pores could constitute only a very small percentage of the total cross-sectional area of the cell walls we examined.

Assuming that the pore diameters we measured represent the maximum size for free exchange between the plasma membrane and the environment, this value becomes an important consideration for many physiological functions as well as scientific manipulations. To cite a few examples, pore size may limit the effective size of toxins that function through binding to the plasma membrane (*19*) and of elicitors of phytoalexin synthesis (*20*), and pore size probably plays a role in a variety of other host-pathogen interactions, including recognition phenomena involving lectins (*21*). A limiting pore diameter must also be considered by researchers in their design of experiments involving the uptake of molecules (nucleic acids, proteins, oligosaccharides) by intact plant cells.

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8. Seeds of *R. sativus* were germinated on water-saturated filter paper. The radicle tips containing extending epidermal hair cells were excised and stained with neutral red to enhance the visibility of plasmolysis. Unfertilized ovules of *G. hirsutum* were cultured in vitro according to C. A. Beasley and I. P. Ting [*Am. J. Bot.* **60**, 130 (1973)]. Intact extending fibers were used after 8 days of culture and also were stained with neutral red. Fibers at this age possess only a primary wall. Palisade parenchyma cells of *X. strumarium* were isolated according to T. D. Sharkey and R. Raschke (*Plant Physiol.*, in press), and those of *C. communis* were obtained by peeling away the lower epidermis and scraping the cells gently into water with a fine dissecting needle. The palisade parenchyma cells were obtained from fully expanded leaves.
9. It should be noted that of the cell types examined here, only the leaf cells were obtained by mechanical disruption of the plant tissue; thus, we cannot exclude the possibilty that the larger pore size observed by us may have resulted from the isolation procedure.
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22. This work was supported by DOE contract EY-76-02-C-1338 and by Cotton Incorporated.

22 February 1979; revised 4 June 1979

[^{3}H]GABA Binding in Brains from Huntington's Chorea Patients: Altered Regulation by Phospholipids?

Abstract. *Binding sites for tritium-labeled γ-aminobutyric acid (GABA) in cerebellar cortex of Huntington's chorea patients have an increased affinity but unaltered maximum capacity as compared to binding sites in tissue from control patients. A similar binding pattern is produced in control membranes by treatment with Triton X-100, phospholipase C, or glycerophosphoethanolamine. Thus, it is likely that phospholipids or their metabolites regulate the accessibility of the GABA binding site and that this regulation is abnormal in Huntington's chorea.*

In the striatum and some other brain areas from Huntington's chorea patients, there is a large loss of neurons that contain γ-aminobutyric acid (GABA) and its synthesizing enzyme L-glutamic acid decarboxylase (*1*). Pharmacological attempts at GABA replacement therapy in Huntington's chorea have been mainly inconclusive (*2*), although some success in treatment of recently diagnosed patients with a GABA-mimetic agent has been reported (*3*). The potential success of GABA replacement therapy is dependent on the integrity of GABA receptors. Initial reports on GABA receptors (as estimated by [^{3}H]GABA binding) in Huntington's disease indicated that these receptors are largely intact (*4*). However, subsequent studies have demonstrated that in the striatum there is a severe loss of GABA receptors in this disease (*5, 6*). Despite this large loss of striatal GABA receptors, GABA replacement therapy may still be efficacious (i) if GABA receptors outside the striatum are relevant to treatment of the disease or (ii) if the remaining GABA receptors are supersensitive. The present report focuses on the second possibility.

The cerebellar cortex was chosen for study because (i) a large amount of homogeneous tissue is available, whereas the degeneration and gliosis occurring in the striatum often do not leave enough tissue for detailed study; (ii) clinical signs of cerebellar dysfunction are often present in Huntington's disease (*7*); and (iii) several of the neurochemical changes typical in Huntington's disease occur in the cerebellum, although they are less marked than in the striatum (*1, 8*). Despite the neurochemical and clinical observations of cerebellar dysfunction in Huntington's disease, the tissue appears normal. However, in the juvenile form of the disease, severe cerebellar degeneration may occur (*9*). These results suggest that in the adult form changes occur in the cerebellum which are not detected in light microscopic examination.

The kinetics of GABA receptors were studied by the [^{3}H]GABA binding technique as adapted in our laboratory (*10*). The materials from control and Huntington's patients were matched for age and postmortem time. Tissue from the same brains was used for all experiments. The clinical diagnosis of Huntington's disease was verified by histopathological examination in all cases. Concentrations of GABA for the binding assay (*10*) ranged from 5×10^{-9} to $5 \times 10^{-7} M$, and binding affinity was estimated from the dose-response, Scatchard, or Lineweaver-Burk plots. As all methods of data analysis yielded very similar results, only the kinetic parameters from the Scatchard analyses are reported. For all patients, only one slope and intercept was observed in the Scatchard plot.

The affinity of [^{3}H]GABA binding to cerebellar membranes (Table 1) is increased (decreased K_d) threefold in membranes prepared from Huntington's cerebellum as compared to controls; the tendency toward an increased number of binding sites is not statistically signifi-

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cant. The decreased K_d is in agreement with the decrease in the 50 percent inhibition concentration (IC_{50}) reported by us for a different series of patients (*5*). These results indicate that the increase in binding of 25 n*M* [^{3}H]GABA previously reported (*5*) is due to the shift in the binding curve. Such a shift resembles to some extent the alterations induced by Triton X-100 in rat brain preparations (*11*). Thus, homogenates from the current series of brains were incubated for 30 minutes at 37°C with 0.02 percent Triton X-100 and then the membranes were prepared as described (*10*). In preparations from normal brains, Triton X-100 treatment decreases the K_d for [^{3}H]-GABA binding without significantly altering B_{max} (Table 1). The kinetic parameters for [^{3}H]GABA binding to cerebellar membranes from Huntington's patients are unaltered by the Triton X-100 treatment. The K_d and B_{max} for [^{3}H]GABA binding to the Triton-treated normal membranes are virtually identical to those for the untreated membranes from Huntington's chorea patients. This observation, together with the lack of effect of Triton X-100 on the [^{3}H]GABA binding to membranes from Huntington's patients, suggests that the change (or changes) induced by Triton treatment of control membranes has already occurred in the Huntington's patients. Although most patients had been treated with neuroleptics, this is unlikely to be a factor in the changes in [^{3}H]GABA binding because treatment of rats for 6 months with either haloperidol or clozapine only slightly alters the [^{3}H]GABA binding to membranes prepared from cerebellar cortex (*9*). Also, two patients who had never received neuroleptics showed the same alterations in [^{3}H]GABA binding as did the drug-treated patients.

A likely change in membrane structure induced by Triton X-100 is an alteration in the lipid components. In an attempt to reproduce this change by a more physiological means, homogenates were incubated with phospholipase C (0.001 unit), in a manner similar to that for Triton X-100, before membrane preparation. This treatment reproduced those changes observed with Triton X-100 (Table 1): K_d was reduced but B_{max} was not significantly altered for [^{3}H]GABA binding in control membranes, whereas the kinetic parameters for membranes from Huntington's patients were unaltered.

Of the phospholipids that are abundant in cerebral membranes, phosphatidylethanolamine is more likely to be involved with GABA receptor function than is either phosphatidylcholine or phosphatidylserine (*12, 13*). Phospholipase C hydrolysis of this substance would produce *o*-phosphoethanolamine. When incubated with human cerebellar membranes *o*-phosphoethanolamine slightly but not significantly altered the K_d for [^{3}H]GABA binding in control and Huntington's chorea patients and was without effect on the changes induced by phospholipase C in the control membranes (Table 2). In contrast, glycerophosphoethanolamine (reaction product of phospholipase A_1 plus A_2 hydrolysis) decreased the K_d of [^{3}H]GABA binding to normal membranes (Table 2). It did not alter the phospholipase C effect nor did it change the [^{3}H]GABA binding kinetics for membranes from Huntington's patients.

These results suggest that (i) membrane phospholipids, especially phosphatidylethanolamine and its metabolites, may be involved in the pathology of Huntington's disease and (ii) normally, phosphatidylethanolamine or a closely related substance has a regulatory action on the affinity of (or access to) the [^{3}H]GABA binding site. With regard to (i), the quantity of phospholipids has been reported to be low in striatal tissue but not in cortex from Huntington's chorea patients (*14*). To our knowledge, cerebellar cortex has not been examined. Other biochemical abnormalities observed in the cerebellar cortex reflect, to a lesser extent, the changes occurring in the striatum and substantia nigra (*1*).

The question of [^{3}H]GABA binding interactions with phospholipids and related substances has been considered (*12, 13*). Phospholipase C but not phospholipase A or D increases GABA binding of a single, high concentration of [^{3}H]GABA to cerebellar junctional complexes, and phosphatidylethanolamine (but not phosphatidylcholine or phosphatidylserine) inhibits [^{3}H]GABA binding. Phospholipase C could act either by removing a phospholipid regulator (inhibitor) from the membrane receptor or by producing a reaction product that functions as an activator. From this and previous studies, it appears that both possibilities may occur. Glycerophosphoethanolamine content is elevated in postmortem material from brains from patients with Huntington's chorea (*1, 9*); this may partially explain the alteration in [^{3}H]GABA binding (Table 2). At present we do not think that phospholipids are inhibitors acting directly at the binding site, but rather suspect that they con-

Table 1. Effect of Triton X-100 or phospholipase C on [^{3}H]GABA binding to cerebellar membranes. Results are expressed as means ± standard error of mean (S.E.M.). Kinetic parameters were calculated from Scatchard plots; statistical significance was determined by Student's *t*-test, two-tailed.

Treatment	Control patients (N = 8)		Huntington's chorea patients (N = 8)	
	K_d (n*M*)	B_{max} (fmole per mg of tissue)	K_d (n*M*)	B_{max} (fmole per mg of tissue)
Untreated tissue	101 ± 8	76 ± 13	37 ± 4*	90 ± 8
Triton X-100 (0.02 percent)	38 ± 3†	98 ± 15	31 ± 4	131 ± 15
Phospholipase C (0.001 unit)	44 ± 11	67 ± 9	31 ± 4	107 ± 11

*P < .001 compared to controls. †P < .001 compared to untreated tissue.

Table 2. Effect of *o*-phosphoethanolamine or glycerophosphoethanolamine on [^{3}H]GABA binding to membranes from cerebellar cortex of control or Huntington's chorea patients. Results are expressed as means ± S.E.M. Data were analyzed by means of Scatchard plots and Student's two-tailed *t*-test. Data for untreated tissue are given in Table 1; N, number of brains.

Treatment	Control patients			Huntington's chorea patients		
	K_d (n*M*)	B_{max} (fmole per mg of tissue)	N	K_d (n*M*)	B_{max} (fmole per mg of tissue)	N
o-Phosphoethanolamine (2 μ*M*)	81 ± 7	78 ± 9	7	50 ± 4*	126 ± 16†	7
o-Phosphoethanolamine (2 μ*M*) + phospholipase (0.001 unit)	41 ± 8‡	65 ± 8	7	37 ± 4	104 ± 17	6
Glycerophosphoethanolamine (0.5 μ*M*)	56 ± 13§	60 ± 14	8	43 ± 6	93 ± 13	8
Glycerophosphoethanolamine (0.5 μ*M*) + phospholipase C (0.001 unit)	57 ± 15§	60 ± 14	8	36 ± 5	114 ± 14	7

*P < .05 compared to controls. †P < .001 compared to controls. ‡P < .001 compared to untreated tissue. §P < .05 compared to untreated tissue.

trol the access of hydrophilic molecules (such as GABA or muscimol) to the binding site by increasing lipid content in the environment. This contention is supported by the observation that Triton X-100 treatment increases the affinity for hydrophilic (that is, lipophobic) ligands such as GABA or muscimol but does not alter the affinity of lipophylic molecules (such as SL 76 002, a GABA-mimetic benzophenone) for the binding site (*3*, *12*).

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19 January 1979; revised 10 April 1979

Vibrations: Their Signal Function for a Spider Kleptoparasite

Abstract. *The stealing behavior of* Argyrodes elevatus *suggests that this kleptoparasitic spider monitors the movements and the hunting success of its web-building host. Wrapping of prey by the host regularly elicits raids from the kleptoparasite. The prey-catching activities of the host generate vibrations that were recorded with a position-sensing photodiode. The recordings indicated that wrapping movements produce a characteristic pattern of vibrations.*

Tropical spiders of the theridiid genus *Argyrodes* Simon inhabit the webs of other spiders (*1*). The kleptoparasitic *Argyrodes elevatus* Taczanowski constructs no web of its own but primarily uses the snares of the orb-weaving spiders *Nephila clavipes* and *Argiope argentata* to secure its food. Fine threads connect its resting place, 20 to 30 cm outside the host's capture area (*2*), with the hub and several radii of the host's web. The kleptoparasites move along these lines either in search of small insects entangled in the sticky spiral, but neglected by the host, or to steal large prey items caught and stored by the host (*3*, *3a*, *4*). Raids for stored prey packets are triggered by the host's prey-catching movements, and a distinct stealing behavior is displayed by the kleptoparasites, indicating a high degree of specialization toward either host species (*4*).

While studying the stealing behavior of *Argyrodes* (*4*), I subjected hosts and kleptoparasites to experiments in which dead prey was presented on the tip of a vibrator but often retrieved from the web before the host reached and "caught" it, thus simulating prey escape. Only in a few instances (9 percent; $N = 100$) did the kleptoparasites venture into a raid if the host ran toward the prey and searched but did not succeed in catching it. In contrast, a raid was regularly elicited (86 percent; $N = 518$) if the prey was captured by the host. Close observations, as well as film analysis of the movements of hosts and kleptoparasites during the experiments, showed that in most cases the kleptoparasites started a raid the instant the prey was wrapped by the host. Since the vision of most web-building spiders is poor (*5*) and the use of acute olfaction has not been demonstrated, it is generally assumed (*6*) that vibrations are of major importance for these spiders. My experiments suggest that vibrations generated by the host during the wrapping sequence of prey capture were of crucial significance to the kleptoparasites. The prominence of these wrapping vibrations in the vibratory pattern of prey capture will be demonstrated.

I employed three methods for recording the vibrations of silken threads to investigate the pattern of vibrations generated by the host spiders during prey catching (*7*, *8*). The best results—recording of large as well as small translocations of a single web strand—were obtained with a position-sensing photodetector (*9*).

A beam of parallel light is sent onto a reflector plate (3M Scotch-Lite High Gain Foil) placed at the hub of the spider web. The returned beam passes through a 100-mm macrolens onto the position-sensing silicon photodiode (Scotty Barrier PIN-SC 10, United Tech.) housed in a camera case. Movements of the reflector are displayed as magnified alterations of a cathode-ray beam. Since the reflecting angle is 0° to 1°, small twists of the reflector influence only the signal amplitude, but do not otherwise alter the signals. To minimize inertial effects, the reflector plate (0.5 mg, 1 to 2 mm^2) is not fixed directly to the *Argyrodes* signal line but is attached to the intersection of a radius and spiral thread at points where signal threads originate. It is assumed (*10*) that the taut signal thread transmits vibrations from its point of attachment at the radius to the receiver with little alteration. Although vibrations in the spider web travel along the radii as longitudinal, transversal, and torsion translations (*11*), I measured only the longitudinal vector (*12*).

Figure 1 shows the pattern of vibra-

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