm, 150 mW), directed from behind and above onto the fly's head and thorax. The laser beam is pulsed (approx. 200ms pulse width at ~4Hz) and its intensity reduced to assure the survival of the fly.

Experiments

Pattern learning

For the traditional pattern-learning experiment (Dill and Heisenberg, 1995; Dill et al., 1993, 1995; Liu et al., 2006; Liu et al., 1998; Liu et al., 1999; Wolf and Heisenberg, 1991), four black, T-shaped patterns of alternating orientation (i.e. two upright and two inverted) are evenly spaced on the arena wall (pattern width ψ=40°, height θ=40°, width of bars=14°, as seen from the position of the fly). A computer program divides the 360° of the arena into 4 virtual 90° quadrants, the centers of which are denoted by the patterns. The flies control the angular position of the patterns with its yaw torque (flight simulator situation). During training, heat punishment is made contiguous with the appearance of one of the pattern orientations in the frontal visual field. Reinforcement of each pattern is always equalized within groups. During test, the heat is permanently switched off and the fly's pattern preference recorded.

Color learning

Color learning is performed as described before (Brembs and Heisenberg, 2000; Brembs and Hempel de Ibarra, 2006; Brembs and Wiener, 2006; Wolf and Heisenberg, 1997). The arena is divided into four virtual 90° quadrants, the centers of which are denoted by four identical vertical stripes (width ψ=14°, height θ=40°). The fly is controlling the angular position of the four identical stripes with its yaw torque as described for the T-shaped patterns above. The color of the illumination of the whole arena is changed whenever one of the virtual quadrant borders passes a point in front of the fly. During training, heat punishment is made contingent on one of the two colors. During test, the heat is permanently switched off and he fly's color preference recorded. Of course, the colors can be combined with patterns for compound conditioning (Brembs and Heisenberg, 2001).

Yaw torque learning

Yaw torque learning is performed as previously described (Brembs and Heisenberg, 2000; Heisenberg and Wolf, 1993). The fly's spontaneous yaw torque range is divided into a ‘left' and ‘right‘ domain, approximately corresponding to either left or right turns. There are no patterns on the arena wall. During training, heat is applied whenever the fly's yaw torque is in one domain and switched off when the torque passes into the other. In the test phases, heat is permanently switched off and the fly's choice of yaw torque domains is recorded.

Composite learning

Composite learning is an extension of yaw torque learning, as described before (Brembs and Heisenberg, 2000). Basically, yaw torque learning and color learning are combined in an experiment with equivalent operant (yaw torque) and classical (colors) predictors. During training, the fly is heated whenever the fly's yaw torque passes into the domain associated with punishment. Whenever the fly switches yaw torque domains, not only temperature but also arena coloration is changed (from green to blue or vice versa). Thus, yaw torque domain and color serve as equivalent predictors of heat. In the test phases, heat is permanently switched off and only the fly's choice of yaw torque domains/colors is recorded.

Discussion

This experimental setup combines superb control over experimental circumstances with an advanced genetic model organism. Using the procedures described in this presentation, the molecular and neurobiological underpinnings of a variety of behavioral traits can be investigated, including, but not limited to, the mechanisms of spontaneous behavior generation, operant and classical conditioning, pattern recognition, color vision or course control.

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