mus-101$^+$ as well as two other *mus* loci (*mus-105*$^+$ and *mus-109*$^+$) (*6*) encode essential functions indicate that a substantial fraction of eukaryotic mutagen-sensitive and repair-defective mutants may be leaky mutants in essential loci.

The cytological analysis of *mus-101* mutants demonstrates that the condensation of euchromatic and heterochromatic portions of the genome is, at least in part, under separate genetic control. That other functions required for mitotic contraction of both euchromatin and heterochromatin can be identified is indicated by a cytological screening of a large collection of lethal mutants, which has led to the isolation of six other mutants affecting chromosome condensation (*8*). Thus, a cytogenetic approach to identifying genes whose products are responsible for the condensation and longitudinal differentiation of chromosomes is feasible. Mutations in these genes should provide probes that will aid in deciphering the organization and function of the eukaryotic chromosome.

MAURIZIO GATTI
Dipartimento di Genetica e Biologia Molecolare, Università di Roma, Piazzale A. Moro 5, 00185 Rome, Italy

DAVID A. SMITH
BRUCE S. BAKER
Biology Department, University of California at San Diego, La Jolla 92093

References and Notes

1. "Chromatin," in *Cold Spring Harbor Symp. Quant. Biol.* **42**, part 1 (1978).
2. J. R. Pringle and L. H. Hartwell, in *The Molecular Biology of the Yeast Saccharomyces*, J. N. Strathern, E. W. Jones, J. R. Broach, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1981), pp. 97–142; G. Simchen, *Annu. Rev. Genet.* **12**, 161 (1978).
3. B. S. Baker, A. T. C. Carpenter, P. Ripoll, *Genetics* **90**, 531 (1978).
4. B. S. Baker and D. A. Smith, *ibid.* **92**, 833 (1979).
5. M. Gatti, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1377 (1979).
6. B. S. Baker, D. A. Smith, M. Gatti, *ibid.* **79**, 1205 (1982).
7. D. A. Smith, B. S. Baker, M. Gatti, in preparation.
8. B. S. Baker, M. Gatti, D. A. Smith, in preparation.
9. J. B. Boyd, M. D. Golino, T. D. Nguyen, M. M. Green, *Genetics* **84**, 485 (1976); T. D. Nguyen, J. B. Boyd, M. M. Green, *Mutat. Res.* **63**, 67 (1979).
10. J. B. Boyd and R. B. Setlow, *Genetics* **84**, 507 (1976); T. C. Brown and J. B. Boyd, *Mol. Gen. Genet.* **183**, 356 (1981); *ibid.*, p. 363.
11. J. M. Mason, M. M. Green, K. E. Smith, J. B. Boyd, *Mutat. Res.* **81**, 329 (1981).
12. M. Gatti, C. Tanzarella, G. Olivieri, *Genetics* **77**, 701 (1974).
13. S. Pimpinelli, M. Gatti, A. DeMarco, *Nature (London)* **256**, 335 (1975).
14. M. Gatti, S. Pimpinelli, B. S. Baker, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1575 (1980).
15. J. R. Merriam, *Drosophila Inf. Serv.* **43**, 64 (1968).
16. Supported by Euratom grants BIO-E-400 and BIO-E-450, by Public Health Service grant GM23345, by Italy–United States cooperative research grant INT-8203086, and by NATO grant 134.82.

10 March 1983

Inhibitory Conductance Changes at Synapses in the Lamprey Brainstem

Abstract. *Although the conductance and kinetic behavior of inhibitory synaptic channels have been studied in a number of nerve and muscle cells, there has been little if any detailed study of such channels at synapses in the vertebrate central nervous system or of the relation of such channels to natural synaptic events. In the experiments reported here, current noise measurements were used to obtain such information at synapses on Müller cells in the lamprey brainstem. Application of glycine to the cells activated synaptic channels with large conductances and relaxation time constants (70 picosiemens and 33 milliseconds, respectively, at 3° to 10°C). Spontaneous inhibitory synaptic currents had a mean conductance of 107 nanosiemens and decayed with the same time constant. In addition, the glycine responses and the spontaneous currents had the same reversal potential and both were abolished by strychnine. These results support the idea that glycine is the natural inhibitory transmitter at these synapses and suggest that one quantum of transmitter activates about 1500 elementary conductance channels.*

The characteristics of channels activated by various putative inhibitory synaptic transmitter substances have been studied in a number of nerve and muscle cells (*1*, *2*). However, except for a study of inhibitory voltage noise (*3*), we know of no detailed analysis of such channels at synapses in the vertebrate central nervous system. Conductance changes underlying inhibitory potentials at such synapses tend to be large and of long duration (*4*), and it would be of interest to know whether these properties reflect the characteristics of individual inhibitory channels. In addition, the characteristics of drug-activated channels can indicate whether the drug under consideration is the natural inhibitory neurotransmitter. Specifically, in addition to having the same pharmacological properties as the natural transmitter, the drug should activate channels with the same reversal potential as the naturally occurring inhibitory events and with compatible kinetic characteristics. Glycine has been shown to increase Cl^- conductance in neurons of the lamprey central nervous system and to mimic the natural inhibitory transmitter pharmacologically (*5*). We now report that in brainstem reticu-

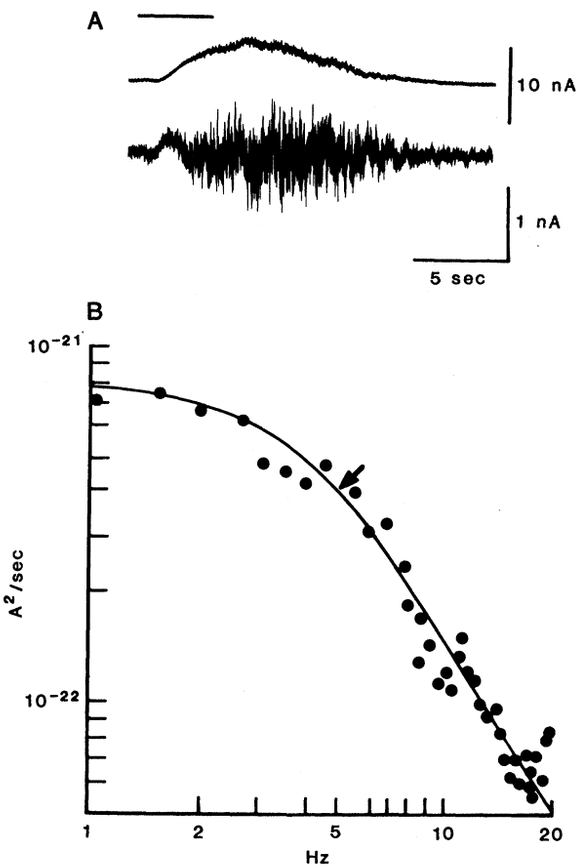

Fig. 1. (A) Current noise produced by a brief exposure of a Müller cell to glycine. Drug application (bar) produced a peak outward current of 5.6 nA (upper trace). The accompanying increase in baseline noise (lower trace) was obtained at a higher gain through an a-c amplifier with a bandpass response of 0.75 to 140 Hz (half-power points). (B) Spectral density distribution from the same cell as in (A). Data points represent the mean of 16 difference spectra, smoothed by the weighted averaging of adjacent points in groups of five. Smooth curve of the form $S(f) = S(0)[1 + (f/f_c)^2]^{-1}$ was fitted by eye with $S(0) = 8 \times 10^{-22}$ A^2/sec and $f_c = 5$ Hz. Holding potential, reversal potential, and mean current were −60 mV, −70 mV, and 6.6 nA, respectively. Calculated channel conductance was 95 pS and mean open time 31.8 msec (3°C).

lospinal neurons (Müller cells), glycine opens channels with the same relaxation time constant and reversal potential as spontaneously occurring inhibitory postsynaptic currents (IPSC's). In addition, the spontaneous inhibitory events appear to result from the synchronous activation of about 1500 elementary conductance channels, suggesting that they are due to the presynaptic release of single quanta of transmitter.

Isolated brains from adult lampreys (*Petromyzon marinus* or *Lampetra lamottenii* anesthetized with tricaine methylsulfonate at 0.5 g/ml) were perfused with oxygenated saline containing 104.5 m*M* NaCl, 2.0 m*M* KCl, 5.0 m*M* $CaCl_2$, 5.0 m*M* $MgCl_2$, 4.0 m*M* glucose, and 2.0 m*M* Hepes, and adjusted to *p*H 7.4. To measure current noise, we added 1.0 μ*M* tetrodotoxin and 1.0 m*M* 4-aminopyridine to the solution to abolish spontaneous synaptic activity and reduce delayed rectification. The perfusate was cooled to about 4°C in most experiments; a few experiments were done at 7° to 10°C. Müller cells on the floor of the fourth ventricle were impaled with two electrodes for voltage clamping. Both the voltage and current electrodes were filled with 3*M* potassium acetate and had resistances of 20 to 30 megohms. The voltage clamp amplifier had a bandpass response of 0 to 1.8 kHz and a gain variable between 5,000 and 10,000. Theoretical clamp efficiency was greater than 98 percent at all relevant frequencies, and no loss of voltage control was detected during drug application or spontaneous synaptic events. The latter appeared to be effectively space-clamped as judged from their smooth exponential decay and from a lack of correlation between their amplitudes and decay time constants. Glycine was applied to the cells near the base of the dendrites by iontophoresis or by pressure pulses from a third pipette filled with a 0.2 to 1.0*M* solution of the drug. Drug application produced membrane currents in the voltage-clamped cells which were then amplified by a low-gain d-c and a higher gain a-c amplifier and stored on two channels of an FM tape recorder for subsequent analysis. The membrane potentials of the cells were clamped 10 to 20 mV from the reversal potential for the glycine response in either the hyperpolarized or depolarized direction.

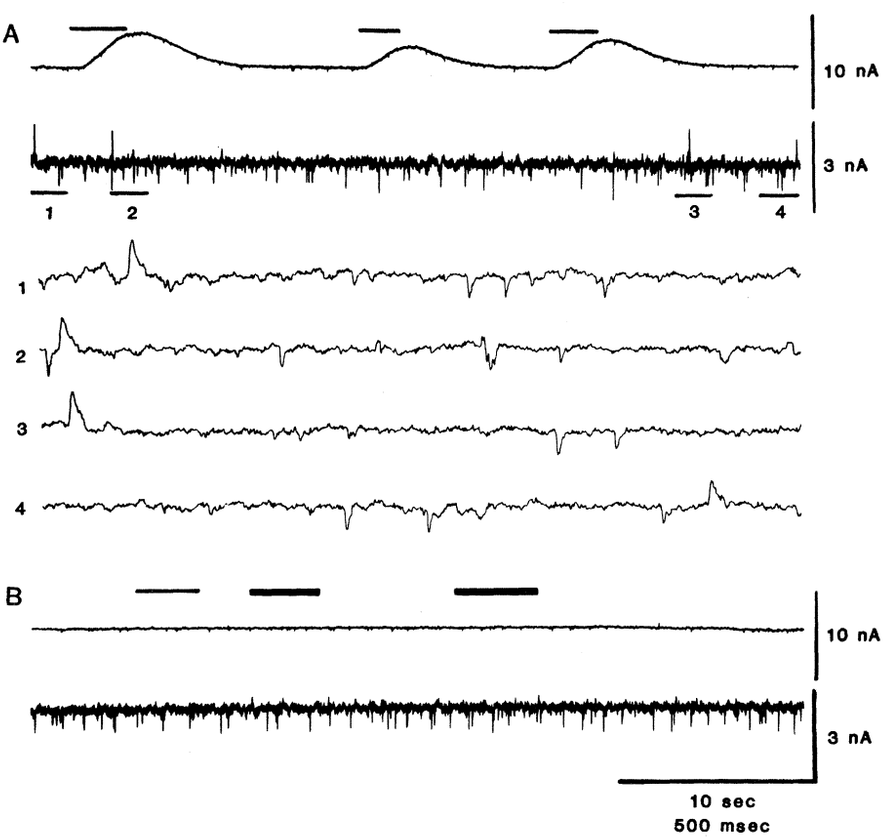

Fig. 2. Effect of strychnine on glycine responses and spontaneous synaptic activity. The upper trace in (A) shows the outward current produced by three applications of glycine (bars) at a pressure of 1 lb/in^2. The second trace shows the higher gain (a-c) record of baseline activity, illustrating spontaneous excitatory (downward) and inhibitory (upward) currents. Indicated segments are expanded in the four subsequent traces to show the time course of IPSC's. (B) After strychnine (20 μ*M*) was added to the bathing solution, glycine application at 1 lb/in^2 (thin bar) and then 10 lb/in^2 (thicker bars) failed to produce any inhibitory current (upper trace), and IPSC's were abolished (lower trace). Holding potential, −55 mV, reversal potential, −65 mV (5°C). The 500-msec time calibration applies to the expanded traces in (A); the 10-second scale applies to the remainder.

Application of glycine produced a maximum outward current of about 6 nA (upper trace in Fig. 1A) and a marked increase in baseline noise (lower trace). More prolonged records with steady d-c levels were used to obtain the spectral density distribution for the same cell (Fig. 1B). Distributions were calculated as the Fourier transforms of 1024 point samples from the experimental records digitized at the rate of 512 or 1024 points per second. Spectra of baseline fluctuations immediately before drug application were subtracted from those obtained during the response and 8 to 24 such difference spectra were averaged to obtain the final distribution. The spectra were described by a single Lorentzian of the form $S(f) = S(0)[1 + (f/f_c)^2]^{-1}$, consistent with the idea that the drug opened channels whose lifetimes were distributed exponentially, with a mean duration $\tau = 1/2\pi f_c$ (*7*). The conductance of individual channels (γ) was calculated from the relation $\gamma = S(0)/4\tau I\Delta V$, where $S(0)/4\tau$ is the area under the distribution, I is the mean current, and ΔV is the difference between the reversal potential for the drug effect and the holding potential. This estimate of γ was independent of mean current over a range of currents at least twice as large as that normally used. From the spectral density distribution in Fig. 1B, τ was estimated to be about 32 msec and γ to be 95 picosiemens (pS). Similar results from a total of 14 cells resulted in overall averages of 70 ± 15 pS (mean ± standard deviation) for channel conductance and 33 ± 9 msec for time open. The mean reversal potential for the drug effect was −65 mV. This was somewhat more positive than reported previously for these cells (*5*), although resting potentials (60 to 70 mV) and input resistances (about 2 megohms) were comparable. There was no obvious dependence of γ or τ on membrane potential over the observed range of −50 to −80 mV. However, experiments over a wider range might be required to reveal any such voltage dependence.

In preparations in which no tetrodotoxin or 4-aminopyridine was added to the bathing solution, cells showed both excitatory and inhibitory synaptic activi-

ty, presumably because of the occurrence of presynaptic action potentials. Records from one such cell are shown in Fig. 2. The upper trace illustrates the response of the cell to glycine; the second trace, obtained at a higher gain, shows the spontaneous activity. The bulk of the spontaneous synaptic currents are inward (downward deflections), corresponding to excitation, but several large inhibitory events (upward deflections) can also be seen. The numbered segments are expanded on the four subsequent traces to show the time course of the inhibitory currents. Strychnine abolished the response to glycine in these cells (upper trace in Fig. 2B) (*5*) and the spontaneous inhibitory currents as well (lower trace). A total of 44 IPSC's from four different cells had amplitudes that were distributed unimodally, with a mean corresponding to a peak conductance of 107 ± 62 nS, and decayed exponentially with a mean time constant of 32 ± 11 msec, the same as the mean relaxation time obtained for the glycine-activated channels. The average reversal potential for the IPSC's was −67 mV and, in individual cells, was indistinguishable from the reversal potential for the responses to glycine.

In summary, both the glycine responses and the IPSC's were blocked by strychnine, both had the same reversal potential, and the time constant of decay of the IPSC's was identical to the mean relaxation time constant of the glycine-activated channels. These observations support glycine as the inhibitory transmitter in these cells. The channel conductance reported here is two to five times greater than that reported for chloride channels in other cells (*1*), but is consistent with the large conductance underlying the IPSC's. Thus the spontaneous inhibitory events each appear to be associated with the synchronous activation of about 1500 channels, which is similar to the number of excitatory channels activated by a quantum of acetylcholine at the neuromuscular junction of the frog (*6, 7*). This, in turn, suggests that the spontaneous IPSC's are due to the release of single quanta of inhibitory transmitter. In support of this idea, we have preliminary evidence that spontaneous IPSC's produced in tetrodotoxin-blocked preparations by potassium depolarization have equivalent amplitudes.

MICHAEL R. GOLD
A. R. MARTIN
Department of Physiology, C240, University of Colorado School of Medicine, Denver 80262

References and Notes

1. J. Dudel, W. Finger, H. Stettmeier, *Pfluegers Arch.* **387**, 143 (1980); D. Gardner and C. F. Stevens, *J. Physiol. (London)* **304**, 145 (1980); S. G. Cull-Candy and R. Miledi, *Proc. R. Soc. London Ser. B* **211**, 527 (1981); J. L. Barker, *et al. J. Physiol. (London)* **322**, 356 (1982); M. Simonneau, L. Tauc, G. Baux, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1661 (1980).
2. W. Osterreider, A. Noma, W. Trautwein, *Pfluegers Arch.* **386**, 101 (1980).
3. D. S. Faber and H. Korn, *J. Neurophysiol.* **48**, 654 (1982).
4. J. S. Coombs, J. C. Eccles, P. Fatt, *J. Physiol. (London)* **130**, 326 (1955); T. Araki and C. A. Terzulo, *J. Neurophysiol.* **25**, 772 (1962).
5. S. Homma and C. M. Rovainen, *J. Physiol. (London)* **279**, 231 (1978); G. Matthews and W. O. Wickelgren, *ibid.* **293**, 393 (1979).
6. B. Katz and R. Miledi, *ibid.* **224**, 669 (1972).
7. C. R. Anderson and C. F. Stevens, *ibid.* **235**, 655 (1973).
8. Supported by research grant NS-09660 and fellowship NS-06283 from the National Institutes of Health.

25 October 1982

Mutual Coupling Between the Ocular Circadian Pacemakers of *Bulla gouldiana*

Abstract. *The ocular circadian pacemakers of* Bulla gouldiana *were found to be mutually coupled, and their interaction could be observed in an isolated nervous system maintained in vitro. Experimentally induced phase separations between the two ocular pacemakers were reduced when the eyes were allowed to interact for 48 hours. The reduction in phase separation did not occur however when the cerebral commissure was severed, indicating that this neural tract is a critical pathway coupling these two circadian clocks.*

Most circadian behaviors analyzed to date are under the control of multiple pacemakers (*1*). For example, Page (*2*) has described the effects on locomotor behavior of shifting the phase of one member of a bilaterally distributed pacemaker pair in the cockroach circadian system. These elegant experiments, and the work of others (*3*), indicate that, in the cockroach, locomotor rhythmicity is controlled by two mutually coupled circadian pacemakers located in the optic lobes. Similarly, in the rodent, the dissociation of the locomotor rhythm into two independent free-running components ("splitting") suggests that at least two pacemakers control locomotor activity in these animals (*4*). Because of the apparent multi-oscillator nature of circadian systems, a complete understanding

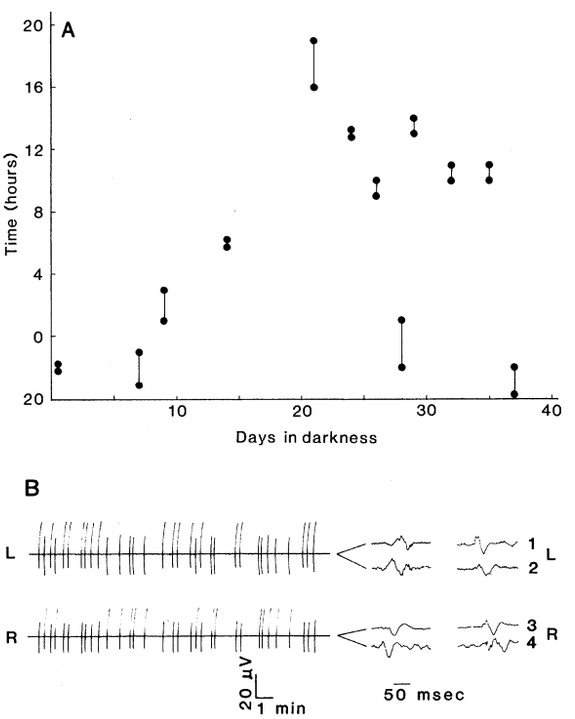

Fig. 1. (A) Phase separations in vivo between the ocular pacemakers of *Bulla*. After a specified time in darkness, both eyes of *Bulla* were removed and the ocular rhythms recorded. Each vertical line in the figure connects the phase reference points of the two ocular rhythms from a single animal. The ordinate shows the time of day (old dawn, 0 hours). As the time in darkness increases, the two ocular rhythms drift relative to clock time (that is, the eyes are free-running), but maintain a relatively constant phase relation to one another, never deviating by more than 3 hours [mean phase separation, 1.45 ± 1.03 (S.D.) hours.] (B) (Left) Recording from both optic nerves of an intact nervous system maintained in vitro. Each optic nerve potential is followed one for one by an efferent signal in the contralateral optic nerve. (Right) Oscilloscope recordings from an isolated *Bulla* nervous system, made with four electrodes, two on each optic nerve: (traces 1 and 2), left optic nerve; (traces 3 and 4), right optic nerve. Electrodes 2 and 3 are closest to the cerebral ganglia. The temporal ordering of the signals (4-3-2-1 or 1-2-3-4) demonstrate that the impulses pass down one optic nerve and up the contralateral optic nerve.