RESEARCH

Analysis of Conditioned Courtship in *dusky* – *Andante* Rhythm Mutants of *Drosophila*

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Abstract

Genetic connections between learning and rhythmicity were suggested to have been established in a previous study, in part because the *dusky* ^{*Andante*} (*dy^{And}*) mutation in *Drosophila* disrupted both behaviors. *dy^{And}* , isolated as a slow-clock variant, was reported to cause an approximately fourfold decrement in courtship-suppression conditioning. These effects have been reexamined; the experiments were buttressed by testing the effects of several recently isolated mutations at the *dusky* locus, along with the original *And* allele that had been induced there. The reexamination was also prompted by anatomical concerns, certain of which have recently focused on *dy* -induced decrements in cell size, but only in terms of wing morphology. Another anatomical issue involves the discovery of a neuronal pathway that seems to connect circadian pacemaker cells to a structure in the *Drosophila* brain that is involved in learning. In observer-blind experiments, however, it was found that neither pacemaker-slowness (*Andante* -like) *dy* mutations nor others that cause no rhythm defects produced subnormal conditioned courtship. Moreover, in the adult brain of a slow-clock *dy^{And}* mutant, no axonal pathway defects were readily discernible and putative pacemaker neurons appeared to be normal in size.

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Introduction

Courtship-suppression conditioning in *Drosophila* has been used to test a variety of genetic variations beyond those involved in reproductive behavior alone. For example, the classic learning and memory mutants yielded diminished aftereffects of a male's previous experiences with reproductively unreceptive flies (for reviews, see Siegel et al. 1984; Hall 1994a; Greenspan 1995). Consequently, this behavioral assay has proved useful for asking whether other kinds of mutants, strains, and molecularly manipulated transgenics would exhibit learning defects (Heisenberg 1980; Collins and Hewitt 1981; Cowan and Siegel 1984; Tempel et al. 1984; Zawistowski and Richmond 1985, 1988; Zawistowski 1988; Gailey and Siegel 1989; Griffith et al. 1993, 1994; Bouhouche and Choulli 1994; O'Dell 1994).

Perhaps the most surprising effects on courtship suppression were those reported for rhythm mutations, such as *period* , *Andante* (*And*), and "phase angle" (*psi*). These X-chromosomal and autosomal mutants, isolated on the basis of rhythm abnormalities (and all exhibiting longer-than-normal periodicities), were found to be defective in courtship suppression (Jackson et al. 1983). Such decrements were at least as great as those caused by *dunce* , *rutabaga* , or *amnesiac* mutations (cf. Gailey et al. 1982, 1984). It was as if a clock that runs slowly would not allow information to be stored or retrieved in an optimal manner. More generally, the possibility that rhythm-related mechanisms might subserve animal learning has long been entertained (e.g., Jenkins and Dallenbach 1924; Smith 1985; Stone et al. 1989).

The most salient rhythm variants in *Drosophila* are the *period* (*per*) mutants, which include strains whose circadian-clock periods are several hours longer than normal (for review, see

Konopka 1987). Males expressing *per-long* (*per^L*) were among those found to exhibit mediocre conditioned courtship suppression (Jackson et al. 1983). Yet, such learning defects for the *per^L* mutants were found not to be extendible in two ways: First, *per* transgenics with ~27-hr periods (vs. the normal value of 24 hr) exhibited normal courtship conditioning. Second, neither *per^L* nor other kinds of *per* mutants (short-period or arrhythmic) showed abnormal learning or memory scores in a Pavlovian olfactory learning task (Gailey et al. 1991). The only *per*-related finding from the earlier study (Jackson et al. 1983) that was reproducible involved a *per^{L1}*-mediated decrement in male learning, as is usually effected by his courtship of fertilized females (Gailey et al. 1991). Extended conditioning periods, however, eliminated any difference between *per^{L1}* and wild-type controls. Learning in the *per^{L1}* mutant also was normal when tested with immature males; furthermore, the *per^{L2}* mutant's learning in both the fertilized-female and immature-male assays was normal (Gailey et al. 1991). The one instance of a *per^L*-associated learning abnormality appeared to result from a genetic-background effect, primarily because the intragenic site change (cf. Baylies et al. 1987; Huang et al. 1993) in the *per^{L1}* mutant was found to be identical to that of *per^{L2}* (Gailey et al. 1991).

In the earlier experiments involving rhythm defects and learning, the most dramatically defective mutant was *And*, notwithstanding the fact that this mutant's circadian clock runs only 1–2 hr more slowly than normal (Konopka et al. 1991; Newby et al. 1991). A set of novel *And*-like mutants was induced by mutagenesis, and the isolation of these variants was accompanied by analysis of their pleiotropic effects (Newby et al. 1991). In this regard, it was definitively established that *And*-like mutants occur at the *dusky* (*dy*) locus (Newby et al. 1991; cf. Konopka et al. 1991). Yet, only some of the new mutants, with their *dy*-wing defects, had longer-than-normal rhythm periods. It was suggested, nevertheless, that the “small wing-cell” phenotype (which is part of *dy*'s pleiotropy) might extend to brain cells underlying the fly's rhythms. Presumably, a smaller-than-normal volume for such “pacemaker neurons” would cause the clock to run at a relatively slow pace (Newby et al. 1991).

The existence of the new *dy* mutants—both the normal and slow-rhythm types—is one reason that prompted an attempt to replicate and extend the original *dy^{And}* learning defects. Moreover, sub-

sequent to the earliest learning experiments on *And* itself, *Drosophila*'s circadian pacemaker cells have been identified as a result of genetic-mosaic and *per*-transgenic experiments (Ewer et al. 1992; Frisch et al. 1994). In addition, axonal projections emanating from a subset of the *per*-expressing neurons send processes into the CNS proper, where they terminate near the calyces of the mushroom bodies (Helfrich-Förster and Homberg 1993; Helfrich-Förster 1995). Those structures are involved in the control of (olfactory) associative learning (for review, see Davis 1993; also see de Belle and Heisenberg 1994). These recent anatomical observations suggest anew that a “clock mutant could be a learning mutant”. *dy^{And}* flies and their allelic relatives might have brain-structural abnormalities after all (in addition to exhibiting their externally visible wing defects). Thus, according to an inference reasonably drawn from the results of Helfrich-Förster and Homberg (1993), *dy^{And}*-related brain anomalies could at once produce a defective biological clock and abnormal learning machinery. Extensive behavioral and neuroanatomical analyses of the original *And* mutation and the newly isolated *dy* mutants have, however, shown this hypothesis to be invalid.

Materials and Methods

DROSOPHILA STRAINS AND CULTURE CONDITIONS

Flies were raised on cornmeal, sugar, and yeast medium. Most *dy*-mutant strains were obtained from F.R. Jackson (Worcester Foundation for Experimental Biology, Shrewsbury, MA). They had been induced by either chemical or gamma-ray mutagenesis (Newby et al. 1991). The ionizing-radiation treatments, followed by screening for novel *dy*-wing variants, yielded four new mutant alleles: *dyⁿ¹*, *dyⁿ²*, *dyⁿ³*, and *dyⁿ⁴*. Similarly, the ethylmethane sulfonate treatments led to the *dy^{r2}*, *dy^{r3}*, *dy^{r4}*, and *dy^{r5}* alleles. One *dy* mutant reported by Newby et al. (1991), *dyⁿ²*, was not investigated in the present behavioral experiments owing to its near-normal appearance (see description of “blind-testing” procedure, below). The original *dy*-related rhythm variant, *dy^{And}*, had been maintained here for many years (having been obtained from R.J. Konopka, a long time before it was reported; Konopka et al. 1991). It, along with the first eight *dy*'s listed above, is identifiable by wings that are smaller and darker than in wild-type

adults. This darkening is not a pigmentation problem but is caused by the fact that individual wing cells of *dy* flies are smaller than normal (see Newby et al. 1991 and references therein).

The eight recently isolated *dy* mutants were maintained (in true-breeding stocks) whose females are attached-X, *y f* females. *dy* males from each such line were outcrossed to Canton-S wild type for two “recombination cycles”. The resulting recombinant lines, extracted by simply selecting for *dy*-wing males and (homozygous) females, were the ones used in all behavioral tests (see below).

For certain of the histochemical experiments (see below), the *dy^{And}* mutation was introduced into stocks that also carried a fusion gene involving the 5' half of *Drosophila's period* locus, ligated in-frame to *lacZ* from *Escherichia coli* (Liu et al. 1988, 1991); this transgene is located on chromosome 2 and is homozygous viable.

BEHAVIORAL PROCEDURES

CIRCADIAN RHYTHMS

Adult males were collected soon after eclosion and entrained for 3 days to a 12:12 light–dark (LD) cycle in a constant-condition room (25°C, 75% humidity). Flies were then individually placed in a circadian rhythm monitoring device (see Hamblen et al. 1986, for details), in constant darkness (DD); each fly's activity was recorded automatically for 2 weeks. Statistical analysis of the locomotor activity events was done on a VAX/VMS computer system (cf. Hamblen-Coyle et al. 1992; Wheeler et al. 1993).

COURTSHIP

The courtship-suppression paradigm, involving male behavior as affected by interactions with fertilized females, was used to determine whether there are different learning abilities among the *dy*-lines. In all cases, a given strain was coded as to the *dy* allele it carried, to militate against observer bias. The “blind controls” could not be wild-type (hence normal-winged) animals and were, instead, deemed to be *dy* mutants that do not have an altered circadian-clock pace (see Introduction and below; Table 1), with the “experimentals” being

Table 1: Circadian rhythms of dusky mutants

Genotype	Circadian τ (h $\pm$ S.E.M.)
<i>dy^{And}</i>	26.2 $\pm$ 0.3
<i>dyⁿ¹</i>	26.0 $\pm$ 0.4
<i>dyⁿ³</i>	25.5 $\pm$ 0.3
<i>dyⁿ⁴</i>	26.0 $\pm$ 0.4
<i>dy^{r2}</i>	24.3 $\pm$ 0.3
<i>dy^{r3}</i>	24.4 $\pm$ 0.2
<i>dy^{r4}</i>	24.2 $\pm$ 0.3
<i>dy^{r5}</i>	24.4 $\pm$ 0.4
Wild type	24.1 $\pm$ 0.3

Free-running circadian periodicities, resulting from monitorings of adult locomotor activity, were determined as described in Materials and Methods, which include reference to the χ^2 periodogram analyses used to provide best estimates of these period (τ) values. Eight to 10 individuals (all of whom behaved in a significantly periodic manner) were tested for each of these nine genotypes; the final one listed refers to males from a Canton-S strain.

dy mutants that exhibit longer-than-normal rhythm periods as well as aberrant wing morphology.

During certain (rather wide) windows of time in 12:12 LD schedules, 5-day-old test males were paired and “conditioned” with 5-day-old fertilized Canton-S (wild-type) females. Almost all such tests were performed within 5 hr after lights-on and within 2 hr before lights-off; these windows are near the times of general locomotion-maxima for flies of this species (e.g., Hamblen-Coyle et al. 1992; Wheeler et al. 1993). Jackson et al. (1983), however, has indicated that courtship-suppression scores seemed not to be affected by the times—throughout the daylight hours—when their tests were performed.

Male–female pairings (in the current experiments) were effected for 30-min training (or control) periods, in cleaned porcelain observation wells covered by glass slides (cf. Gailey et al. 1986, 1991). The fertilized females used in these tests had each been observed to mate with one Canton-S male, 18–24 hr previously. In the control paradigm, 5-day-old males were placed alone in the observation wells for 30 min (cf. Jackson et al. 1983, whose controls involved pairing a previously naive male with an immature virgin female for 30 min).

For testing, conditioned or control males were gently aspirated into new individual obser-

vation wells (immediately after the 30 min of pairing or solitary confinement). There—within 1–2 min—they were presented with a 5-day-old etherized, Canton-S virgin female. A courtship index (CI) was then measured for each male by accumulating (using electronic timers) the proportion of the observation periods during which the test male directed any courtship actions toward the ether-immobilized female (see reviews by Tompkins 1984, 1989; Hall 1994a, for further details about CI determinations). An observation period was terminated as soon as the etherized female regained mobility, which was usually in the range of 6–15 min.

CI values were processed by angular (arcsine) transformations (Sokal and Rohlf 1981, p. 427), to correct for the abnormal distribution of residuals in such raw data. This unevenness may be caused by “floor” and “ceiling” effects, whereby data points at the high and low extremes exert disproportionate leverage on the regression. This minidiscussion is provided, against a background of floor and ceiling effects (mainly the former) not having been corrected for in previous courtship-

conditioning experiments—notably those involving rhythm mutants (Jackson et al. 1983).

CI values were further processed by two different methods: (1) One way to express the extent to which males of a given genotype learned is simply to consider the difference between the CI_{cont} and CI_{exp} values (though this metric is subject to the aforementioned floor effects, i.e., when CI_{cont} values are relatively low); the standard errors of the mean associated with such differences (Table 2) were estimated as in Sokal and Rohlf (1981, p. 226). (2) The courtship learning index (λ_C) was computed for each individual mutant:

$$\lambda_C = [1 - (CI_{exp}/CI_{cont})]100$$

as originally devised by Gailey et al. (1982, 1984). Because the estimate of the CI_{exp}/CI_{cont} ratio is biased (cf. Grossman and Norton 1980), it was corrected by $(CI_{exp}/CI_{cont}) \times [1 + \text{Var } CI_{cont}/(CI_{cont})^2]$. After subtracting the corrected ratio from 1.0, the resulting λ_C has a value of 0 in a case for which the behaviors of experimental and control

Table 2: Courtship suppression assays of long-period (L) and circadian-normal (N) dusky mutants

Mutants	Raw		Transformed		$CI_{cont}-CI_{exp}$	λ_C
	CI_{cont}	CI_{exp}	CI_{cont}	CI_{exp}		
dy^{And} (L)	43 ± 8	12 ± 5	37 ± 6	14 ± 4	23 ± 7	61 ± 12
dy^{n1} (L)	47 ± 8	15 ± 4	41 ± 6	18 ± 3	23 ± 7	55 ± 10
dy^{n3} (L)	22 ± 7	5 ± 3	20 ± 5*	6 ± 3*	14 ± 6	68 ± 17
dy^{n4} (L)	24 ± 6	5 ± 4	23 ± 5*	7 ± 3*	16 ± 6	68 ± 15
dy^{r2} (N)	48 ± 9	20 ± 7	41 ± 7	19 ± 6	22 ± 9	52 ± 17
dy^{r3} (N)	50 ± 8	26 ± 7	43 ± 6	24 ± 6	19 ± 8	43 ± 16
dy^{r4} (N)	48 ± 8	26 ± 6	40 ± 6	23 ± 5	17 ± 8	41 ± 15
dy^{r5} (N)	50 ± 9	23 ± 7	42 ± 7	22 ± 6	20 ± 9	46 ± 17
Wild type (N)	67 ± 6	29 ± 8	56 ± 4	28 ± 6	28 ± 7	50 ± 11

Courtship indices ($CI \pm \text{s.e.m.}$) for control (cont) and experimental (exp) tests of eight independently isolated *dy* mutants and wild-type (Canton-S) flies were determined as described in Materials and Methods; $n = 20$ for each of the values in the first four data columns. (L) A long-period mutant; (N) a strain that exhibits normal circadian τ 's (cf. Table 1). Raw CI values ($\pm \text{s.e.m.}$) are given in the first two data columns; arcsine-transformed versions of such values (see Materials and Methods) are in the third and fourth columns. Using transformed CI's, control vs. experimental values (whose differences appear in the fifth column) were compared statistically; all were significantly different: P values for the nine intragenotype comparisons ranged from 0.02 to 4×10^{-4} . Courtship learning indices (λ_C) were calculated (using the transformed CI_{cont} and CI_{exp} values), as described in the text (also see Gailey et al. 1982, 1984). Pairwise, intergenotype comparisons of these λ_C 's indicated no significant differences (P values were 0.21 or higher). dy^{n3} and dy^{n4} exhibited significantly low courtship vigor in general [by ANOVA; see asterisks (*); cf. Materials and Methods] and lower-than-normal locomotor-activity levels (see Table 1); this made the $CI_{cont}-CI_{exp}$ values relatively low for these two *dy* mutants. Yet, intergenotype pairwise comparisons among all the CI-difference values indicated none was significant: P values were 0.07 or higher, and—owing to the large number of pairwise comparisons made— α had to be corrected to a value much lower than the benchmark one of 0.05, i.e., by the function $1 - (.95)^{1/k}$ (where k is the number of such comparisons; Sokal and Rohlf 1981, p. 241).

males of a given genotype, tested in parallel, are alike within a line; thus, “no learning” is inferred to have occurred; “perfect learning” (100) is computed in a case where courtship-suppression, as a result of training in the presence of the fertilized female, is absolute ($CI_{\text{exp}} = 0$), with appreciable CI_{cont} values being obtained (as is always the case). The standard errors associated with the learning indices could not be computed directly (owing to the subtraction of one group mean from another: see above) and so were estimated by the function: $(\text{Mean } CI_{\text{exp}})^2 / (\text{Mean } CI_{\text{cont}})^2 \times [\text{Var } CI_{\text{exp}} / (\text{Mean } CI_{\text{exp}})^2 + \text{Var } CI_{\text{cont}} / (\text{Mean } CI_{\text{cont}})^2]$ (cf. Tully and Hirsch 1982).

CI values (of the transformed variety) were compared among genotypes, using a two-way analysis of variance; the “main effects” considered in these ANOVAs were genotype and paradigm (control or experimental); a significant F-ratio determination was followed by “planned” comparisons between genotypes. Owing to the fact that there is no series of individual λ_C or $CI_{\text{cont}} - CI_{\text{exp}}$ values for a given genotype, pairwise comparisons between learning indices and the control versus experimental CI differences were performed by *t*-tests (Sokal and Rohlf 1981). Intragenotype differences between CI_{cont} s and CI_{exp} s were also compared by *t*-tests.

HISTOCHEMISTRY

Stainings of *per* gene-expressing cells in the CNS and PNS of adult heads was performed by cryostat sectioning (10 μm , horizontal plane), followed by (1) application—to sections of flies from the *dy^{And}*-carrying, *per-lacZ* transgenic strain (see above)—of an α - β GAL antibody (see Liu et al. 1991 for procedural details) [specifically, we found that an α - β GAL from mouse (Promega) gave sharper staining of “*per* neurons” than did other commercially available antibodies made against this bacterial enzyme (e.g., as made in rabbits, purchased from Cappel)] and (2) use of an anti-ARRESTIN-2 antibody (α -ARR2) (cf. Dolph et al. 1993), which happens to mark all photoreceptor cells and CNS neurons that express *per* (B. Frisch, J. Wagner, B. van Swinderen, M. Kaneko, and J.C. Hall, in prep.). This antibody was a gift of P. Dolph and C.S. Zuker (University of California, San Diego, La Jolla). The α -ARR2 reagent had been made in rabbit against a synthetic peptide (P. Dolph and C.S. Zuker, pers. comm.). We applied this antibody

to frozen sections and stained them as in Siwicki et al. (1988). Some of the sections were prepared from fly heads that had been fixed overnight in paraformaldehyde; this yielded better labeling of neuronal projections (see below). Dolph et al. (1993) and B. Frisch, J. Wagner, B. van Swinderen, M. Kaneko, and J.C. Hall (in prep.) have shown that the *arr2* gene’s normal, bona fide product is expressed in photoreceptors of the compound eye and ocelli and in a putative extraocular photoreceptive structure (cf. Hofbauer and Buchner 1989). α -ARR2 also leads to staining of all or nearly all *per* neurons and many of the projections from them (B. Frisch, J. Wagner, B. van Swinderen, M. Kaneko, and J.C. Hall, in prep.; see Table 3 footnote). That the perikarya and neurites are *per*-expressing neuronal entities in the adult brain is inferred not only from the similar cellular positions and qualities that are observed when applying α -PER or α -ARR2 in separate studies, but also as a result of performing double-label experiments (B. Frisch, J. Wagner, B. van Swinderen, M. Kaneko, and J.C. Hall, in prep.). However, the latter antigen seems to be cross-reacting material owing to the continued presence of CNS signals in an *arr2*-null mutant (B. Frisch, J. Wagner, B. van Swinderen, M. Kaneko, and J.C. Hall, in prep.), which had been isolated by Dolph et al. (1993).

Results

CONDITIONED BEHAVIOR OF *dy* MUTANT MALES

Certain *dy* mutations were reported to produce relatively long-period rhythms (>25 hr), whereas others were ostensibly normal (Konopka et al. 1991; Newby et al. 1991). Retesting outcrossed versions of these particular mutants yielded circadian-rhythm periodicities (exemplified in Fig. 1) similar to those reported previously (cf. Newby et al. 1991). Thus, four lines (*dy^{And}*, *dyⁿ¹*, *dyⁿ³*, *dyⁿ⁴*) continue to display longer-than-normal periods, and four others (*dy^{r2}*, *dy^{r3}*, *dy^{r4}*, *dy^{r5}*) still give normally rhythmic behavior (Table 1). Within the slow-clock category, however, certain variations were noted: *dyⁿ³* and *dyⁿ⁴* seemed less active than *dyⁿ¹* or *dy^{And}* (exemplified in Fig. 1), although the ~25- to 26-hr rhythmicities computed for all four of these types were robust (in terms of analytical determination of the relevant “ τ ” values; Table 1).

dy^{And}, née *And*, is the one long-period *dy* mu-

Table 3: α -ARR2 immunohistochemistry of dy^{And} mutant adult brains

	Present?	Diameter (μ m)	Number	Vertical dispersal (μ m)
Dorsal posterior cells	Yes*	4–8	1–2	20
Dorsal–lateral cells	Yes*	4–6	1–2	10
Ventral–lateral cells	Yes	10–12	4–6	~35
Central posterior cells	Yes	2–8	4–6	30
H–B (photoreceptor?) cells	Yes	—	—	—
Ventrolateral $\rightarrow$ central connections	Yes	—	—	—
Dorsal loop	Yes	—	—	—

Five dy^{And} males were stained with α -ARR2 using the same protocol as applied to wild-type controls (see below). The stained structures listed in the first three rows of the left-most column correspond to the *per*-expressing dorsal neurons (DNs) and lateral neurons (LNs) of Siwicki et al. (1988), Zerr et al. (1990); and Fisch et al. (1994); the Hofbauer–Buchner cells are putative extraocular photoreceptors (Hofbauer and Buchner 1989), which express *per*, as do all other external photoreceptors (Siwicki et al. 1988; Ewer et al. 1992). The various cell types were observed in all five mutant individuals prepared for these histochemical observations, except for the two types denoted by asterisks (*); these were not seen in one fly (further information on these seven anatomical features is given in Materials and Methods). The ranges implied by dashes are the extreme interanimal values for these anatomical measurements. Values in the right-most column represent the roughly averaged extents—in the dorsal-to-ventral direction—over which these four groups of cells were distributed. The ventral cluster of lateral neurons sends an apparent projection to the region of cells stained in a relatively “central posterior” region: from the region of the latter, a “loop” of stained axon bundles projects in a dorsal direction, terminating in a neuropil region near that of the mushroom bodies. The several elements of these cell-body and neurite stainings were first established in sections of nine wild-type heads (methodological and informational details to be reported elsewhere; B. Frisch, J. Wagner, B. van Swinderen, M. Kaneko, and J. C. Hall, in prep.). As implied by the tabulated entries and the foregoing statements in this legend, the staining patterns and associated numerical features (see the three data columns above) were essentially identical between these normal preparations and those of the dy^{And} mutant heads.

tant for which learning has been assayed (Jackson et al. 1983). The courtship learning index in a previous test of wild-type males (circadian τ for that strain, 23.8 hr)—involving mated females and untransformed CI values—was 91, with the parallel test of one of the above mutants, dy^{And} [τ (then) = 25.5 hr], yielding a λ_C of 23 (Jackson et al. 1983). Note that the current control λ_C value (50) is considerably lower than that of Jackson et al. (1983). This is not uncommon in courtship suppression tests (see Hall 1994a for the pertinent literature), performed with different strains (irrespective of mutants) and at widely separate times. The key comparisons would involve courtship suppression scores determined contemporaneously from testing flies with similar genetic backgrounds (see Materials and Methods).

Using this kind of genetic design and in light of the problematical nature of *per^L*-associated learning phenotypes (Gailey et al. 1991), we reexamined and extended the original experiments on *dy*'s learning abilities. The extension included an internal control: Courtship-suppression phenotypes of the long-period and clock-normal *dy* mutants were compared in an experimenter-blind

manner (possible, because all of these lines have the *dy*-wing phenotype).

Table 2 summarizes results from the current courtship experiments. First, all differences in arcsine-transformed CI values from control (CI_{cont}) versus experimental (CI_{exp} , i.e., postmated female) groups within a genotype were significant (see Table 2 footnote). Second, transformed CI_{exp} values for most of these *dy* mutants were not significantly different from the wild-type value nor from one another. The exceptions involved dy^{n3} and dy^{n4} flies, for which both CI_{exp} s and CI_{cont} s were significantly lower than the corresponding values for the other mutant or wild-type strains (Table 2, see asterisks)—notwithstanding the fact that these and all of these *dy* alleles had been outcrossed to wild type and had true-breeding mutant animals reextracted before commencing the behavioral experiments (see Materials and Methods). The relatively nonvigorous courtship performances of the dy^{n3} and dy^{n4} mutants produced a floor effect when calculating the absolute differences between the CI_{cont} and CI_{exp} values (Table 2) for these two *dy* mutants. This difference is another way to reflect the extent to which a given

Figure 1: Actograms displaying normal and mutant circadian rhythms of locomotor activity. Each plot shows the activity of one male fly, recorded in DD over a period of ~2 weeks. “TIME” axes represent two consecutive 24-hr blocks, such that the horizontal “darkness” bars at the top of each “double plot” represent 48 hr. Double plotting means that days 1–2 of DD behavior are displayed on the top line of a given actogram, days 2–3 on the next line, etc. The rough peaks of activity-event values occurred vertically with respect to one another, in the wild-type record and those of the four *dy* mutants at left, which in general have normally paced clocks (Table 1). In the four right-hand plots, the major peaks occur appreciably later on consecutive days, typifying the >25-hr rhythm periods that are caused by these four *dy* alleles (Table 1).

male learned from his experience with a fertilized female (see Materials and Methods and below). Nevertheless, none of the mutant $CI_{cont}-CI_{exp}$ difference values were different from one another (see Table 2 footnote). The latter $CI_{cont}-CI_{exp}$ value for wild type was 28—nominally (yet not significantly) higher than the (interstrain) averages for the long-period and rhythm-normal *dy* mutants; these were very similar to each other, 19 ($n = 4$ strains) and 20 ($n = 4$), respectively (Table 2).

The courtship-learning index (λ_C) was developed in part to reflect relative, as opposed to absolute, differences between CI_{cont} and CI_{exp} values. Here again, mutant λ_C s did not differ significantly from each other or from those of the wild type (see Table 2 footnote). Moreover, ranking the nominally highest to lowest λ_C (e.g., dy^{n3} , dy^{n4} versus dy^{r3} , dy^{r4}) revealed no correlation with

these mutants’ period values (cf. Table 1). In fact, the interstrain λ_C averages for the normally periodic mutants (46) was nominally worse than the overall index (63) for the slow-clock lines. In this regard, the $CI_{cont}-CI_{exp}$ difference values (and the statistic newly applied for comparing them, Table 2) may better reflect the rather uniform courtship-learning abilities exhibited by these two categories of mutants.

BRAIN ANATOMY OF A LONG-PERIOD *dy* MUTANT

The *dy* mutants have wing-cell sizes that are markedly smaller than normal; this led Newby et al. (1991) to hypothesize that brain cells involved in clock control might be similarly anomalous in morphology, for the subset of the *dy* mutants whose circadian pacemakers run too slowly. We

therefore examined elements of CNS anatomy in the canonical *dy^{And}* mutant. First, a general tissue stain revealed no gross abnormalities in the morphology of ganglia in the adult head, neither in the central brain nor the four pairs of optic lobes flanking it (Fig. 2). Using these same sections, the overall volumes of wild-type and mutant brains were estimated and found to be indistinguishable (see legend to Fig. 2). Second, we scrutinized certain anatomical details associated with particular clusters of brain neurons in the brain, which comprise significant components of the fly's circadian pacemaker system (Siwicki et al. 1988; Zerr et al. 1990; Ewer et al. 1992; Frisch et al. 1994). The ~25 pairs of such neurons, distributed among 3 pairs of bilaterally symmetrical clusters, are conveniently marked by the reporter factor contained in a *per-lacZ* fusion gene; β GAL activity or immunoreactivity stains mostly the cell bodies of these putative pacemaker neurons (e.g., Liu et al. 1988, 1991; Ewer et al. 1992; Helfrich-Förster 1995).

The *per*-expressing pacemakers also are stained fortuitously by application of an anti-ARRESTIN antibody (see Materials and Methods), which marks the cell bodies and also neuronal processes emanating from these “*per*-neuronal” perikarya.

The approximate numbers and intrabrain positions of *per* neurons in *dy^{And}* individuals were scored; these assessments were augmented by measurements of the perikaryal diameters. Typically, these relatively large cell bodies—most notably in lateral-brain regions near the anterior rim of the medulla optic lobe—are ~6–10 μ m in diameter (e.g., Ewer et al. 1992 and size estimates done in the current experiments on sections through *dy⁺* heads). From scrutinizing serial sections prepared from five *dy^{And}; per-lacZ* adults, no abnormalities in numbers, positions, or lateral-neuronal diameters could be discerned (Fig. 3; see its legend for information on *per-lacZ*-mediated signals in controls). The cell-body measurements were made on 15 such neurons, with two to four

Figure 2: General brain morphology of the *dy^{And}* mutant. (A) A horizontal section from a *dy^{And}* male's adult head was stained with Toluidine blue. Notwithstanding the low resolution nature of this image, the overall morphology of the brain and the various ganglia and lobes within it look normal; the same was so for six additional *dy^{And}* males sectioned and stained in this manner or with Methylene blue. (B) A drawing of a horizontal brain section from a normal adult (representing eight such controls sectioned and stained in this manner), which indicates the major substructures within the central brain and visual system. The distance between the most distal regions of the paired lamina optic lobes is ~450 μ m. Actual measurements of *dy^{And}* and *dy⁺* CNS widths were made—at this rather central position (re a series of horizontal sections), and at every fifth section, proceeding more dorsally and more ventrally from it. The average width for the entire CNS/optic lobe complex (measured from just inside the retinal layers on either side of the brain) was 406 ± 46 (S.E.M.) μ m for wild type ($n=8$) and 399 ± 37 μ m for *dy^{And}* ($n=7$); these values are means of means, i.e., based on measurements of seven horizontal sections per specimen (the intrafly variability, with respect to standard deviations; for such measurements were ~20% of the per-fly mean values, reflecting much smaller widths in the dorsal-most sections). Average widths for the CNS proper were 169 ± 17 μ m for wild type and 160 ± 13 μ m for *dy^{And}* (intrafly variability among planes of section was ~30% of the means, owing to the fact that the central brain, ignoring the flanking optic lobes, is relatively spherical, giving low values in both dorsal and ventral regions).

Figure 3: Overall and rhythm-related anatomy of brains. (A) A horizontal section through the head of a *per-lacZ* fusion transgenic male, compared with one from a *dy^{And}; per-lacZ* male; both were stained with anti- β -galactosidase at approximately the same level as the Toluidine-blue stain in Fig. 2. (Arrows) Lateral neurons (cf. Table 3), which are larger and fuzzier in appearance than neighboring smaller cells, the latter being glia (cf. Ewer et al. 1992). The control (*dy⁺*) fly, representing 12 adults of this genotype that were processed in this manner, carried two copies of the reporter–fusion gene, which is noticeable by its darker stain. Lateral neurons are nevertheless evident in the *dy^{And}* fly whose section is shown here, as they were in the others of this genotype that were sectioned and stained ($n = 10$). Bar, for the *dy⁺* section, 40 μm ; that from the *dy* fly is shown at 1.2 $\times$ the control image. (B) Lateral neurons (cf. Table 3) in control *per-lacZ* and *dy^{And}; per-lacZ* flies. These cells, observed in all five *And* flies assayed, had diameters ranging from 6 to 10 μm (with respect to 15 cells sampled)—essentially identical to what was observed in contemporary controls and in previous studies (notably Ewer et al. 1992). Bar, (bottom left), 14 μm ; the other control section directly above is shown at 0.75 $\times$ this magnification. The two *dy* images are at 1.5 $\times$ that of the scale-barred magnification.

perikarya being sampled per fly, from among five separate adults of the genotype noted above; all such diameters were in the normal 6- to 10- μm range. Five additional *dy^{And}* adults were sectioned and stained using α -ARR2. The antigen (a cross-reacting one, as opposed to the *arr2*'s gene product; see Materials and Methods) was distributed in CNS patterns similar to those observed after application of this antibody to head sections of wild-type adults (Fig. 4; see its legend for information on control immunoreactivity). Moreover, various *per*-neuronal “parameters” were determined for

the α -ARR2-stained controls and mutants (Table 3). These quasinumerical details—referring to cell bodies and axonal projections stained by this antibody, including those that seem to be aimed at the dorsal mushroom bodies (see Table 3 footnote; cf. Helfrich-Förster and Homberg 1993)—showed no *dy^{And}*-induced abnormalities.

Discussion

The *dy* mutants of *Drosophila melanogaster* are anatomical ones by definition. In this regard,

Figure 4: ARRESTIN-2-like immunoreactivity in dy^{And} . Two consecutive horizontal sections, at 140–150 μm from the dorsal-most part of the brain, are depicted, having been exposed to the α -ARR2 reagent that leads to staining of bona fide ARRESTIN-2 in the eye and of cross-reacting material in the CNS, including the LN cells shown. Bar, 50 μm .

the number of mutants in this species with abnormalities of external appearance and internal anatomy is legion (Bate and Martinez-Arias 1993). This would be one perspective from which to surmise that the dy^{And} mutation could produce both learning and rhythm defects, in addition to considering the more specific hypothesis about relationships that may exist between the mechanisms subserving conditioning and timing phenomena. Furthermore, such a pleiotropic effect could now be rationalized, given the observation that elements of the fly's CNS involved in learning and rhythms are, although spatially distinct, literally connected (Helfrich-Förster and Homberg 1993; Helfrich-Förster 1995; cf. Davis 1993; Hall 1995).

Another less interesting possibility is that general brain damage, associated with the more pleiotropically acting dy mutations (dy^{And} and the like; Table 1), would disrupt the neural substrates of both courtship-related learning and circadian rhythms in a rather gratuitous fashion, as the mutational effects “swept through” a variety of brain structures. Two further points about dy -related pleiotropy should be mentioned in this regard: The relevant slow-clock mutations not only “should” affect something in the head (as well as of course causing defects in the development of a thoracic structure); in addition, a genetic-mosaic analysis has shown that dy^{And} does indeed cause its circadian-rhythm abnormalities through a direct head effect of the mutation, with the aberrant wing morphology being autonomous to that relatively posterior appendage (Newby and Jackson 1995). Moreover, this very mutation was shown by Kyriacou et al. (1993) to cause a lengthening of the male's courtship song-rhythm period (which normally has an ~ 55 -sec cycle duration); that behavioral defect can be inferred to have a thoracic

etiology (again, from mosaic experiments) (see Hall 1984, 1995; Hall and Kyriacou 1990).

Some of the issues just discussed are moot, because none of the dy mutations was found to cause decrements in conditioned courtship behavior. We should note, however, that Jackson et al. (1983) also examined courtship suppression in the immature-male assay (cf. Gailey et al. 1982; Tompkins 1989), and there, as well as in the case of mated females, found dy^{And} to be conditioned subnormally. Though we did not perform tests involving training of long-period dy mutants in the presence of such immature males, our results (Table 2) further weaken the notion that, in *Drosophila*, the control of rhythms and learning share underlying genetic factors, that is, in addition to the weakening that resulted from a reexamination of slow-clock *per* mutants in courtship-suppression assays (Gailey et al. 1991).

An irony here is that chronomnemonic connections recently have been reformed in the opposite genetic direction: *dunce* learning/memory mutations were shown to cause a mild alteration of free-running rhythm periodicity and a more substantial one relating to light-pulse pacemaker-resetting dynamics (Levine et al. 1994). Moreover, *DCO* mutants, which show reductions of protein kinase A activity and olfactory learning abilities (Skoulakis et al. 1993), are also rhythm defective (Levine et al. 1994).

The dy mutants' pleiotropies, which are well established insofar as external morphology and circadian rhythms are concerned, could have been reasonably predicted to extend into other areas, just as *dunce*'s pleiotropy goes even beyond the phenotypes noted above (for review, see Hall 1994b). Thus, dy mutants might have been demonstrable as globally defective genetic variants

that happen to encompass learning abnormalities among their several impairments. This is a somewhat different view of the gene's hypothetically broad effects, compared with one that would focus on potentially more discrete overlaps between the fly's timing and learning "machineries". In any case, neither *dy* nor any of the other salient rhythm mutations are as behaviorally pleiotropic as the literature on them has implied (summarized most recently by Jackson 1993). And the absence of detectable *dy*-induced brain damage (Figs. 2–4), especially within fiber pathways that putatively connect circadian-pacemaker cells to other brain regions (Table 3; cf. Helfrich-Förster and Homberg 1993), once more militates against wide-ranging pleiotropy being a hallmark of the phenotypes influenced by this gene.

What about the extent to which products of the *dy* locus are expressed, possibly in several tissue types and at various stages of the life cycle? This will have to await more definitive findings on the nature of the transcription units present at this locus: *dy*'s molecular genetics has been problematical (Jackson and Newby 1993). Once the complexities in question are unraveled—and against a background of *dy* mutants being wing only or wings and rhythms—it may turn out that the gene products are found in rather discrete locations. The results of our reexamination of the possibility that *dy*'s effects spread beyond its clear areas of action are consistent with the idea that this gene's pleiotropy, and hence its directly revealed expression patterns, may turn out to be rather limited.

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References

- Bate, M. and A. Martinez-Arias, ed. 1993. *The Development of Drosophila melanogaster*, Vols. I and II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Baylies, M.K., T.A. Bargiello, F.R. Jackson, and M.W. Young. 1987. Changes in abundance or structure of the *per* gene product can alter periodicity of the *Drosophila* clock. *Nature* **326**: 390–392.
- Bouhouche, A. and M.K. Choulli. 1994. Étude de l'inhibition sexuelle acquise chez un mutant neurologique, *no-bridge*^{K549}, appartenant à l'espèce *Drosophila melanogaster*. *Can. J. Zool.* **72**: 1376–1382.
- Collins, M.F. and J.K. Hewitt. 1981. A biometrical analysis of a reported conditioned response in the courtship behaviour of male *Drosophila melanogaster*. *Behav. Genet.* **11**: 595–596.
- Cowan, T.M. and R.W. Siegel. 1984. Mutational and pharmacological alterations of neuronal membrane function disrupt conditioning in *Drosophila*. *J. Neurogenet.* **1**: 333–344.
- Davis, R.L. 1993. Mushroom bodies and *Drosophila* learning. *Neuron* **11**: 1–14.
- de Belle, J.S. and M. Heisenberg. 1994. Associative odor learning in *Drosophila* abolished by chemical ablation of mushroom bodies. *Science* **263**: 692–695.
- Dolph, P.J., R. Ranganathan, N.J. Colley, R.W. Hardy, M. Socolich, and C.S. Zuker. 1993. Arrestin function in inactivation of G protein-coupled receptor rhodopsin in vivo. *Science* **260**: 1910–1916.
- Ewer, J., B. Frisch, M.J. Hamblen-Coyle, M. Rosbash, and J.C. Hall. 1992. Expression of the *period* clock gene within different cell types in the brain of *Drosophila* adults and mosaic analysis of these cells' influence on circadian behavioral rhythms. *J. Neurosci.* **12**: 3321–3349.
- Frisch, B., P.E. Hardin, M.J. Hamblen-Coyle, M. Rosbash, and J.C. Hall. 1994. A promoterless *period* gene mediates behavioral rhythmicity and cyclical *per* expression in a restricted subset of the *Drosophila* nervous system. *Neuron* **12**: 555–570.
- Gailey, D.A. and R.W. Siegel. 1989. A mutant strain in *Drosophila melanogaster* that is defective in courtship behavioural cues. *Anim. Behav.* **38**: 163–169.
- Gailey, D.A., F.R. Jackson, and R.W. Siegel. 1982. Male courtship in *Drosophila*: The conditioned response to immature males and its genetic control. *Genetics* **102**: 771–782.
- . 1984. Conditioning mutations in *Drosophila melanogaster* affect an experience-dependent behavioral modification in courting males. *Genetics* **106**: 613–623.
- Gailey, D.A., R.C. Lacaille, and J.C. Hall. 1986. Chemosensory elements of courtship in normal and mutant, olfaction-deficient *Drosophila melanogaster*. *Behav. Genet.* **16**: 375–405.
- Gailey, D.A., A. Vilella, and T. Tully. 1991. Reassessment of the effect of biological rhythm mutations on learning in *Drosophila melanogaster*. *J. Comp. Physiol. A* **169**: 685–697.

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- Greenspan, R.J. 1995. Understanding the genetic construction of behavior. *Sci. Amer.* **272**(4): 88–93.
- Griffith, L.C., L.M. Verselis, K.M. Aitken, C.P. Kyriacou, W. Danho, and R.J. Greenspan. 1993. Inhibition of calcium/calmodulin-dependent protein kinase in *Drosophila* disrupts behavioral plasticity. *Neuron* **10**: 501–509.
- Griffith, L.C., J. Wang, Y. Zhong, C.-F. Wu, and R.J. Greenspan. 1994. Calcium/calmodulin-dependent protein kinase II and potassium channel subunit Eag similarly affect plasticity in *Drosophila*. *Proc. Nat. Acad. Sci.* **91**: 10044–10048.
- Grossman, M. and H.W. Norton. 1980. Approximate intrinsic bias in estimates of heritability based on variance component analysis. *J. Hered.* **71**: 295–297.
- Hall, J.C. 1984. Complex brain and behavioral functions disrupted by mutations in *Drosophila*. *Dev. Genet.* **4**: 355–378.
- . 1994a. The mating of a fly. *Science* **264**: 1702–1714.
- . 1994b. Pleiotropy of behavioral genes. In *Flexibility and constraint in behavioral systems* (ed. R.J. Greenspan and C.P. Kyriacou), pp. 15–27. Wiley, New York.
- . 1995. Tripping along the trail to the molecular mechanisms of biological clocks. *Trends Neurosci.* **18**: 230–240.
- Hall, J.C. and C.P. Kyriacou. 1990. Genetics of biological rhythms in *Drosophila*. *Adv. Insect Physiol.* **22**: 221–298.
- Hamblen, M., W.A. Zehring, C.P. Kyriacou, P. Reddy, Q. Yu, D.A. Wheeler, L.J. Zwiebel, R.J. Konopka, M. Rosbash, and J.C. Hall. 1986. Germ-line transformation involving DNA from the *period* locus in *Drosophila melanogaster*: Overlapping genetic fragments that restore circadian and ultradian rhythmicity to *per*⁰ and *per*[–] mutants. *J. Neurogenet.* **3**: 249–291.
- Hamblen-Coyle, M.J., D.A. Wheeler, J.E. Rutila, M. Rosbash, and J.C. Hall. 1992. Behavior of period-altered circadian rhythm mutants of *Drosophila* in light:dark cycles (Diptera: Drosophilidae). *J. Insect Behav.* **5**: 417–446.
- Heisenberg, M. 1980. Mutants of brain structure and function: What is the significance of the mushroom bodies for behavior? In *Development and neurobiology of Drosophila* (ed. O. Siddiqi, P. Babu, L.M. Hall, and J.C. Hall), pp. 373–390. Plenum Press, New York.
- Helfrich-Förster, C. 1995. The *period* clock gene is expressed in CNS neurons which also produce a neuropeptide that reveals the projections of pacemaker cells within the brain of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **92**: 612–616.
- Helfrich-Förster, C. and U. Homberg. 1993. Pigment-dispersing hormone-immunoreactive neurons in the nervous system of wild-type *Drosophila melanogaster* and of several mutants with altered circadian rhythmicity. *J. Comp. Neurol.* **337**: 177–190.
- Hofbauer, A. and E. Buchner. 1989. Does *Drosophila* have seven eyes? *Naturwissenschaften* **76**: 335–336.
- Huang, J.Z., I. Edery, and M. Rosbash. 1993. PAS is a dimerization domain common to *Drosophila* Period and several transcription factors. *Nature* **364**: 259–262.
- Jackson, F.R. 1993. Circadian rhythm mutants of *Drosophila*. In *Molecular genetics of biological rhythms* (ed. M.W. Young), pp. 91–121. Marcel Dekker, New York.
- Jackson, F.R. and L.M. Newby. 1993. Products of the *Drosophila miniature-dusky* gene complex function in circadian rhythmicity and wing development. *Comp. Biochem. Physiol.* **104A**: 749–756.
- Jackson, F.R., D.A. Gailey, and R.W. Siegel. 1983. Biological rhythm mutations affect an experience-dependent modification of male courtship behavior in *Drosophila melanogaster*. *J. Comp. Physiol. A* **151**: 545–552.
- Jenkins, J. and K. Dallenbach. 1924. Oblivescence during sleep and waking. *Amer. J. Psychol.* **35**: 605–612.
- Konopka, R.J. 1987. Genetics of biological rhythms in *Drosophila*. *Annu. Rev. Genet.* **21**: 227–236.
- Konopka, R.J., R.F. Smith, and D. Orr. 1991. Characterization of *Andante*, a new *Drosophila* clock mutant, and its interactions with other clock mutants. *J. Neurogenet.* **7**: 103–114.
- Kyriacou, C.P., M.L. Greenacre, J.R. Thackeray, and J.C. Hall. 1993. Genetic and molecular analysis of song rhythms in *Drosophila*. In *Molecular genetics of biological rhythms* (ed. M.W. Young), pp. 171–193. Marcel Dekker, New York.
- Levine, J.D., C.I. Casey, D.D. Kalderon, and F.R. Jackson. 1994. Altered circadian pacemaker functions and cyclic AMP rhythms in the *Drosophila* learning mutant *dunce*. *Neuron* **13**: 967–974.
- Liu, X., L. Lorenz, Q. Yu, J.C. Hall, and M. Rosbash. 1988. Spatial and temporal expression of the *period* gene in *Drosophila melanogaster*. *Genes & Dev.* **2**: 228–238.
- Liu, X., Q. Yu, Z. Huang, L.J. Zwiebel, J.C. Hall, and M. Rosbash. 1991. The strength and periodicity of *D. melanogaster* circadian rhythms are differentially affected by alterations in *period* gene expression. *Neuron* **6**: 753–766.
- Newby, L.M. and F.R. Jackson. 1995. Developmental and genetic mosaic analysis of *Drosophila m-dy* mutants: Tissue foci behavioral and morphogenetic defects. *Dev. Genet.* **16**: 85–93.
- Newby, L.M., L. White, S.M. DiBartolomeis, B.J. Walker,

LEARNING IN DUSKY RHYTHM MUTANTS

- H.B. Dowse, J.M. Ringo, N. Khuda, and F.R. Jackson. 1991. Mutational analysis of the *Drosophila* miniature-dusky (*m-dy*) locus: Effects on cell size and circadian rhythms. *Genetics* **128**: 571–582.
- O'Dell, K.M.C. 1994. The *inactive* mutation leads to abnormal experience-dependent courtship modification in male *Drosophila melanogaster*. *Behav. Genet.* **24**: 381–388.
- Siegel, R.W., J.C. Hall, D.A. Gailey, and C.P. Kyriacou. 1984. Genetic elements of courtship in *Drosophila*: Mosaics and learning mutants. *Behav. Genet.* **14**: 383–410.
- Siwicki, K.K., C. Eastman, G. Petersen, M. Rosbash, and J.C. Hall. 1988. Antibodies to the *period* gene product of *Drosophila* reveal diverse tissue distribution and rhythmic changes in the visual system. *Neuron* **1**: 141–150.
- Skoulakis, E.M., D. Kalderon, and R.L. Davis. 1993. Preferential expression in mushroom bodies of the catalytic subunit of protein kinase A and its role in learning and memory. *Neuron* **11**: 197–208.
- Smith, C. 1985. Sleep states and learning: A review of the animal literature. *Neurosci. Behav. Rev.* **9**: 157–168.
- Sokal, R.R. and F.J. Rohlf. 1981. *Biometry*, 2nd ed. p. 241, pp. 427–428, W.H. Freeman & Co., New York.
- Stone, W.S., H.J. Altman, R.F. Berman, D.F. Caldwell, and M.M. Kilbey. 1989. Association of sleep parameters and memory in intact old rats and young rats with lesions in the nucleus basalis magnocellularis. *Behav. Neurosci.* **103**: 755–764.
- Tempel, B.L., M.S. Livingstone, and W.G. Quinn. 1984. Mutations in the dopa decarboxylase gene affect learning in *Drosophila*. *Proc. Natl. Acad. Sci.* **81**: 3577–3581.
- Tompkins, L. 1984. Genetic analysis of sex appeal in *Drosophila melanogaster*. *Behav. Genet.* **14**: 453–482.
- . 1989. Homosexual courtship in *Drosophila*. In *Perspectives in neural systems and behavior* (ed. T.J. Carew and D. Kelley), pp. 229–248. Alan R. Liss, New York.
- Tully, T. and J. Hirsch. 1982. Behaviour-genetic analysis of *Phormia regina* II. Detection of a single, major-gene effect from behavioural variation for central excitatory state (CES) using hybrid crosses. *Anim. Behav.* **30**: 1193–1202.
- Wheeler, D.A., M.J. Hamblen-Coyle, M.S. Dushay, and J.C. Hall. 1993. Behavior in light-dark cycles of *Drosophila* mutants that are blind, arrhythmic, or both. *J. Biol. Rhythms* **8**: 67–94.
- Zawistowski, S. 1988. A replication demonstrating reduced courtship of *Drosophila melanogaster* by associative learning. *J. Comp. Psychol.* **102**: 174–176.
- Zawistowski, S. and R.C. Richmond. 1985. Experience mediated courtship reduction for males by male *Drosophila melanogaster*. *Behav. Genet.* **15**: 561–569.
- . 1988. Experience-dependent reduction in courtship of *Drosophila melanogaster* in large and small chambers. *J. Comp. Psychol.* **101**: 90–93.
- Zerr, D.M., J.C. Hall, M. Rosbash, and K.K. Siwicki. 1990. Circadian fluctuations of the *period* protein immunoreactivity in the CNS and the visual system of *Drosophila*. *J. Neurosci.* **10**: 2749–2762.

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References

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