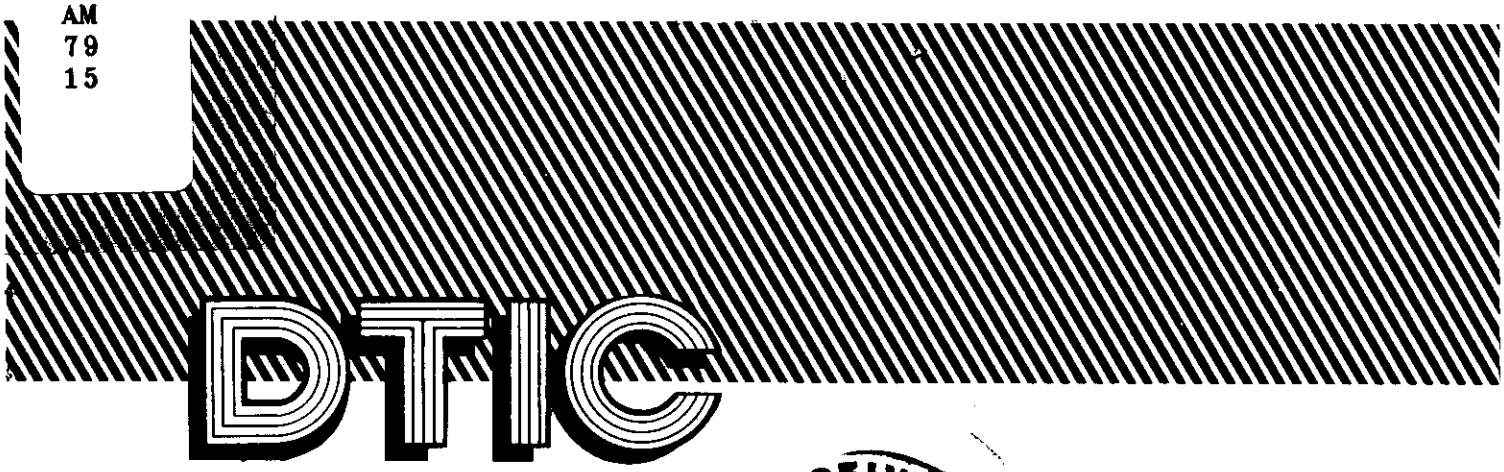

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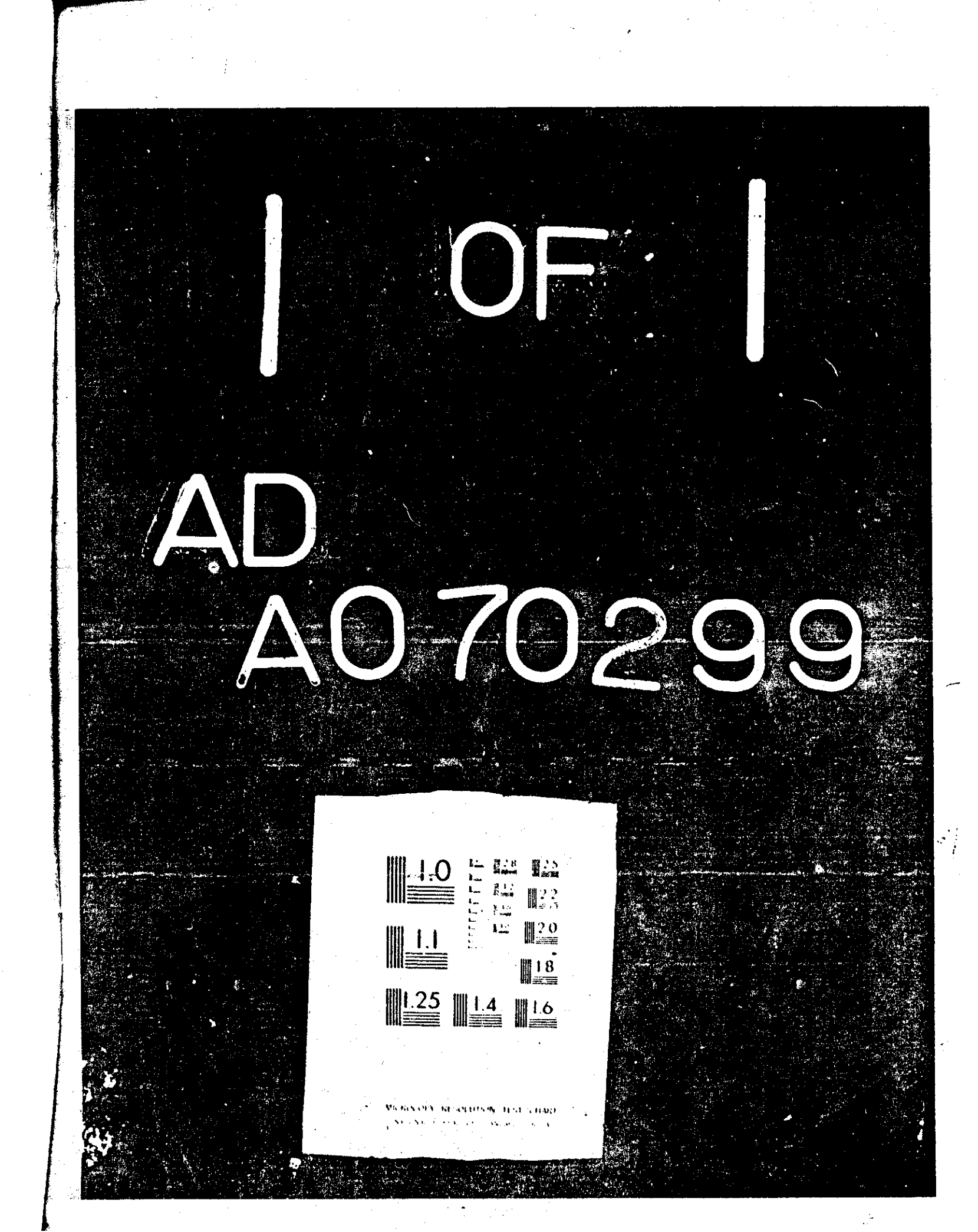
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DEVELOPMENT OF ELECTROPHYSIOLOGICAL INDICES OF NEUROLOGICAL TOXICITY FOR ORGANOPHOSPHATE PESTICIDES AND DEPRESSANT DRUGS

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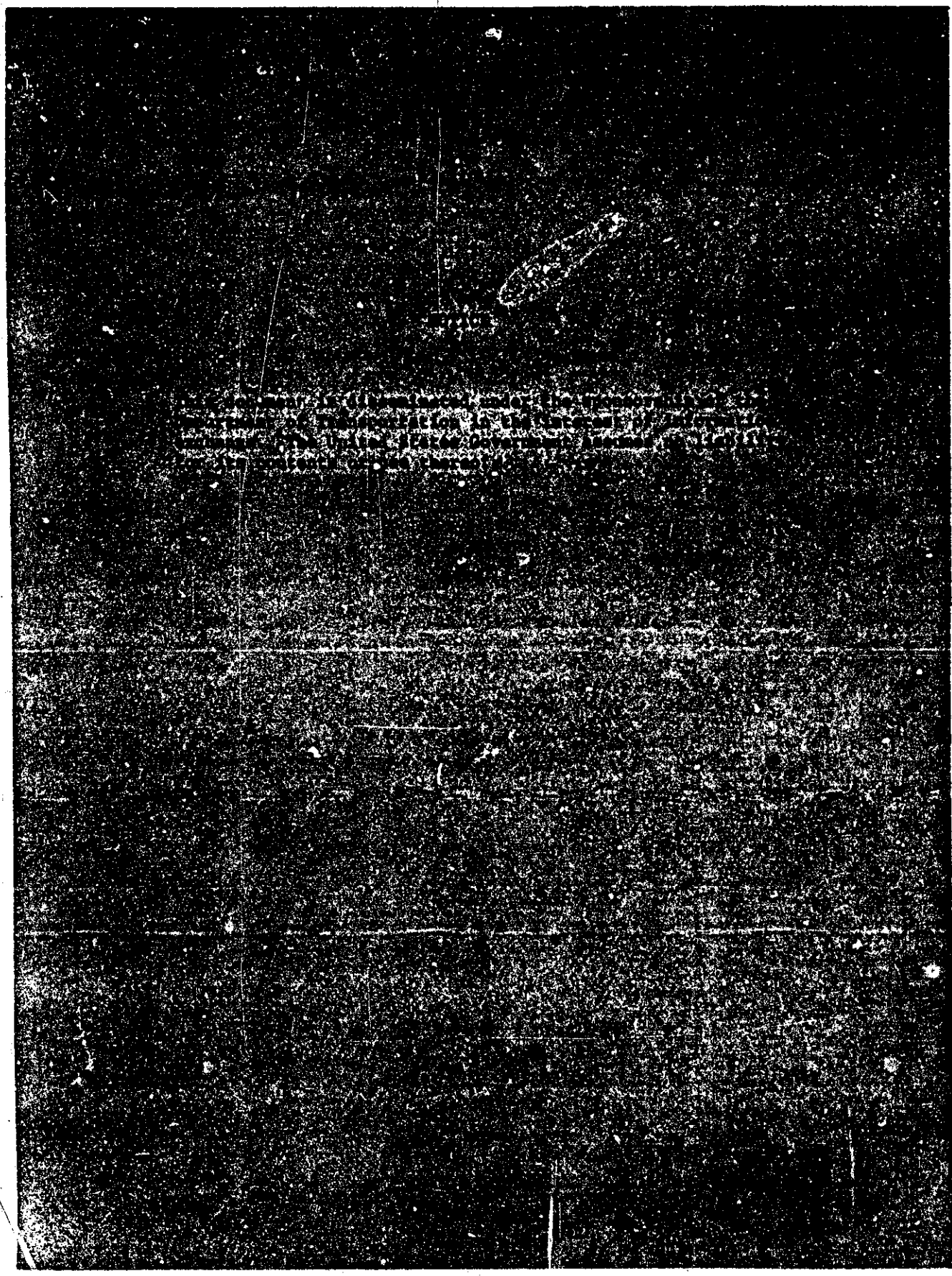

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16. Abstract
Accurate control of eye movements and rapid detection of unexpected events in the periphery of the visual field are critically important in the aviation environment. We have studied the effects of certain drugs and environmental pollutants on brain mechanisms controlling these visual reflexes, using single nerve cell recordings in animal model systems. Most agents studied deleteriously affected reflex functions at extremely low doses. This was especially true for organophosphate pesticides, related cholinergic compounds, and ethyl alcohol. Some drugs, such as imipramine and amphetamine, seemed to have little deleterious effect. Overall, the results suggest that some drugs and environmental pollutants can impair visual functions at doses not normally considered hazardous, and thus indicate the need for extreme caution in evaluating the safety margins of such materials in aviation medicine.

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DEVELOPMENT OF ELECTROPHYSIOLOGICAL INDICES OF NEUROLOGICAL TOXICITY FOR ORGANOPHOSPHATE PESTICIDES AND DEPRESSANT DRUGS

I. Introduction.

The end point of toxicology is risk assessment. For some purposes, risk may be established if a toxic agent produces any change in behavior or other neurological functions. Such simple criteria are not always sufficient. For example, it has long been known that organophosphate pesticides (OP) and related cholinergic agents can affect brain and behavior. However, the economic importance of these toxic substances precludes their complete banishment. It is also difficult to convince aerial applicator personnel and agricultural users, the major populations at risk, that exposure to even low levels of OPs can be hazardous. In this situation any recommendations concerning exposure limitations must be supported by data clearly demonstrating that exposure to these agents at "real world" levels can result in performance deficits or risk. Furthermore, the results of our research must be evaluated, and appropriate actions taken, by regulatory or legislative people who may understand little of our arcane techniques but do need to understand why, and in what way, our measures translate into a risk for exposed people. Ideally, therefore, our test systems should measure phenomena whose importance is readily appreciated by nonspecialists.

The fact that we are dealing with toxic agents means that the use of human subjects must be limited, and animal models used. However, for the model to have maximum utility it must involve functions or systems that are similar or identical to the homologous function or system in man. This is a general consideration in all research using animal models but is particularly pertinent in toxicology, with its direct applicability to human well-being. Unfortunately, the process of selecting a suitable model system is not trivial because the demonstration of homology is difficult and controversial (4). However, much current evidence indicates that the structure and functions of the visual projections from retina through mesencephalon and thalamus to the telencephalon are similar in all diurnal vertebrates (1,13, 20,21,31). Thus, results from experiments on these visual projections can, quite reasonably, be extrapolated from animals to man. This evolutionary constancy of the early stages of visual processing encourages research in the area of visual toxicology. Furthermore, there is an immense amount of fundamental information about visual system neurophysiology (3,7,14,28). Thus, we can select, for toxicological studies, neurophysiological phenomena that are well understood, stable, predictable, and readily manipulable. Therefore, changes in response patterns elicited by drugs or toxic agents may be explicable in terms of known mechanisms and may help predict types of functional impairments associated with the specific changes (8,10,11,22, 23,29,30,33). Finally, we are highly visual animals, and the hazards of impaired visual function are clearly understood by everyone, especially those who fly airplanes or drive automobiles.

There are many behavioral and electrophysiological approaches to the evaluation of visual toxicology. All are open to the same basic question: In what way is a change in the measured phenomenon hazardous? This can be an embarrassingly difficult and complex question to answer. For example, changes in simple schedule-driven behaviors, in reaction times to simple visual stimuli, or in the amplitude of flash-evoked gross potentials at the brain surface are difficult to interpret because the phenomena measured are very complex. Though it is likely that changes in these phenomena do signal functional alterations in the brain, any change by itself does not predict whether, or in what way, these alterations can lead to impaired performance or hazard in the specific environments with which we are concerned. However, more direct measures of changes in central nervous system (CNS) function produced by toxic agents are available. The processing and transfer of visual information in the brain is accomplished by suitable patterned sequences of discharges (firing) of single neurones in the visual projection areas. In the early stages of visual processing, e.g., from retina through the first telencephalic synapses, the response pattern of any given visual neurone to a variety of visual stimuli is characteristic, stable, and predictable (7,9,19,25,34). Thus, any change in the neurone's firing pattern resulting from a toxic agent directly and unambiguously indicates that we have altered the output of the neurone in response to a given input. Therefore, the unit has been made to carry erroneous information and this is, clearly, a deleterious change in CNS function. For example, many neurones in the optic tectum (superior colliculus) are sensitive to direction of movement of visual stimuli (5,9,16,25,26,32,34). A change in these properties would signal impairment of the ability to direct attention to an object moving in a certain direction (11,18,22). If the change were produced by some toxic agent, we could immediately conclude that this agent presented a hazard to aircraft and automobile operators, for such people need properly functioning control of attention and eye movements. However, it is conceivable that specific effects of toxic agents on brain functions would not result in any significant performance effects in the "real world" since compensation for the toxic effects could occur.

We do not believe that compensation for visual deficits can occur. There are several bases for this admittedly heterodox position. The major one is drawn from recordings done on single units in the early stages of visual processing both in this laboratory (25,26,27) and in many others (5,9,16,22,29,32,34). If one tests visual neurones with a wide variety of stimuli, differing in size, shape, color, distance, velocity, position, etc., each neurone appears to have a unique and stable pattern of responses. Therefore, it looks as if each visual neurone has some special and unique function in visual perception. Consequently, compensation for dysfunctions of some neurones cannot occur since any strategy used by the brain to correct errors would involve changing or reinterpreting the responses of other neurones--whose functions in turn would be lost. It is not now clear whether similar arguments can be applied to other brain functions. Nevertheless, it does appear that errors induced by toxic agents in early stages of visual processing are unlikely to be without functional consequences, so that such errors, if they occur, are of considerable toxicological significance.

Thus, the study of the changes induced by toxic agents in evoked discharge patterns of defined types of visual neurones in the early stages of visual processing satisfies the criteria discussed in the preceding paragraphs: (i) changes signal hazardous phenomena; (ii) whatever happens in animals will also happen in man; (iii) these data can readily be explained to, and comprehended by, interested parties, scientists or not.

The major projections from the retina are directed to two structures in all vertebrate brains, the dorsal thalamus and the optic tectum (16,20,21, 31). The functions of the optic tectum are basic to regulation of visual attention and involve detection of significant moving stimuli, particularly in peripheral vision, and the generation of appropriate foveating or following eye movements (5,6,7,9,16,19,28,34). Conversations with aerial applicators exposed to OPs suggested that dysfunctions in peripheral vision might be an early sign of poisoning. Furthermore, our own initial studies (24) together with later literature data (10,17,22,29,30,33) suggested that visual responses in the optic tectum and its rostral projections to dorsal thalamus and telencephalon were unusually sensitive to a variety of drugs and toxic agents. Therefore, we directed our studies at this projection system. Similar recent studies on the pharmacology of single unit responses in visual (10,22,29,30,33) and auditory (8) cortex suggest that measurement of cortical phenomena would also be useful, and support the general utility of this approach. These studies also emphasize the critical importance of studying populations of neurones having distinct and characteristic response patterns (8,22) rather than looking for statistical effects in a random sample of uncharacterized, or uncharacterizable, neurones in some anatomically defined brain area.

The selection of a suitable experimental animal presented few problems. Since the functional properties of the early stages of the visual system are similar in all diurnal vertebrates, the test animals could be selected on the basis of convenience. We chose the pigeon. It is inexpensive to buy, is inexpensive to maintain, is easy to handle, is free from disease, adapts well to the laboratory, and has been used in enough behavioral and physiological studies that procedural and methodological problems in experimental manipulations are minimal because someone else has already solved them.

Thus, our search for a good experimental system for assaying the neurobehavioral toxicity of environmental pollutants and other toxic agents has resulted in the measurement of patterns of discharge of single nerve cells of specified functional properties in the optic tectum of the pigeon. A standardized procedure has evolved which we will now describe.

II. Methods.

We used the highly inbred white carneau pigeon exclusively. The birds were anesthetized with urethane, 1.2-1.8 gm/kg, injected very slowly into the brachial vein. The anesthetic was injected "to effect," the end point being a quite characteristic caudad movement of the legs of the animal.

In some experiments α-chloralose (70 mg/kg) was used instead of urethane. The experimental results were identical. Both anesthetics produced very stable anesthesia levels lasting for about 5 hours for chloralose and 9 hours for urethane. Since both anesthetics are nephrotoxic and hepatotoxic, the experiments were terminated after 8 hours to prevent any metabolic abnormality from biasing our results. The birds were placed in a special stereotactic instrument oriented according to the Karten and Hodos coordinates (12). The apparatus was so constructed that there was no obstruction in the visual field of the left eye of the pigeon. The apparatus and bird could also be pivoted about three orthogonal axes through the nodal point of the left eye. The visual stimuli were presented against a tangent screen placed 1.5 meters from the animal. The pivot arrangement permitted us to examine the entire visual field of the bird without moving the tangent screen or projectors. Stimuli consisting of white or dark spots or bars of various shapes and sizes could be projected on the light-gray surface of the tangent screen. White or black cardboard targets about 3 mm thick were, however, most frequently used in examining the responses of the units. These targets were moved by hand or by a hydraulic cylinder linkage system and proved to elicit more selective, stable, and predictable responses than the projected two-dimensional images. The targets were usually circular, and their sizes ranged from 0.5 to 10.0 degrees of arc. For study of responses to very large targets one of the experimenters would move in front of the animal, a simple and effective tactic. Target velocity was varied by timing arm and hand movements with a metronome or by manipulating a needle valve in the hydraulic cylinder's control system. Velocities used ranged from $1^{o}/s$ to $300^{o}/s$, but the most used target velocity was $40^{o}/s$. The recording electrodes were micropipettes with tip diameters of about 3 micrometers and were driven by a hydraulic microdrive. They were filled with a 5:1 mixture of 3M NaCl and KCl. This filling solution seemed to produce less long-term shift in discharge patterns than either pure NaCl or KCl solutions. Experiments using micropipettes were usually more successful than those using metal microelectrodes, presumably because the pipettes had more flexible tips and a gentler tip taper and so caused less local tissue damage and distortion. Amplifying, display, and recording apparatus was conventional, based on the use of Neurolog amplifying and logic modules and a Sony cassette tape recorder. With suitable brain stabilization procedures including a sealed calvarium, infiltrated with dental acrylic for rigidity, and extremely slow electrode movement, to give time for tissue stabilization, we could consistently record from single neurones for periods of 2.0-4.5 hours. This was sufficiently long that there was no problem in recording the effects of a toxic agent over time in one cell. Once a suitable spontaneously active unit was found and characterized, procedures which could take 2-3 hours, control recordings were taken for at least 45 minutes. If the unit's responses were stable throughout the control period, the toxic agent under study was administered by intramuscular or intravenous injection. The response patterns of the cell were then followed for as long as we could record from the cell. Evaluations of the unit's responses were usually aural, as discussed by Bishop (3). This simple approach, used with caution, gave stable and readily reproducible results.

Eye movements can be a confounding variable in this kind of study. Under the conditions of these studies eye movements never exceeded 1.5 degrees and were usually less than 0.5 degrees, measured by microscopic observation of movements of corneal marks. Since the visual field of the units studied ranged from 120 to 170 degrees (25,26), and normal stimulus movements covered 30-50 degrees, it does not seem likely that eye movement artifact presented any problems. During the experiment the eyelids of the pigeon were mechanically retracted, but normal activity of the nictitating membrane kept the cornea in excellent condition without further intervention from the experimenter. Significant alterations in pupillary diameter were not seen in these experiments.

III. Results.

Response Patterns. The rostrad efferent discharges of the optic tectum (superior colliculus) are relayed in the posterior thalamus (1,2,5,9,16,19, 21,27). The specific thalamic nuclei involved carry a number of names (e.g., lateralis posterior-pulvinar complex in mammals (9), nucleus rotundus in birds (27)) but all serve to relay visual information from the optic tectum to the telencephalon. In the bird, long-term single unit recording from the nucleus rotundus is a good deal simpler than recording from tectum so our data were taken from rotundal neurones. The response patterns of the units in rotundus have been extensively studied (25,26). In general, rotundal units respond only to moving visual stimuli. Visual fields are very large, most units having field sizes of 150-170 degrees. One class of cell, located mainly in posterior portions of rotundus (2,26), responds vigorously and nonselectively to any movement of the retinal image. A second group of cells, distributed mainly in the anterior areas of rotundus (2,26), show complex mixtures of responses selective for one or more abstract characteristics of the moving stimuli: size, direction of movement, velocity of movement, albedo, etc. The two commonest neuronal response patterns seen were the "respond to anything moving" cells, or "posterior rotundal" units, and units directionally selective for small dark targets moving quickly (more than 20^{o}/s), found mostly in anterior rotundal areas. Most units studied have been of these two types.

Cholinesterase-Inhibiting Pesticides and Related Compounds. A variety of substances that affect cholinergic neurotransmission were tested first, since tectum contains large amounts of localized acetylcholinesterase (14,15,23) and we were interested in OP effects. The results are summarized in Figure 1 and Table 1. As can be seen, any compound capable of affecting central cholinergic functions blocked directional selectivity in any rotundal neurone showing this characteristic. Other response characteristics were relatively unaffected (Table 1). That these effects were the result of central actions is indicated by the lack of effect of the compounds that do not readily penetrate the blood-brain barrier, atropine methyl bromide and the cholinesterase reactivator, pralidoxime. These had effects only at extremely high doses suggesting that their effects on

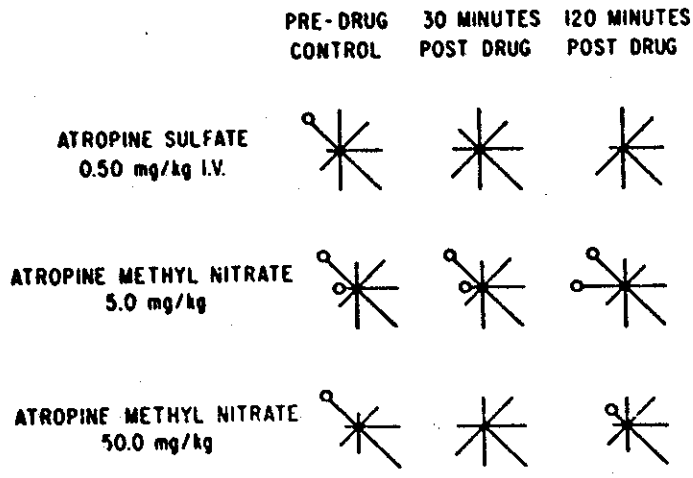

Figure 1. Effects of some muscarinic cholinergic agonists on directional selectivity characteristics of rotundal units. The targets, 2°-diameter black cardboard circles, were moved in the eight indicated directions. The length of the vector is proportional to the intensity of the response. A vector with a circle at the end indicates an inhibitory response; ongoing activity is partly or fully inhibited. Atropine sulfate blocks the inhibitory, or "null" component of the response within 5-10 minutes of intramuscular injection, with partial recovery, in this unit, seen by 120 minutes post-injection. Methyl atropine, which does not readily pass the blood-brain barrier, has no effect at 10 times the dose of atropine sulfate. At 100 times the dose, methyl atropine tends to block directional selectivity just as atropine sulfate does. Other cholinergic drugs, such as mevinphos, block directional selectivity just as atropine sulfate does.

TABLE 1. Effects of Cholinergic Agents on Rotundal Unit Activity

Toxic Agent Dose, mg/kg	Number of Units[a]	Dir. Sel.[b]	Size Pref.	Dark vs. Light Targets	Vel.	Ongoing Rate	Notes
Mevinphos							
0.050	16	-	0	0	0	0	c
0.100	17	B	0	0	0	0	d
0.200	7	B	0	0	0	+	e
Pilocarpine Nitrate							
2.00	4	B	0	0	0	+	
Eserine							
0.10-5.00	8	B	0	0	0	0	
Atropine Sulfate							
0.20-1.00	21	B	0	0	0	0	
2.00-4.00	6	B	0	0	0	-	
Scopolamine Hydrobromide							
0.1	4	B	0	0	0	0	
Atropine Methyl Bromide							
2.0-40.0	7	0	0	0	0	0	
45.0	3	-	0	0	0	0	
Pralidoxime							
1.0-20.0	6	0	0	0	0	0	
10.0	2	-	-	0	-	-	f

a Some units were used for more than one dose of an agent and/or for more than one agent.

b B indicates complete block of directional selectivity.

c At near threshold doses the directional selectivity "null" was abolished but the directional response patterns were strongly asymmetrical.

d Directional response patterns were nearly symmetrical; no clear directional preferences were seen.

e Maximum usable dose; higher doses caused seizures.

f Inhibition of directional selectivity and other effects seen in two sick birds (see text).

response patterns could only be seen when sufficient amounts of the drug crossed the blood-brain barrier. The two exceptions recorded for pralidoxime were extremely interesting. Anecdotal accounts from farming acquaintances suggested that the effects of some OPs are exacerbated in the presence of a "cold" or other viral infection. During the course of these experiments two birds developed a moderate viral pneumonitis. The birds were given pralidoxime at a dose which, though high, was usually without effect. As can be seen in Table 1, the pralidoxime had a substantial effect on unit response patterns in these sick birds. Again, mevinphos (an OP), pilocarpine (a cholinergic agonist), eserine (a carbamate acetylcholinesterase inhibitor), and atropine and scopolamine (muscarinic cholinergic antagonists) all block directional selectivity at relatively low dose levels. No peripheral parasympathomimetic signs were seen, either in anesthetized or normal animals, at OP doses that blocked directional selectivity. Thus a substantial degree of malfunction of peripheral vision may be present after OP exposure without any peripheral signs of poisoning. Also, since all cholinergic drugs have similar effects, presumably due to a block of a cholinergic neurone necessary to the generation of the directional null, effective therapy of this aspect of OP poisoning is probably not possible.

Psychoactive Substances. The data in Table 1 show that all cholinergic drugs have a common pattern of action on rotundal response patterns. This suggests that each class of centrally acting agents will show some unique pattern of action on rotundal responses. To verify this idea, we have tested a variety of depressant and stimulant drugs. The results are summarized in Figure 2 and Table 2. These results are rather more complex than those just reported for the cholinergic agents, but the data summarized in Table 2 suggest that, indeed, each type of drug has a unique effect on rotundal responses.

For example, the stimulants amphetamine and imipramine both have similar effects on directional selectivity and spontaneous rate (Figure 2). The effects on directional selectivity are probably not specific. A directionally selective response pattern is defined (3) as one showing a maximum excitatory response to the direction of target movement and an inhibition of ongoing activity, or "null," in another direction, usually directly opposite to that producing maximum excitation. Other directions of movement produce intermediate excitatory responses (Figures 1 and 2). The overall response patterns (Figures 1 and 2) look as if an inhibitory response to one direction of movement is summed with an overall nondirectional excitatory response pattern to produce the measured overall characteristic. This is consistent with the effects of the cholinergic agents, which seem to inhibit the inhibitory response, resulting in an apparent loss of directional selectivity. However, the null is usually quite narrow in the sense that the inhibitory response can only be elicited over a range of $\pm 10^{o}$ to $\pm 15^{o}$ from the maximum null. This angular extent of the null, or its included angle, is the variable used in Table 2, under "Dir. Sel." However, the interpretation of a change in directional selectivity is not immediately obvious. For example, a reduction in the

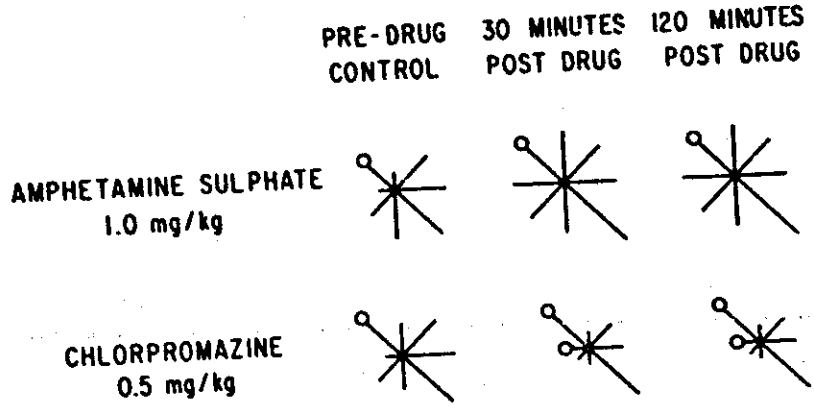

Figure 2. Effects of amphetamine sulfate and chlorpromazine on directional selectivity of rotundal neurones. Graphing conventions are those of Figure 1. Amphetamine increases the intensity of excitatory components of the evoked response. Spontaneous rate is also increased which accounts for the apparent increase in the amplitude of the inhibitory response; ongoing activity is inhibited, but starting from a higher level. Imipramine produces identical effects. Chlorpromazine increases the extent of the inhibitory response. This is seen both in terms of the widened angle over which inhibition is observed and in terms of the decreased excitatory responses in movement vectors adjacent to those giving a null.' Note, however, that the intensity of the maximum response is unchanged, as is the ongoing discharge rate.

TABLE 2. Effects of Some Depressant and Stimulant Drugs on Rotundal Unit Activity

Drug or Toxic Agent Dose, mg/kg	Number of Units[a]	Dir. Sel.[b]	Size Pref.	Dark vs. Light Targets	Vel.	Rate	Notes
Amphetamine Sulfate							
0.25-2.00	15	-	0	?	0	+	c
Imipramine							
1.0-5.0	11	-	0	?	0	+	
Chlorpromazine							
0.1-0.5[d]	6	+	0	0	0	?	e
Clonidine							
1.00	4	0	-	?	0	-	
Chlorpheniramine maleate							
0.30-2.00	18	0	0	-	-	-	f
Diphenhydramine							
0.10-0.50	3	0	0	-	?	-	
Ethanol[g]							
Anterior Rotundus							
0.050	24	0	0	0	0	-	
0.10-0.40	10	+	0	0	0	-	h
Posterior Rotundus							
0.050-0.100	15	n.a.[i]	n.a.	n.a.	n.a.	+	
0.200-0.400	11	n.a.	n.a.	n.a.	n.a.	-	
Dorsal Thalamic Visual							
0.050-0.250	6	n.a.	0	0	0	0	
0.400	4		0	0	0	-	

a Some units were used more than one dose of a drug and/or for more than one drug.

b Change in directional selectivity is expressed as a change in the included angle of the null; (+) = an increase in the included angle. NOTE that general changes in excitability can cause apparent changes in the angle (e.g., a decrease in excitability, by reducing all response vectors, can give the illusion of an increase in the included angle of the null response).

c The reduction in Dir. Sel. is probably a result of an increase in overall excitability (see note b).

d High doses of chlorpromazine depress all neurone responses and so seem to further increase Dir. Sel. (note b).

e The change in Dir. Sel. is apparently real and not due to a change in general excitability.

f Chlorpheniramine frequently induces a brief (2-7 minute) period of hyper-excitability immediately following injection.

g Ethanol doses = ml 95% alcohol per kg.

h The apparent increase in selectivity is a consequence of the general decrease in neurone excitability (see note b).

i Not applicable, these units were not selective for the indicated variable.

included angle of the null can be due, inter alia, to an inhibition of the inhibitory response or a facilitation of the excitatory response, since the effects of these on the summed response pattern would be similar. As stated, imipramine and amphetamine reduce the included angle of the null of directionally selective rotundal neurones. They also increase the amplitude of the excitatory movement responses (Figure 2) and increase spontaneous discharge rate. These findings suggest that amphetamine and imipramine simply increase the level of excitation in rotundus. Thus, ongoing activity and the amplitude of the excitatory response to target movement will both increase, giving an apparent reduction in the null angle.

The smaller doses of chlorpromazine (CPZ), on the other hand, increase the size of the null angle (Figure 2) without much significant change in the other variables measured (Table 2). This suggests a simple, specific facilitation of the inhibitory response to movement. At higher doses of CPZ, ongoing rate and the size of the maximum movement-evoked responses are inhibited together with a reduction in selectivity of all other specific response patterns. Thus, the higher doses of CPZ seem to produce a general inhibition of rotundal excitability in addition to the apparently specific effect on the inhibitory component of the movement response seen at lower doses. The actions of CPZ seem, in part at least, opposite to the effects of amphetamine and imipramine.

We have also looked at two drugs widely used as antihistamines, chlorphentramine maleate and diphenhydramine. Both had relatively little effect on movement-evoked responses. Those units which responded differently to small dark vs. small light targets were affected to some extent, the response differences being decreased. This effect was not clearly seen with any other compound tested. Ongoing activity, or spontaneous discharge rate, of all units tested was reduced by these drugs, this reduction in rate being much greater than the reductions in stimulus-elicited responses. The doses required were, however, rather high.

Ethanol is the most widely used and misused psychoactive drug. As such, it probably qualifies, with caffeine, as an environmental pollutant. The effects of ethanol have been intensively studied for many years by a large number of investigators. Despite the thousands of papers resulting from this activity, there are wide areas of uncertainty about the actions of ethanol. For example, there is little agreement on the threshold dose for "significant" performance decrement, though this is a critical element in evaluating the effects of alcohol on safe aircraft or automobile operation.

Ethanol, in rotundus, has little effect on movement-evoked responses. However, spontaneous activity is profoundly affected by very low doses of the drug (Table 2). In directionally selective, "anterior rotundal" neurones alcohol is purely inhibitory. Threshold dose is 0.050 ml/kg, which is very low. Duration of effect of threshold amounts is 30-50 minutes. Doses of 0.100 ml/kg produced effects lasting for about 2 hours. Doses of 0.200-0.400 ml/kg usually completely inhibit spontaneous activity, with some general inhibitory effects (increasing null angle and decreased excitatory

response amplitude) also seen in the directionally selective neurones. Duration of effect of the higher doses is indeterminate, since we have not been able to record from single units for long enough to measure this. Ethanol has more complex effects on posterior rotundal neurones. Low doses, in the range of 0.050-0.100 ml/kg, cause an increase in firing rate. Higher doses produce a complex alteration in firing rates: an initial increase lasting 3-5 minutes is followed by a prolonged decrease lasting for the "life" of the neurone. Two units tested with 0.200 ml/kg did return to control rates, and, perhaps, overshot, some 150 minutes post-injection, but the units were "lost" shortly thereafter so the increase in rate could have been a membrane damage artifact. Several visually driven units in dorsal thalamus were also tested. These had small visual fields, were responsive to stationary or moving stimuli, had ON-center, OFF-surround characteristics, and otherwise looked rather like cells from cat lateral geniculate nucleus. Ethanol inhibited both ongoing and evoked activity in these cells, but the threshold dose for this effect was very high, about 5 times rotundal thresholds. Duration of effect was indeterminate, but greater than 2 hours.

The effects of ethanol on rotundal units suggest that low doses of alcohol will affect peripheral vision, as suggested by several recent studies (17,18,35). The complexities of the effects of alcohol are emphasized by the higher threshold doses for dorsal thalamic depression (Table 2) and for depression of other brain structures (11), suggesting that the effects of this substance on performance are a very complex function of the dose ingested. It is also interesting that the antihistamines and ethanol produced roughly similar changes in response patterns.

IV. Discussion.

We have discussed some of the considerations that went into the design and development of a system for assaying neurobehavioral toxicity of environmental pollutants, described in some detail the system as it has been used, and detailed some of the results obtained using the system. In general, the design criteria have been met. The system is extremely sensitive to many toxic agents, notably cholinergic drugs and ethanol. It is a selective system in that different classes of drugs or toxic agents have characteristic patterns of action on functionally different types of neurones in nucleus rotundus. This aspect of the results emphasizes, in a unique way, that the neurobehavioral effects of any toxic agent cannot be characterized in any simple, definitive fashion. The effects seen vary as a function of the specific phenomena being tested and the dose of, and mode of exposure to, the specific toxic agent. Further, the data, and their significance for, say, aerial applicators, have proven, in informal conversations, etc., to be easily comprehended, even by nontechnical or nonbiological observers. Thus, as stated, the initial design criteria appear to have been met.

This general approach to neurobehavioral toxicology is not, however, a general panacea. For one thing the experiments are extremely difficult and time consuming. Because, among other things, electrode tips break, and units

are "lost" in the middle of a recording, about two experiments out of three are failures. Thus single unit studies cannot easily be used for "screening" or for rapid assessments of possible hazards of toxic agents.

However, in many circumstances detailed assessments of the effects of toxic agents on brain mechanisms are necessary. In a sense, the single unit approach enables one to talk to individual neurones and ask them, directly, about the effects of a toxic agent. Their responses can be extremely useful to the neurobehavioral toxicologist.

One major practical point emerges from this research. We must, in the future, be very much more cautious about exposure/dose recommendations for any drug or environmental pollutant known to affect brain function. The reasons for this are clear.

The results from, for example, the mevinphos and ethanol studies indicate that drugs or toxic chemicals may have substantial effects on some brain functions at doses well below those now believed hazardous. Mevinphos can affect systems mediating reflex phenomena in peripheral vision at doses (0.05 mg/kg) roughly one-third to one-quarter those needed to elicit peripheral parasympathomimetic symptoms or skeletal muscle tremor. Alcohol acts at doses (0.05 ml/kg) more than an order of magnitude less than those recognized as "safe" for driving by most states. Under normal driving or flying conditions the effects produced by low doses of these, and other, agents may not be significant--though a reduced responsiveness to events in peripheral vision (side-road traffic or aircraft flying intersecting flight paths) should always be of concern. However, under emergency conditions, or when there is heavy traffic--in fact, when we need all of our faculties operating properly--the perceptual or information-processing deficits become disproportionately important and could lead to an accident.

Evaluation of the deficits, or prediction of hazard, is complex because not all sensory systems, or brain systems, are affected equally, as shown by the range of threshold doses for different systems summarized in Tables 1 and 2. There is no reason to assume that similar kinds of differential drug effects are not present in other brain structures as well (8,22). Thus, the specific performance deficits produced by any given toxic agent will depend on the dose, since the dose determines which sets of brain functions are disturbed. Furthermore, prediction of performance deficits, at any low dose level, may be very difficult since the tests used must probe those functions sensitive to the agent. Clearly, tests measuring central visual functions, as most do, would not be nearly as sensitive to mevinphos or ethanol as tests measuring peripheral visual functions (17,18,35).

Therefore, the author feels that it may be necessary to review and revise current recommendations regarding "safe" centrally acting drugs or toxic agents, "safe" levels or doses of such agents, or minimum time interval from exposure to task performance.

REFERENCES

1. Benevento, L. A., and J. H. Fallon: The Ascending Projections of the Superior Colliculus in the Rhesus Monkey. (Macaca mulatta). J. COMP. NEUROL., 160:339-362, 1975.

2. Benowitz, L. I., and H. J. Karten: Organization of the Tectofugal Visual Pathway in the Pigeon. J. COMP. NEUROL., 167:503-520, 1976.

3. Bishop, P. O.: Central Nervous System: Afferent Mechanisms and Perception. ANN. REV. PHYSIOL., 29:427-484, 1967.

4. Campbell, C. B. G., and W. Hodos: The Concept of Homology and the Evolution of the Nervous System. BRAIN BEHAV. EVOL., 3:353-367, 1970.

5. Chalupa, L. M., and S. E. Fish: Response Characteristics of Visual and Extravisual Neurones in the Pulvinar and Lateral Posterior Nuclei of the Cat. EXP. NEUROL., 61:96-120, 1978.

6. Denny-Brown, D., and E. G. Fischer: Physiological Aspects of Visual Perception. II. The Subcortical Visual Direction of Behavior. ARCH. NEUROL., 33:228-242, 1976.

7. Dow, B. M.: Central Mechanisms of Vision: Parallel Processing. FED. PROC., 35:54-59, 1976.

8. Foote, S. L., R. Freedman, and A. P. Oliver: Effects of Putative Neurotransmitters on Neuronal Activity in Monkey Auditory Cortex. BRAIN RES., 86:229-242, 1975.

9. Godfraind, J. M., M. Meulders, and C. Veraart: Visual Properties of Neurons in Pulvinar, Lateralis Posterior and Nucleus Suprageniculatus Thalami in the Cat. BRAIN RES., 44:503-526, 1972.

10. Hess, R., and K. Murata: Effects of Glutamate and GABA on Specific Response Properties of Neurones in the Visual Cortex. EXP. BRAIN RES., 21:285-297, 1974.

11. Kalant, H.: Ethanol and the Nervous System. Experimental Neurophysiological Aspects. INT. J. NEUROL., 9:111-124, 1974.

12. Karten, H. J., and W. Hodos: A Stereotaxic Atlas of the Brain of the Pigeon (Columba livia). Baltimore, Johns Hopkins Press, 1967.

13. Karten, H. J., W. Hodos, W. J. H. Nauta, and A. M. Revzin: Neural Connections of the "Visual Wulst" of the Avian Telencephalon. Experimental Studies in the Pigeon (Columba livia) and the Owl (Speotyto cunicularia). J. COMP. NEUROL., 150:253-278, 1973.

14. Krnjevic, K.: Chemical Nature of Synaptic Transmission in Vertebrates. PHYSIOL. REV., 54:418-540, 1974.

15. Kusonoki, T.: The Chemoarchitectonics of the Avian Brain. J. HIRNFORSCH., 11:477-497, 1969.

16. McIlwain, J. T.: Central Vision: Visual Cortex and Superior Colliculus. ANN. REV. PHYSIOL., 34:291-314, 1972.

17. Moskowitz, H., and S. Sharma: Effects of Alcohol on Peripheral Vision as a Function of Attention. HUM. FACTORS, 16:174-180, 1974.

18. Moskowitz, H., J. Ziedman, and S. Sharma: Visual Search Behavior While Viewing Driving Scenes Under the Influence of Alcohol and Marijuana. HUM. FACTORS, 18:417-431, 1976.

19. Motter, B. C., and D. B. Lindsley: Response Properties of Cells in the Nucleus Lateralis Posterior of the Cat Associated With Visual Orientation. NEUROSCIENCE ABSTRACTS, 3:570, 1977.

20. Nauta, W. J. H., and H. J. Karten: A General Profile of the Vertebrate Brain With Sidelights on the Ancestry of Cerebral Cortex. In F. O. Schmitt (Ed.), Neurosciences 2nd Study Program. New York, Rockefeller University Press, pp. 7-26, 1970.

21. Nieuwenhuys, R.: The Brain of the Lamprey in a Comparative Perspective. ANN. N.Y. ACAD. SCI., 299:97-145, 1977.

22. Pettigrew, J. D., and J. D. Daniels: GABA Antagonism in Visual Cortex: Different Effects on Simple, Complex and Hypercomplex Neurons. SCIENCE, 182:81-83, 1973.

23. Phyllis, J. W.: The Pharmacology of Thalamic and Geniculate Neurones. INT. REV. NEUROBIOL., 14:1-48, 1971.

24. Revzin, A. M.: Effects of Endrin on Telencephalic Function in the Pigeon. TOXICOL. APPL. PHARMACOL., 9:75-83, 1966.

25. Revzin, A. M.: Some Characteristics of Wide-Field Units in the Brain of the Pigeon. BRAIN BEHAV. EVOL., 3:195-204, 1970.

26. Revzin, A. M.: Functional Localization in the Nucleus Rotundus. In A. Granda (Ed.), Neural Mechanisms of Behavior in the Pigeon. New York, Plenum, 1979.

27. Revzin, A. M., and H. J. Karten: Rostral Projections of the Optic Tectum and Nucleus Rotundus in the Pigeon. BRAIN RES., 3:264-276, 1966/67.

28. Richards, W.: Motion Detection in Man and Other Animals. BRAIN BEHAV. EVOL., 4:162-181, 1971.

29. Rose, D., and C. Blakemore: Effects of Bicuculline on Functions of Inhibition in Visual Cortex. NATURE, 249:375-377, 1974.

30. Sillito, A. M.: The Effectiveness of Biculline as an Antagonist of GABA and Visually Evoked Inhibition in the Cat's Striate Cortex. J. PHYSIOL. (LONDON), 250:287-304, 1975.

31. Snyder, M.: The Evolution of Mammalian Visual Mechanisms. In R. Jung (Ed.), Handbook of Sensory Physiology, Vol. VII/3,A. New York, Springer-Verlag, 1973.

32. Stein, B. E.: Development and Organization of Multimodal Representation in Cat Superior Colliculus. FED. PROC., 37:2240-2245, 1978.

33. Tsumoto, T., W. Eckart, and O. D. Crutzfeldt: Modification of Orientation Sensitivity of Cat Visual Cortex Neurone by Removal of GABA-Mediated Inhibition. EXP. BRAIN RES., 14:351-363, 1979.

34. Wurtz, R. H., and C. Mohler: Organization of Monkey Superior Colliculus: Enhanced Visual Response of Superficial Layer Cells. J. NEUROPHYSIOL., 39:745-765, 1976.

35. Zunder, P. M.: Effects of Alcohol and Prediction Outcome on Extrafoveal Signal Detection. INT. STUD. ALCOHOL, 38:392-402, 1977.