Neurophysiological Genetics in *Drosophila melanogaster*

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SYNOPSIS. Neurophysiological genetics is the study of the mechanisms by which genes control nervous function and behavior. The transduction of genetic information into neural information is studied at the level of the neuron through genetic and physiological techniques.

The neurons responsible for the leg-shaking action specific to a single-gene mutant of *Drosophila melanogaster, Hk*^{1}, have been located in three pairs of small regions in the thoracic ganglion. The activity pattern of these neurons is coded by the mutant *Hk*^{1} gene. The center for the specifically patterned leg-shaking action is composed of several motor neurons whose activity is governed by the pacemaking activity of at least one interneuron. As it is most likely that the mutant gene is expressed autonomously in this interneuron, there is a possibility of investigating ways in which genes may influence the properties of neurons. The activity of the mutant neuron was monitored intracellularly, and the pattern formation mechanism was studied. The amplitude, duration, and periodicity of the pacemaker potential and the spike initiation site determine the activity pattern resulting in the specific leg-shaking action.

INTRODUCTION

The development of classical genetics has been supported mainly by data obtained on the relationship between genes and resulting morphological phenotypes. Morphological phenotypes have been quite useful for this purpose because relatively simple observations can provide the description necessary for the distinction between one phenotype and another.

Behavior genetics, on the other hand, has been slow to develop. It is more difficult to identify behavorial than morphological phenotypes. Even a simple behavior is the product of integration of its genetic background, environment, and intrinsic morphological and physiological conditions. The development of behavior genetics, therefore, is on one hand dependent on the availability of precise ethological data for animals of genetic interest. The animals for which intriguing ethological data are available, however, are not necessarily animals suitable for genetic research. Widely accepted methods in classical behavior genetics for overcoming this situation are, for instance, the crossing and segregation of behavioral phenotypes, selection, and the observation of phenotypes which appear in inbred and hybrid strains. These approaches have clearly shown the importance of genetics in behavioral research. However, the feasibility of explaining gene-behavior relationships is not inherent within these kinds of approaches because starting from the observation of inbred or hybrid strains and tracing a pathway to the responsible gene might well end in an unanalyzable genic structure. Thus, it is desirable to establish a workable strategy for study of the gene-behavior relationship by

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starting at a completely different basis from that of classical behavior genetics. Precise genetic information must be available to determine which specific genes are responsible for a particular behavior and upon which cells they act. With this knowledge, physiology can be introduced into behavior genetics to bridge the gap between gene and behavior.

Neurophysiological genetics can be defined as a study of the mechanism of the genetic determination of nervous function. The mechanism for transduction of genetic information to a neural code is studied at the neural level. The neurophysiologically decoded genetic information may then relate the gene action to the resulting nervous function or behavior.

SOME CRITERIA FOR NEUROPHYSIOLOGICAL GENETICS

From the standpoint of neurophysiological genetics, a living organism is an information-processing machine which works as a decoder of the genetic code. The decoded results are expressed as either structural (morphological) or functional (behavioral) phenotypes. Neurophysiological genetics deals with the decoding mechanism which results in the latter. Thus, the feasibility of neurophysiological genetics must be examined at those three levels, i.e., genetic input, intermediating decoder, and behavioral output levels.

1) *The genetic input level.* The search for a suitable genic condition starting from an inbred strain with interesting behavior is hardly feasible because even if the target behavior should fortunately appear to be the expression of a single gene, the abnormality or ambiguity of another genome provides a complicated situation. Instead of selecting first an interesting behavior which appears in an inbred or hybrid strain and tracing back to the genic structure, the direct approach would be to choose the gene first and trace it to the behavior. A single-gene mutation resulting in a specific behavior provides suitable material. The target single gene should reside in an otherwise normal genome; thus, a single-gene mutation produced in a normal background is the most desirable. The gene should then be located. For further genetic manipulation, a recessive, sex-linked gene provides an additional advantage. Because of the availability of simple techniques for gene localization and further genetic manipulation, *Dorsophila melanogaster* emerges as the most promising organism.

2) *Intermediating decoder's level.* The pathways from gene to behavioral phenotype can be many. A genetic change, present in every cell and tissue, may still result in a specific behavior. The diverse effects of a genetic change expressed in various cells or tissues will cause serious difficulty in physiologically surveying for the mechanism; therefore, it should be avoided. On the other hand, a genetic change could be expressed in a particular cell or tissue but affect the behavior in an indirect manner. It could also appear in a cell or tissue which is directly related to the behavior. The most preferable situation is the genetic change which is expressed in certain cells or tissues which are directly related to the specific behavior. Among various systems in a living organism, the nervous system and the endocrine system play essential roles for information processing. Other systems such as the respiratory, circulatory, etc., play supporting roles. The genetic code expressed in the supporting systems might well affect the function of the nervous or endocrine systems and result in a particular behavior. However, if the genetic change is directly upon the nervous or endocrine system, the analysis of the mechanism will be easier. In comparing these two systems, the expression which appears in the nervous system is preferable because the input-output relation is far more discrete. One would prefer the mutant expression of the nervous system to be functional rather than structural. Genetically induced structural changes may be the cause of a specific behavior. The transduction of genetic code in this case, however, is developmental and behaviorally uninteresting. It will provide interesting material for developmental genetic studies but not for neurophysiological genetics.

On the other hand, when the mutant expression is in the nervous function, study right at the genetic site of action may be possible.

The living organism receives information from the environment through its sensory organs. It is then processed by the nervous system, and finally expressed as behavior by the effector organs. A mutation affecting a sensory organ (extreme input side) or an effector organ (extreme output side) should be avoided in many cases; for example, blindness caused by the lack of photosensitive pigment or flightlessness caused by the lack of wings would not be a good subject for neurophysiological genetics.

In the nervous system, the genetic change could occur either in the sensory, integrative, or motor systems. Although it is possible to detect the expression in the sensory or integrative systems, these expressions are not easily related to the behavior because a number of neural processings must be involved between them and the resulting behavior. A mutant affecting the motor system is preferred because the final output for the behavior is determined by that system. Therefore, the neurophysiological study of the motor mechanism underlying the behavior is the most feasible and meaningful. A relatively simple motor nervous system is needed; from the standpoint of structure and the availability of neurophysiological information, the insect nervous system offers one of the best possibilities.

3) *Behavioral output level.* The preferred behavior should be one which directly and distinctly reflects the genetic background but remains relatively independent of environmental conditions. If the behavior is affected by the environment, the genetic effect may be obscured even though the capability of reacting to the environment is the product of heredity as well. Therefore, behaviors which largely depend upon environmental conditions, such as adaptation, habituation, learning, memory, etc., should be avoided at least until such time that better techniques than are presently available may be brought to bear upon them. It is apparent, therefore, that endogenously patterned, instinctive behavior is the best. In this respect, the insect is an outstanding subject for study. Most insect behavior is endogenously patterned (a direct reflection of genetics), including very sophisticated behaviors.

The most feasible approach to the genetically coded behavior as a first step in neurophysiological genetics, therefore, is to use material in which the behavioral pattern is endogenous and autonomously coded by a single gene at the transduction site within the motor system.

INSECTS AS MATERIAL FOR NEUROPHYSIOLOGICAL GENETICS

Insects offer good systems not only for the study of ethology itself but also for study of the neural mechanisms underlying behavior. The rationale for the former is that instinctive behavior (Tinbergen, 1951), fixed action patterns (Hess, 1962), or endogenously patterned behavior (Ikeda and Kaplan, 1970*a*) are best expressed in insects, and these are the types of behavior most feasible for ethological analysis (Manning, 1967). The rationale for the latter is that an insect behavioral action can be established by a relatively small number of neurons, yet maintain a high level in its function (Hoyle, 1970). The small number of neural units and the distinct function of each unit make possible neurophysiological analysis of neural activities governing a behavorial action. The insect nervous system thus presents an ideal preparation for the study of endogenously patterned behavior which is a direct reflection of heredity. Remarkable achievements in recording neural activity of a single identified neuron in relation to a behavior were shown in insect nervous systems (Rowe, 1969; Young, 1969; Hoyle and Burrows, 1970). The functional organization of neural architecture relating to a particular behavior can be more precisely studied in the insect than in other animals. Good examples revealing the neural basis for behavior in the cricket (Huber, 1960), grasshopper (Elsner and Huber, 1969; Elsner, 1969), locust (Hoyle and Burrows, 1973*a,b;* Burrows and Hoyle, 1973) have been shown. Activities found in motor

neurons of insects have suggested the existence of a built-in pattern for motor output (Fielden and Hughes, 1962; Mill, 1963; Knights, 1965). The bilaterally coordinated sound production of the cicada was interpreted in terms of the linking function of an interneuron and the patterned activity of bilateral motor neurons (Hagiwara and Watanabe, 1956). Roeder and collaborators (Roeder, 1935, 1937; Roeder et al., 1960) disclosed completely stereotyped movements in the sexual behavior of the praying mantis. A built-in program of the thoracico-abdominal ganglia which control the mating behavior is released when connection to the brain is severed. The flight motor center of the locust (Wilson, 1961; Wilson and Weis-Fogh, 1962) is known to be driven by a general excitatory state and is not phasically driven by any input (Wilson and Wyman, 1965). The lepidopteran flight center appears to operate similarly (Kammer, 1968, 1970; Kammer and Nachtigall, 1973). In agreement with this, motor units firing spontaneously have been reported (Neville, 1963). These studies clearly indicate that physiological investigation of endogenously patterned neural activity is feasible, especially in the insect nervous system. However, none of these cited studies has been conducted in direct relation with genetics.

Insect behavior has attracted the interest of geneticists as well as physiologists. A great deal of work on selection for behavior characteristics has been carried out, especially in *Drosophila,* as shown in a fine review by Ewing and Manning (1967). Those studies, of course, carry their own significance; however, the purpose of the present study is to establish a more direct approach to the gene-behavior relationship. Many genes of *Drosophila melanogaster* have been known to have effects on sexual behavior, e.g., *white* (Reed and Reed, 1950; Petit, 1958; Geer and Green, 1962), *ebony* and *vestigial* (Rendel, 1951), and *yellow* (Bastock, 1956). An effect of gene action upon behavior has been shown, but direct control of the activity pattern cannot be expected in these cases. Elegant work on genetic control of patterned behavior has been done with other insects. Röthenbuhler's work (1964) on the honey bee and von Hörmann-Heck's paper (1957) on the cricket clearly show patterned behavior derived from the action of a single gene. However, the neurophysiological basis for those behaviors has never been investigated. It is clear now that neurophysiological studies of the mechanism underlying genetically coded behaviors will unveil the gene-behavior relationship, and the animal which is suitable for neurophysiological genetics is the insect. Among insects, *Drosophila melanogaster* is the one for which the most extensive genetic information is available and on whom various techniques can be applied.

Hyperkinetic^1, *Hk*^{1}, A MUTANT *Drosophila melanogaster*

The single-gene mutant of *Drosophila melanogaster, Hk*^{1}, satisfies the conditions necessary for the study of neurophysiological genetics as discussed in previous sections. This mutant was found in the *Canton-S* wild-type stock of *Drosophila melanogaster* following treatment with the mutagen ethyl methane sulphonate. Detailed procedures for mutagenesis and controlling the genetic background were described in a previous paper (Ikeda and Kaplan, 1970*a*). During anesthetization by ether, *Hk*^{1} shows a discretely patterned leg-shaking action, while *Canton-S* wild-type flies are completely paralyzed. The behavior of this mutant has been studied by Kaplan and Trout (1969), and the gene was located at 30.9 on the X chromosome. The phenotype was originally described as semi-dominant but has since become recessive.

Ikeda and Kaplan (1969, 1970*a,b,c,* 1971) have investigated the phenotype by means of neurophysiological techniques. The single unit rhythmic discharges of motor neurons which result in the characteristic leg movement were recorded from motor areas of the thoracic ganglion. The rhythmic activity was found to be endogenous within the thoracic ganglion, because the activity remained unchanged even after total deafferentation or complete iso-

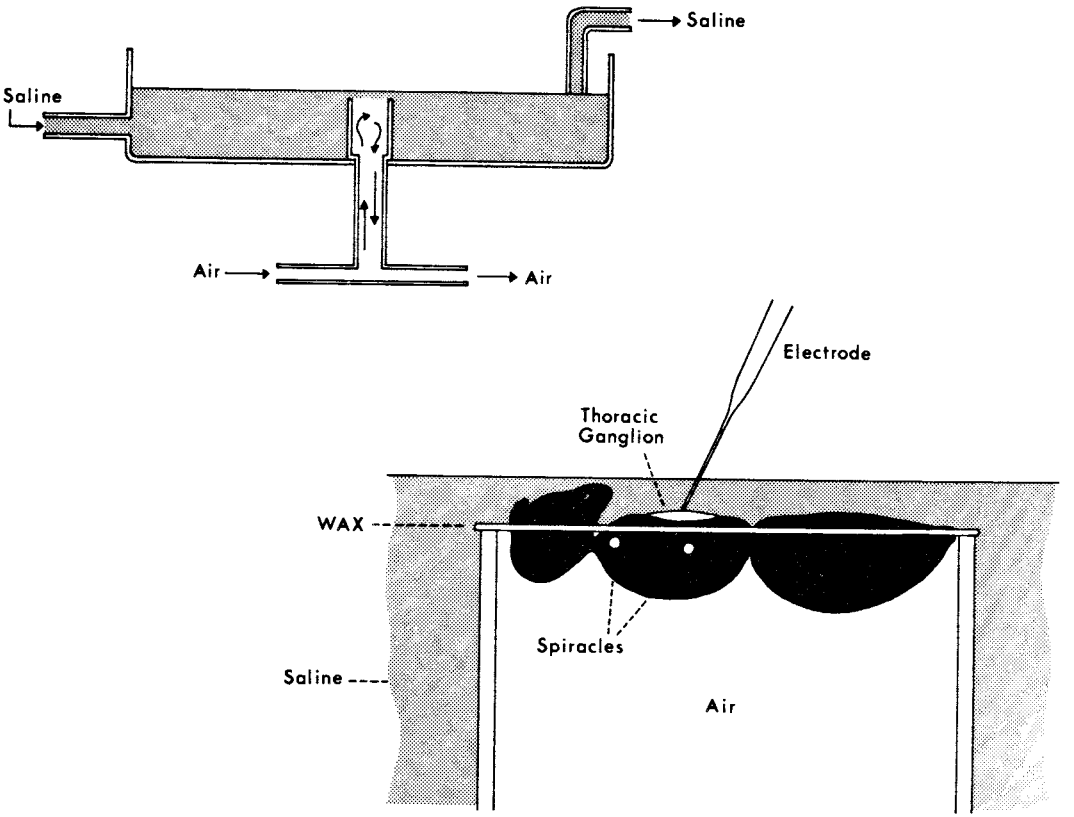

FIG. 1. Mounting *Drosophila* for intracellular recording from a neuron in the thoracic ganglion. The upper drawing shows a perfusion chamber with teflon tubing for the air supply. The fly is mounted ventral side up on the opening of the tube. The lower drawing shows magnified view of the top of the tube with mounted fly.

lation of the thoracic ganglion from the rest of the nervous system. This excludes the possibility that the mutation is acting in some peripheral areas, such as the neuromuscular system, and that positive feedback inflow was elicited by way of a reflex arc. The neurons responsible for the leg-shaking action were located in the three pairs of motor regions in the thoracic ganglion. The corresponding motor neurons of the control fly, *Canton-S* strain, were not active under the same conditions.

As the gene is sex-linked and recessive, the heterozygous female does not show the above activity. Gynandromorphs mosaic for Hk^1 were made in order to record the activity of the female neuron (heterozygous for this gene) and that of the male neuron (hemizygous for the mutant gene) in the same fly. Gynandromorphs which are male on one side and female on the other side of the thoracic ganglion can be obtained. The leg-shaking activity described was obtained on the male side of the gynandromorph, and the activity of both motor and pacemaker-type neurons was recorded. However, no similarly patterned activity was obtained on the female side. This finding eliminates the possibilty of humoral regulation of this specific activity (Ikeda and Kaplan, 1970*b*, *c*). The study of flies with more complicated gynandric patterns revealed that the pattern formation center for each leg operated independently. Thus, the genetic change is autonomous at each center.

THE MUTANT ACTION OF Hk^1 NEURONS

A group of neurons forming a mutant activity center in each leg motor region was

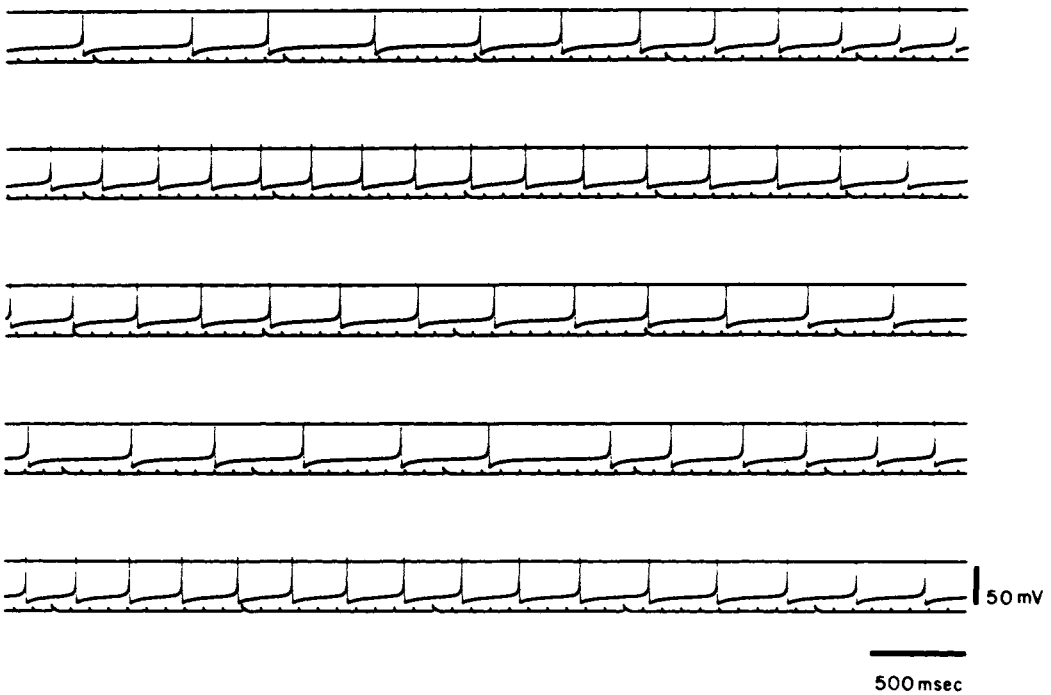

FIG. 2. Discharge pattern recorded intracellularly from a *Type 2* neuron. Right mesothoracic region of 4-day-old homozygous *Hk¹ Drosophila melanogaster* female. Each action potential is preceded by slowly rising depolarization.

found to be composed of several motor neurons, *Type 1,* and at least one pacemaker-type neuron, *Type 2* (Ikeda and Kaplan, 1970*a*). When antidromic stimulation was applied to the leg nerve, the invading spike potential was recorded at the soma of *Type 1* neurons while the *Type 2* neuron showed no response. When a *Type 1* neuron was destroyed after the intracellular recording, the activity of other motor neurons observed extracellularly at the leg nerve remained unchanged. However, when the *Type 2* neuron was destroyed, the motor output specific to the mutant disappeared. These findings suggest that the activity of *Type 1* neurons is controlled by the *Type 2* neuron. It is most likely, therefore, that the *Type 2* neuron is the one which is directly affected by the mutation: It follows that the mechanism underlying the mutant phenotype should be sought in the *Type 2* neuron, and the pattern formation mechanism of the specific motor output should be investigated in the system composed of this neuron and its associated motor neurons.

The present paper will describe the pacemaking activity of the *Type 2* neuron. For the recording of electrical activity, a 4-day-old female homozygous *Hk¹* fly was placed ventral side up in the opening of a small teflon tube (Fig. 1). The space between the fly and the tube was sealed with a thin layer of wax. The ventral half of the fly with the ganglion exposed lay above the film of wax and was covered with saline. The dorsal portion of the fly lay below the wax and was in contact with circulating air. The composition of the saline was as follows: NaCl, 128 mM; KCl, 4.7 mM; $CaCl_2$, 1.8 mM; Na_2HPO_4, 0.74 mM; KH_2PO_4, 0.35mM. As the spiracles were exposed to the air inside the tube, the fly was able to breathe even though the thoracic ganglion was under saline. With air supplied by a respirator, the fly could be kept in good physiological condition for more than 24 hr. When the tube was connected to a 1000-ml flask containing 3.4% v/v diethyl ether, the mutant fly's leg-shaking continued with a specific pattern for more than 24 hr. The *Canton-S*

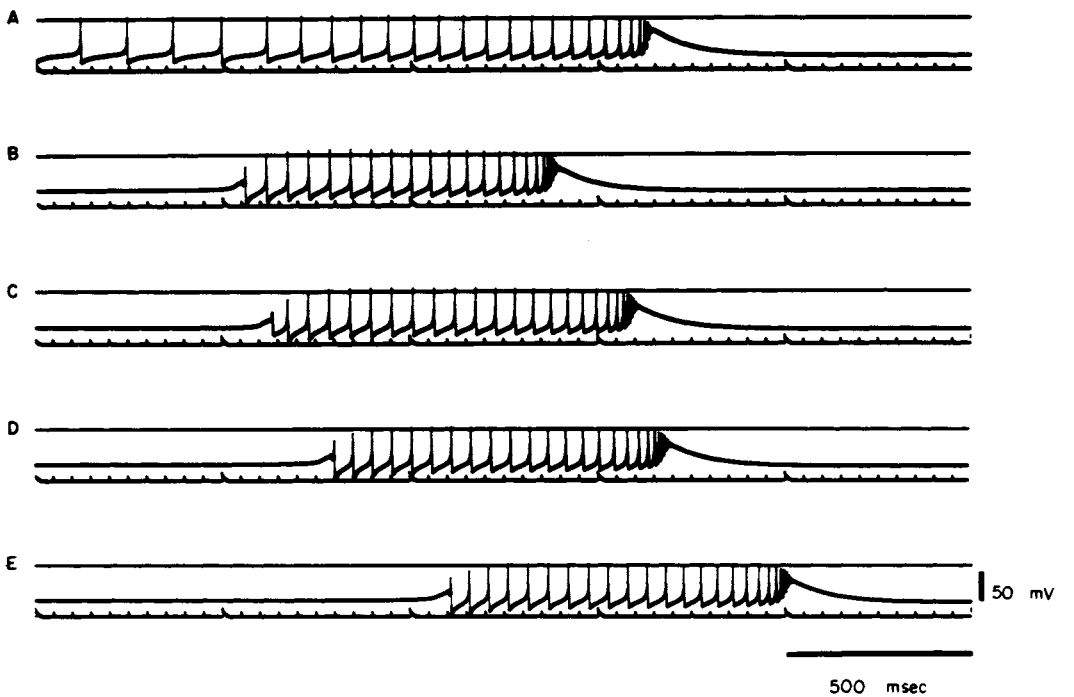

FIG. 3. Shift of discharge pattern from continuous to intermittent. Recorded intracellularly from a *Type 2* neuron in the right prothoracic region of of 4-day-old homozygous *Hk¹ Drosophila melanogaster* female. This neuron has been continuously firing with a pattern similar to the first 500 msec of record *A*. The pattern then shifted to intermittent at record *A*. *B*, *C*, *D*, and *E* are successive bursting discharges following *A*. A silent period of exactly 7 sec was cut off from the original recording between every record here.

control fly, however, was completely paralyzed under the same conditions.

In order to expose the thoracic ganglion ventrally, the prothoracic and mesothoracic legs were removed at their sterno-coxal joints. The mesopreepisternum and the posterior process of proepisternum were then dissected out. After making lateral cuts on pro- and meso-furca, the furca were removed. The ganglion was thus exposed and in contact with the saline solution. The approximate location of the neurons studied has already been mapped in a previous paper (Ikeda and Kaplan, 1970*a*). A glass pipet of small tip diameter was filled with either 3 M KCl or 3 M K-propionate and used for the recording. Electrodes which showed resistance of less than 200 M ohm when filled with 3 M KCl were rejected. The recording electrode was connected to a high impedance amplifier via Ag-AgCl wire immersed in the solution in the pipet. An indifferent Ag-AgCl wire electrode was immersed in a bath of the saline solution. After the operation, the preparation was perfused with the saline solution for 30 min at the rate of 6 cm^3/min. All experiments were performed at temperatures of 23 to 24 C.

A typical activity recorded intracellularly from *Type 2* neurons is shown in Figure 2. Each action potential is preceded by a slowly rising depolarization. The pattern changes from time to time within a certain range. The frequency usually changes in a waxing and waning fashion within the range of 2 to 16 per second. The waxing and waning of frequency occurs every 10 to 20 sec. This pattern is specific to *Hk¹* and matches the leg-shaking pattern obtained separately by Trout and Kaplan (1973). Usually, the discharge continues for more than 10 min with the fluctuation shown in this record, but sometimes the pattern shifts

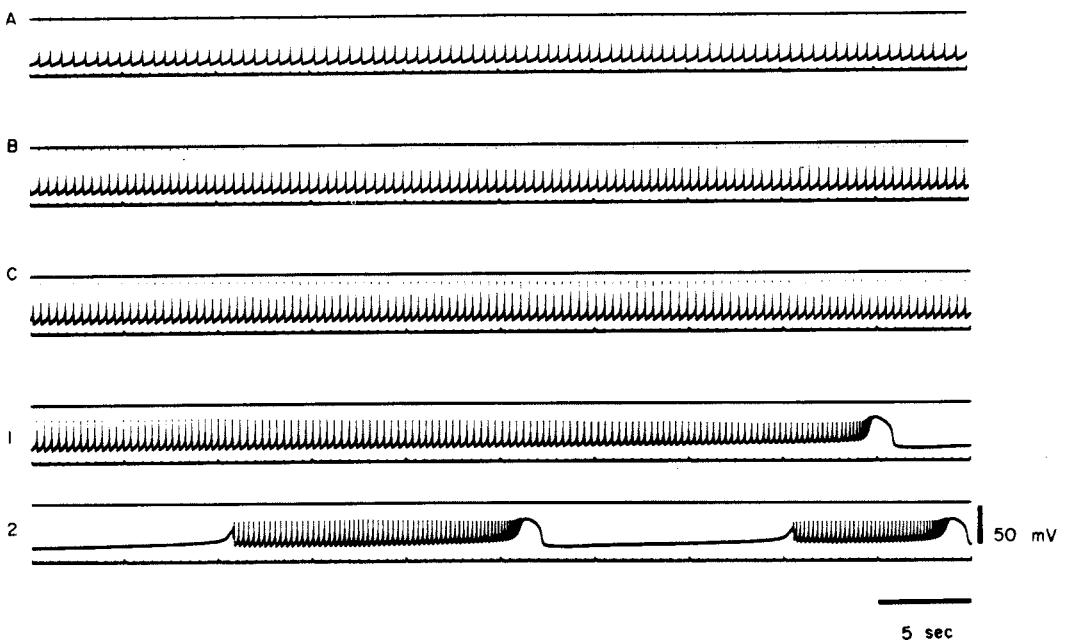

FIG. 4. Shift of discharge pattern from continuous to intermittent. Recorded intracellularly from a *Type 2* neuron in the right mesothoracic region of 4-day-old homozygous *Hk¹ Drosophila melanogaster* female. *A*, *B*, and *C* are continuous recordings. Between *C* and *1*, an exact 3-min period was cut off from the original recording. *1* and *2* are continuous recordings.

gradually to intermittent discharges (Fig. 3). The neuron in this figure had been discharging continuously for about 3 min before the recording shown in Figure 3*A* and then discharged in intermittent bursts. The bursting occurred about every 10 sec (the silent period of exactly 7 sec was cut off between each record). In most cases, the pattern became continuous after several bursts, after which the entire sequence was repeated.

Another example is shown in Figures 4 and 5. In these Figures, *A*, *B*, and *C* are continuous records. An exact 3-min period was cut off from the original record between the end of record *C* and the beginning of record *1* because the change in pattern was small. The records shown from *1* to *8* are continuous. This neuron had been discharging continuously as shown in *A*, *B*, and *C* until it stopped at the end of record *1*. After that, the pattern became intermittent. The discharge of action potentials stopped suddenly, leaving a large generator potential. After repolarization, a slowly rising depolarization occurred and resulted in the next burst (Figs. 4,*2*; 5,*3*; 5,*4*; 5,*5*). As the generator potential became smaller, the number of action potentials and their amplitudes was reduced, and finally only generator potentials remained. The change in the duration of a generator potential was much greater than that of its amplitude. Usually, the generator potential became larger again and extended its duration, after which the pattern shifted to continuous discharges. The entire sequence was repeatable.

The generator potentials occur every 10 to 20 sec, corresponding to the waxing and waning period of the continuously discharging phase, suggesting that the membrane potential change caught here as generator potential is the source of the waxing and waning pattern characteristic of *Hk¹*. A prominent character of this neuron is that action potentials disappear even when the generator potential is quite large. When the activity becomes lower, the amplitude of the action potential becomes smaller and the firing level higher. The recording electrode is most likely in the soma. This

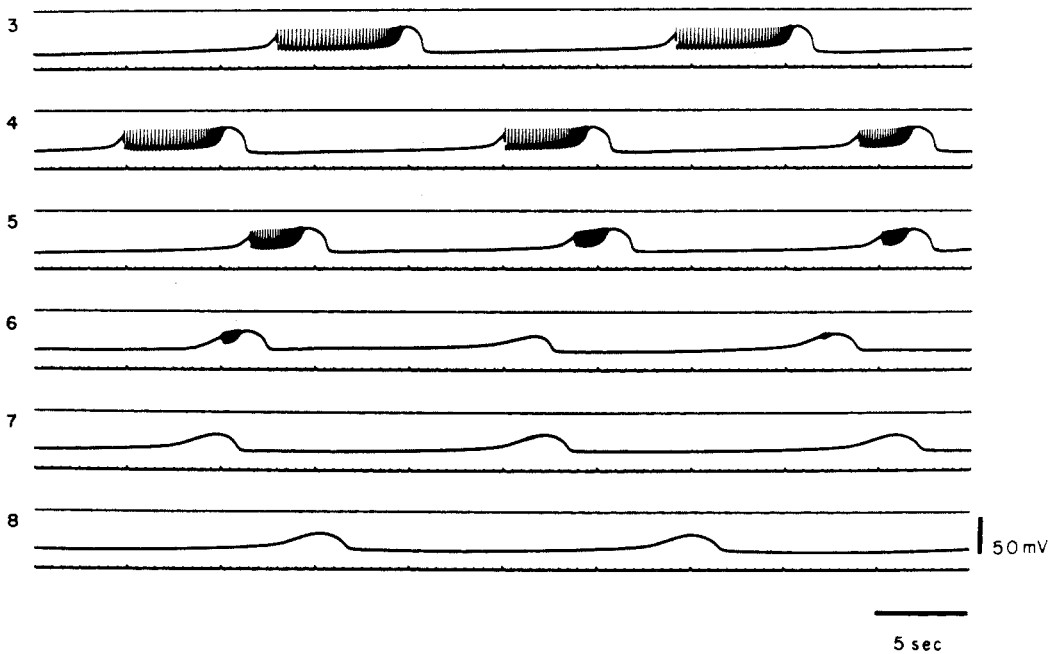

FIG. 5. Shift of dlischarge pattern from intermittent bursts to generator potentials only. Continuous recording following Figure 4. Recorded intracellularly from a *Type 2* neuron in the right mesothoracic region of 4-day-old homozygous *Hk¹ Drosophila melanogaster* female (same neuron and fly as in Fig. 4). All recordings are continuous.

point has not been confirmed, but considerations of the size of the neuron suggest it is unlikely that successful penetration of the electrode can be made into another part of the neuron. Therefore, it is reasonable to assume that the membrane of the soma initiates the generator potential, resulting in the large recorded size, while action potentials appear to be initiated somewhere on the axon away from the soma. The site of the action potential initiation on the axon seems to move away from the soma as the threshold becomes higher, resulting in smaller action potentials and a higher firing level observed at the soma. Thus, besides the amplitude, duration, and periodicity of the generator potential, the shift of the site of action potential initiation along the axon seems to be a factor which determines the pattern. When the initiation site is close to the soma, the pattern can be continuous with fluctuation in frequency depending on the phase of the generator potential. When it is far from the soma, it may become intermittent.

From physiological evidence, it is certain that there are three pairs of centers in the thoracic ganglion which furnish the leg-shaking specific to *Hk¹*. Each center is composed of at least one interneuron which has pacemaking activity and several motor neurons which are driven by the former. Insofar as this specific activity is concerned, each center operates independently, and no communication is detected among the centers. Mosaic material has proved that when a ganglion is composed of a hemizygous part and a heterozygous part, only the centers in the hemizygous part produce the specific activity. Even when a small part including one center is hemizygous and the rest of the thoracic ganglion is heterozygous, the hemizygous center shows the specific activity which results in the leg-shaking. These facts suggest that the mutant phenotype is expressed in a small region including those neurons and that the specific activity is endogenous. Thus, as far as physiological evidence is concerned, the endogenous quality may be limited to a center composed of

at least one interneuron and several motor neurons.

Although observations of the activity of *Type 2* neurons appear to support the contention that the *Type 2* neuron is the pacemaker which directly reflects the genetic change, morphological identification of those neurons has not yet been achieved because of technical difficulties in injecting dye into a neuron with the high resistance electrode required for penetration. This problem should be overcome in the future.

Although the mosiac material having a hemizygous center closely surrounded by heterozygous tissue gives strong evidence that no input to the *Type 2* neurons is necessary to produce this specific activity, it does not completely eliminate the possibility that some other neuron located very close to the *Type 2* neuron might send information to set *Type 2* neuron's rhythmicity. A physiological experiment such as resetting the pace by stimulation of the *Type 2* neuron will provide further information. The best evidence will be given by mosaic material in which only those neurons involved are Hk^1 genotype and they are surrounded by neurons of other genotypes. For this purpose, identification of genotypes at the cellular level is necessary. This point remains for future investigation.

Since the pacemaker neuron, *Type 2*, appears to be directly affected by the Hk^1 gene autonomously expressing the genetically coded activity pattern, it follows that the study of the mechanism underlying the pacemaking activity of this neuron will reveal the finer localization of the genetic change in this neuron. The generator mechanism is dependent on membrane permeability and the ionic-pump mechanism of the neuron. Physiological investigations of this aspect will be the next step. Among various volatile anesthetics so far investigated, chloroform, fluothane, penthrane, and trilene paralyzed Hk^1 flies as well as control *Canton-S* flies without inducing leg-shaking. Those causing leg-shaking action of Hk^1 and complete paralysis of *Canton-S* flies are diethyl ether, ethyl-vinyl ether, and divinyl ether, which indicates the expression of the specific activity is ether-dependent. The mechanism of ether action on this material has not been investigated. However, Hk^1 flies when unetherized do not have disorders in walking, and the shaking appears only when the fly is anesthetized by ether. It may be speculated, therefore, that the specific activity of the *Type 2* of the Hk^1 flies ordinarily is inhibited by some neuron. When this neuron is suppressed by ether, the endogenous activity of the *Type 2* neuron can be released and results in the leg-shaking action. Under the same conditions, the *Canton-S* fly remains paralyzed because it does not have the endogenous activity of *Type 2* neurons.

The present status of research is still immature for discussion of the gene-behavior relationship at the molecular level; however, further physiological investigation of the membrane of *Type 2* neurons will lead the study in that direction.

NEUROPHYSIOLOGIAL GENETICS AS A BASIC SCIENCE

In addition to its great contribution to classical genetics, *Drosphila melanogaster* is now being rediscovered as an outstanding material for neurophysiological genetics because of the availability of many techniques for genetic manipulation. Only a few genetic techniques have been employed in the present study. It is certain that new forms of biology will be developed in the coming decade which may be applied to *Drosophila* genetics. From this point of view, recent work on temperature-sensitive paralysis (Suzuki, 1970), optic response (Hotta and Benzer, 1969; Pak et al., 1969; Alawi and Pak, 1971; Wong et al., 1972), mosaics (Hotta and Benzer, 1970, 1972; Suzuki et al., 1971), sexual behavior (Connolly and Cook, 1973), and cell culture (Seecof et al., 1971, 1972) are of particular interest. The discussion here, however, will be limited to aspects relevant to neurophysiological genetics.

Despite the obvious importance of behavorial genetics, its present status as a basic science is not satisfactory. Mere accumulation of behavioral data on materials of genetics interest can even obscure the *rai-*

son d'être of behavior genetics as a science. Behavior genetics can become a science only when a logical approach to the gene-behavior relationship is established. Provided with proper material, a specific gene and its background can be manipulated and the behavior can be analyzed. It is impossible, however, to relate a gene and the behavior it controls unless a mode for processing of genetic information is unveiled. Numerous steps are involved in the processing, starting from the transcription of DNA molecules to the expression of behavior. Therefore, it is unapproachable unless the processing step performing the most essential decoding for the particular gene action may be studied. This point cannot be handled by classical methods of behavior genetics, and this is the reason that physiology must be introduced into the study. Neurophysiological genetics investigates the processing mechanism of the genetic code by decoding at the neural level. The basic mechanism underlying genetically coded behavior is analyzed in order to disclose the pathway of processing. Previous papers (Ikeda and Kaplan, 1970*a,b,* 1971) and the present work have shown that neurophysiological decoding of genetic information is possible, at least when genetic expression is autonomous within the motor system. The decoded gene action at the neural level hopefully may be traced back at the molecular level to reach the particular gene.

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