The effects on locomotor activity and reactivity of the *hypoactive* and *inactive* mutations of *Drosophila melanogaster*

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Locomotor activity, expressed as walking and running, is reduced in adult flies by the mutant genes *inactive*, *inactive*², *hypoactive-C* and *hypoactive-E*. The mutations *iav* and *hypoC* reduce both the amount and speed whereas *hypoE* reduces the speed but not the amount of locomotion. Stimulated activity, reactivity, and spontaneous activity are affected in different ways by these mutations. The frequency of jumping is greatly reduced by all four mutations, and the threshold for the jumping response appears to be related to speed of locomotor activity. The developmental profiles for locomotor activity of *hypoactive* and *inactive* mutants is described.

INTRODUCTION

Several mutations have been isolated on the basis of aberrant locomotor activity. The *inactive* mutation was described by Kaplan (1977). Seven non-allelic *hypoactive* mutations, described by Homyk and Sheppard (1977) and Homyk *et al.* (1980), were isolated using a screen for mutants of reduced flight ability. Homyk (1977) described the *hypoactive* subset of these mutants as difficult to arouse into the excited state necessary for flight. They have low spontaneous activity and soon become inactive following any disturbance that has excited them. O'Dell and Burnet (1986) reported that the mutant genes *hypoactive-B* and *inactive* are alleles.

Changes in the level of expression of the activity of an individual over a short time period have been attributed to reactivity, but the links have yet to be worked out between reactivity, and spontaneous activity expressed when a fly is in a settled state. Individuals may show an initially high level of activity in response to disturbance which Meehan and Wilson (1987) refer to as stimulated activity. Connolly (1967) used a measure of reactivity based on repeated sampling of the behaviour of a fly after transfer to constant conditions. Individuals ceased to respond to extraneous features of the environment as they attained a

settled state. He used the rate of change in activity as a measure of reactivity. Differences in the expression of reactivity in lines selected for changes in locomotor activity have indicated that spontaneous activity and reactivity are at least partially under the control of different genes (Connolly, 1967; van Dijken, 1982). More recently Burnet *et al.* (1988) partitioned locomotor activity into two independent but related measures of amount and speed which, though correlated, were also found to differ in their underlying genetic control. Flies reacted to disturbance by a startle response characterised by jumping, increased speed of locomotion, and a reduced rate of preening. Their reactivity was found to be expressed more in terms of changes in speed than amount of movement.

Longer-term changes in activity have been investigated in connection with circadian rhythm mutations such as *period* (Konopka and Benzer, 1971) which is perhaps the most intensively studied behavioural mutation in *Drosophila* (Ashburner, 1987; Yu *et al.*, 1987). Rather less attention has been directed towards developmental changes in locomotor activity, although Cobb *et al.* (1987) found a relationship with age in their comparative study of different species of the *melanogaster* species subgroup.

Neurological mutations could potentially affect one or more of the different aspects of expression of locomotor activity in *Drosophila*, and detailed

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description of their effects is a first step toward recognising groups of genes involved in control of specific functional systems. Here we describe the effects of three such genes *hypoactive-C*, *hypoactive-E* and *inactive* (*hypoactive-B*).

MATERIALS AND METHODS

The Novosibirsk isogenic inbred wild type stock has been kept under a system of full sib mating for many years (Eastwood and Burnet, 1977). The Sierra Leone Isogenic inbred wild type (line 23) stock was derived from a single wild caught female and has been maintained under constant brother sister mating since 1982.

Inbred strains *hypoactive-C*¹, *hypoactive-E* and *inactive*² (formerly *hypoactive-B*¹, O'Dell and Burnet, 1986) all share an Oregon-R wild type background. They were isolated by Homyk and Sheppard (1977), and Homyk *et al.* (1980). Further information about the mutant genes is given by Hall (1982), and by Lindsley and Zimm (1985).

The *inactive* strain is described by Kaplan (1977). Two isogenic inbred stocks, Novosibirsk *iav* and Sierra Leone *iav*, were synthesised. The mutant allele *iav* (X:18.8) was first incorporated by crossing-over into a multiply marked first chromosome: *y iav v f car*. Using inversion-containing balancer chromosomes with dominant marker genes the homozygous autosomal background of each of the isogenic wild type stocks was then constructed round the multiply marked X-chromosome. The *iav* mutant allele was then transferred from the multiply marked X into the appropriate wild type first chromosome background by crossing-over. Serial backcrossing into the corresponding isogenic inbred wild type strain and reisolation of the *iav* mutant allele was then carried out over several generations to yield *inactive* strains with the same associated genetic background as the wild type allele. These were designated *Niav*⁺ and *Niav*⁻, and *Siav*⁺ and *Siav*⁻.

For the analysis of reactivity (figs. 1 and 2) the original inactive stock of Kaplan (1977) was used. Reciprocal F₁ hybrids between the isogenised stocks *NSiav*⁺, and *SNiav*⁺, also between *NSiav*⁻ and *SNiav*⁻, were used for analysis of age-related changes (figs. 3 and 4). Analyses of variance revealed no statistically significant differences between the reciprocals so the results were pooled for further analysis.

Stocks were maintained at 25±0.5°C on a 12:12 hour night day cycle at constant humidity

on standard oatmeal and treacle medium seeded with live bakers yeast. Sexes were separated within six hours of eclosion and kept in groups of 10–12 individuals. Flies kept for effects of age were transferred to fresh food vials on alternate days. On the day preceding each behavioural trial all flies were transferred to fresh food vials, and behavioural observations were made in the constant temperature room in which the flies were cultured.

Locomotor activity was observed in an open field chamber (10×10×1 cm) made of white perspex fitted with a clear lid engraved with a grid of 1×1 cm squares. A single fly was transferred into the chamber through a closable entry port using an aspirator. The chamber was then tapped 10 times in quick succession before beginning observations. Behaviour was recorded using an Apple II microcomputer interfaced with manually operated keyboards. The frequency and duration of key presses on each independent channel was continuously recorded.

The amount of movement was computed as the proportion of time spent moving in each successive minute of the observation period and then transformed to angles (arcsine). Speed of movement is calculated from the number of squares entered. Weighting is necessary because differences in the amount of time spent moving affects the accuracy of the speed score. For a group of *r* individuals the mean weighted speed *s* is calculated as the mean number of squares entered *d* divided by the untransformed mean time spent moving *t*. Because the mean speed score is actually a ratio estimator its standard deviation is:

$$\frac{d}{t} \sqrt{\frac{\text{var } d}{d} + \frac{\text{var } t}{t} - 2 \frac{\text{cov } dt}{dt}}$$

where var *d* is the variance of *d*, var *t* is the variance of *t*, and cov *dt* is the covariance of *dt*. The standard error follows in the usual way.

For analysis of age-related changes in activity the flies were given a settling down period of 5 min after entry into the observation chamber. Activity was then recorded for the following 5 min.

In this manner locomotor activity was scored at six ages: 1, 2, 4, 8, 16 and 32 days old. In any session, 14 5-min trials were conducted using male and females from seven genotype groups: *hypoC*, *hypoE*, *iav*², *NSiav*⁺, *SNiav*⁺, *NSiav*⁻ and *SNiav*⁻. Within each age group a single set of 14 trials was completed in a two and a half hour session during the afternoon. Ten replications of each age group were made over a period of three months.

A problem associated with attempting to record activity in older flies is the variation in survival observed between the wild type and mutant stocks. The *hypoC* females show a particularly poor rate of survival. Observations on the *hypoC*, *hypoE* and *iav*² mutants were not continued to 32 days of age for this reason.

Figure 1 Amount of movement by female flies. Novosibirsk wild type $\blacktriangle$, Sierra Leone wild type $\blacksquare$, *hypoactive-C* $\square$, *hypoactive-E* $\bullet$, *inactive* $\circ$, and *inactive*² $\triangle$.

RESULTS

Amount and speed of movement

Locomotor activity of female flies observed singly in an open field activity chamber is shown in fig. 1. Wild type flies (Novosibirsk and Sierra Leone) and *hypoactive-E* mutants were similar in that the

amount of movement remained relatively stable over the first 10 min of the observation period then became more variable in the period 10–20 min.

Regression constants in table 1 provide an estimate of the mean initial amount of movement during min 1 after disturbance on entry into the observation chamber. They show that these three stocks were initially similar. The amount of movement of *hypoE* mutant females remained steady over the full 20 min observation period whereas the regression coefficients for the wild types showed a small but significant decline in movement with time.

The *hypoactive-C* mutants moved as much as the wild types initially but they became less active during the first 10 min and reached a lower level in the period 10–20 min, significantly below that of the wild types.

In the first minute of observation females homozygous for the *inactive* or *inactive*² mutant alleles spent less time in locomotion than flies of any of the other genotype groups. The amount of movement declined during the first 10 min. In the succeeding period (10–20 min) the amount of movement was rather variable around what appears to be a settled level significantly lower than that for *hypoE* and the wild types.

Table 1 shows the regression constants and coefficients for amount of movement over 10 min for the strains *hypoC*, *iav* and *iav*² and for the three hybrid strains derived from crosses between them. These may be compared with the values for *hypoE* and the two inbred wild type strains computed over a 20 min period, as shown in fig. 1. Heterozygotes for *hypoC/iav* and *hypoC/iav*² show a high initial amount of movement and a small but significant fall over time (*hypoC/iav*²), or no significant change (*hypoC/iav*). Heterozygotes for the allelic mutations (*iav/iav*²) have a

Table 1 Regression constants and coefficients for change in the amount and speed of locomotion. *P* denotes the associated probability that the regression slope differs from zero due to chance

	Amount of locomotion			Speed of locomotion		
	Constant	Coefficient	<i>P</i>	Constant	Coefficient	<i>P</i>
<i>iav</i>	0.9534 $\pm$ 0.0844	-0.4041 $\pm$ 0.1168	<0.01	0.4208 $\pm$ 0.0290	-0.1068 $\pm$ 0.0401	<0.05
<i>iav</i> ²	0.7655 $\pm$ 0.0764	-0.3753 $\pm$ 0.1058	<0.01	0.4068 $\pm$ 0.0149	-0.0477 $\pm$ 0.0207	<0.05
<i>hypoC</i>	1.1385 $\pm$ 0.0515	-0.4461 $\pm$ 0.0714	<0.001	0.6059 $\pm$ 0.0310	-0.1520 $\pm$ 0.0429	<0.01
<i>iav/iav</i> ²	0.9910 $\pm$ 0.0678	-0.4344 $\pm$ 0.0938	>0.01	0.5216 $\pm$ 0.0321	-0.0446 $\pm$ 0.0444	ns
<i>iav/hypoC</i>	1.1732 $\pm$ 0.0292	-0.0329 $\pm$ 0.0404	ns	1.5398 $\pm$ 0.0530	-0.2140 $\pm$ 0.0734	<0.05
<i>iav</i> ² / <i>hypoC</i>	1.2643 $\pm$ 0.0205	-0.1281 $\pm$ 0.0284	<0.01	1.3400 $\pm$ 0.0424	-0.1017 $\pm$ 0.0587	ns
<i>hypoE</i>	1.1968 $\pm$ 0.0181	-0.0277 $\pm$ 0.0184	ns	0.4524 $\pm$ 0.0064	-0.1197 $\pm$ 0.0065	<0.001
Novosibirsk	1.1906 $\pm$ 0.0080	-0.0900 $\pm$ 0.0081	<0.001	1.1628 $\pm$ 0.0132	-0.1560 $\pm$ 0.0135	<0.001
Sierra Leone	1.1964 $\pm$ 0.0190	-0.1919 $\pm$ 0.0193	<0.01	1.1588 $\pm$ 0.0123	-0.0261 $\pm$ 0.0128	n.s.

low initial level of activity similar to *iav* homozygotes. Regression coefficients, which reflect decay of activity over time, are similar for *hypoC*, *iav*, and *iav*² homozygotes, and *iav/iav*² heterozygotes.

There is a clear dichotomy between wild types and mutants for speed of locomotion (fig. 2). The regression constants, which estimate initial speed of locomotion, are similar for the two wild type strains (table 1). Sierra Leone females showed no significant change in speed over the 20 min observation period, whereas speed of the Novosibirsk females decreased significantly. Speed of locomotion of all four mutant strains was initially less than half that of the wild types, and tended to decrease towards a settled level. The regression coefficients in table 1 show that this reduction in speed with time is significant for each of the *hypoactive* and *inactive* mutants but not for the heterozygote *iav/iav*².

Figure 2 Speed of locomotion by female flies. Novosibirsk wild type $\blacktriangle$, Sierra Leone wild type $\blacksquare$, *hypoactive-C* $\square$, *hypoactive-E* $\bullet$, *inactive* $\circ$, and *inactive*² $\triangle$.

Developmental profile of locomotor activity

Fig. 3 shows the mean expression of predominantly settled activity in relation to age. Wild type flies of both sexes exhibited a steady decline in the amount of movement with age from day 1 to day 32. The *inactive* flies reached a maximum activity at day 2 but thereafter, there was a fall in activity until day 32. There were no statistically significant differences in the amount of time spent moving as between males and females of the same genotype group. Consequently observations were pooled for

the analysis of variance in table 2 which confirms the significance of differences in amount of locomotion between genotypes. The *inactive* flies are consistently less active than wild type at all ages. In the wild type group the reduction in activity below the level at day 1 is significant at day 8. Increase in activity of *inactive* flies between day 1 and 2 is not significant, but the decrease from the peak reached at day 2 is significant at days 8, 16 and 32.

Figure 3 Change in amount of movement with age. Wild type males $\blacksquare$ and females $\bullet$, *inactive* males $\square$ and females $\circ$. Each point represents the mean $\pm$ standard error.

Figure 4 Relationship between age and speed of movement. Wild type males $\blacksquare$ and females $\bullet$, *inactive* males $\square$ and females $\circ$. Each point represents the mean $\pm$ standard error.

Table 2 Analysis of variance for effects of age and genotype on amount of movement by wild type and inactive flies

Source	Males			Females		
	df	ms	F	df	ms	F
Genotype	1	11.86	107.6***	1	6.928	75.6***
Age	5	1.024	9.3***	5	0.955	10.4***
Interaction	5	0.053	0.5	5	0.116	1.3
Error	228	0.110		228	0.092	
Total	239			239		

*** Denotes value of F significant at $P < 0.001$.

Table 3 Analysis of variance for the effects of age on the speed of locomotion of wild type and inactive flies

Wild type						
Source	Males			Females		
	df	ms	F	df	ms	F
Age	5	1.300	27.2***	5	1.610	32.0***
Error	112	0.048		114	0.050	

Inactive						
Source	Males			Females		
	df	ms	F	df	ms	F
Age	5	0.117	3.3**	5	0.172	9.0***
Error	98	0.036		102	0.019	

*** and ** denote values of F significant at $P < 0.001$ and $P < 0.01$, respectively.

Fig. 5 shows that the amount of movement by *hypoactive-E* mutants was comparable to that of wild type flies at 1–16 days of age, and shows a similar age related decrease (cf. fig. 3). The *iav*² strain was the least active at each age, and *hypoC* mutants were consistently intermediate until day 16 at which there was overlap with *iav*². Analysis of variance (table 4) confirms significant differences in the amount of movement between these genotype groups. There were significant differences in activity with age, and also interaction reflecting the increase in amount of movement between days 1 and 2 in *hypoC* and *iav*² which was not observed in the *hypoE* mutants.

Speed of locomotion was significantly higher in males than in females in all genotype groups. The *inactive* mutants were significantly slower than wild type at all ages up to 32 days after eclosion (fig. 4). Wild type flies got slower as they aged from day 8 onwards (table 3), whereas *inactive* mutants showed a significant but less pronounced age-related change.

Figure 5 Change in amount of movement with age. *hypoactive-C* males ■ and females □, *hypoactive-E* males ● and females ○, *inactive*² males ▲ and females △.

Figure 6 Relationship between age and speed of movement. *hypoactive-C* males ■ and females □, *hypoactive-E* males ● and females ○, *inactive*² males ▲ and females △.

The mutant alleles *hypoC*, *hypoE* and *iav*² cause reduced speed of movement at all ages from day 1–16 (fig. 6). In both sexes the mutants differed significantly from one another in speed (table 5). The *hypoE* mutants are the slowest flies. The speed of movement of *hypoC* and *iav*² flies increased between days 1 and 2 in concert with the increase

in amount of movement (fig. 5), suggesting that the processes underlying the developmental profiles for activity of these two mutants are linked.

Jumping

Flight is constrained by the low roof of the observation chamber so that jumping, most frequently observed during the first three minutes after disturbance, may sometimes actually be aborted take-off. Jumping is one facet of a startle response reflecting a high state of excitation (Burnet *et al.*, 1988). Over a 5 min observation period mutant flies of both sexes jumped significantly less frequently than wild type flies (fig. 7). The marked dichotomy between wild-type and mutant flies suggests that the threshold of excitation associated with jumping is infrequently attained in mutant flies. The attenuation of jumping which occurs in *hypoE* mutants implies that the threshold for expression of jumping is in some way correlated with speed of locomotion, because they are abnormally slow (fig. 2), whilst they are indistinguishable from wild type in amount of movement (fig. 1).

The frequency of jumping was sustained at a very low level as age increased in *inactive* and *hypoactive* mutants. Wild type flies showed significant age related change from high frequency between days 1–8, falling thereafter to negligible frequency similar to that in the mutants.

Table 4 Analysis of variance for the effects of age and genotype on amount of movement by *hypoactive-C*, *hypoactive-E*, and *inactive*² flies

Source	Males			Females		
	df	ms	F	df	ms	F
Genotype	2	4.75	54.3***	2	4.29	61.2***
Age	4	0.446	5.1***	4	0.639	9.1***
Interaction	8	0.224	2.6*	8	0.278	4.0***
Error	135	0.088		135	0.070	

*** and * denote values of *F* significant at $P < 0.001$ and $P < 0.05$, respectively.

Table 5 Analysis of variance for the effects of genotype on speed of locomotion of *hypoactive-C*, *hypoactive-E* and *inactive*² flies

Source	Males			Females		
	df	ms	F	df	ms	F
Genotype	2	0.600	16.9***	2	0.289	18.1***
Error	139	0.035		139	0.016	

*** denotes values of *F* significant at $P < 0.001$.

Figure 7 Change in amount of jumping with age. Each point represents the number of jumps measured over a 5 min period. Wild type males ● and females ○, *hypoactive-C* males ■ and females □, *hypoactive* males ▼ and females ▽, *inactive* males ▲ and females △.

DISCUSSION

There is a clear distinction between the mutations *iav* and *hypoC*, which affect both the amount and speed of adult locomotor activity, and *hypoE* which affects only speed of locomotion, so that it is perhaps more appropriate to describe the phenotype of *hypoE* mutants as slow rather than hypoactive. This difference, together with the observation that males and females differ significantly for speed, but not amount, of locomotor activity, supports the conclusions of Burnet *et al.* (1988) that amount and speed of locomotion are largely under independent genetic control.

Cobb *et al.* (1987) using the same measure of adult activity as that previously used by Connolly (1967) observed sex differences within different species of the *Drosophila melanogaster* species subgroup. Since this measure confounds two largely independent components, only one of which shows significant sex differences in *D. melanogaster*, it is probable that the species differences described by Cobb *et al.* (1987) are largely due to differences in speed rather than amount of locomotion.

Reactivity is a kinesis in the sense that it expresses itself as a change in the rate of locomotor activity measured in terms of amount or speed of locomotion (Schöne, 1984). In the present context the magnitude of the response to disturbance is

reflected by the initial response and by the rate of decay towards a settled or spontaneous level. Meehan and Wilson (1987) regard the initial response, which they term stimulated activity, as a category of locomotor activity distinct from spontaneous activity and reactivity. van Dijken *et al.* (1987) measured locomotor activity in adult *D. melanogaster* in a locometer, an apparatus which represents a modification of the method used by Ewing (1963). Reactivity was defined as the regression of locomotor activity on density, using groups of individuals of varying size. From an analysis of the effects of different combinations of homozygous chromosomes derived from selected lines van Dijken (1982) concluded that spontaneous activity and reactivity to other flies of the same sex are at least partially controlled by different genes.

Wild type flies show a relatively low rate of decay in amount and speed of locomotion over a 10 min period below the level of initial response. The mutations *iav* and *hypoC* cause a rapid decay in amount of movement, and to this extent are less reactive than wild type. However the mutations differ in their effects on the magnitude of stimulated activity which is not affected by *hypoC* but is depressed by *iav*. The *hypoE* mutation causes no change in expression of reactivity as expressed through amount of movement. The effects of *iav* are the reverse of those of the *Tyr-1* mutation which causes an increase in stimulated activity (Meehan and Wilson, 1987). All three mutant genes attenuate the initial speed of locomotion, but the rates of change measuring reactivity are essentially similar to wild type. These findings show that stimulated activity, reactivity, and spontaneous activity may be separately distinguished in terms of amount and speed of locomotion, and that different mutant genes affect the separate aspects of activity in different ways.

Although at separate gene loci, the *iav* and *hypoC* mutations are remarkably similar in their range of phenotypic effects. Homyk (1977) has located different mutant foci for these mutations in the thoracic and suboesophageal ganglia using mosaic mapping procedures. However, as Hall (1985) has pointed out, since the mapping procedures depended upon mutant versus normal tissues only with respect to the external cuticle, and without additional internal markers, only indirect inferences are possible with respect to the neural defects. An extension of the search for the foci of these mutations, and of that for *hypoE*, using internal tissue markers may provide further evidence concerning their mode of action. O'Dell

et al. (1987) have detected a significant deficit in octopamine levels in *iav* mutants. Whether this reflects a direct effect of the *iav* gene, or an indirect consequence of reduced locomotor activity is as yet unclear. This is additional *prima facie* evidence that the *iav* mutation has a neural focus. It is not yet known what effect, if any, the *hypoactive* mutations may have on octopamine titres. The *Tyr-1* mutation is associated with a reduction in dopamine levels (Burnell and Daly, 1982). Altered catecholamine balances may be a mechanism linking the effects of all of these mutations on differing aspects of locomotor activity.

Mutations which have been found to cause abnormalities of the jumping response are *bendless* (Thomas, 1980; Thomas and Wyman, 1982), *jumpless* (Hall, 1982), and *non-jumper* (Thomas, 1980), which are associated with abnormalities affecting the giant nerve fibre. Another mutation, *acclinal* (Deak, 1976) has an unknown focus. The threshold of reactivity necessary for induction of the jumping response is rarely attained in *inactive* and *hypoactive* flies at all ages, whereas wild type flies exhibit a clear age-related profile in which the jumping response shown by younger flies steadily declines with age. The near absence of a jumping response by *hypoE* females suggests that the threshold for this activity may be associated with the mechanism controlling speed of locomotion.

Wild type flies show a developmental profile with respect to amount and speed locomotion characterised by high levels of expression during days 1-8 after eclosion from the pupa followed by decay in the levels of expression through days 16-32. Actually, the high levels of activity in wild type flies at one day of age preceded by significantly lower levels of expression on the day of eclosion (O'Dell and Jallon, unpub.). The developmental trajectory in locomotion by *hypoE* mutants resembles that of wild type, whereas the *iav* and *hypoC* mutations appear to effect a change in the developmental profile by causing a delay in attaining the age-related maximum, without apparently postponing a decay of expression in older flies. Leffelaar and Grigliatti (1980a, b) argued that the characteristics of developmental profiles of specific behaviours may aid genetic studies of premature ageing. In their search for adult lethal mutations which exert their effects by accelerating the ageing process, they pointed out that there are few behaviours in *Drosophila* that are consistently age related. Further studies on the developmental profiles of wild type adult locomotor activity as described here may prove informative in that context.

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