V. Current Status of Neurotoxicity Screening Procedures

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National Institutes of Health

RAPID SCREENING OF NEUROTOXIC AGENTS BY IN VIVO AND IN VITRO MEANS

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I would like to tell you about the work of our group, the Neurochemistry Section within the laboratory of Behavorial and Neurological Toxicology, NIEHS, which is within the National Institutes of Health and is located at the Research Triangle Park. We have existed for just about a year now, and so much of our data is preliminary. I would like to talk about our plans for the future and give you a few examples of our current work. Rather than taking a broad approach, we have decided to put all our eggs in one basket and, in many ways, take a rather narrow approach to get one relatively specialized technique working effectively.

The major interest of most people at this meeting seems to be agents which are either especially or selectively neurotoxic, and there has been a tendency to shun chemicals which have a very general toxicity, and I think that is a desirable approach. These chemicals that are selective are likely to attack the more specific features of brain or nerve metabolism. There are two kinds of specialized features of brain metabolism. There is the quantitatively specific aspect such as high oxygen and glucose consumption, which one may consider specific because it is so much in excess of nutrient consumption by most other tissues, and there are qualitatively specific features such as myelin and neurotransmitter-related metabolism.

It is the neurotransmitter-related aspects that are especially interesting to use with emphasis on the synapse, which could be considered a weak link between axons. In the synaptic cleft, there is a certain amount of neurotransmitter, and various processes govern its concentration there. The presynaptic release and re-uptake mechanisms regulate cleft transmitter levels. These levels in turn determine the proportion of specific binding sites which are activated by formation of complexes with the transmitters. Such sites can be both pre- and postsynaptic.

Our initial approach has been to ask whether the consequences of neurotoxicity can be detected by altered characteristics of neurotransmitter receptor sites. The assay of neurotransmitter binding is technically rather simple, but the interpretation is often baffling and every week a new question arises in the literature as to the specificity or location of a binding reaction.

Neurotransmitter receptors have, in my opinion, been neglected by toxicology, but they have not been neglected by disease nor by pharmacology. For instance, many disease states exist where receptors are directly or secondarily abnormal. There are suspected to be dopamine receptor abnormalities in schizophrenia and in Huntington's disease.[1] There seem to be norepinephrine receptor abnormalities in a variety of conditions such as, say, terminal shock, where after somebody is in acute shock, with very low blood pressure, when one injects norepinephrine, there is no response, suggesting refractory receptors.

Myasthenia gravis is a good example of a single receptor involvement disease where apparently antibodies to the nicotonic receptors are made, and block neuromuscular conduction.[2] The kind of epilepsy that

follows months after trauma to the brain may well turn out to be a receptor problem related to denervation supersensitivity. After preparation of isolated cortical slabs, by careful cutting, leaving the blood supply intact one can show the onset of the supersensitive condition in animals.[3]

There can be distinct changes in neurotransmitter pathways in disease, and receptors can change secondarily in response to hyperactivity or inactivity of the neuronal circuit. If I may oversimplify, the concentration of a receptor seems to be inversely proportional to the concentration of the corresponding neurotransmitter around it. This presumably reflects the body's attempt to maintain some kind of homeostatis. Changes in presynaptic events such as uptake and release can also affect receptor density,and altered activity of a specific neuronal pathway might ultimately be reflected in changes in the receptors of that pathway.

Many pharmacological agents act either secondarily or primarily on receptors. The primary ones include morphine, which seems to act on an opiate receptor, the minor tranquilizers, the benzodiazepines, which seem to act by binding to a specific receptor in the brain, and the neuroleptics such as haloperidol, which bind to the dopamine receptor and thereby block dopaminergic function and antagonize the dopamine receptor. Other agents include atropine and muscimol, a GABA agonist which is used in the treatment of Huntington's disease. Then there are the classical drugs which influence heart rate and blood pressure: the alpha and beta-blockers such as phentolamine and propanolol—all of these are thought to act directly on receptors, and many of them we actually use in our assays. There also exist other specific pharmacological agents such as LSD and strychnine, also which seem to act by binding to specific receptor sites.[4] It seems reasonable to suppose that other less specific neurotoxic chemicals perhaps can also influence receptors. We have got one problem with this, though, and that is that when we test agents found in the environment as a result of industrial activity, we cannot expect as much specificity as we can with drugs.

Pharmacological agents and natural toxins are designed, either by man or nature, for a very selective process. The snake has very skillfully built alpha-bungarotoxin in a few million years, and we are much quicker in the pharmacological world, but these are all attempts to produce a specific effect and there does not seem to be much sense or design to industrial wastes or many food additives.

What are the features that might make binding studies useful? One is that receptors can respond rapidly to physiological changes. Fifteen minutes after a seizure, one can detect changed diazepam receptor sites in the brain.[5] Such changes in receptor density could reflect allosteric unfolding of a protein molecule or it could represent new synthesis.

Another feature about receptors is that one can find conditions where there are changes in receptor density, without changes in the gross levels of neurotransmitters. For instance, there are conditions where chlorpromazine administration will increase the density of the dopamine receptor but will not alter dopamine levels.[6] Thus, receptors could be a very selective and sensitive index of perturbation of a specific neuronal circuit, even if we do not know the exact location or the function of the receptors.[7]

I feel that screening for defects of distinctive neuronal species might be possible using this method. Whether the effects that we detect are primary or secondary may not be so important; it does not make the system less valuable that many effects are probably not due to a toxic agent directly affecting the receptor.

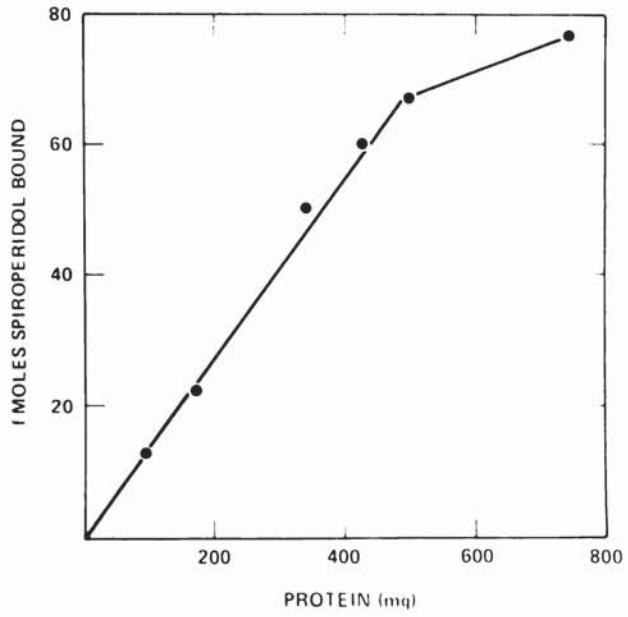
Once we get a clue that a certain nerve pathway is involved, then we can proceed to detailed uptake, release, enzyme and turnover measurements of a specific transmitter. I view the receptor approach as a useful preliminary screen and not as an end in itself.

Most of our effort in the laboratory so far has been in just setting up the basic criteria of a reasonable assay because we have to establish this rigorously before we can use these methods in toxicology. There are certain criteria for high affinity interactions, and we have worked them out for several postulated neurotransmitter species. We have also established baselines for some ligands that are not transmitters, such as diazepam.

I would like to illustrate our approach using the dopamine receptor as an example.

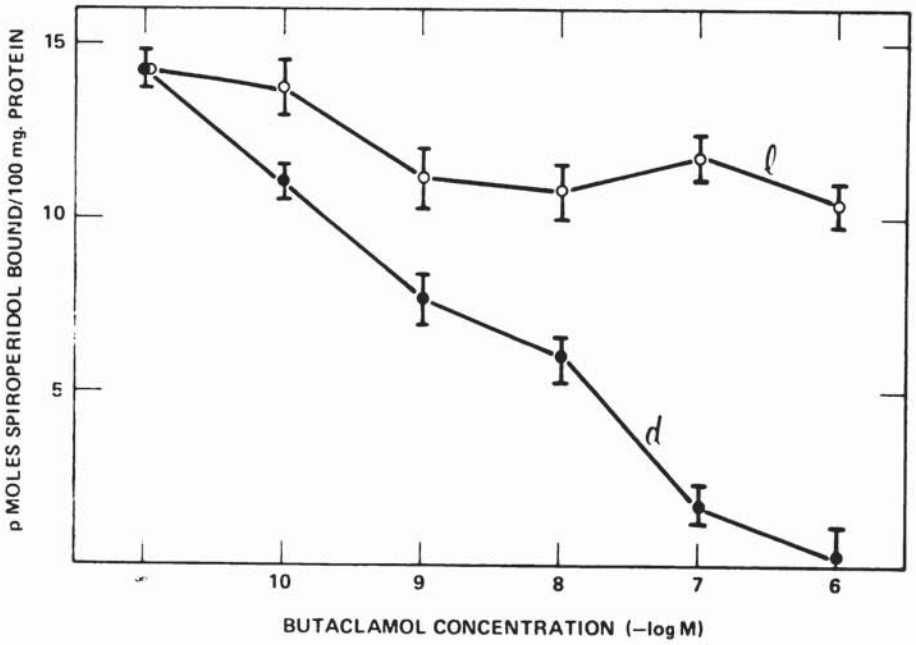
The amount of binding of tritiated spiroperidol that we get is proportional to the amount of striatal membrane preparation in our test tubes (Figure 1). This is high

Figure 1. Binding of ^{3}H-spiroperidol to striatal membranes of rat brain.



Each point represents the mean of three determinations.

Figure 2. Binding of ^{3}H-spiroperidol to striatal membranes in the presence of varying amounts of d- and l-butaclamol.



Standard errors of the mean are presented.

affinity specific binding to isolated membranes. Spiroperidol is a dopamine antagonist and thought to bind to some classes of dopamine receptor. This binding is stereospecific in that the competition of spiroperidol binding with D-butaclamol is much greater than with L-butaclamol (Figure 2).

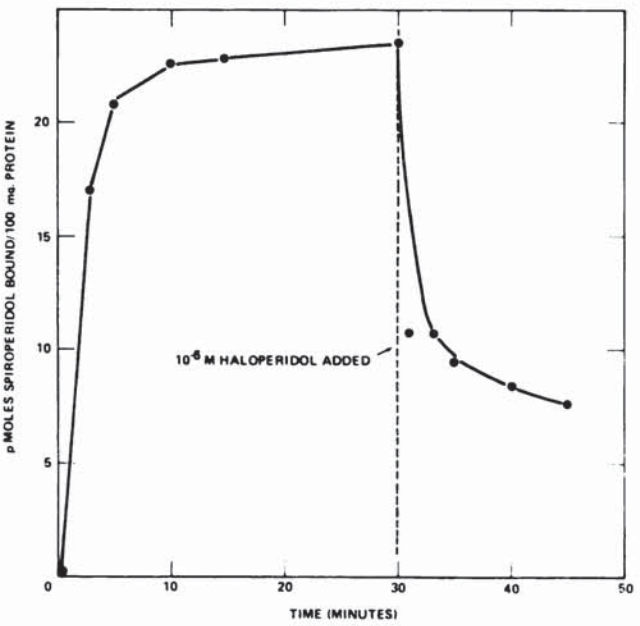
The time course of spiroperidol binding suggests that it is not an enzyme reaction; but rather an equilibrium (Figure 3). Because it is an equilibrium, if we add an excess of nonradioactive competitor later, we are able to displace the radioactive compound. This reversibility is important because the irreversible formation of covalent linkages between the receptor molecule and the lingand would invalidate results. It is necessary to make all these preliminary surveys before using neurotransmitter binding techniques in neurotoxicological studies.

Another survey that confirms the validity of these assays is regional specificity of binding. Areas where there are thought to be dopamine synapses like the striatum have more ^{3}H-spiroperidol binding activity than areas thought to possess no or few dopamine receptors (Figure 4).

This kind of data encourages us to think that we are measuring a specific and functionally meaningful phenomenon. The use of three different kinds of tritiated ligands allows the assay of dopamine, beta, and alpha-noradrenergic binding sites. The competition between these chemicals and inappropriate competitors illustrates the specificity of binding under the conditions we use. The spiroperidol is displaced by haloperidol but not by either alprenolol or ergocryptine. The radioactive dihydroergocryptine, which should bind to alpha receptors, is displaced by nonradioactive ergocryptine and not by the haloperidol or alprenolol (Figure 5).

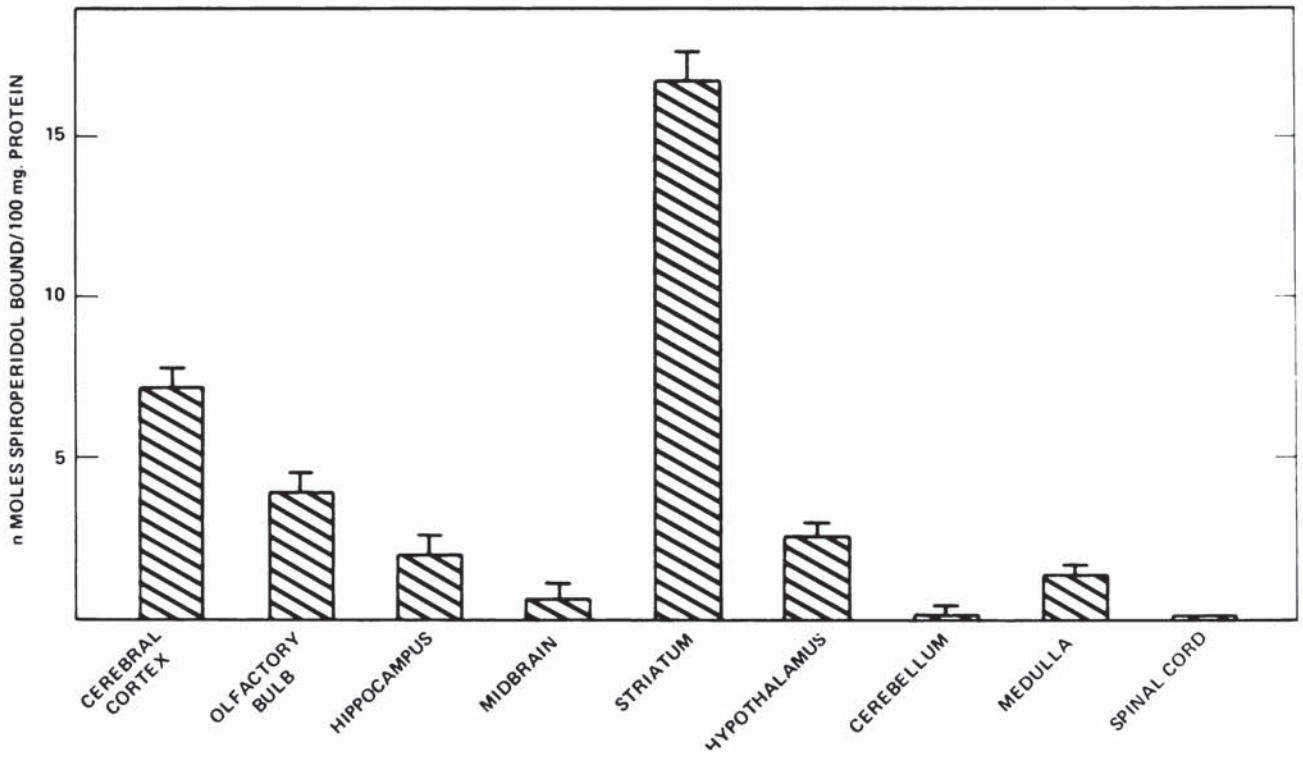
Another procedure that we plan to develop is to link this binding to a physiological response such as stimulation of adenylcyclase in the case of dopamine, or assay

Figure 3. Time course of ^{3}H-spiroperidol binding to striatal membranes.



After 30 minutes, 10^{-6}M haloperidol was added to remaining incubation tubes. Each point represents the mean of three determinations.

Figure 4. Regional distribution of ^{3}H-spiroperidol binding to rat brain.



Standard errors of the mean are presented.

of the rate of neurotransmitter release thus demonstrating presynaptic feedback regulation. We are in the process of developing these methods now because we realize that binding by itself is only a preliminary technique.

Our approach has been to study receptor-ligand interaction without prejudice and without too much of a theory about which of the neurotransmitters is going to be affected by a given toxic substance. Now, obviously our series of binding legends is not entirely unbiased since we are not assaying all neurally relevant binding phenomena. We have left our histamine, endorphins, and other peptides. Our basic screening system is shown in Table 1. We use very low concentrations of the radioactive compound, and we consider specific binding

Figure 5. Specificity of catecholamine binding to striatal membranes.

^{3}H LIGAND (10^{-9}M)	UNLABELED COMPETITOR (10^{-6}M)			
	NONE	HALOPERIDOL	ERGOCRYPTINE	ALPRENOLOL
SPIROPERIDOL	22.9	5.1	20.3	23.2
DIHYDROERGOCRYPTINE	22.6	19.2	10.1	24.5
DIHYDROALPRENOLOL	11.0	11.1	11.7	5.4

BINDING EXPRESSED AS pmoles LABELED LIGAND BOUND PER 100mg PROTEIN

Each value represents the mean of three determinations.

Table 1. Pharmacological agents used in binding studies.

PRESUMPTIVE ENDOGENOUS LIGAND	LABELED LIGAND (nM)		UNLABELED COMPETITOR (μM)	
GABA	MUSCIMOL	1.0	GABA	10
ACETYLCHOLINE (MUSCARINIC)	QNB	1.0	ATROPINE	10
α-NOREPINEPHRINE	DIHYDRO-ERGOCRYPTINE	1.3	ERGOCRYPTINE	1.0
β-NOREPINEPHRINE	DIHYDRO-ALPRENOLOL	0.7	ALPRENOLOL	1.0
DOPAMINE	SPIROPERIDOL	1.0	HALOPERIDOL	1.0
INOSINE ?	DIAZEPAM	0.75	DIAZEPAM	3.0
GLYCINE	STRYCHNINE	1.0	STRYCHNINE	10.0

that which can be displaced by a thousand-fold excess concentration of an unlabeled competitor.

We plan to use pharmacological agents rather than natural neurotransmitters for several reasons. One is, they are more stable. They are not degraded by catabolic enzymes like monoamine oxidase. The second is they tend to have higher affinities than endogenous compounds. That is why they are often pharmacologically active. For example, strychnine has a higher affinity for the glycine receptor than glycine itself does. The third reason is that synthetic ligands are often more specific—obviously, alpha and beta noradrenergic binders are more specific than norepinephrine, which will bind to either receptor type.

Regional studies show there is some specificity to this approach. The glycine receptors are more concentrated in the hind brain and the spinal cord, with very little binding within the cortex (Figure 6). The benzodiazepine receptors are widely distributed (Figure 7). In this case, it is not clear whether these sites are neuronal or glial. Since behavior is very much affected by benzodiazepenes, we thought it would be appropriate to include diasepam in our preliminary screen. The cholinergic receptors tend to be in the frontal areas of the brain with less density in the hind brain (Figure 8). There are two directions one can proceed with binding studies. One is to examine binding in animals that have been treated **in vivo** with toxic agents by testing the receptors after dosing; otherwise, we can add toxic compounds right into the test tube for an **in vitro** study where the direct effects of toxicants can be evaluated. We can certainly control the conditions better but important indirect and secondary effects would not be seen in **in vitro** work. As Dr. Silbergeld has said, there may be occasions when the two approaches will complement each other.

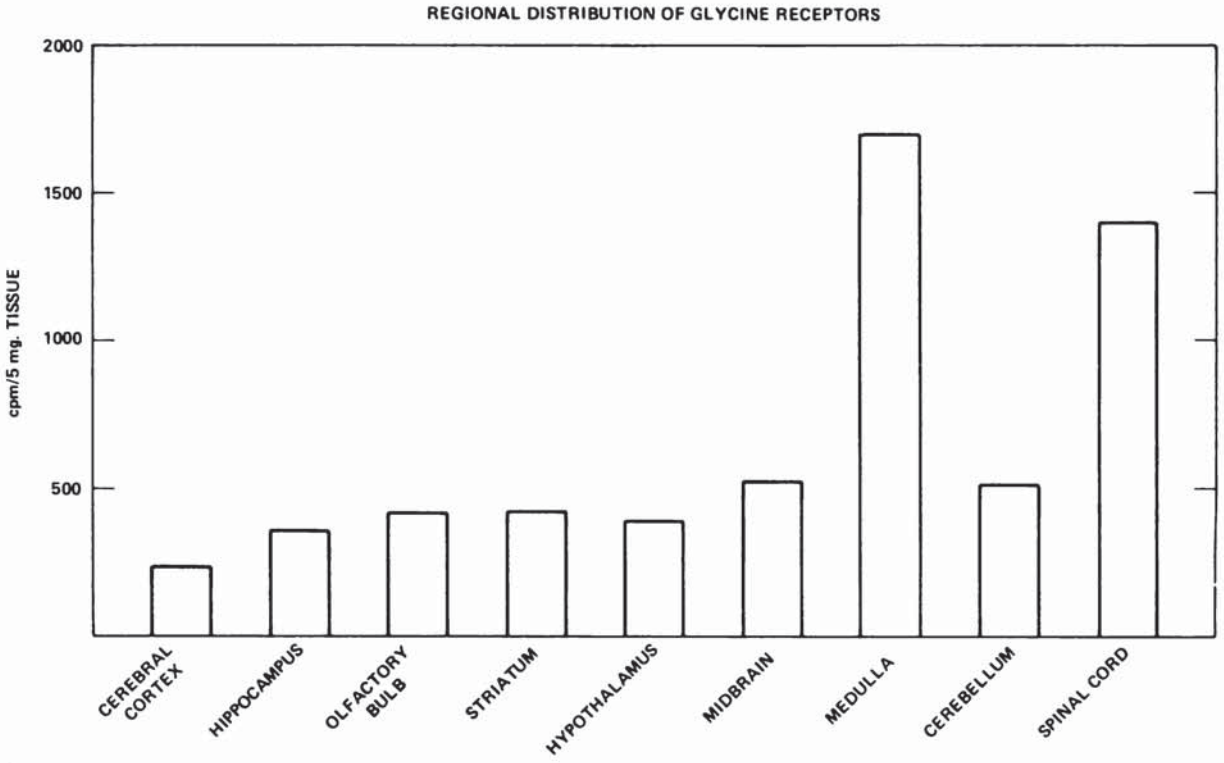
I would like to give you an example of some preliminary work that has been done, largely by Dr. Ashok Agrawal in our lab, with the toxic agent, acrylamide. This compound has been long known to be a peripheral nervous system toxicant which causes "dying back" axonopathy. However, more recently, several workers have shown that it actually acts on the CNS as well.[8-10] We started by doing a survey on the effect of acrylamide on the dopamine receptor in the striatum, as there are some behavioral clues that suggest this system might be especially vulnerable.

Striatal spiroperidol binding was measured in 6 week old rats that had been treated with a single oral administration of acrylamide. The striatum was removed by the standard dissection of Iversen and Glowinski.[11] After 24 hours, there was significant increase in the overall binding of spiroperidol to striatal membranes of animals treated with a single dose of acrylamide, at several levels (Figure 9).

When one finds more radioactivity binding to one membrane than another, it could be due to either a change in site density or the affinity of binding. To distinguish between these, it is necessary to carry out a Scatchard plot, measuring binding over a wide range of ligand concentrations. Scatchard analysis of treated animals revealed that ligand binding was tighter, that is K_D (dissociation constant) was reduced in the treated animals (Figure 10).

In addition we carried out studies with repeated administration of lower doses of acrylamide. We gave 10 doses of acrylamide over two weeks and then we waited

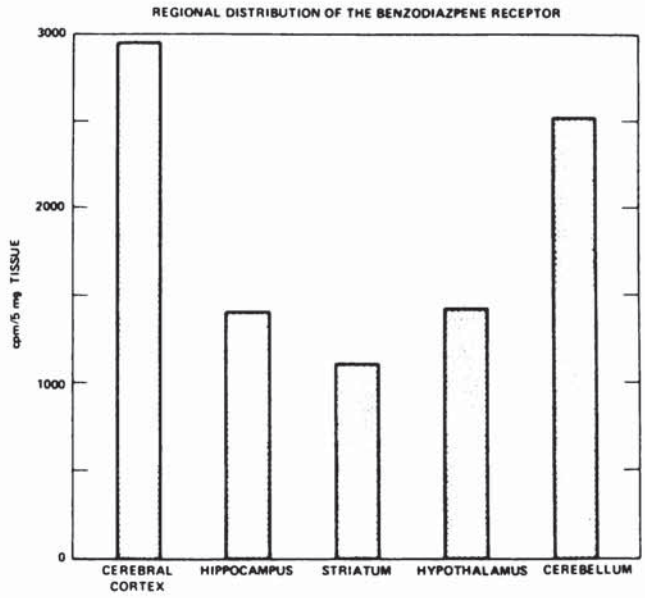
Figure 6. Regional distribution of ^{3}H-strychnine binding to rat brain.



Each value represents the mean of three determinations.

a day before measuring binding sites and we also waited a further week to determine whether observed changes were reversible. There was again a significant increase of ^{3}H-spiroperidol binding in treated animals. Thus, with repeated administration of acrylamide, there was an increased binding that was even more marked than in the acute situation but this effect was totally reversible in a week (Figure 11).

Figure 7. Regional distribution of ^{3}H-diazepam binding to rat brain.



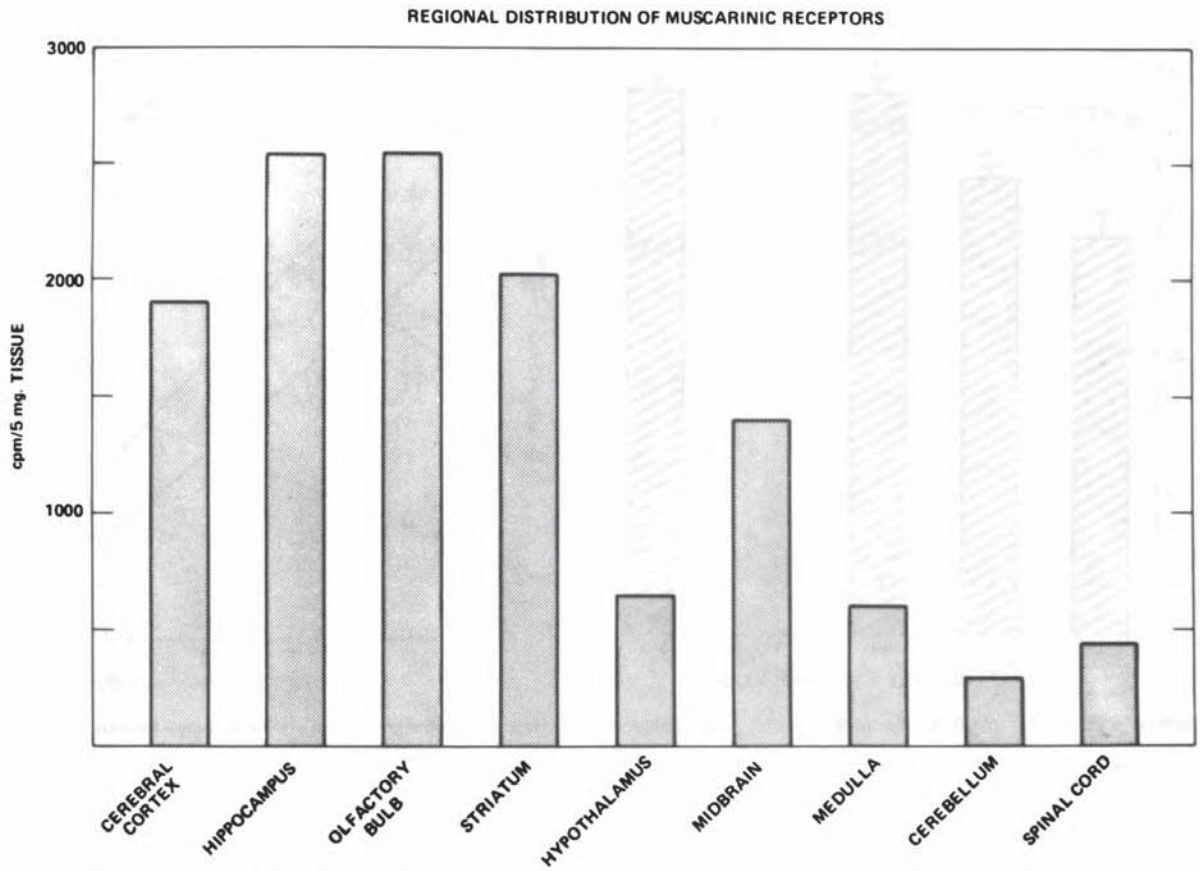
Each value represents the mean of three determinations.

The Scatchard plot of these data indicated that the treated animals may actually have more sites as well as tighter binding toward ^{3}H-spiroperidol (Figure 12). In this case, both receptor density and binding affinity were altered by a acrylamide treatment. We don't yet know how specific this is. We have to test other neurotransmitter binding systems. Since data are expressed on a protein basis, these results cannot be due to merely nonspecific nutritional effects.

We plan to assay other regions and ultimately perhaps data like these might be correlated with morphological changes like spine density. We have also started a third dopamine-acrylamide project, a developmental study, where we injected pregnant mothers between days 5 and 15 of ingestion every day with acrylamide, and then we raised the pups (some of which were cross fostered) and killed the pups at 2 or 3 weeks after birth.

Two weeks after birth, striatal membranes of male offspring of treated rats had a reduced spiroperidol binding ability (Table 2). This was found in cross-fostered and non cross-fostered pups. A lesser effect was seen in female progeny. After a further week, binding in all offspring appeared restored to normal values. Thus, the deficit incurred was largely reversible. Cross fostering studies suggested that the prenatal toxic effect was a

Figure 8. Regional distribution of ^{3}H-QNB binding to rat brain.



Each value represents the mean of three determinations.

greater factor than the postnatal effect. This latter response may have been the result of transfer of acrylamide or its metabolites during lactation.

Since there was a depression in body weight in treated animals, these were not the best doses to look for very subtle effects. However, the data suggest that dopamine sites may be unusually vulnerable to acrylamide compared to overall brain protein. Since we cannot detect any permanent loss of receptors, these results may be due to a delay in receptor development in animals derived from treated mothers.

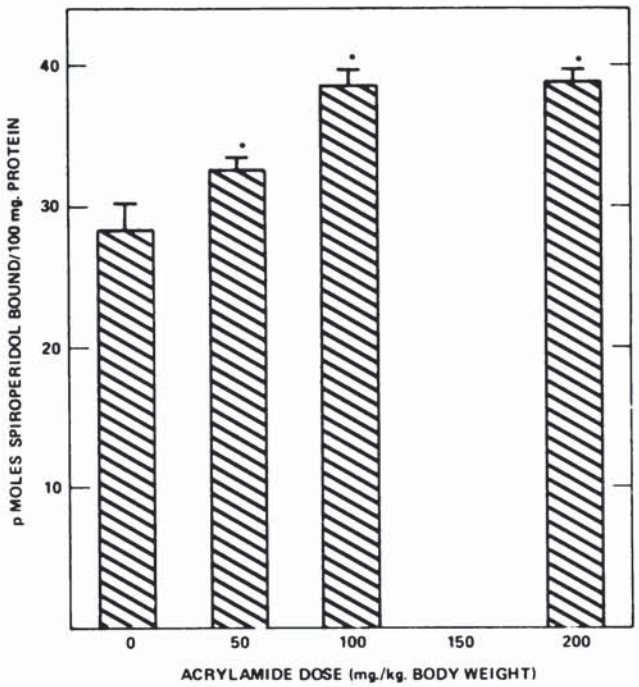
More information is needed concerning the effects of acrylamide on a variety of transmitter binding sites. We also need more dose-response data. At present, we are carrying out a neonatal study where pups are treated with acrylamide shortly after birth.

Table 2. Spiroperidol binding to striatal membranes of neonatal rats.

	pmoles ^{3}H-spiroperidol bound/100 mg protein			
	Male		Female	
Fostering Treatment	2 Weeks	3Weeks	2 Weeks	3 Weeks
Untreated dams with own pups	32.2 ± 1.8	37.6 ± 1.6	26.5 ± 1.3	34.1 ± 2.4
Untreated dams with pups with treated dams	24.6* ± 2.3	33.3 ± 1.5	24.1 ± 1.8	33.4 ± 2.6
Treated dams with pups from untreated dams	32.7 ± 2.1	36.3 ± 2.0	20.8 ± 1.7	31.7 ± 1.1
Treated dams with own pups	25.2* ± 0.9	34.7 ± 2.2	21.5* ± 0.7	37.5 ± 1.3

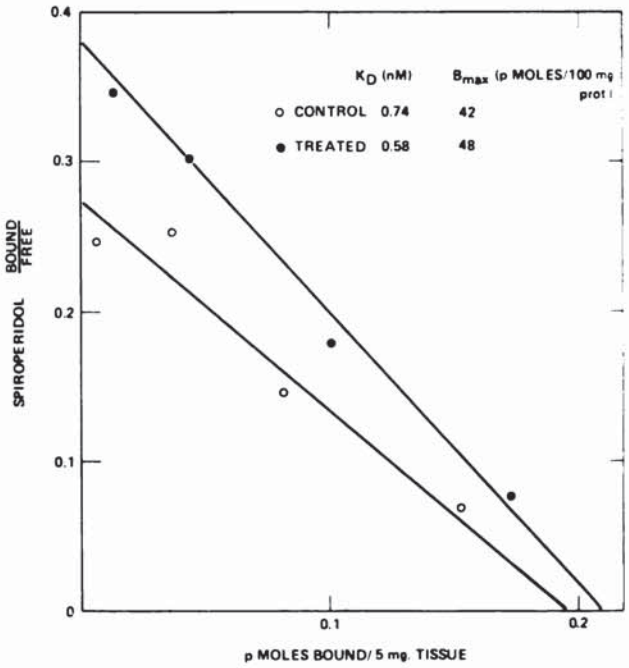
Pregnant rats of the strain received ten doses of 20 mg/kg acrylamide daily between days 5-15 of gestation.
*Experimental differs significantly from corresponding control (Analysis of Variance, $P<0.05$ two-tailed, LSD-test).

Figure 9. Effect of a single acrylamide treatment upon the striatal binding of ^{3}H-spiroperidol.



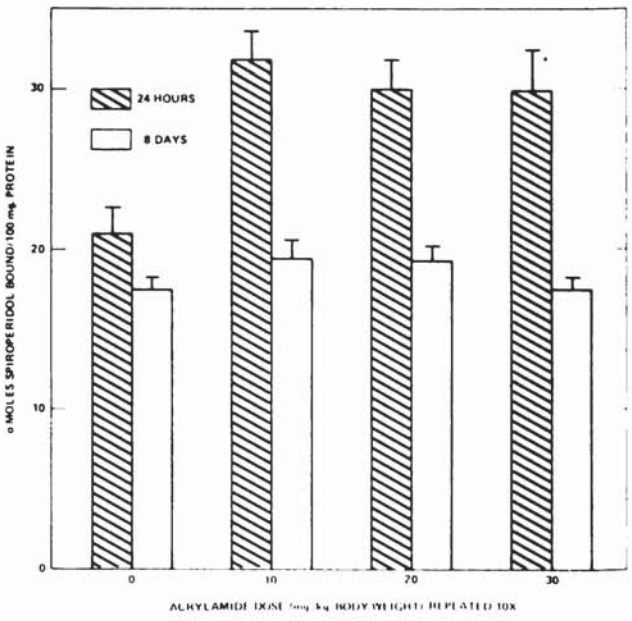
Assays were carried out 24 hours after administration of acrylamide to 6-week old male Sprague-Dawley rats. Bars indicate standard error of the mean. Eight rats were used in each treatment group. *Differs significantly from control value (Fisher's Least Significant Difference Test, $P < 0.05$).

Figure 10. Scatchard analysis of striatal binding of ^{3}H-spiroperidol to membranes prepared from rats 24 hours after administration of a single dose of acrylamide (100 mg/kg body weight).



Data are from eight animals in each group. ● - experimental rats, o = control rats. Curve derived from linear regression analysis.

Figure 11. Effect of repeated treatment with acrylamide upon the striatal binding of spiroperidol.

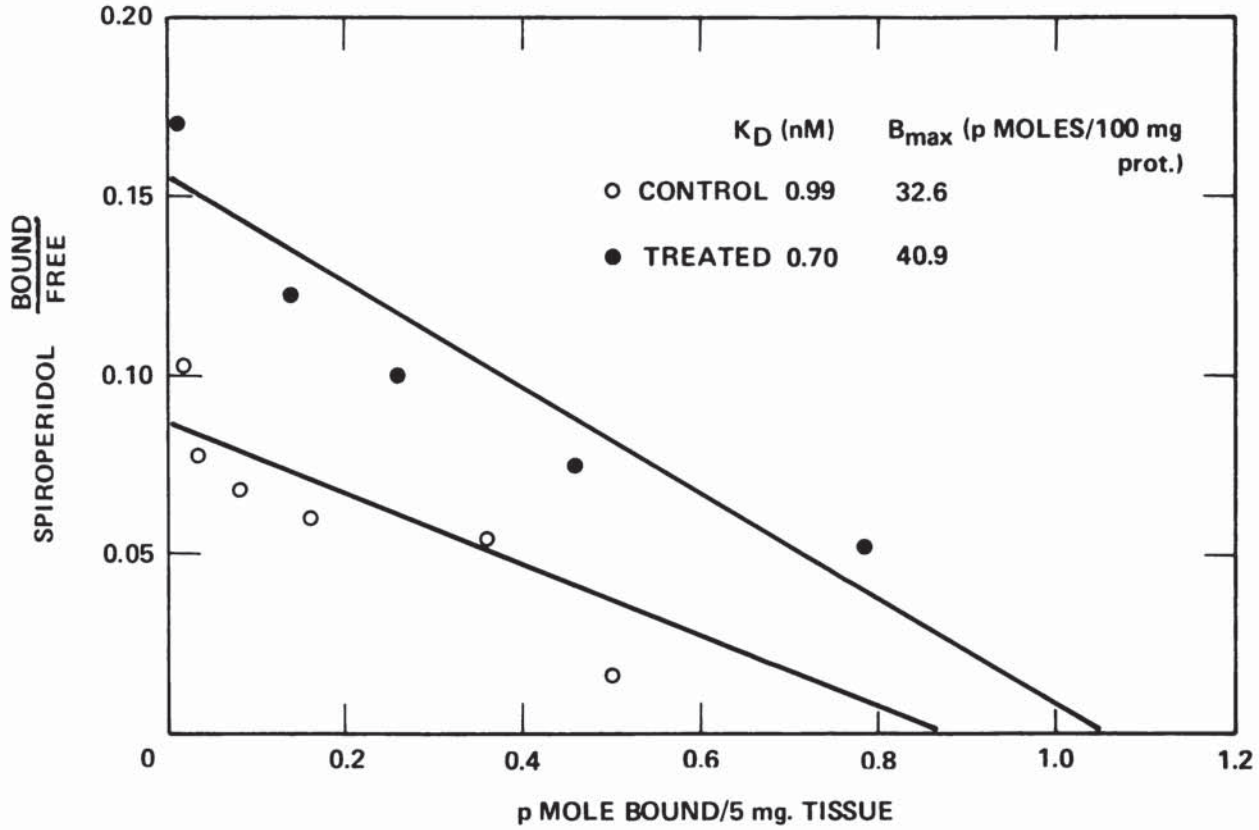


Assays were performed 24 hours or 8 days after completion of a course of 10 doses of acrylamide over a 2-week period to 6-week old male Fisher rats. Eight rats were used in each group. Standard errors of the mean are given. *Differs significantly from corresponding value for untreated rats (Fisher's Least Significant Difference Test, $P < 0.05$). Solid bars; 24 hours after completion of treatment, hatched bars; eight days after completion of treatment.

I would like to point out that the direction of binding changes found in the developmental study is opposite to the direction in the adult study. The receptors go down in binding capacity in the young, whereas in the adult study they went up. This suggests that neonatal chemistry is different and sometimes opposite to adult chemistry. There is also an interesting parallel here with haloperidol, which in the adult will increase receptor density, but if given to a pregnant rat, one obtains a decrease of dopamine receptors in the offspring.[12] Thus, the effect of haloperidol is reminiscent of that of acrylamide. We also tested acrylamide for direct effects on the dopamine receptor. At a concentration of 10^{-5}M there was no measurable effect of acrylamide on the interaction between membranes and tritiated spiroperidol. What we are reporting here is then an indirect effect.

Turning to **in vitro** studies, there are certain types of problems that might best be initially approached in this manner. In this case, we measure the binding of a radioactive ligand to membranes in the presence of a toxic agent and one of the studies that we are interested in is a comparison of the toxicity of organic and inorganic metals. To do this **in vivo** is difficult because of problems not only with the blood-drain barrier, but with renal clearance rates. For instance, the effect of organic lead triethyl compounds upon transmitter binding cannot be easily measured in a living animal because

Figure 12. Scatchard analysis of striatal binding of ^{3}H-spiroperidol to membranes prepared from rats 24 hours after completion of a course often doses of acrylamide (10 mg/kg body weight each time) over a two-week period, to six-week old male Fisher rats.



Data are from 8 animals in each group. ● = experimental rats, 0 = control rats. Curve derived from linear regression analysis.

the kidney clears this compound so rapidly that one can never get the plasma level high and it is hard to demonstrate any neuropathological changes at all.[13]

We have been studying four different metal compounds, two mercury, two lead. Two compounds are more ionic and two are more covalent. We have been looking **in vitro** at how they affect ligand-receptor interactions. We have measured the binding of tritiated compounds in the presence of different concentrations of these lead and mercury compounds.

I would like to give you three examples of the kind of results we are obtaining (Figures 13-15). The inhibitory effects of the two mercury compounds upon spiroperidol binding are rather similar. This suggests that the mechanism is probably not through a sulfhydryl enzyme. Sulfhydryl enzymes tend to be more inhibited by inorganic mercury than organic derivatives of mercury.

We get a much larger difference between the lead acetate, the more water-soluble hydrophilic compound, and the more hydrophobic, the tributyl lead acetate. This large difference is also found using labeled alpha adrenergic or muscarinic antagonists. In the case of α-adrenergic receptors, the mercuric chloride is more toxic than the organic mercury compound. This could reflect an attack on a sulfhydryl group near the active site of the receptor.

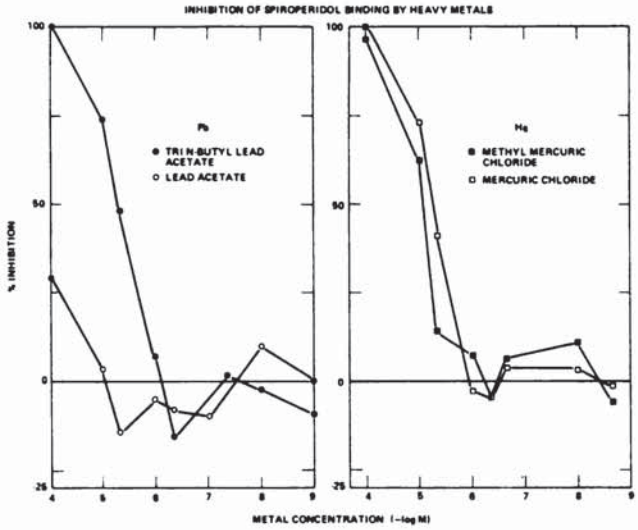
Tri-n-butyl lead acetate is a large organic molecule with hydrophobic chains. This may enable such a chemical to penetrate into a lipid matrix and interfere with protein configuration, whereas methyl mercuric chloride is not nearly so hydrophobic. We are planning to look at a larger range of organometallic compounds so that we can kind of try and get a better understanding of this kind of inhibition.

Now, the Laboratory of Behaviorial and Nuerological Toxicology is particularly interested in the correlation of biochemistry and behavior, and so I would like to mention a few of the problems that I see in bringing these two disciplines together, differences in approach that sometimes make for a difficulty in dialogue.

It seems to me that neurochemistry has a wider range of reasonable things to study than behavior. We have many possible assays, and we are a long way away from getting close to any kind of primary screen or any neurochemical equivalent of the Ames test for mutagenicity. In behavior, we may be a little bit closer to the possibility of a behavioral comprehensive test battery.

One of the features of current neurochemistry—I don't know if it is a problem or a blessing—is that we have a high rate of emergence of new methods. All our important techniques are fairly recent, obsolescence is frighteningly rapid, new ideas are coming along all the time. For example, there is much research and discus-

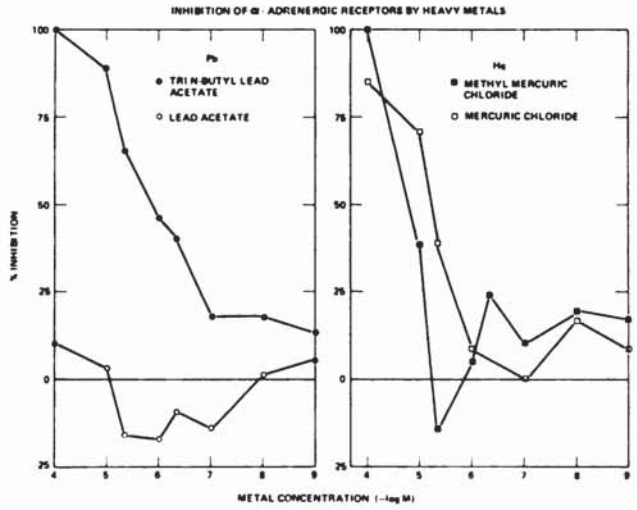
Figure 13. Inhibition of ^{3}H-spiroperidol binding to striatal membranes by the presence of various concentrations of heavy metal compounds in the incubation mixture.



Each point represents the mean of 6-9 determinations.

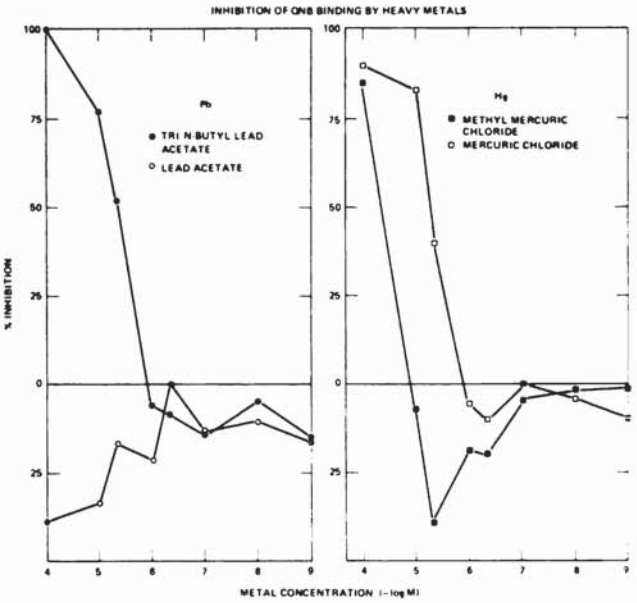
sion currently concerning classes of receptors. Many transmitter receptors are known to be heterogeneous and the full extent of this diversity is not yet known. Another debated issue is the question whether catecholamines are largely neuromodulators rather than neurotransmitters. We are constantly faced with changing understanding of basic mechanisms. Methods are often criticized, and general consensus is rare. This makes the development of an acceptable neurochemical screen for use in neurotoxicology difficult.

Figure 14. Inhibition of binding of an α-adrenergic ligand to striatal membranes (^{3}H-dihydroergocryptine) in the presence of various concentrations of heavy metal compounds in the incubation mixture.



Each point represents the mean of 6-9 determinations.

Figure 15. Inhibition of binding of a cholinergic muscarinic antagonist (^{3}H-quinuclidynyl benzilate, QNB) to cortical membranes in the presence of various concentrations of heavy metal compounds in the incubation mixture.



Each point represents the mean of 6-9 determinations.

From the public health aspect, I think that neurochemical changes in the absence of toxic symptoms or behavior change may be irrelevant. They may be fascinating to us, but behavioral tests are really necessary to validate the significance of altered metabolism.

Correlation of chemical and behaviorial data is not readily achieved. For instance, if an animal is hyperactive, such behavioral change could be caused by several different underlying chemical changes in a variety of transmitter systems. Thus, it is difficult to demonstrate a precise relation so that a given behavior can be correlated with a unique biochemical lesion. In addition, one must take into consideration the existence of mechanisms which allow restoration of normal behavior in the presence of chemical derangement. The peripheral and central aspects of many of the behaviors that are tested may not be readily distinguished. For example, impaired motor activity could come from the CNS or from the PNS, or directly from muscle.

Correlations of dose-response are not always very clear. For instance, the maximal anticonvulsant effect of benzodiazepines can be achieved when only 20 percent of the benzodiazepene sites are occupied.[14] Here there is very limited receptor binding concurrent with maximal behaviorial effects.

I would like to finish by reminding you of the dying words of Gertrude Stein. She was fading fast and people were sitting around listening. She muttered to herself, "What is the answer?" And everybody thought those were her last words. But after about another minute, she said one more thing. She said, "What is the question?"

I think that now it is premature to look for answers, and we really have to address ourselves to finding good questions.

DISCUSSION

Mishra: I have two questions. One is, I am very interested in work that you have shown. Could you correlate or explain the toxicity of acrylamide, especially the axonal degeneration, with the work that you have done with receptor binding? Do you think there is some correlation that could explain the toxicity of acrylamide?

The next question is, have you tested any other compound like n-hexane or other compounds which are known to cause similar degeneration of axons?

Bondy: The answers to both of those questions is no. I think that at this stage, it is much too premature to say that what we are observing biochemically has any relation to the observed behavioral changes. As for using other compounds, in many ways what I would like to do is use as few compounds as possible so that we can go into some detail with each. Already with acrylamide, many questions have cropped up concerning the developmental aspect, the reversibility aspect, the regional aspect, that we have really a lot to explore with this one compound before moving on to another.

Barr: What was the nature of the increase in dopamine receptor binding with the acrylamide-treated animals?

Bondy: The Scatchard plot analysis suggests that acrylamide can affect both site density and the dissociation constant of the dopamine receptors.

Barr: Was there any overt behavioral difference in those treated animals?

Bondy: This is till under study but positive effects have been found.

McKenna: You made one comment which I would like for you to clarify a little, if you would. You stated that neurochemical changes in the absence of behavioral changes are irrelevant, and this is a comment that sort of gets neurochemists right where it hurts.

My question is, do you really feel that behaviorial testing has reached the sophistication to uncover everything that we need to uncover as far as functional problems?

Bondy: It was a theoretical remark that I was making. In other words, I don't think that behavior has got to that stage yet, but I do feel it is possible that there are certain redundant features of mammalian biochemistry where enzyme levels can be significantly depressed without harm to the organism. The population itself varies so enormously as to enzyme levels in some cases. I am a neurochemist myself, but one must be objective as to the significance of observed chemical changes.

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