sodium channels in excitable membranes. However, our results suggest an effect at radiation doses one to two orders of magnitude lower than those used in voltage-clamp analysis. In addition to noting differences in the target tissue used, it is necessary to observe that the different types of radiation employed may vary in their relative biological effectiveness. However, preliminary studies in this laboratory with gamma-irradiation suggest a dose-dependent effect on sodium channel function similar to that produced by high-energy electrons.

A radiation effect such as we observed indicates that the mammalian CNS is more sensitive to single sublethal doses of radiation than is generally thought. If this is the case, then a radiation-induced disruption of a fundamental CNS process is possible that could affect individuals at far lower doses than previously believed, perhaps even at doses commonly used for therapeutic purposes. Further studies of discrete receptor sites in the sodium channel may provide additional information on the mechanism by which radiation exerts its effect and on the biological significance of these findings.

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10. Use of this crude synaptosomal suspension for the sodium uptake assay led to no loss of sensitivity compared to highly purified synaptosomal preparations and was substantially more convenient.
11. Freshly prepared synaptosomes that had been suspended in the buffer solution were placed in glass test tubes (4 ml per tube). The tubes were placed in a Plexiglas holder filled with ice water for irradiation by electrons accelerated to an energy of 18 MeV at 0.55 A by the linear accelerator of the Armed Forces Radiobiology Research Institute. Pulses were delivered at a rate of 15 per second (pulse duration, 4 μsec). Dosimetry was performed with tissue-equivalent ion chambers (volume, 0.05 cm^3) placed in empty tubes. The calibration of the ion chambers is traceable to the National Bureau of Standards.
12. Various concentrations of veratridine were added to portions of the irradiated synaptosomal suspension and allowed to incubate for 10 minutes at 36°C. At this time the suspension was diluted again with a solution containing the same toxin concentrations plus 5.4 m*M* KCl, 0.8 m*M* $MgSO_4$, 5.5 m*M* glucose, 50 m*M* Hepes-tris (*p*H 7.4), 128 m*M* choline chloride, 2.66 m*M* NaCl, 5 m*M* ouabain, and 4 μCi of carrier-free [^{22}Na]Cl (New England Nuclear, 9.358 mCi/ml) per milliliter. After a 10-second incubation the reaction was terminated by adding 3 ml of an ice-cold wash solution of 163 m*M* choline chloride, 0.8 m*M* $MgSO_4$, 1.8 m*M* $CaCl_2$, 5 m*M* Hepes-tris (*p*H 7.4), and bovine serum albumin (1 mg/ml). The mixture was then rapidly filtered under vacuum through a Millipore HAWP or HAMK cellulose filter of 0.45-μm mesh. The filter was washed twice with 3-ml portions of the wash solution, and the radioactivity of the resulting sample was measured with a Packard Autogamma scintillation counter. For determination of specific veratridine-stimulated uptake, nonspecific uptake (in the presence of 1 μ*M* tetrodotoxin) was subtracted from the total uptake with veratridine. Initial results showed that the concentration of tissue used was not saturating. The time course of ^{22}Na uptake under these conditions indicated that uptake was linear until 15 seconds after the addition of the radioactive solution.
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Modifying Oculomotor Activity in Awake Subjects Increases the Amplitude of Eye Movements During REM Sleep

Abstract. *The eye movements of human subjects were experimentally modified while they were awake to determine the effect of waking experience on electrooculographic activity during rapid eye movement (REM) sleep. After normal eye movements were monitored under controlled conditions, subjects spent 5 days wearing goggles that contained minification lenses and that curtailed vision to a 5° field. The amplitude and frequency of eye movements decreased when subjects were awake and increased during REM sleep; sleep stage durations and distributions were unaffected. Values returned to normal in the first 24 hours of recovery.*

Theories of the role of rapid eye movement (REM) sleep in the biological economy of mammals frequently focus principally on the spontaneous appearance of oculomotor activity (*1*) and related central nervous system (CNS) excitation (*2*) that occur in this state. Various theories share the premise that the organism benefits from the activation of the visual system during REM sleep: binocular stereopsis is improved (*3*), perceptual motor learning is integrated (*4*), or the ontogenetic development of the CNS is enhanced by endogenous neural stimulation (*5*).

Little is understood of how activity within the visual system during REM sleep is related to waking visual and oculomotor activity (*6*). Similarly, although studies of interactions between the waking state and REM dreaming have shown that meaningful waking experiences (*7*) and recent perceptual activity (*8*) can influence dream content, the regulatory mechanisms by which material is incorporated into dreams remain elusive. Does the phasic event system of the REM state discharge principally under the influence of genetotrophic factors (*9*), or might daily waking experiences affect patterns of neuromuscular activation within the REM state as they affect dreams? If REM sleep is somehow functionally related to behavior of the visual system during the waking state, it should be possible to demonstrate behavioral or physiological interactions within the visual system across these two states. Such interactions might be observed through attributes of dream content or in terms of the characteristics of REM sleep eye movements. We measured the frequency and amplitude of eye movements while subjects were awake and when they were in REM sleep; we now report that phasic events characterizing REM sleep are influenced by experimentally induced modifications of visuomotor behavior while awake.

Six volunteer subjects, 19 to 30 years old, with normal visual acuity, depth perception, and orthophoria continuously participated in a 12-day study. Each subject underwent 2 weeks of home sleep normalization and three laboratory adaptation nights prior to 12 consecutive 24-hour periods in the experiment. During the 12 live-in days (four baseline, five experimental, and three recovery), electrooculographic (EOG) activity was recorded daily with d-c amplification while the subject was awake. REM sleep eye

movements were continuously taped and converted to digital form. We telemetrically recorded the same standardized activities (such as reading or paddle ball) at the same time of day in all experimental phases under free-moving conditions to provide samples of normative ocular activity.

Throughout all waking moments of the experimental phase, subjects wore ophthalmological frames fitted with opaque diaphragms containing 5° apertures. Within each aperture was a reduction lens, oriented straight ahead, that permitted a 30° view in the 5° field. Proper lens alignment permitted binocular convergence from 1 m to infinity. Side shields blocked light from extraaperture sources. Electrodes located immediately adjacent to one eye in horizontal and vertical pairs permitted the recording of two orthogonal d-c EOG channels. A repeated calibration procedure utilizing eye movements of known direction and amplitude enabled us to generate equivalencies between voltage and degrees of visual angle. Calibrations were conducted in normal room illumination at the beginning and end of each daytime recording session and were repeated, after 20 minutes of dark adaptation, in near total darkness before and after each night of sleep.

Eye movement amplitude, the principal dependent variable, was analyzed with separate repeated measures analyses of variance for sleeping and waking data. The mean eye movement amplitude for each REM period during baseline was smaller than that during the experimental phase [$F(1, 5) = 13.68$, $P < .025$] but not significantly different from that during recovery (Fig. 1). Further analysis of the main effect indicated that experimental first, second, and third REM periods differed significantly from baseline, but that fourth REM periods did not. The influence of the minification goggles on amplitude was greatest in REM periods early in the night as indicated by a phase-by-REM-period interaction effect [$F(1, 5) = 16.62$, $P < .025$] (Fig. 1).

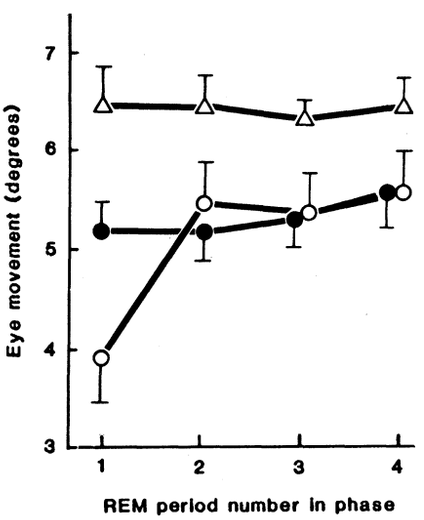

Fig. 1. Eye movement amplitudes during consecutive REM periods of the night and during the three phases of the study (*15*). Amplitude increased in successive nocturnal REM periods in both baseline (●) and recovery (○) conditions. Amplitudes during experimental (△) condition are higher and more constant. Statistically, the strongest effect was in early night REM periods. The first REM period of the recovery phase showed lowered amplitudes, indicating a compensatory aftereffect. Vertical bars represent standard error of the mean.

In each of the six subjects, the eye movement amplitudes of REM sleep increased during the experimental phase, the magnitude of the increase being similar to that of the decrease effected by the goggles when the subjects were awake. Figure 2 pairs individual waking state and REM sleep mean values to facilitate comparisons within 24-hour cycles and across phases. The frequency of eye movements (per minute of REM sleep) increased from baseline to the experimental phase [$F(1, 5) = 17.5$, $P < .025$] but did not interact with REM period number. The only significant comparison between baseline and recovery was a decrease in the amplitude of eye movements in the first REM period of the recovery phase [$F(1, 3) = 35.63$, $P < .025$] (Fig. 2).

There was no significant difference among mean number of minutes in each sleep stage. Neither REM latency nor REM percentage differed significantly from phase to phase. Thus, the effect of the minification goggles on number and frequency of eye movements during sleep occurred in the absence of a parallel effect on the proportion of sleep occupied by any of the stages. The mean value for eye movement amplitudes while awake decreased by about 25 percent in the experimental phase (Fig. 2). Both amplitude and frequency of eye movements during the waking state decreased from baseline to experimental conditions [$F(1, 3) = 37.50$, $P < .025$].

Augmentation of REM sleep components (phasic, tonic, or both) in response to perceptual manipulations while awake could be consistent with learning (*10*), programming (*4*), or perceptual adaptation theories (*11*) of the function of this state. None of these hypotheses, however, specifically predict that waking experience would change the properties of eye movements in the absence of a corresponding effect on the proportion of REM sleep. When subjects continuously wear goggles containing wave band–specific red filters, the greatest alteration of dreamed colors occurs in awakenings from REM periods early in the night (*8*). Accordingly, the greatest effect of daytime alterations of the visual system found in investigations of both dream content and REM eye movement seems to occur in the early REM sleep. In the dream-content study, however, dreamed colors mimicked the effect of the red filters, unlike the inverse effect on amplitude and frequency of eye movements during REM sleep that results from the minification goggles.

The responsiveness of the phasic event system of REM sleep to waking oculomotor manipulations implies plastic capabilities within the system and a physiological interaction between REM sleep and the waking state. Our evidence suggests an inverse physiological influence of waking experience on REM sleep that is specific to the oculomotor system. In a finding similar to ours, Berger (*12*) conditioned monkeys to lower the frequency of their eye movements during the day; he observed an increase in frequency during REM sleep. He also observed increases in CNS visual system monophasic waves. Thus, Berger's manipulation of frequency and our modification of amplitude produced similar inverse effects on oculomotor activity during REM sleep.

Minification lenses alter the relation-

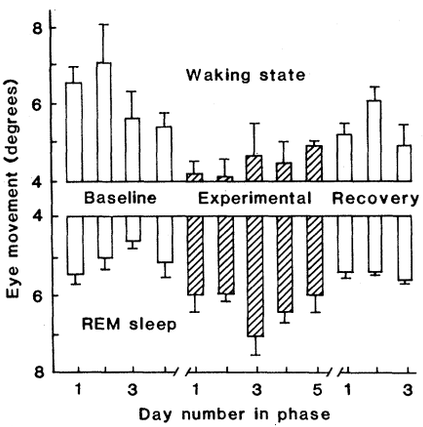

Fig. 2. Eye movement amplitude during the waking state and during REM sleep in three subjects. Waking values were obtained during 20 minutes of recording controlled activity; tasks remained constant throughout baseline, experimental, and recovery phases. Epochs with muscle or high-frequency artifact were eliminated. Sections of REM sleep interrupted by body movements or brief arousal were also removed. Amplitude during waking decreased as REM sleep amplitude increased.

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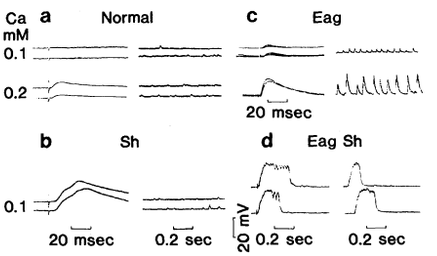
Potassium Currents in *Drosophila*: Different Components Affected by Mutations of Two Genes

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



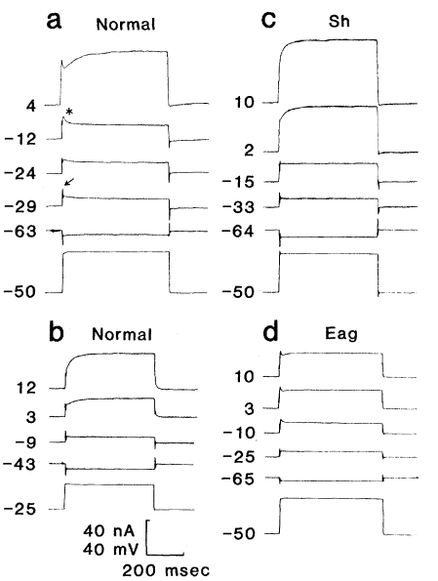


Fig. 1 (left). Excitatory junctional potentials (EJPS) obtained from larval neuromuscular junctions in normal (a), Sh^{KS133} (b), *Eag* (c), and the double mutant *Eag* Sh^{KS133} (d) larvae at the Ca^{2+} concentrations indicated. EJPS were evoked by stimulating the segmental nerve cut close to ganglion (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.