REVIEWS

Into the mind of a fly

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Where do animal behaviours come from and are they controlled by genes? This is the fundamental question posed by the field of neurogenetics. Pioneering work from the 1960s in Seymour Benzer's laboratory demonstrated for the first time that *Drosophila melanogaster* fruitflies could be mutated to obtain animals with insomnia, learning disabilities and homosexual courtship behaviours.

Forty years ago, the field of *Drosophila* neurogenetics (defined in Box 1) was born in Seymour Benzer's laboratory at Caltech[1]. In the mid-1960s, Benzer made an abrupt and orthogonal turn from his early ground-breaking work defining the fine structure of the gene in bacteriophage[2] to the heretical idea that single genes could control behaviour in complex animals. A paper appearing in the September 1967 issue of *Proc. Natl Acad. Sci.* presented the first evidence that mutant flies defective in phototaxis behaviour, or locomotor responses to light, could be identified[1]. The premise of neurogenetics—widely disbelieved at the time—was that complex behaviours such as the ability to learn and remember, the internal biological rhythms of the body, and courtship and sexuality could all be under genetic control.

Drosophila melanogaster as an experimental organism has contributed much to contemporary neurobiology. The first cloning of a structural gene for a potassium channel was achieved by Benzer's trainees, Yuh Nung Jan and Lily Jan, when they isolated the gene corresponding to the *shaker* (*sh*) mutant[3]. The founding member of the now enormous *transient receptor potential* (*trp*) ion channel family had its origins as a fly mutant defective in light-evoked retinal electrophysiology[4]. Vertebrate and invertebrate TRP channels have since turned up in biological processes as diverse as the sensation of odours, tastes, pungent compounds such as wasabi, capsaicin and menthol, cold, heat, touch and hearing, among others (reviewed in ref. 5). Beginning in the late 1960s, William Pak amassed a large collection of mutants defective in visual signal transduction, such as the *neither inactivation nor afterpotential* (*nina*) mutants[6]. This genetic dissection of phototransduction in *Drosophila* enabled later molecular analysis of the molecules underlying visual signal transduction in the laboratories of Pak, Gerald Rubin, Charles Zuker and others (reviewed in ref. 7).

The cloning of *sh* and *trp* are excellent examples of the power of neurogenetics. Both arose from genetic screens designed to test the hypothesis that studying shaky flies or flies with altered retinal physiology would lead to interesting insights into neural function. The tools of this discipline are simple and require only a suitable behavioural paradigm (three are shown in Fig. 1), a means to make flies with mutations in single genes, and standard molecular genetic techniques to progress from a mutant phenotype to a genotype.

This Review article will discuss how the revolution started by Benzer and his students in 1967 has spread to many fields of neurobiological investigation in *Drosophila*, from whence it jumped to mice, zebrafish and other species, including humans. Here I will focus specifically on three original discoveries in *Drosophila* neurogenetics and behaviour—biological rhythms, sexual courtship and chemoreception—and how these have blossomed in the last 40 yr.

The genetics of circadian rhythms in the fly

Flies, like all other animals and plants on earth, have a daily routine in synchrony with the rhythms of the Sun and Earth. Like humans, flies tend to wake up around dawn, enjoy a siesta in the afternoon, and are largely inactive after nightfall[8]. The biological rhythm in locomotor activity recurs on a roughly 24 h cycle, hence it is termed a circadian (*circa diem*—around a day) rhythm. This modulation of locomotor behaviour is driven by external environmental rhythms, but can also persist in flies raised for generations in the dark[9].

Ronald Konopka in Benzer's laboratory provided the first evidence that the biological clock was under genetic control and could be broken by mutagenesis[10]. In an elegant and simple screen for flies with altered hatching and locomotor rhythms, using activity-monitoring devices such as those in Fig. 1b, Konopka and Benzer

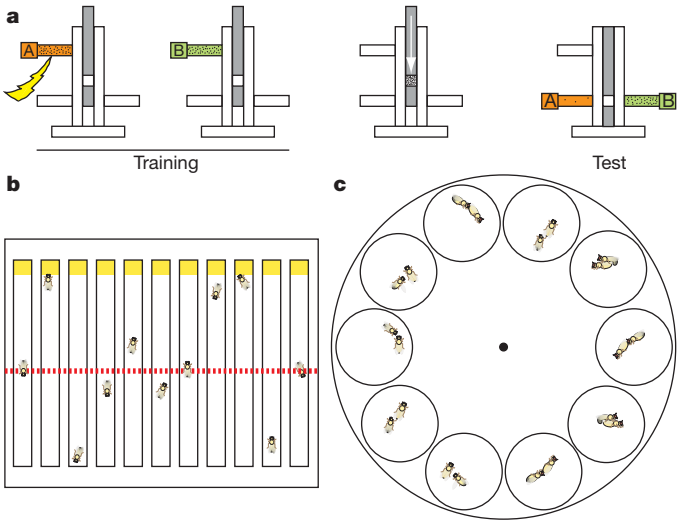

Figure 1 | A diversity of behaviour paradigms is used to measure *Drosophila* behaviour in the laboratory. a. The olfactory T-maze is used for Pavlovian olfactory conditioning[40,64]. Flies are trained to associate odour A (orange) with electric shock (left). During testing, these flies avoid odour A (right). The assay is carried out with reciprocal training, such that only one half of the paradigm is depicted here. Flies are depicted as small black dots. **b**, Circadian-activity monitors measure locomotor activity of individual flies using an infrared beam (red dotted line)[10]. An external computer tracks the number of times the fly breaks the beam, allowing continual monitoring of fly locomotor activity over a period of weeks. **c**, The courtship wheel permits the observation of up to 10 fly couples in which the male engages in such stereotyped sexual activity as the following: genital licking, wing vibration to produce a species-specific song, and copulation. Graphic in **a** is adapted from figure 1 of ref. 65 with permission from Elsevier (copyright 2004).

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Box 1 | The subject

Neurogenetics merges concepts and techniques from neurobiology and genetics to study the genetic basis of behaviour and neural function. By generating and studying mutant animals that exhibit abnormal behaviour, mistakes in neural wiring, or anomalies in the structure or function of neurons, neurogeneticists can track down the genes responsible for these phenotypes, thereby understanding the function of the genes in producing a normal brain and its associated behaviours. In 1967, Seymour Benzer suggested that neurogenetics could act as a 'microsurgical tool' to study the brain:

"Thus, use of mutation as a microsurgical tool could conceivably lead to the identification of the various transmitters, about which little is presently known. The counter-current procedure is obviously adaptable to a wide range of stimuli, such as gravity, odour, sound and special visual patterns, thus lending itself to the isolation of many kinds of behavioural mutants, including ones in which the wiring pattern of the nervous system is affected. Furthermore, as preliminary experiments have shown, the speed of the procedure permits its use in the study of short-term modifications of behavior."[1]

isolated three different mutant alleles of the same gene, called *period* (*per*). per^0 flies are insomniac, per^s flies live a short day, and per^l flies a long day[10]. An amazing parallel with humans was uncovered with the recent identification of mutations in a human *period* homologue (*PER2*) as the genetic culprit behind the per^s-like phenotype in familial advanced sleep-phase syndrome[11]

How a single gene could both be necessary for the clock but also set its running speed remained a mystery until the age of molecular cloning and the isolation of other clock genes. The groups of Michael Rosbash and Jeffrey Hall, and the group of Michael Young cloned *per* in 1984 (refs 12, 13). Both *per* messenger RNA and PER protein were subsequently shown to cycle with a circadian rhythm, and show a rhythmic nuclear accumulation, prompting a model in which PER acts as a feedback suppressor to control the clock[14]. *per* turned out to be just the tip of an enormous iceberg of clock genes, clock accessory genes, and clock-controlled genes. The present model for the *Drosophila* clock includes a host of core clock components that include a positive transcriptional feedback loop (*Clock* (*Clk*), *cycle* (*cyc*) and *vrille* (*vri*)), a negative transcriptional feedback loop (*per* and *timeless* (*tim*)), and factors that modulate the light-regulated accumulation and output of the core clock genes (*double-time* (*dbt*; also known as discs overgrown or *dco*), *shaggy* (*sgg*), *cryptochrome* (*cry*), *Pigment-dispersing factor* (*Pdf*) and others; reviewed in ref. 8). Some recent surprises in the clock field include a somewhat mysterious cytoplasmic timing mechanism that regulates the delay in nuclear accumulation of period and timeless[15], as well as the discovery that the clock protein has chromatin-remodelling activity[16].

Homologues of many of the core clock genes have been identified in vertebrates, further validating the fly as a model for circadian biology. Microarray studies by Young[17] and others have identified several hundred genes under circadian control, the analysis of which promises to provide an integrated view of how the physiology of the entire organism is synchronized to the daily rhythms of the planet.

fruitless and its power to shape sexual behaviour

Copulation in *Drosophila* is preceded by an intricate series of sexually dimorphic pre-copulatory courtship behaviours between the male and female fly[18] (Fig. 1c). Benzer's trainees Hall and Yoshiki Hotta (Box 2) used genetic mosaic analysis to define portions of the central nervous system required for male courtship behaviour[19,20] and genes that governed heterosexual behaviour[21]. One gene named *fruitless* (*fru*), identified in 1963 by K. S. Gill and cloned over 30 yr later by Daisuke Yamamoto[22]—and separately by the group effort of Hall, Bruce Baker and Barbara Taylor[23]—is now known to be a master regulator of sexuality in the fly[23,24]. The transcription factor encoded by the *fru* gene is expressed in a subset of central, peripheral, sensory and motor neurons in the adult fly, which are likely to comprise a circuit controlling sexually dimorphic behaviour[25–27]. Mutant *fru* males show homosexual courtship behaviour in which large groups form chains of males courting each other. In a remarkable experiment, Barry Dickson showed recently that male courtship behaviour directed at females can be induced in chromosomally female flies simply by expressing the male-specific isoform of *fru* in the female brain[24]. Recent work in the mouse from Catherine Dulac's group suggests a similar underlying latency in the female mouse to exhibit male behaviours on manipulation of a single gene[28]. A major goal in this field is to define the molecular targets of *fru* and define the neural circuits that drive both male and female sexual behaviours.

Olfactory communication in the fly

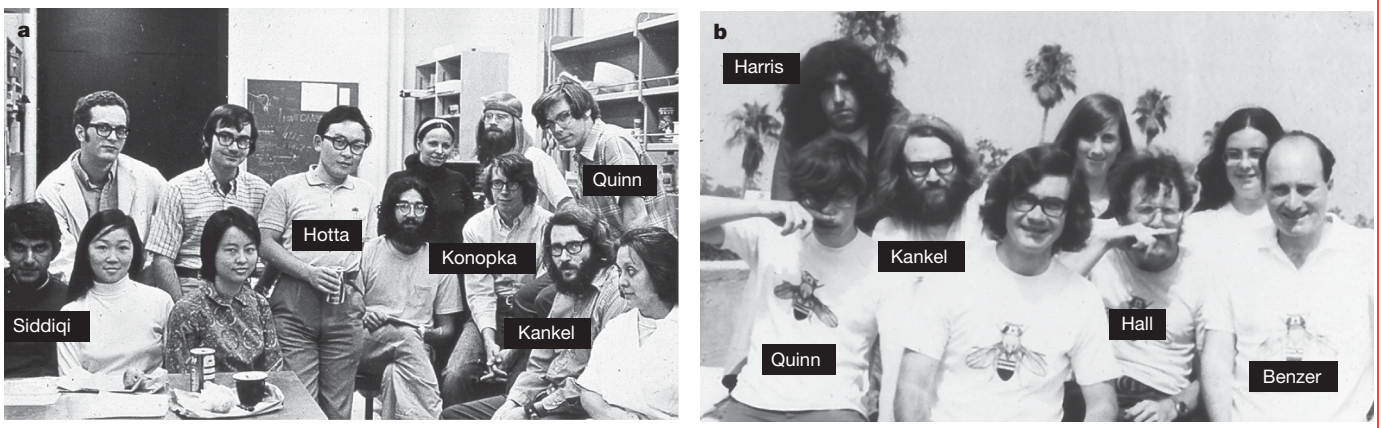
Fruitflies are strongly attracted to the smell of vinegar, yeast, rotting fruit and to each other. The genetic basis of this chemosensory behaviour was first studied by Obaid Siddiqi, a Benzer trainee (Box 2). Mutants defective in the olfactory T-maze, using the device in Fig. 1a but omitting electric shock, as well as other olfactory behaviour paradigms were collected throughout the 1980s by Siddiqi[29], and later by John Carlson[30], and others. One of the Carlson mutants, *acj6*, proved to be a key transcription factor necessary for the regulation of a subset of odorant receptor genes[31]. The availability of the genome sequence of *Drosophila melanogaster* opened this system to rapid molecular analysis by Carlson, Dean Smith, Liqun Luo, Dickson, Richard Axel and a number of former Axel trainees, resulting in the complete description of the sequence and expression of all 62 odorant receptors and 68 taste receptors (reviewed in ref. 32), the complete map of connectivity of primary olfactory centres[33,34], an initial view of how primary olfactory information is mapped in the higher brain[35,36], and a comprehensive survey of ligand tuning of a majority of the odorant receptors[37], including those tuned to pheromones[38,39]. A major effort in this growing field is to understand the underlying central mechanisms by which a fly discriminates among all the odours it is able to detect and how the circuitry underlying pheromone perception leads to stereotyped behaviours.

A myriad of other complex behaviours

Beyond these brief examples from the original neurogenetic studies to emerge from the Benzer laboratory, many other behaviours have been productively dissected with genetic and behavioural tools in *Drosophila*. The seminal work of William Quinn, William Harris and Benzer (Box 2) demonstrating that flies can be conditioned to avoid an odour paired with shock (Fig. 1a)[40], was followed by the identification of a series of mutant flies that either could not learn this task or rapidly forgot it (reviewed in ref. 41). Subsequent genetic analysis by Quinn, Ronald Davis, Tim Tully and others produced the provocative finding that many learning and memory defective mutations in the fly affect the cyclic AMP pathway (reviewed in ref. 41), the same signalling pathway implicated in conditioned behaviours in *Aplysia* and the mouse[42,43]. Subsequent genetic screens for learning mutants by Tully and others, in one case combined with microarray analysis, produced a host of other candidate memory genes, including several involved in local control of mRNA translation[44].

The cloning of the *dunce* (*dnc*) gene[45] and its enrichment in a part of the fly brain called the mushroom body[46] allowed the field to move from the genetic to the cellular level. Davis, Martin Heisenberg and others carried out a series of genetic and ablation studies strongly implicating this olfactory processing centre in the fly as the seat of memory[47,48]. Current work in the field is zeroing in on how fly-brain microcircuitry processes paired odour and shock input[49], how the circuitry is modulated by conditioning[50], and how the processes of learning and retrieval of memories are compartmentalized[51]. Neurogenetics has also enabled scientists to localize memory to smaller and smaller areas of the fly brain. A particularly elegant recent example comes from Heisenberg, Li Liu and co-workers, who localized circuits that learn certain visual features to two groups of

Box 2 | The people



Box 2 Figure | The birth of neurogenetics in the Benzer laboratory at Caltech. a, Benzer laboratory at Caltech, November 1971. Front row (left to right): O. Siddiqi; research technicians, Y.-H. Jing and J.-Y. Yu; M. Deniro; R. Konopka; D. Kankel; and laboratory manager, E. Eichenberger. Back row (left to right): T. Hanson, D. Edgington, Y. Hotta, J. Lewis, P. Christensen and W. Quinn. **b, Benzer laboratory at Caltech, around 1972.** Front row (left to right): W. Quinn, D. Edgington, J. Hall, S. Benzer. Back row (left to right): W. Harris, D. Kankel and research technicians, J. Gorn and B. Butler. Photos courtesy of S. Benzer, Caltech.

Career paths of selected Benzer laboratory members (interested readers can learn more about the history of neurogenetics and the Benzer laboratory by consulting J. Weiner's celebrated book[65]):

- Seymour Benzer: still active and scientifically prolific at the age of 86 as the James Griffin Boswell Professor of Neuroscience, Emeritus (active) at Caltech. In recent years, his group has studied longevity, brain degeneration, fear, and feeding behaviours in *Drosophila*.
- Jeffrey Hall: Professor at Brandeis University, who was inducted into the US National Academy of Sciences in 2003 for his comprehensive work on the neurogenetics of circadian, courtship and social behaviours in *Drosophila*.
- William Harris: fellow of the Royal Society and Head of the Neuroscience Department at the University of Cambridge, UK, with a group working on the molecular embryogenesis of the vertebrate visual system.
- Yoshiki Hotta: Director of the National Institute of Genetics in Japan, Hotta went on to a prominent career in *Drosophila* neural development.
- Douglas Kankel: Professor at Yale University investigating the neurogenetics of visual and nervous system development in *Drosophila*.
- Ronald Konopka: Continued to work on biological clocks at Clarkson University before retiring from science.
- William Quinn: Professor in the Department of Brain and Cognitive Sciences at Massachusetts Institute of Technology has continued his seminal work on learning and memory in *Drosophila*.
- Obaid Siddiqi: founding Director of the TIFR National Centre for Biological Sciences at Bangalore and inducted in 2003 as a foreign member of the US National Academy of Sciences, Siddiqi pioneered the field of behaviour genetics of *Drosophila* olfaction after leaving the Benzer laboratory.

neurons in a structure called the fan-shaped body[52]. Although the small size of *Drosophila* central-brain neurons has hindered electrophysiological access, recent work from Rachel Wilson and Gilles Laurent suggests that this barrier is not insurmountable[53], and a more detailed functional analysis of memory at the level of single mushroom-bodies seems likely.

Ulrike Heberlein has turned the fly into a genetic model for alcohol intoxication, demonstrating that flies exhibit progressive and eerily human-like responses to acute alcohol exposure: first, they become hyperexcitable, then they lose coordination, and finally they pass out[54]. Some of the same cAMP pathway genes required for learning and memory affect a fly's sensitivity to alcohol[55].

Both Edward Kravitz, who studies aggression in lobster, and Ralph Greenspan have recently turned their attention to fly aggression. Flies, both male and female, exhibit aggressive behaviours, with males fighting other males in the presence of a female and females jousting with females over food resources[56]. Kravitz has shown that fighting style differs between the sexes and is controlled by *fru* (ref. 57). Multigenerational selection for aggressive or docile strains has been achieved by Greenspan, and such strains have been analysed by whole-genome microarray to identify a large number of genes, the expression of which is modulated differentially in aggressive strains[58]. These genes will provide avenues for future investigation into the genetic basis of aggression.

A behavioural paradigm recently pioneered by Roland Strauss is that of gap-crossing (Fig. 2). Flies are presented with gaps of varying widths, from narrow and easy to cross to unbreachable chasms, and make sophisticated estimations of which gaps can be reasonably crossed[59].This goal-directed climbing behaviour is useful to dissect motor planning and coordination, and to identify the circuits in the fly brain that estimate distance, but could, in principle, also lead to mutants with altered appetite for risk. It is conceivable that both risk-averse flies, capable of crossing a gap but choosing not to, and reckless flies, those choosing to cross impossibly wide gaps, could be identified through genetic screens.

Unlike the classic eusocial insects such as ants and bees, flies are not typically known for their group dynamics. This view has been changing somewhat on closer behavioural investigation, which has revealed some surprising evidence of social interactions in *Drosophila*. For instance, Joel Levine and Hall have shown that circadian rhythms can be phase-shifted by the odour of flies living in another time zone or flies of another genotype[60]. Hubert Amrein showed that normal circadian locomotor activity of a male is drastically affected by the presence of a female[61]. These experiments hint at as yet unknown volatile chemical substances produced by other flies and detected by the olfactory system, and suggest that social interactions shape group interactions in the fly. In fact there is a growing trend to monitor *Drosophila* behaviour in more natural and enriched contextual environments that mimic those they might encounter in the real world. For instance, Levine has been observing fly social interactions in groups in the presence of food (Fig. 3). Free from the constraints of courting a single female in an austere Plexiglas chamber, as is the norm for observing courtship behaviour (Fig. 1c), *Drosophila* males in group situations seem to engage in complex group sex that combines foreplay, copulation and feeding behaviours (Fig. 3). It will be interesting to study the regulation and modulation of such group social behaviours and the importance of context in regulating them.

Figure 2 | Goal-oriented behaviour is measured in a gap-climbing paradigm, in which the fly estimates the width of the gap and judges if it seems feasible to cross. Photo by S. Pick, kindly provided by R. Strauss. Reprinted from ref. 59 with permission from Elsevier (copyright 2005).

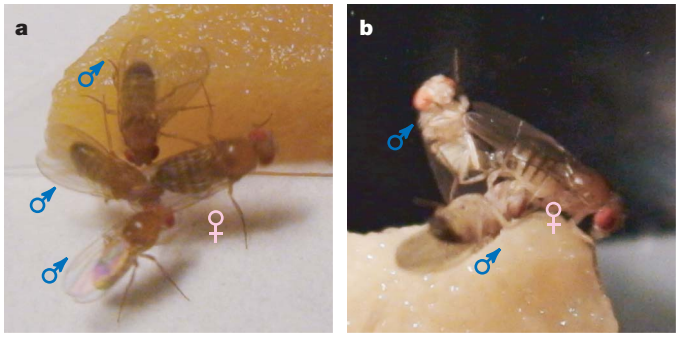


Figure 3 | The contextual courtship assay measures sexual behaviour under more naturalistic conditions in group situations on food. **a**, Three males courting a single virgin female near a wedge of food. **b**, Male with forelegs raised high copulates with a female, while another male, on his back, touches and licks her abdomen. This occurs on top of a wedge of food. Note that the female's right foreleg stretches out across the surface of the food as does the left foreleg of the male beneath her. Such sexual behaviour is affected by the presence or absence of food in the assay. Gustatory receptors on the tarsi, the part of the foreleg in contact with the sweet food, are in a good position to sample food and may play a mechanistic role in this sexual interaction. Photo by N. Stepek and J.-C. Billeter, kindly provided by J. D. Levine, Univ. of Toronto, Mississauga.

A related example of a behaviour that emerges in groups is the innate avoidance that flies show for an empty tube previously occupied by flies that experienced stress. Avoidance by naive flies of tubes previously occupied by shaken flies was first noticed by Benzer in 1967 (ref. 1), and subsequently investigated by Greg Suh, working with the Benzer laboratory and the groups of David Anderson and Axel, as an innate olfactory avoidance of a *Drosophila* stress odor, dSO (ref. 62). This robust behaviour, resulting from the recognition and avoidance of the smell of a fellow fly in trouble, will be useful in future studies of the circuitry of anxiety, stress and innate fear.

Concluding remarks

Significant advances in our understanding of the biological clock, sensory systems, learning and memory, sexual courtship and many other behaviours have been made through neurogenetic research in *Drosophila*. With these successes behind us, some adventurous *Drosophila* neurogeneticists are moving beyond these original neurogenetics questions, which may in hindsight represent the low-hanging fruit—robust behaviours amenable to investigation in laboratory-based behavioural paradigms. It now seems possible to approach in the fly more complex behaviours and even emotions, the neurobiological basis of which are not well understood at the genetic or functional level in any animal: sociality, common sense, altruism, empathy, frustration, motivation, hatred, jealousy, peer pressure, and so on. The only a priori limitation to studying any of these traits is the belief that flies can show such emotions and the design of a plausible behavioural paradigm to measure them.

This Progress article accompanies the release of complete genomes of eleven additional *Drosophila* species (*D. ananassae, D. erecta, D. grimshawi, D. mojavensis, D. pseudoobscura, D. simulans, D. virilis, D. yakuba, D. persimilis, D. sechellia, D. willistoni*), with vastly different ecologies and lifestyles to *Drosophila melanogaster*. What will be the impact of these additional *Drosophila* genomes on neurogenetics and behaviour research? Such information may begin to provide clues to differences in pheromonal communication and species recognition among these flies, some of which occupy overlapping ecological niches and need to pay careful attention to which species they are courting. A second area of interest will be in food preference and how this might be influenced by the evolution of smell and taste receptors. Are there functional differences in chemosensory reception of a fly with omnivorous taste as compared to a fly species with more specialized tastes? Hints that such phenomena are both existent and genetically tractable come from recent work in Bill Hansson's group, which found that the Seychelles island species *D. sechellia* has an olfactory system specialized to sense its preferred food, the Noni fruit[63].

The little vinegar fly *Drosophila melanogaster*, along with its sister species, promises to reveal many more surprises about how the nervous system produces complex behaviours in the next 40 yr.

1. Benzer, S. Behavioral mutants of *Drosophila* isolated by countercurrent distribution. *Proc. Natl Acad. Sci. USA* **58,** 1112–1119 (1967).
2. Benzer, S. Fine Structure of a genetic region in bacteriophage. *Proc. Natl Acad. Sci. USA* **41,** 344–354 (1955).
3. Papazian, D. M., Schwarz, T. L., Tempel, B. L., Jan, Y. N. & Jan, L. Y. Cloning of genomic and complementary DNA from *Shaker*, a putative potassium channel gene from *Drosophila*. *Science* **237,** 749–753 (1987).
4. Cosens, D. J. & Manning, A. Abnormal electroretinogram from a *Drosophila* mutant. *Nature* **224,** 285–287 (1969).
5. Montell, C. The TRP superfamily of cation channels. *Sci. STKE* **2005,** re3 (2005).
6. Pak, W. L., Grossfield, J. & Arnold, K. S. Mutants of the visual pathway of *Drosophila melanogaster*. *Nature* **227,** 518–520 (1970).
7. Smith, D. P., Stamnes, M. A. & Zuker, C. S. Signal transduction in the visual system of *Drosophila*. *Annu. Rev. Cell Biol.* **7,** 161–190 (1991).
8. Hardin, P. E. The circadian timekeeping system of *Drosophila*. *Curr. Biol.* **15,** R714–R722 (2005).
9. Sehgal, A., Price, J. & Young, M. W. Ontogeny of a biological clock in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **89,** 1423–1427 (1992).
10. Konopka, R. J. & Benzer, S. Clock mutants of *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **68,** 2112–2116 (1971).
11. Toh, K. L. *et al.* An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science* **291,** 1040–1043 (2001).
12. Bargiello, T. A., Jackson, F. R. & Young, M. W. Restoration of circadian behavioural rhythms by gene transfer in *Drosophila*. *Nature* **312,** 752–754 (1984).
13. Zehring, W. A. *et al.* P-element transformation with *period* locus DNA restores rhythmicity to mutant, arrhythmic *Drosophila melanogaster*. *Cell* **39,** 369–376 (1984).
14. Hardin, P. E., Hall, J. C. & Rosbash, M. Feedback of the *Drosophila* period gene product on circadian cycling of its messenger RNA levels. *Nature* **343,** 536–540 (1990).
15. Meyer, P., Saez, L. & Young, M. W. PER–TIM interactions in living *Drosophila* cells: an interval timer for the circadian clock. *Science* **311,** 226–229 (2006).
16. Doi, M., Hirayama, J. & Sassone-Corsi, P. Circadian regulator CLOCK is a histone acetyltransferase. *Cell* **125,** 497–508 (2006).
17. Claridge-Chang, A. *et al.* Circadian regulation of gene expression systems in the *Drosophila* head. *Neuron* **32,** 657–671 (2001).
18. Greenspan, R. J. & Ferveur, J. F. Courtship in *Drosophila*. *Annu. Rev. Genet.* **34,** 205–232 (2000).

19. Hotta, Y. & Benzer, S. Courtship in *Drosophila* mosaics: sex-specific foci for sequential action patterns. *Proc. Natl Acad. Sci. USA* **73,** 4154–4158 (1976).
20. Hall, J. C. Control of male reproductive behavior by the central nervous system of *Drosophila*: dissection of a courtship pathway by genetic mosaics. *Genetics* **92,** 437–457 (1979).
21. Hall, J. C. Courtship among males due to a male-sterile mutation in *Drosophila melanogaster*. *Behav. Genet.* **8,** 124–141 (1978).
22. Ito, H. *et al.* Sexual orientation in *Drosophila* is altered by the *satori* mutation in the sex-determination gene *fruitless* that encodes a zinc finger protein with a BTB domain. *Proc. Natl Acad. Sci. USA* **93,** 9687–9692 (1996).
23. Ryner, L. C. *et al.* Control of male sexual behavior and sexual orientation in *Drosophila* by the *fruitless* gene. *Cell* **87,** 1079–1089 (1996).
24. Demir, E. & Dickson, B. J. *fruitless* splicing specifies male courtship behavior in *Drosophila*. *Cell* **121,** 785–794 (2005).
25. Kimura, K., Ote, M., Tazawa, T. & Yamamoto, D. Fruitless specifies sexually dimorphic neural circuitry in the *Drosophila* brain. *Nature* **438,** 229–233 (2005).
26. Manoli, D. S. *et al.* Male-specific fruitless specifies the neural substrates of *Drosophila* courtship behaviour. *Nature* **436,** 395–400 (2005).
27. Stockinger, P., Kvitsiani, D., Rotkopf, S., Tirian, L. & Dickson, B. J. Neural circuitry that governs *Drosophila* male courtship behavior. *Cell* **121,** 795–807 (2005).
28. Kimchi, T., Xu, J. & Dulac, C. A functional circuit underlying male sexual behaviour in the female mouse brain. *Nature* **448,** 1009–1014 (2007).
29. Ayyub, C., Paranjape, J., Rodrigues, V. & Siddiqi, O. Genetics of olfactory behavior in *Drosophila melanogaster*. *J. Neurogenet.* **6,** 243–262 (1990).
30. Carlson, J. Olfaction in *Drosophila*: genetic and molecular analysis. *Trends Neurosci.* **14,** 520–524 (1991).
31. Clyne, P. J. *et al.* The odor specificities of a subset of olfactory receptor neurons are governed by *Acj6*, a POU-domain transcription factor. *Neuron* **22,** 339–347 (1999).
32. Vosshall, L. B. & Stocker, R. F. Molecular architecture of smell and taste in *Drosophila*. *Annu. Rev. Neurosci.* **30,** 505–533 (2007).
33. Couto, A., Alenius, M. & Dickson, B. J. Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. *Curr. Biol.* **15,** 1535–1547 (2005).
34. Fishilevich, E. & Vosshall, L. B. Genetic and functional subdivision of the *Drosophila* antennal lobe. *Curr. Biol.* **15,** 1548–1553 (2005).
35. Lin, H. H., Lai, J. S., Chin, A. L., Chen, Y. C. & Chiang, A. S. A map of olfactory representation in the *Drosophila* mushroom body. *Cell* **128,** 1205–1217 (2007).
36. Jefferis, G. S. *et al.* Comprehensive maps of *Drosophila* higher olfactory centers: spatially segregated fruit and pheromone representation. *Cell* **128,** 1187–1203 (2007).
37. Hallem, E. A. & Carlson, J. R. Coding of odors by a receptor repertoire. *Cell* **125,** 143–160 (2006).
38. van der Goes van Naters, W. & Carlson, J. R. Receptors and neurons for fly odors in *Drosophila*. *Curr. Biol.* **17,** 606–612 (2007).
39. Kurtovic, A., Widmer, A. & Dickson, B. J. A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. *Nature* **446,** 542–546 (2007).
40. Quinn, W. G., Harris, W. A. & Benzer, S. Conditioned behavior in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **71,** 708–712 (1974).
41. Davis, R. L. Olfactory memory formation in *Drosophila*: from molecular to systems neuroscience. *Annu. Rev. Neurosci.* **28,** 275–302 (2005).
42. Bourtchuladze, R. *et al.* Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell* **79,** 59–68 (1994).
43. Schacher, S., Castellucci, V. F. & Kandel, E. R. cAMP evokes long-term facilitation in *Aplysia* sensory neurons that requires new protein synthesis. *Science* **240,** 1667–1669 (1988).
44. Dubnau, J. *et al.* The *staufen/pumilio* pathway is involved in *Drosophila* long-term memory. *Curr. Biol.* **13,** 286–296 (2003).
45. Byers, D., Davis, R. L. & Kiger, J. A. Jr. Defect in cyclic AMP phosphodiesterase due to the dunce mutation of learning in *Drosophila melanogaster*. *Nature* **289,** 79–81 (1981).
46. Nighorn, A., Healy, M. J. & Davis, R. L. The cyclic AMP phosphodiesterase encoded by the *Drosophila dunce* gene is concentrated in the mushroom body neuropil. *Neuron* **6,** 455–467 (1991).
47. Heisenberg, M., Borst, A., Wagner, S. & Byers, D. *Drosophila* mushroom body mutants are deficient in olfactory learning. *J. Neurogenet.* **2,** 1–30 (1985).
48. Han, P. L., Levin, L. R., Reed, R. R. & Davis, R. L. Preferential expression of the *Drosophila rutabaga* gene in mushroom bodies, neural centers for learning in insects. *Neuron* **9,** 619–627 (1992).
49. Yu, D., Keene, A. C., Srivatsan, A., Waddell, S. & Davis, R. L. *Drosophila* DPM neurons form a delayed and branch-specific memory trace after olfactory classical conditioning. *Cell* **123,** 945–957 (2005).
50. Yu, D., Ponomarev, A. & Davis, R. L. Altered representation of the spatial code for odors after olfactory classical conditioning; memory trace formation by synaptic recruitment. *Neuron* **42,** 437–449 (2004).
51. Krashes, M. J., Keene, A. C., Leung, B., Armstrong, J. D. & Waddell, S. Sequential use of mushroom body neuron subsets during *Drosophila* odor memory processing. *Neuron* **53,** 103–115 (2007).
52. Liu, G. *et al.* Distinct memory traces for two visual features in the *Drosophila* brain. *Nature* **439,** 551–556 (2006).
53. Wilson, R. I., Turner, G. C. & Laurent, G. Transformation of olfactory representations in the *Drosophila* antennal lobe. *Science* **303,** 366–370 (2004).
54. Wolf, F. W., Rodan, A. R., Tsai, L. T. & Heberlein, U. High-resolution analysis of ethanol-induced locomotor stimulation in *Drosophila*. *J. Neurosci.* **22,** 11035–11044 (2002).
55. Moore, M. S. *et al.* Ethanol intoxication in *Drosophila*: Genetic and pharmacological evidence for regulation by the cAMP signaling pathway. *Cell* **93,** 997–1007 (1998).
56. Chen, S., Lee, A. Y., Bowens, N. M., Huber, R. & Kravitz, E. A. Fighting fruit flies: a model system for the study of aggression. *Proc. Natl Acad. Sci. USA* **99,** 5664–5668 (2002).
57. Vrontou, E., Nilsen, S. P., Demir, E., Kravitz, E. A. & Dickson, B. J. *fruitless* regulates aggression and dominance in *Drosophila*. *Nature Neurosci.* **9,** 1469–1471 (2006).
58. Dierick, H. A. & Greenspan, R. J. Molecular analysis of flies selected for aggressive behavior. *Nature Genet.* **38,** 1023–1031 (2006).
59. Pick, S. & Strauss, R. Goal-driven behavioral adaptations in gap-climbing *Drosophila*. *Curr. Biol.* **15,** 1473–1478 (2005).
60. Levine, J. D., Funes, P., Dowse, H. B. & Hall, J. C. Resetting the circadian clock by social experience in *Drosophila melanogaster*. *Science* **298,** 2010–2012 (2002).
61. Fujii, S., Krishnan, P., Hardin, P. & Amrein, H. Nocturnal male sex drive in *Drosophila*. *Curr. Biol.* **17,** 244–251 (2007).
62. Suh, G. S. *et al.* A single population of olfactory sensory neurons mediates an innate avoidance behaviour in *Drosophila*. *Nature* **431,** 854–859 (2004).
63. Dekker, T., Ibba, I., Siju, K. P., Stensmyr, M. C. & Hansson, B. S. Olfactory shifts parallel superspecialism for toxic fruit in *Drosophila melanogaster* sibling, *D. sechellia*. *Curr. Biol.* **16,** 101–109 (2006).
64. Tully, T. & Quinn, W. G. Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. *J. Comp. Physiol.* **157,** 263–277 (1985).
65. Gerber, B., Tanimoto, H. & Heisenberg, M. Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. *Curr. Opin. Neurobiol.* **14,** 737–744 (2004).
66. Weiner, J. Time, Love, Memory: A Great Biologist and His Quest for the Origins of Behavior (Alfred A. Knopf, Inc., New York, 1999).

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