ship between the sizes of eye and head movements, thus affecting the vestibulo-ocular reflex. Modification of retinal displacement by magnification or minification goggles leads to rapid adaptation in the vestibular system in humans (*13*), and vestibular-ocular interactions could play some role in the effect we observed. It would be helpful to replicate the minification study while examining another phasic system [middle ear muscle activity (*14*)] to determine (i) whether the effect on eye movements might be generalized to other sensory systems and (ii) whether increasing the amplitude of eye movements while awake would have a similar inverse effect on REM sleep.

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15. The first three subjects' REM sleep EOG's were recorded with a-c coupled amplification. The interaction between a-c high-pass filtering and eye movement velocity artifactually underestimated the amplitudes of REM sleep eye movements because of their reduced velocity. Subsequent trials were recorded by d-c EOG. A test for homogeneity of variance, however, demonstrated no interaction between recording technique and phase or REM period number. Therefore, all six subjects were included in the analyses of variance. Because of the less artifactual condition of d-c amplification, Fig. 1 illustrates only subjects recorded by this technique.
16. We thank D. Barker for help in software development, M. E. Taylor and R. Boys for technical assistance, C. Hamm for aid in statistical analysis, and M. Hirshkowitz for comments. Supported by the Biohumanics Foundation and NIMH grant MH 3414 to J.H.H.

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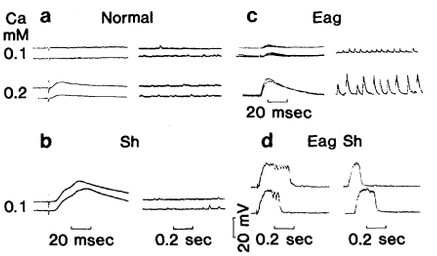
Potassium Currents in *Drosophila*: Different Components Affected by Mutations of Two Genes

Abstract. *Electrophysiological analysis of the* Drosophila *behavioral mutants* Eag *and* Sh *and the double mutant* Eag Sh *indicates that the products of both genes take part in the control of potassium currents in the membranes of both nerve and muscle. In voltage-clamped larval muscle fibers,* Sh *affects the transient A current, whereas* Eag *reduces the delayed rectification and, to a lesser extent, the A current.*

Mutations can be used to perturb excitable membranes to analyze their physiological and developmental processes (*1–4*). In *Drosophila*, analysis of behavioral mutants *Shaker* (*Sh*) suggested defective potassium (K^+) currents (*2, 3*). Voltage-clamp studies in developing flight muscles (*4*) showed that *Sh* affects the transient potassium A current (*5*), I_A, but not the delayed rectification potassium current (*6*), I_K. Therefore, other genes must exist that affect I_K. In addition, with some *Sh* alleles, I_A was absent in developing flight muscles but not mature ones (*4*). We now report on the effect of *Sh* at other developmental stages.

We have found that *Eag*, a mutation previously identified by abnormal leg-shaking behavior (*7*) similar to that of *Sh*, also affects larval neuromuscular transmission. In *Sh* larvae, transmitter release at the neuromuscular junction (*8, 9*) is more readily triggered than in normal (Canton-S) larvae, resulting in excitatory junctional potentials (EJPS) of increased amplitude and duration (*2*). Similarly, in all *Eag* larvae examined ($N > 20$), EJPS



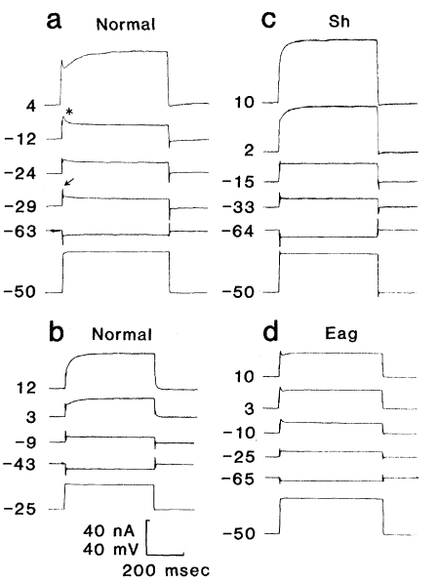


Fig. 1 (left). Excitatory junctional potentials (EJPS) obtained from larval neuromuscular junctions in normal (a), Sh^{KS133} (b), *Eag* (c), and the double mutant *Eag* Sh^{KS133} (d) larvae at the Ca^{2+} concentrations indicated. EJPS were evoked by stimulating the segmental nerve cut close to ganglion (left traces in a to d) or occurred spontaneously in the same preparation without stimulation (right traces). Spontaneous EJPS occurred frequently in *Eag* but only miniature EJPS were seen in normal and Sh^{KS133}. In the double mutant *Eag* Sh^{KS133}, greatly prolonged EJPS occurred both spontaneously and in response to nerve stimulation. Fig. 2 (right). Outward membrane currents in normal (Canton-S) and mutant larval muscles obtained by a conventional two-microelectrode voltage clamp at 15°C in 0 m*M* Ca^{2+} Ringer (*9*). In (a) to (d), the total membrane currents are shown, and a single voltage trace is given below to indicate the time of voltage steps and conditions of membrane voltage control. Numbers to the left of current traces indicate the amplitude of the voltage step in millivolts and those to the left of voltage traces indicate the holding potential, V_H. (a) Normal, $V_H = -50$ mV. The transient I_A (asterisk) and delayed steady I_K can be seen. Capacitive currents (arrow) were reduced by the limited frequency response of the strip-chart recorder. However, I_A and I_K were faithfully reproduced as compared to oscilloscope recordings (not shown). (b) Normal, $V_H = -25$ mV. I_A is inactivated at this V_H. (c) Sh^{KS133}, $V_H = -50$ mV. The I_A is absent. (d) *Eag*, $V_H = -50$ mV. There is a reduction in I_K and I_A.

evoked at low Ca^{2+} concentrations had a greater amplitude than that in normal larvae. However, a striking feature distinguishing *Eag* from both *Sh* and normal is the high frequency of spontaneously occurring EJPS (Fig. 1). These EJPS were similar in time course to those evoked by nerve stimulation and were blocked by tetrodotoxin (not shown), indicating abnormal spontaneous repetitive firing in *Eag* motoneurons.

When *Eag* and *Sh* are combined in a double mutant, there is a strong synergistic effect. Transmitter release in the double mutant (Fig. 1) is prolonged by nearly an order of magnitude compared to either single mutant and these qualitatively different EJPS occur both spontaneously and in response to nerve stimulation. This synergism could result from a combined effect of both *Sh* and *Eag* on membrane repolarization.

This notion can be directly examined in the larval muscle fibers under current clamp conditions (*8, 9*). At 2 m*M* K^+ and 0.8 m*M* Ca^{2+}, regenerative Ca^{2+} potentials are prevented by the outward K^+ currents in normal larval muscles (*10*). Increasing external K^+ concentrations from 2 to 30 m*M* reduces K^+ currents, and regenerative Ca^{2+} potentials can then be evoked (not shown). In contrast, regenerative Ca^{2+} potentials can be evoked in both *Sh* and *Eag* larval muscles by depolarizing current pulses at 2 m*M* K^+ (not shown). These results suggest that *Eag*, like *Sh*, disrupts membrane repolarization in both nerve and muscle.

To further analyze the effects of the mutations on membrane currents, conventional two-microelectrode voltage-clamp experiments (*11*) were performed on the larval muscles (*9*). In normal (Canton-S) larvae, as in the adult (*4*), there are an inward Ca^{2+} current and outward K^+ currents. The Ca^{2+} current can be eliminated if Ca^{2+}-free saline is used (*9*). The remaining outward current has a fast transient phase followed by a delayed steady phase (Fig. 2a). These currents are most likely carried by K^+, as judged by the reversal potential (~ −45 mV) and the fact that both phases are sensitive to the K^+ channel blocker tetraethylammonium (30 m*M*). The two components of K^+ current can be separated by their difference in voltage threshold and inactivation properties. Stepping the membrane potential to about −25 mV from a hyperpolarizing holding potential (−50 mV) activates the transient component (Fig. 2a). The steady component is only initiated at a more depolarized level (about −15 mV). Because of this difference in threshold and the rapid inactivation kinetics of the transient component, at holding potentials above −30 mV only the steady component is induced by depolarizing steps (Fig. 2b). The two components apparently correspond to the transient I_A and the delayed I_K described in adult flight muscles (*4*). However, the threshold of both components in the adult is about −40 mV, and separation of the currents was based on the difference in developmental onset and pharmacological sensitivity (*4*).

In larval muscles, as in the developing flight muscles (*4*), Sh^{KS133} completely eliminates I_A, and only leakage current is present before I_K is activated (Figs. 2c and 3b). However, I_K is apparently normal, as indicated by the pooled current-voltage (*I-V*) relations (Fig. 3a) of the active currents (*12*), the reversal potentials, and kinetics of current tails (not shown). Sh^{102}, a different allele, has an effect similar to Sh^{KS133}, while two other alleles (Sh^5 and Sh^{rKO120}) reduce I_A but do not eliminate it (*13*). Whereas I_A eventually appears in the mature flight muscles of Sh^{KS133} adults (*4*), it does not do so in fully developed larval muscles, suggesting the possibility of different genetic control of I_A in these developmentally distinct muscles.

The *I-V* relation from pooled voltage-clamp data of *Eag* shows that this allele reduces the amplitude of I_K but does not eliminate it (Figs. 2d and 3a). Other parameters such as the reversal potential and kinetics of current tails appear normal (not shown). However, the *I-V* relations of I_A (Fig. 3b) suggest that *Eag* also reduces I_A but to a lesser extent than I_K (*14*). The effects of *Eag* and Sh^{KS133} on K^+ currents is additive in the double mutant. Therefore, their strong synergistic effect on EJPS (Fig. 1) presumably derives from nonlinear effects of altered K^+ currents on subsequent events. The effect of *Eag* suggests that a normal allele controls the synthesis or processing of I_K channels and perhaps I_A channels as well. Voltage-clamp experiments to examine Ca^{2+} channels did not indicate differences from normal. However, we cannot completely exclude the possibility that other channels are affected.

The genetic control of K^+ currents may be complex since several types of K^+ channels are known which may vary in distribution among different cell types (*15*) at different developmental stages.

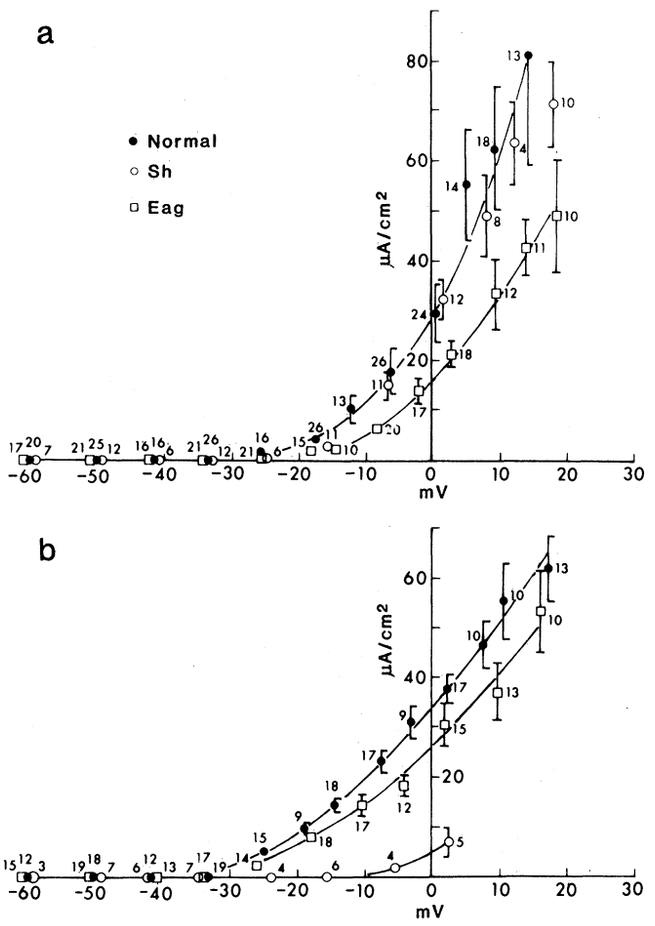


Fig. 3. (a) Current-voltage relations of I_K measured at the steady state in normal (●), Sh^{KS133} (○), and *Eag* (□) larval muscles at $V_H = -50$ mV. The active current density (mean ± S.E.M.) is determined from the number of fibers indicated (*12*). For each point, there is a small variation in the amplitudes of voltage steps around the given membrane potential (within ± 2 mV). I_K is not significantly different from normal in Sh^{KS133} but is significantly decreased in *Eag*. (b) Outward active current (*12*) at peak of I_A. Other conditions as in (a). I_A appears in *Eag* but is entirely missing in Sh^{KS133}. For Sh^{KS133} the active currents at 20 msec after the depolarizing step (time to peak I_A in normal) are plotted. The active current at this time is significant only at membrane voltages above 0 mV because of the rapid rise of I_K (not plotted).

Our results demonstrate that at least two genes, *Sh* and *Eag*, are involved in this control. It is likely that additional genes controlling the K^+ currents remain to be identified. Finally it should be noted that, although *Sh* and *Eag* affect both nerve and muscle, the K^+ currents in *Drosophila* nerve remain to be analyzed. The parameters of these currents and their alterations in the mutants may differ in detail from those in muscle.

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Hybrid Tree Frogs: Vocalizations of Males and Selective Phonotaxis of Females

Abstract. *Male hybrids of reciprocal crosses between gray and pinewoods tree frogs* (Hyla chrysoscelis × H. femoralis) *that were raised to sexual maturity in the laboratory produced distinctive vocalizations. Hybrid females preferred the calls of hybrids to calls of gray tree frogs and also chose synthetic calls with a pulse repetition rate typical of the hybrids in preference to calls with a rate typical of pinewoods tree frogs.*

Information about the extent to which sound production and recognition are matched or coordinated in interspecific hybrids may help to elucidate the mechanisms and evolution of animal communication. Most studies of the acoustic behavior of F_1 hybrids have dealt with the analysis of sound production (*1*), and data on the selective responsiveness of hybrids to acoustic signals have been available only for insects (*2*, *3*). We report that female hybrids between two species of tree frogs preferred the calls of male hybrids to those of one of the parental species. Females also discriminated between synthetic calls that differed in a temporal property thought to be critical for signal recognition; they preferred a stimulus typical of calls produced by hybrids.

Reciprocal hybrids between the gray tree frog (*Hyla chrysoscelis*) and the pinewoods tree frog (*H. femoralis*) were obtained by switching the males of mating pairs before oviposition began. The rates of fertilization and survival to sexual maturity were high, and males first began calling 7.5 months later. Sounds of male hybrids were recorded in a semianechoic, temperature-regulated chamber; selective phonotaxis of female hybrids was tested in two-speaker playback experiments conducted at 23° to 25°C in the same chamber (*4*).

Vocalizations of the parental species have been characterized (*5*). Males of *H. chrysoscelis* produce discrete trains of sound pulses. The pulse repetition rate is stereotyped and averages 44 sec^{-1} in populations in eastern Georgia at about 24°C. Males of *H. femoralis* produce pulses in an irregular, often continuous fashion for several minutes; the pulse repetition rate varies between 6 and 12

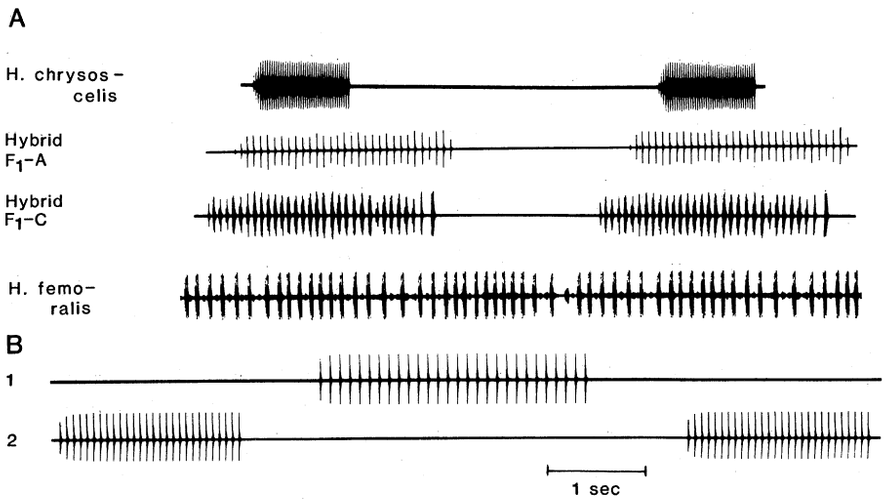

Fig. 1. (A) Oscillograms of representative vocalizations of *Hyla chrysoscelis*, *H. femoralis*, and F_1 hybrids recorded at 23° to 25°C. These calls were used in playback experiments with female hybrids. The calls of the parental species were recorded (tape recorder, Nagra or Uher; microphone, Sony C-22 or Sennheiser 815) in eastern Georgia, the region from which the parents of the hybrids were collected. Hybrid calls were categorized into three types. F_1-A calls had shorter pulses than F_1-C calls; F_1-B calls (not illustrated) had both short and long pulses (*4*). Only part of the vocalization of *H. femoralis* is shown; males of this species often call more or less continuously; *H. chrysoscelis* and hybrids, by contrast, organize pulses into discrete trains such as those shown. (B) Oscillograms of synthetic calls modeled after the F_1-A hybrid call. The two acoustic stimuli differed only in pulse repetition rate, duty cycle (ratio of pulse duration to pulse period), and call duration. The pulse repetition rates were chosen to approximate the mean rates of *H. femoralis* (oscillogram 1, 8 sec^{-1}) and F_1 hybrids (oscillogram 2, 15 sec^{-1}) at 24° to 25°C. The timing of the two calls during presentation to female hybrids was as shown. Each was recorded on and played back from a separate track of a stereophonic recorder (Nagra IV-S). When the "fast" root-mean-square sound pressure level (SPL) of the two stimuli was equalized, the discrepancy in their peak SPL was only 2 decibels.