agent is also observed when chemical carcinogens are used as the transforming agents (*10*). Therefore, studies of the function of genes responsible for differential susceptibility—which are segregated, depressed, or induced in the variant cells—will make it possible to identify crucial factors in the neoplastic transformation by physical and chemical agents. The isolation of variants reported here also suggests a possible way to develop a better system for rapid assay of environmental carcinogens.

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(−)Pentobarbital Opens Ion Channels of Long Duration in Cultured Mouse Spinal Neurons

Abstract. *Intracellular recordings from voltage-clamped mouse spinal neurons in tissue culture were used to study the membrane mechanisms underlying inhibitory responses to γ-aminobutyric acid and the (−) isomer of pentobarbital. Fluctuation analysis suggested that both substances activated ion channels in the membranes. However, the channels activated by pentobarbital remained open five times longer than those activated by γ-aminobutyric acid.*

Racemic pentobarbital is used clinically to induce general anesthesia. Studies of the cellular mechanisms underlying this pharmacologic action have revealed that the drug depresses neuronal excitability in a variety of ways (*1–4*). Two of these depressant effects appear to be stereospecific and are caused primarily by the (−) isomer of the drug (*5*). One of these actions involves an increase in Cl^- conductance (*3*, *5*), an effect that is blocked by drugs that also antagonize Cl^- conductance evoked by the putative neurotransmitter γ-aminobutyric acid (GABA) (*2*, *3*). It is thus possible that the effects of (−)pentobarbital are mediated through activation of GABA receptors (*2*, *3*). The recent application of fluctuation analysis and single-channel recording techniques to membrane responses induced by endogenous ligands revealed the statistical nature of the underlying elementary events and their associated electrical properties (*6*). We applied fluctuation analysis to membrane current responses evoked in cultured mouse spinal neurons by GABA and (−)pentobarbital, and we report that the drug opens ion channels whose conductance is similar to that of GABA-activated channels but which remain open five times as long.

We grew mouse spinal neurons in tissue culture (*3*) and, at the time of the experiment, replaced the normal maintenance medium with Hanks balanced salt solution containing 1 m*M* $CaCl_2$, 10 m*M* $MgCl_2$, and 0.5 μ*M* tetrodotoxin to eliminate all evoked synaptic activity and allow clearer study of the pharmacologic responses. Using the voltage clamp technique, we made intracellular recordings on the modified stage of an inverted phase microscope with two electrodes filled with 3*M* KCl. The recordings were made at room temperature (25° ± 1°C). The GABA was applied to individual cells by iontophoresis from pipettes containing 1*M* solutions, and the (−) isomer of pentobarbital was applied by pressure from pipettes containing 100 μ*M* solutions. Analysis of current fluctuations was performed with the aid of PDP 11/10 and 11/40 computers.

Fourteen cells were studied with GABA alone; two, with (−)pentobarbital alone; and seven, with both agonists. Application of GABA or (−)pentobarbital induced membrane current responses in all of the cells tested. The responses to GABA and (−)pentobarbital were accompanied by additional membrane current variance (Fig. 1). Variances of membrane current fluctuations during 30-second samples of baseline records and of agonist responses were averaged, and the former were subtracted from the latter to yield the variance induced by the drug. Agonist-induced variance was directly proportional to the amplitude of the current response over the 0.3- to 3.0-nA response range. Assuming that the variance reflects statistical fluctuation in the number of open ion channels about a mean number of open channels, the average conductance γ of a single channel can be estimated from the relation $\gamma = \sigma^2/[\Delta I(V_c - E_R)]$, where σ^2 is the variance associated with agonist-induced changes in membrane current ΔI, E_R is the reversal potential of the agonist response, and V_c is the holding potential under voltage clamp. Estimates of γ for GABA, 14.5 ± 7 pS (mean ± S.D., $N = 21$ cells), and (−)pentobarbital responses, 14.7 ± 9 pS ($N = 9$ cells), were not significantly different ($P > .90$, Student's *t*-test), indicating that both substances open channels of similar conductance.

Estimates of the average lifetime of open channels were obtained from the power spectral density of current fluctuations in the presence of the agonists. The spectra were calculated as the fast Fourier transform of 6144-point samples of membrane current, digitized at 5 msec per point. Spectral analysis of baseline fluctuations in all of the cells showed that, in the absence of agonist, power spectral density was proportional to the inverse of frequency *f* over the entire frequency range of the spectrum and over the membrane potential range used (−50 to −90 mV) (Fig. 2A). Similar $1/f$ spectra have been reported in a number of excitable tissues studied in the absence of chemical stimulation, but the physical basis of the $1/f$ spectrum remains unclear (*7*).

Spectra of baseline fluctuations immediately before or after agonist responses were subtracted from spectra obtained during agonist responses, giving difference spectra of agonist-induced fluctuations. We obtained 139 spectra of GABA responses in 21 cells and 75 spectra of (−)pentobarbital responses in nine cells. For holding potentials over the −50 to −90 mV range, the difference spectra can be described by a single Lorentzian term, $S(f)/S(0) = 1/[1 + (f/f_c)^2]$, where $S(f)$ is the spectral density as a function of frequency and f_c is the half-power frequency of the spectrum (Fig. 2B) (*6*). The Lorentzian character of the spectra suggests that both GABA and (−)pentobarbital open an array of channels whose lifetimes are exponentially distributed around an average duration τ, where $\tau = 1/2\pi f_c$ (*6*). The estimated mean lifetime of (−)pentobarbital-activated channels,

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153.4 ± 27 msec, is five times that of GABA-induced channels, 30.7 ± 7 msec ($P < .001$). Channel lifetime appeared to vary little for either agonist over the −50 to −90 mV membrane potential range.

The results show that, like the responses to GABA, the membrane responses to (−)pentobarbital were associated with the appearance of additional current fluctuations whose behavior can be described by a single Lorentzian term. A simple interpretation of this observation is that the drug opens ion channels of similar conductance and five times the lifetime of those activated by GABA from synaptosomal membrane veal the mechanism by which the drug activates the channel. Barbiturates potentiate the pharmacologic responses of exogenously applied GABA and synaptic events mediated by GABA (*2, 3*), apparently by prolonging the average lifetime of GABA-activated channels (*8*). The $1/f$ character of baseline spectra we obtained indicates little, if any, detectable contribution of either exogenously (*9*) or endogenously released GABA in the absence of (−)pentobarbital (*10*). It is conceivable that the drug either induces release of GABA or blocks uptake of ambient GABA and then prolongs GABA-activated channels. If induced release of GABA does occur, it cannot be resolved as discrete quantal events. There is no evidence to indicate that barbiturates induce the release or inhibit the uptake of GABA from synaptosomal membrane preparations of mammalian brain (*11–13*). Thus it seems unlikely that the drug effects are due to prolongation of GABA-activated channels. Alternatively, the drug effects may not require the presence of GABA, but may result from direct activation of membrane conductance through engagement of receptors for a natural ligand or interaction with ion channels per se.

It is difficult to conclude that the drug directly engages the agonist binding site of the GABA receptor, since 100 μM (−)pentobarbital does not always directly affect cultured neuron membranes or other excitable membranes sensitive to GABA (*4*), and since in binding assays barbiturates do not compete with GABA for receptor sites (*14*). Such a competitive interaction has, however, been demonstrated between barbiturates and picrotoxin (*15*), which is thought to be a noncompetitive antagonist of GABA responses in vertebrate neurons (*16*). It is possible, therefore, that (−)pentobarbital may activate membrane channels permeable to Cl^- by causing them to open transiently (*17*). It should be noted that endogenous ligands such as purines, pyrimidines (*18*), and peptides (*19*) can also activate Cl^- conductance mechanisms in cultured spinal neurons. These observations raise the possibility that GABA receptors may not be involved in mediating the effects of (−)pentobarbital, but that receptors for other endogenous ligands may. Thus, although the data suggest that (−)pentobarbital opens ion channels analogous to these activated by a neurotransmitter, it is not yet clear how this occurs.

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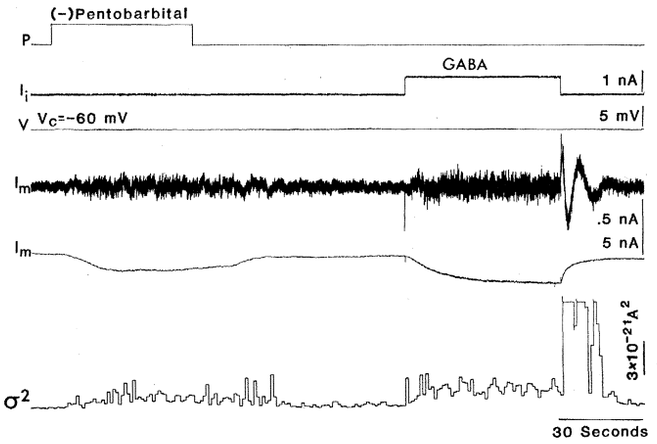

Fig. 1. Membrane responses to (−)pentobarbital and GABA, as recorded with KCl electrodes from a cultured mouse spinal neuron voltage-clamped to −60 mV. The 100 μm (−)pentobarbital was applied by pressure from a pipette during the period indicated on trace *P*. The GABA was iontophoresed from a 1*M* solution at the current shown in trace I_i. The high-grain, a-c-coupled membrane current record (I_m) is illustrated above the low-gain, d-c-coupled record (I_m). Membrane current variance σ^2 (converted to a voltage) is displayed at the bottom. Both substances evoke membrane current responses associated with a thickening of the current tracings and a coincident increase in variance.

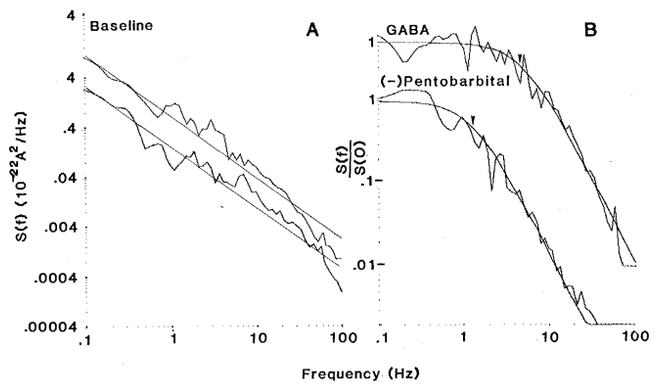

Fig. 2. Power spectral density spectra of membrane current fluctuations in a cultured mouse spinal neuron. (A) Each of the baseline spectra observed under control conditions at −70 mV (immediately preceding application of the agonist) can be approximated by a straight line that delineates a $1/f$ relationship between power spectral density and frequency. (B) These spectra were obtained as the difference between the spectra derived from baseline fluctuations and those calculated during responses to GABA and (−)pentobarbital. Each is the average of 17 difference spectra. They have been normalized by dividing each spectral density point $S(f)$ by the zero frequency asymptote of the spectrum $S(0)$. Least-squares analysis of the normalized spectra show that both are closely approximated by single Lorentzians (solid lines) described by the equation $S(f)/S(0) = 1/[1 + (f/f_c)^2]$, whose cutoff frequency f_c is 4.72 Hz for GABA and 1.23 Hz for (−)pentobarbital. Since the mean channel lifetime $\tau = 1/2\pi f_c$, τ for GABA = 33.7 msec and τ for (−)pentobarbital = 129.1 msec.

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DDT Contamination at Wheeler National Wildlife Refuge

Abstract. *Disposal of industrial waste resulted in massive DDT contamination at Wheeler National Wildlife Refuge, Alabama. Nearly a decade after the cessation of DDT manufacturing at the facility responsible, concentrations of DDT residues in the local fauna are still high enough to suggest avian reproductive impairment and mortality. Populations of fish-eating birds are low, endangered species are being exposed, and muscle lipids of game birds contain up to 6900 parts of DDT (isomers and metabolites) per million.*

The impact of toxic waste on the environment is often difficult to assess (*1*). However, the effects of DDT pollution are well known and have helped spur the environmental movement. In particular, data on DDT residues have been experimentally tied to harmful effects in various wildlife species. In this report we document the effects of massive contamination of an important national resource with industrial DDT.

From 1947 to 1970, DDT was manufactured on a site leased from the U.S. Army at Redstone Arsenal, Alabama. Production reached approximately 10^6 kg per year in 1963 (*2*); more than 6×10^5 kg were produced in 1970 prior to June of that year, when manufacture ceased. A ditch carrying effluent from the factory ran directly into Wheeler National Wildlife Refuge, where it joined Huntsville Spring Branch, a tributary to the Tennessee River, about 1 km from the plant site. The refuge, a 140-km^2 area administered by the U.S. Fish and Wildlife Service, is the major waterfowl refuge in Alabama, with winter populations recently estimated at 80,000 ducks and geese; in the past, population peaks of 150,000 were recorded (*3*).

In the 1960's, attempts were made to contain the effluent by dumping it in holding ponds or hauling it to landfills. Even so, the amount of DDTR (the simple sum of DDT isomers and metabolites) currently present in the sediments of Huntsville Spring Branch within the boundaries of the wildlife refuge has been estimated to be as high as 4×10^6 kg (*4*). Gross contamination is not limited to the aquatic system; in February 1979 we collected fist-sized chunks of crystalline material lying on the ground near the plant site; this material was 21 percent DDT.

Table 1. Concentrations of DDTR in selected fauna collected in February 1979 at Wheeler National Wildlife Refuge and the adjacent Redstone Arsenal. Abbreviations: Max, maximum; GM, geometric mean; and CI, 95 percent confidence interval.

Species and tissue	DDTR (ppm)	
	Wet weight basis	Lipid weight basis
Mallard ducks (N = 27)		
Carcass		
Max	480	6730
GM	4.0	38.0
CI	1.6–9.9	15–49
Muscle		
Max	150	6900
GM	0.67	11.5
CI	0.31–1.4	2.1–49
Crows (N = 14)		
Muscle		
Max	49	1470
GM	4.0	123
CI	1.4–11.2	42–355
Cottontail rabbits (N = 4)		
Muscle		
Max	0.52	79
GM	0.27	21.7
CI	0.12–0.62	0–626
Swamp rabbits (N = 5)		
Muscle		
Max	0.58	79
GM	0.25	18.0
CI	0.10–0.50	1.1–170

Birds, rabbits, and earthworms were collected for residue analysis in February 1979 (*5*), nearly 9 years after the manufacture of DDT at the Redstone site was halted. Mallard ducks (*Anas platyrhynchos*) were collected from flocks entering an evening roosting area on Huntsville Spring Branch approximately 0.5 km downstream from its confluence with the drainage ditch. Crows (*Corvus brachyrhynchos*), cottontail rabbits (*Sylvilagus floridanus*), and swamp rabbits (*Sylvilagus aquaticus*) were collected 0.4 to 0.5 km from the plant site. Composite samples of earthworms (*Bimastos, Dendrobaena,* and *Diplocardia*) were obtained about 0.1, 0.3, and 1.0 km from the factory.

Concentrations of DDTR in these fauna are high (Table 1). On a lipid weight basis, concentrations of residue in the muscle of the rabbits and birds exceeded established tolerances for human consumption of domestic animals in 37 of 50 samples (*6*). The concentration of DDTR in whole carcasses of female mallards was as high as 480 parts per million (ppm) (wet weight), and the geometric mean for DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene) in these females was 3.8 ppm (6.1 ppm DDTR) (*7*). In an experimental study, females of the closely related black duck (*Anas rubripes*), with whole-carcass DDE residues averaging 3.4 ppm, produced eggs with shells nearly 10 percent thinner than those of control eggs (*8*). Also, the overall productivity of these females was found to be significantly lower than that of controls ($P < .05$, χ^2 test). In captive mallard ducks reproductive effects due to DDE occur for periods of at least 11 months after cessation of exposure (*9*) and in captive black ducks for at least 2 years after exposure (*8*).

Waterfowl wintering at Wheeler National Wildlife Refuge migrate from as far north as Ontario (*10*). Our data and those of Longcore and Stendell (*8*) and Haegele and Hudson (*9*) indicate that impaired reproduction on breeding grounds far removed from the refuge is highly likely. In addition, contamination from this one source has had a strong influence on the interpretation of DDT residue data for the entire waterfowl population of Alabama. Nationwide monitoring of pesticides in waterfowl wings since the mid-1960's has shown that DDT and DDE concentrations in Alabama waterfowl are among the highest in the United States. Up to 50 percent of all ducks killed by Alabama hunters come from a tricounty area surrounding the refuge; and the residues found in these animals, high enough to indicate im-

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