Patterned Neural Activity of a
Mutant *Drosophila melanogaster**

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Abstract. Hyperkinetic [1P], a behavioral mutant of *Drosophila melanogaster*, shows rhythmic leg-shaking action derived from mutation of a single, sex-linked gene. The neural mechanism governing this abnormal motor function consists of rhythmic bursts of activity produced by motor neurons located within the thoracic ganglion. The mechanism of the pattern formation was independent both of sensory information and higher centers in the central nervous system but endogenous within the ganglion. Two types of neurons which appear to be involved in the pattern formation were found within six limited regions.

Introduction. Recent progress in the physiology of the nervous system has revealed that general principles of nervous function may be established throughout a wide variety of animals. On the other hand, no animal is free of conditions determined by phylogeny and ontogeny; i.e., the genetic program inherited by the animal. A direct way to understand nervous function in terms of genetic programming is to investigate the relationship between a single gene and the resulting nervous function. *Drosophila melanogaster*, under precise genetic control, provides excellent material for such studies.

Benzer[1] has described a use of visual mutants of *Drosophila melanogaster* as a microsurgical tool for the study of the nervous system. Some physiological studies of the visual system of mutant *Drosophila* have been reported.[2,3]

Gene mutation may, conceivably, affect the motor system of an organism, also. Study of such mutations would provide a basis for understanding the neural mechanism of motor regulation. Kaplan and Trout[4] have described four different single-gene mutants (shakers) of *Drosophila melanogaster* which exhibit abnormalities in motor function. One of these, Hyperkinetic[1P] (HK[1P]), shows specific leg-shaking action during anesthetization with diethyl ether and during recovery phase from it. The legs of HK[1P] shake rapidly in a regular rhythmic fashion. This behavior differentiates this mutant sharply from wild type flies, permitting localization of the mutant gene by means of classical recombinational techniques. The gene was located at 30.9 on the X chromosome.[4] The shaking may be a semidominant character, as in other shaker flies;[5–7] but more precise measurements are needed to define the phenotype of HK[1P] heterozygotes. HK[1P] flies may be kept in a closed container in the presence of 1–8% ether vapor (v/v) and will remain anesthetized and shaking for up to 24 hr. Chloroform under the same conditions does not elicit shaking. Canton-S, Oregon-R, and

several other wild type strains of *Drosophila melanogaster* do not exhibit this phenotype. The present study investigates the neural mechanism underlying this specific motor function.

Materials and Methods. (*a*) **Mutagenesis:** HK^{1P} was produced experimentally in the Canton-S wild type stock of *Drosophila melanogaster*. After feeding upon ethyl methane sulfonate, males were mated to females of the X chromosome balancer stock, FM6K.[8] The F_1 daughters were backcrossed to FM6K males. The FM6K stock had previously had its autosomes replaced with Canton-S chromosomes by a series of controlled crosses. By this device, the genetic background of the mutant which appeared in the F_2 generation was essentially Canton-S. A single F_2 male, carrying the mutant gene in its X chromosome, was used to build an HK^{1P} stock (Fig. 1).

To determine if the observed neurophysiological characteristics of the HK^{1P} were attributable to the single gene change, it was necessary to have the genetic backgrounds of the control Canton-S and the HK^{1P} as similar as possible. Therefore, after the stock was established, it was backcrossed to the Cantonized FM6K stock for 10 generations. The resulting strain became virtually identical to its reference control stock except for the single gene change.[9]

(*b*) **Rearing:** Both HK^{1P} (mutant for the present study) and Canton-S (control) stocks were grown on corn meal, sucrose, yeast, and agar mediums at 25°C in constant light.

(*c*) **Physiological saline solution:** To obtain the most suitable physiological saline solution for *Drosophila melanogaster*, solutions of various ionic compositions were tested by observing
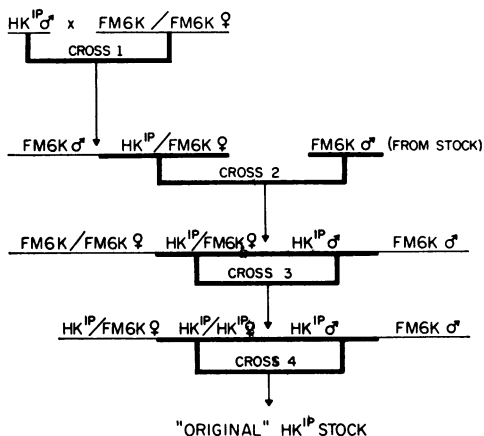

Fig. 1.—Breeding scheme to establish homozygous HK^{1P} stock from a single mutant male.

the electrical activity of nerve and muscles. A slight modification of Bodenstein's saline solution[10] maintained the activity of nerve and muscle in good condition for more than 24 hr when the respiratory system of the fly was undisturbed. This solution had the following composition: NaCl:128 mM, KCl:4.7 mM, $CaCl_2$:1.8 mM, Na_2HPO_4: 0.74 mM, KH_2PO_4:0.35 mM.

(*d*) **Experimental procedure:** Because the behavioral activity of the flies changes after emergence,[4] 4-day-old flies were used throughout this study. Both sexes of the mutant and control flies were used. The electrical activity of neurons during the recovery phase from ether anesthetization was recorded by means of standard neurophysiological techniques. The electrode for extracellular recording was a glass pipet (1- to 2-μ tip diameter) filled with the saline solution. A glass pipet of smaller diameter filled with a 3 M KCl solution was used for intracellular recording. The electrodes were connected to a high input impedance preamplifier via Ag-AgCl wire immersed in the solution of the pipet. A reference Ag-AgCl electrode was immersed in a bath of the saline solution. The fly was mounted in the bath in such a manner that the openings made on the body for recording purposes were in contact with the solution; the spiracles were in contact with air provided from a tube connected to a respirator. Details of this technique will be published elsewhere.

For the recording of nerve impulses from the tibial nerve, the femur and the tibia were held in position with wax. An electrode was inserted into a small hole in the articular corium of the femo-tibial joint. This joint was preferred because of the least disturbance from muscle action potentials. The area however, was too small to apply two

electrodes in measuring the direction of conduction; so the second recording site in these experiments was near the thoracic ganglion.

For recording from the thoracic ganglion, the fly was fixed in position on its back, and the prothoracic and mesothoracic legs were removed at the sterno-coxal joint. The meso-preepisternum and the posterior process of the pro-preepisternum were then dissected out. After making lateral cuts on pro- and meso-furca, the furca were removed. The entire thoracic ganglion was thus exposed, making ventral insertion of electrodes possible. After the operation, the preparation was perfused with the saline solution for 30 min at the rate of about 6 cm³/min. All experiments were performed at a temperature of 23–24°C.

Results. (*a*) **Efferent impulses in the tibial nerve:** The pattern of nerve impulses in the tibial nerve of the mutant showed two types of rhythmic bursts. One, a long-lasting burst of approximately 2-sec duration and slow rhythm (1 burst/10–15 sec), was not characteristic of the mutant, as this activity was also found in the control. The second type, unique to the mutant, was a burst of short duration (20–40 msec) with a faster rhythm (3–10 bursts/sec) and corresponded to the leg-shaking action. The leg movements accompanying this fast rhythmic burst were small but discrete; i.e., typical shaking. Observing the movement of the tarsus of the experimental leg, while listening to nerve impulses with an audiomonitor, demonstrated that one burst corresponded to one stroke of the tarsus.

The recorded results from mutant flies are summarized in Table 1. The

TABLE 1. *Number of HK¹ᴾ preparations (events/legs).*

	Prothoracic		Mesothoracic		Metathoracic		
	Right	Left	Right	Left	Right	Left	Total
Slow rhythmic bursts only	0	2	0	0	1	0	3
Fast rhythmic bursts only	4	4	1	5	4	2	20
Both rhythmic bursts	9	12	12	11	9	10	63
Recording failures	13	8	13	10	12	14	70

numbers in the table indicate the number of preparations which showed the specified events. From 26 flies (providing 156 legs), slow bursts were recorded in 66 cases and fast bursts in 83 cases. Only slow bursts were recorded in three cases, probably because of some technical failure to record the fast bursts, since fast rhythmic movement of the tarsus was observed in each instance. Both slow and fast rhythmic bursts were obtained in 63 cases. The 70 failures most likely reflected nerve damage caused by the operation.

The nerve impulses of the tibial nerve of the control flies were also recorded. Slow rhythmic bursts of the same range as those of the mutant were obtained, but no fast rhythmic bursts were found. In some cases, a few impulses were discharged during the pause between slow rhythmic bursts, but they were not rhythmic and thus were clearly distinguishable from the fast rhythmic bursts of the mutant. Table 2 summarizes the recordings obtained from 43 control flies (258 legs). Slow rhythmic discharges were obtained in 103 cases, but no sign of regular bursts in the range of the fast rhythmic bursts of the mutant was found.

In order to determine the direction of impulse propagation in the mutant, the impulses were recorded at the femo-tibial joint and just outside the thoracic ganglion. Fast and slow rhythmic bursts at the femo-tibial joint always showed

Table 2. *Number of Canton-S preparations (events/legs).*

	Prothoracic		Mesothoracic		Metathoracic		
	Right	Left	Right	Left	Right	Left	Total
Slow rhythmic bursts only	15	17	24	12	23	12	103
Fast rhythmic bursts only	0	0	0	0	0	0	0
Both rhythmic bursts	0	0	0	0	0	0	0
Recording failures	28	26	19	31	20	31	155

a certain delay from those recorded just outside the ganglion.   When the nerve was cut peripheral to the recording point, there were no noticeable changes in either the fast or slow rhythmic bursts; however, when the cut was central, both types disappeared.   These facts confirmed that the impulses of both types of burst were efferent but not afferent.

When the cervical cord of the mutant was cut, the slow rhythmic bursts disappeared, while the fast ones remained unchanged.   The disappearance of the slow rhythmic bursts after cutting the cervical cord was also confirmed in the controls.   This indicates that the cephalic ganglion is involved in coding the slow rhythmic bursts but not the fast ones.   In those cases in Table 1 where only fast rhythmic bursts were obtained, a blockage of information between the cephalic and thoracic ganglia may have taken place.   Figure 2A demonstrates fast rhythmic bursts obtained from the tibial nerve after cutting the cervical cord.
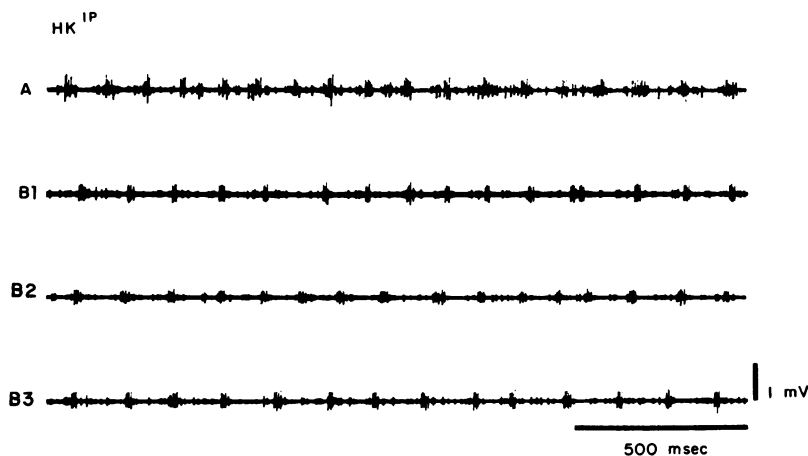

Fig. 2.—Fast rhythmic bursts of nerve impulses corresponding to the leg-shaking action of the mutant. Extracellular recordings from (*A*) right mesothoracic leg nerve, recorded at the femo-tibial joint after cutting the cervical connective; (*B*1) right mesothoracic motor region. Central nervous system intact. Prothoracic and mesothoracic leg nerves were cut; (*B*2) Same preparation after cutting the cervical connective; (*B*3) Same preparation after cutting all nerves from the thoracic ganglion.

(*b*)   **Electrical activity of the thoracic ganglion:** Extracellular recordings were made to locate the neurons responsible for the fast rhythmic bursts in the mutant.   An extensive search was made all over the ganglion, but active areas furnishing the same pattern of nerve impulses as in the fast rhythmic bursts

were limited to paired regions in the pro-, meso-, and metathoracic motor areas. In other parts of the ganglion, some neurons showed regular trains of impulses and some irregular trains. In the posterior medial region, which is functionally the abdominal ganglia but is fused to the thoracic ganglion, several neurons discharged rhythmic bursts; however, their patterns were easily distinguished from the fast rhythmic bursts of present interest by their longer burst period and slower rhythm. No activity similar to the pattern of the fast rhythmic bursts was found away from the three pairs of regions.

Figure 2*B*1 shows the fast rhythmic bursts obtained from the right meso-thoracic region. Here, the nervous system was intact except for the operation necessary for the recording. Figure 2*B*2 shows the rhythmic bursts obtained from the same preparation after cutting the cervical connective. For this purpose, the electrode was withdrawn and then reinserted. The pattern of activity remained unchanged. In the same preparation, all nerves originating in the thoracic ganglion were cut, and the electrode was reinserted. The pattern of activity remained the same (Fig. 2*B*3). These facts showed that the cephalic ganglion was not involved in the formation of this patterned activity, and that the sensory input did not affect the pattern. Therefore, the mechanism furnishing the patterned activity specific to the mutant must be endogenous within the thoracic ganglion.

The electrical activity of the thoracic ganglion of the controls was carefully surveyed. From 37 control flies, no rhythmic activity within the range of the fast rhythmic bursts of the mutant was obtained. Various activities, such as trains of regularly or irregularly spaced impulses, were recorded, but most were not rhythmic bursts. Some rhythmic bursts occurred, but these were clearly distinguishable from the fast rhythmic activity of the mutant because of the longer period of the burst and slower rhythm.

(c) **Electrical activity in single neurons of the mutant:** Within one region, intracellular recordings showed that several neurons were discharging impulses with the same frequency as that of the fast rhythmic bursts recorded extra-cellularly. Two types of such neurons were found. Type 1 discharged action potentials without a sign of prepotential; whereas, in Type 2, an action potential was always preceded by a slowly rising depolarization. When considering the discharge pattern of action potentials, however, the pattern of Type 2 neurons was quite similar to that of Type 1.

Type 1 neurons were encountered about three times more frequently than Type 2. The resting potential of Type 1 ranged from -52 to -63 mV (from 23 stable recordings obtained from 18 preparations which maintained fast rhythmic activity for more than 10 min). Action potentials showing overshoot were observed in only two cases ($+6$ mV and $+2$ mV). The most frequently registered temporal pattern was the repetitive discharge of action potentials with relatively regular intervals (Fig. 3*A*). The short-term fluctuation of interval from one to the next can be seen here, but the over-all frequency was relatively stable as long as the neuron was discharging at 8–10/sec. In some cases, however, a neuron discharged with lower frequency (2–6/sec) with long-term fluctuation; i.e., the average frequency changed with a period of several seconds.
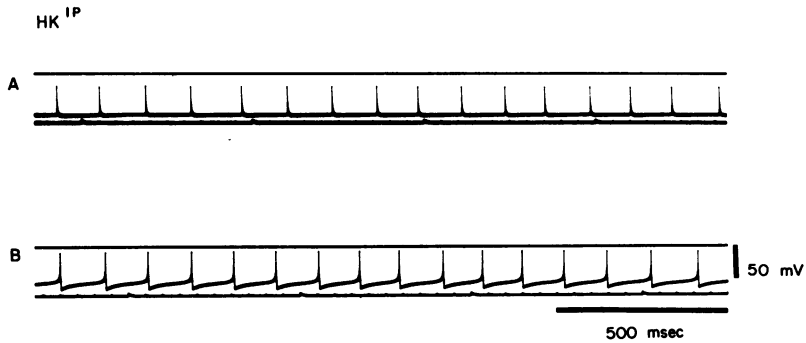
HK^{1P}

FIG. 3.—Two types of neurons in the mesothoracic motor regions of the mutant. Intracellular recordings from (*A*) Type 1 neuron. Action potentials without prepotentials; (*B*) Type 2 neuron. Action potentials preceded by slowly rising depolarizations.

In Type 2 neurons, the resting potential could not be measured because of the prepotential, but it appeared to be near that of Type 1 neurons (Fig. 3*B*). In most cases, the frequencies ranged from 5 to 10/sec; however, when a Type 2 neuron was discharging with lower frequency, long-term fluctuation nearly always occurred, as in Type 1. When the frequency was low, the rate of rise of the slow depolarization was low, and vice versa. When the depolarization reached about −50 mV from 0 level, an action potential was elicited, indicating the critical level for the initiation of action potential and the pacemaking role of the slowly rising depolarization.

(*d*) **The location of the neurons:** In order to determine the location of the neurons involved in the specific activity of the mutant, the sites of each recording were superimposed on the ventral projection of the thoracic ganglion.

In the experiment described in (*b*), fast rhythmic bursts were obtained in at least one of the regions of each of the 41 mutant flies. The following number of preparations showed the fast rhythmic burst at each region: prothoracic right, 12; prothoracic left, 9; mesothoracic right, 15; mesothoracic left, 18; metathoracic right, 5; metathoracic left, 9. Each recording site is marked with a circle in Figure 4*A*.

Type 1 neurons were found at 23 sites from 18 preparations. The location was mapped in the same way (Fig 4*B*). Type 2 neurons, from the same preparations, were found at nine sites (Fig. 4*C*).

Discussion. This study confirmed that the phenotype of HK^{1P} was based upon a neurological change caused by a single gene mutation. The involvement of either sensory organs or neuromuscular systems in the formation of the motor output pattern specific to the mutant was eliminated. The cephalic ganglion, the only central nervous system anatomically separated from the thoracic ganglion, was shown to have no role in the pattern formation; therefore, the neural concomitant of the genetic change must have occurred in the thoracic ganglion itself.

During the course of the experiments, the respiratory and circulatory systems were kept as intact as possible.[11] The possibility that the gene change affects
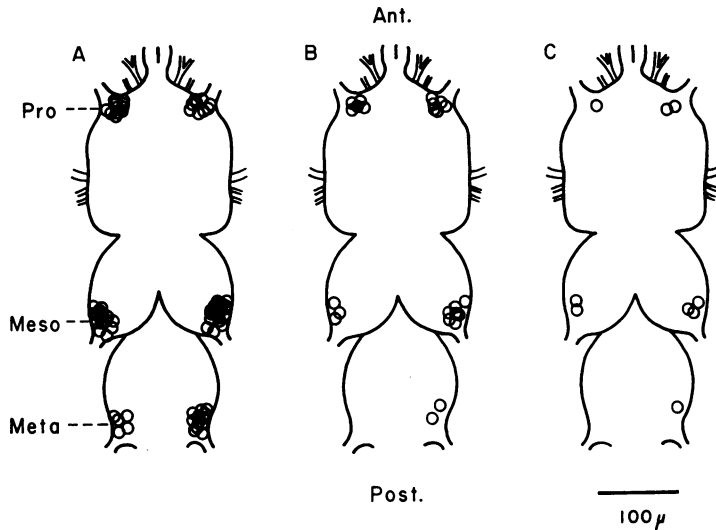
FIG. 4.—Ventral view of the thoracic ganglion with superimposed recording sites of (*A*) extracellular recordings of the fast rhythmic bursts from the mutant; (*B*) intracellular recordings from Type 1 neurons of the mutant; (*C*) intracellular recordings from Type 2 neurons of the mutant. (One circle represents one recording site.)

these systems, which in turn influence the thoracic ganglion, must be taken into account. The circulatory system of the *Drosophila* is an open system, so the saline solution which was perfused through the preparation replaced the hemolymph. Therefore, possible factors carried by the hemolymph have been excluded. Although the respiratory system may be involved, it is unlikely that the change in that system would result in a specifically patterned activity in only particular neurons.

For a control experiment, the lack of a pattern similar to that of HK[1P] should ideally be shown in an identical neuron of Canton-S. Because of the small size of the neurons, insertion of the electrode into an identical cell of different preparations could not be ascertained. A secondary choice had to be employed to show that the same activity pattern as that of the mutant was never detected in the controls. Power[12] presumed that the giant cells of the thoracic ganglion were motor neurons; however, the location of the neurons in this study appeared to be different from that of giant cells.

Type 2 neurons may be regarded as the pacemakers for Type 1 neurons. If so, the Type 2 neuron is the one directly affected by the mutation, while Type 1 are the motor neurons. Further experimentation is needed to establish more evidence for this.

Only a few reports have shown action potentials recorded from neuron somata of insects.[13–16] In this study, action potentials were recorded intracellularly, but the precise location of the tip of the electrode in the neuron was unknown. The recording of intracellular events from a part other than the soma of a neuron as small as the present material is unlikely.[17] This does not imply that the recorded action potential is generated in the membrane of the soma. The action

potential may be initiated in the axon and recorded electrotonically by the electrode placed in the soma.[18]

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[9] Because the method used for isolating the HK[1P] chromosome and establishing the original and the Cantonized stocks maintained the integrity of the treated X chromosome in which the mutation appeared, other mutations induced by the ethyl methane sulfonate treatment may also be present on the X chromosome. Recently, the chromatin material to the left and the right of the HK[1P] locus has been replaced with Canton-S chromatin and subsequent work will make use of this better controlled "completely Cantonized" stock.
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[17] Most neuron somata of *Drosophila melanogaster* are less than 3 μm in diameter. The largest neuron soma (identical to Power's giant cell) in the thoracic ganglion is 8 μm in its long axis. Most axons are beyond the resolution of light microscope, except the giant axon, which is about 5 μm in diameter.
[18] Appreciation is extended to Prof. G. Hoyle, University of Oregon, for the discussion concerning this point.