



**Recovery of the Visual Evoked Response  
in the Cat Following Administration  
of Diisopropylfluorophosphate,  
An Irreversible Cholinesterase Inhibitor  
(Reprint)**

By

**A. W. Kirby  
T. H. Harding  
R. W. Wiley**

**Sensory Research Division**

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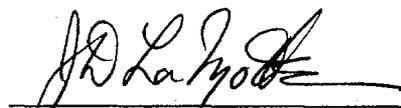


BRUCE C. LEIBRECHT, Ph.D.  
LTC, MS  
Director, Sensory Research  
Division

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RECOVERY OF THE VISUAL EVOKED RESPONSE IN THE CAT FOLLOWING  
ADMINISTRATION OF DIISOPROPYLFLUOROPHOSPHATE, AN  
IRREVERSIBLE CHOLINESTERASE INHIBITOR

A.W. Kirby, T.H. Harding and R.W. Wiley \*

U.S. Army Aeromedical Research Laboratory  
Fort Rucker, Alabama 36362-5292  
USA

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SUMMARY

Visual evoked responses (VER) to counterphased gratings were recorded from area 17 of cat visual cortex prior to and following administration of diisopropylfluorophosphate (DFP). The VER and acetylcholinesterase (AChE) activity of blood, retina, and visual cortex were reduced significantly following DFP administration. Approximately two hours after exposure to 4 mg/kg DFP, the VER began to recover and in some cats returned to base line levels. In contrast, blood, retina, and cortex AChE activity showed little, if any, tendency for recovery throughout the experiment. Since atropine sulfate provided at least partial recovery of the VER following DFP without affecting AChE inhibition, an accumulation of acetylcholine (ACh) probably is involved in the initial visual loss. However, recovery of the VER over time while AChE remained severely inhibited implicates mechanisms other than, or in addition to, accumulation of ACh at receptor sites.

Exposure to organophosphates (OPs) leads to irreversible inhibition of cholinesterase (ChE), and the toxicity which follows is associated with the accumulation of acetylcholine (ACh). Recovery is believed to parallel return of ChE to normal levels, especially in the central nervous system (CNS) (1). There is minimal information concerning the physiological effects of OPs on the visual system. However, ACh has been found or its presence implicated in the retina, lateral geniculate nucleus, and visual cortex (2) so a strong visual effect following OP exposure would be expected. Administration of isopropyl methylphosphonofluoride (sarin) increases the b-wave of the electroretinogram (ERG) in cats (3). Diisopropylfluorophosphate (DFP), an irreversible inhibitor of acetylcholinesterase (AChE), reduces or abolishes the cortical visual evoked response (VER) in rabbits (4). The administration of parathion to awake unrestrained rats produces an increase in the latency of the averaged flash-evoked VER and a decrease in its amplitude (5). The same experiment on monkeys resulted only in a latency increase. Finally, VERs to counterphased gratings were recorded from cats before and after administration of DFP, and responses to low spatial frequencies were preferentially reduced (6). The present experiments investigate recovery of visual function following DFP administration.

### Methods

Adult cats (2.5 to 4.7 kg) were anesthetized with halothane in a 3:1 gas mixture of nitrous oxide and carbogen. Additionally, all areas of surgical incision were infiltrated with lidocaine. Halothane was removed from the gas mixture prior to physiological recording. The trachea, one femoral artery, and two saphenous veins were cannulated. To reduce eye movements, the two sympathetic nerve trunks were cut and the animal was paralyzed by intravenous infusion of 30 mg/kg/hr of gallamine triethiodide in an isotonic glucose solution. The animal was ventilated artificially and end tidal CO<sub>2</sub> was maintained near 4%. The cat was held in a stereotaxic head holder and core temperature was maintained near 37°C. Heart rate, blood pressure, lung resistance, and EEG were monitored continuously.

Atropine sulfate (1%) and phenylephrine hydrochloride (10%) were instilled into the conjunctival sacs to provide cycloplegia and retract the nictitating membranes. Contact lenses having 3 mm diameter artificial pupils were fitted to each eye to keep the cornea moist and to reduce optical aberrations. One eye was focused with an auxiliary lens onto a cathode ray tube (CRT); the other eye was occluded. The CRT subtended a visual angle of 50° by 42° at the viewing distance of 12.7 cm.

VERs were recorded from bone screws over visual (area 17) and parietal cortex. Square wave luminance gratings were generated on the CRT and phase alternated in square wave fashion at 2 Hz. Six spatial frequencies, each having a mean luminance of 82 cd/m<sup>2</sup>, were presented in quasi-random fashion under computer control. Each spatial frequency was presented for 10 s followed by a 1 s equivalent uniform luminance exposure. This continued until cumulative response averages of 120 s were obtained for each frequency. Our response measure was the sum of the amplitudes of the first five even Fourier harmonics of the fundamental less the sum of the first five odd harmonics (7,8). We use the term spatial frequency to refer to the fundamental frequency, even though square wave gratings were used. We showed previously that results with square- and sine-wave gratings were essentially the same (7).

Arterial blood samples were collected from the femoral artery and used to measure blood gases (pO<sub>2</sub>, pCO<sub>2</sub>), pH, and whole blood AChE levels. Blood gases were measured with a Micro 13 blood gas analyzer (Instrumentation Laboratories, Lexington, MA). Whole blood AChE was measured according to a colorimetric method (9). Coincident with initial blood gas and AChE determinations, base line VERs were obtained. Due to normal variation inherent in the VER, five response measures were averaged to obtain a base line. Variability of our base lines ranged from ±7% to ±27% S.D. Following determination of the base line response measure, DFP (Aldrich Chemical Co., Inc., Milwaukee, WI) was administered i.v. over 1-2 min. Response histograms and arterial blood samples were collected at various times after DFP.

A primary systemic effect of AChE inhibition is stimulated secretory activity. If physiological signs of hypoxia were found following DFP, VER recordings were discontinued until normality could be restored by adjusting ventilation stroke volume and/or aspirating the airway. Following completion of an experiment, halothane was added once again to the anesthetic gas mixture and the retinas and samples of visual cortex were removed for AChE assay (9).

### Results

Results are reported here from 17 cats receiving DFP. The largest single dose given was 5 mg/kg, although some animals received multiple doses totaling as much as 10 mg/kg over time. Three of the animals received 0.5 or 1.0 mg/kg

DFP initially and the dose doubled every 2 hr with the final administration being 4.0 mg/kg. VERs were collected from 15 of the animals and, as reported previously, all showed a preferential reduction in responses to low spatial frequencies at moderate doses of DFP, and uniform depression or abolition of responses across all spatial frequencies for those receiving higher doses (6) (cumulative doses in this study). The other two animals were used for AChE assay only.

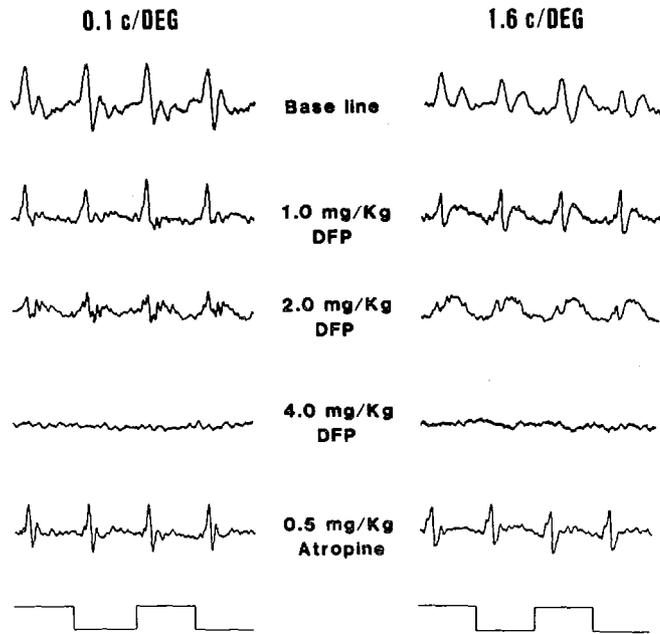


FIG. 1.

Peristimulus histogram averages for two spatial frequencies (0.1 and 1.6 cycle/deg) for base line conditions as well as after administration of 1.0, 2.0, and 4.0 mg/kg DFP given 2 hr apart, and 0.5 mg/kg atropine sulfate given 1 hr after the last DFP. The bottom row depicts the 2-Hz square-wave alternation of the grating pattern. All response averages (120 s collection period with a 1 msec sampling interval) were collected with grating contrast held constant at 0.40 ( $L_{\max} - L_{\min} / L_{\max} + L_{\min}$ , where  $L$  is luminance), and were illustrated because they were closest to the average following that drug dose.

Responses to the 0.1 cycle/deg and 1.6 cycle/deg gratings are shown in Fig. 1 for base line conditions as well as 1.0, 2.0, and 4.0 mg/kg DFP given 2 hr apart, and 0.5 mg/kg atropine sulfate given 1 hr after the last DFP. There were five histograms obtained for each spatial frequency over 2 hr during base line conditions, and following 1.0 and 2.0 mg/kg DFP. There was no light driven response during a 1 hr period following 4.0 mg/kg DFP, and three sets of histograms were obtained during the first hour after atropine. The histograms for each spatial frequency were averaged during each drug condition, and those illustrated were selected because they were closest in amplitude to the average

for that dose. A response peak occurs following each phase reversal of the grating. The most striking feature is the apparent loss of all light-driven response following a cumulative dose of 7.0 mg/kg, and its return following administration of atropine. Note also by comparing the first three histograms in each column that a prominent secondary response peak often was present, especially at higher spatial frequencies (7,6,8).

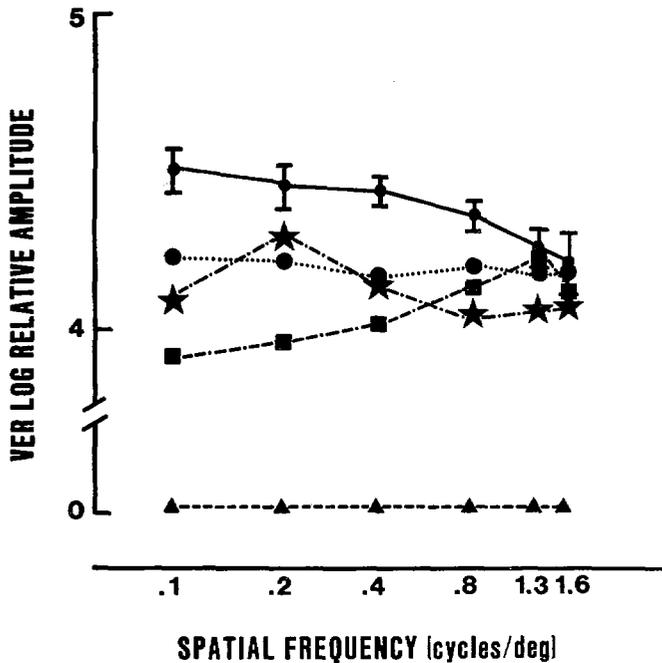


FIG. 2.

Relative amplitude for the VER for each of our six spatial frequencies at each of the five conditions from Fig. 1. Five response measures were averaged to obtain the base line, 1.0 and 2.0 mg/kg DFP curves. Three response measures were averaged to obtain the 4.0 mg/kg DFP (cumulative 7.0 mg/kg DFP) and the 0.5 mg/kg atropine curve. Filled small circles represent the average of five base line measures. Bars show  $\pm 1$  S.D. The 1.0, 2.0, and 4.0 mg/kg DFP curves are represented by the filled large circles, squares, and triangles respectively. The atropine curve is represented by the stars. Grating contrast was held constant at 0.40.

Spatial frequency responsivity curves are shown in Fig. 2 from the same animal as in Fig. 1. Note the preferential reduction in responses to lower spatial frequencies following moderate doses of DFP (1.0 and 2.0 mg/kg), and the uniform response abolition for all spatial frequencies at higher doses (4.0 mg/kg - cumulative dose of 7.0 mg/kg) as reported previously (6). With increasing amounts of DFP, both the response reduction and amount of AChE inhibition increased. Average AChE inhibition following the three drug administrations was 42%, 78%, and 85%. Finally, 0.5 mg/kg atropine quickly reversed the complete loss of response to all spatial frequencies even though AChE inhibition remained high (84%). The other two animals receiving the same dose regimen showed similar results.

Four other animals were given atropine while showing strong reduction in the VER (cumulative DFP doses from 6 to 10 mg/kg). In three receiving from 0.5 to 1.4 mg/kg atropine the VER recovered quickly, at least as well as that demonstrated in Fig. 2. The other animal received two injections of 0.5 mg/kg atropine following a cumulative dose of 7.5 mg/kg DFP and showed only slight VER recovery.

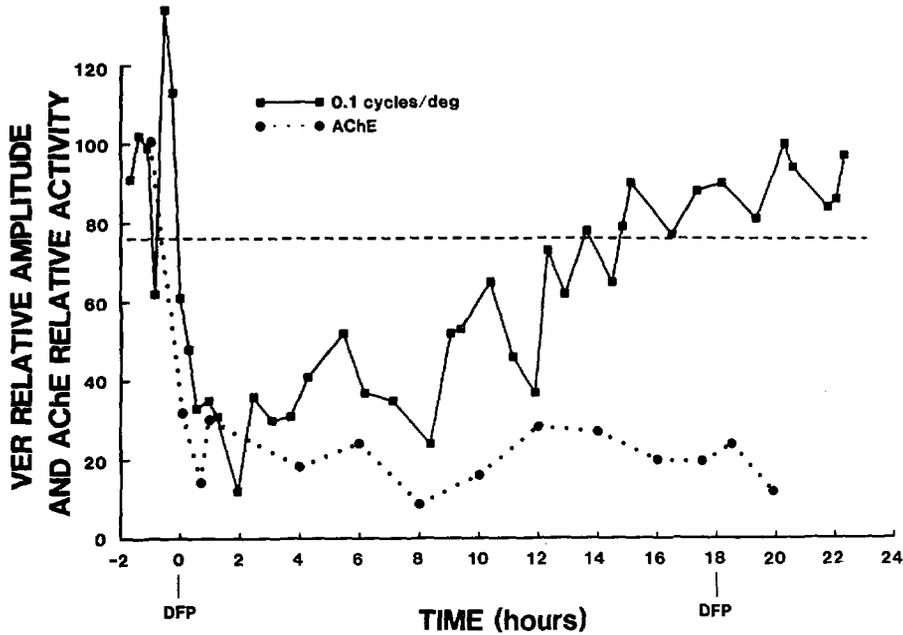


FIG. 3.

Relative amplitudes of the VER (squares) to an 0.1 cycle/deg grating, and relative activity of whole blood AChE (circles), prior to and following 4.0 mg/kg DFP given at 0 and 18 hours. The dashed line represents  $-1$  S.D. from the mean of the VER base line measures. Base line averages were normalized to an ordinate value of 100. Grating contrast was held constant at 0.40.

VER reduction generally reaches its maximum about 2 hr after DFP administration (6). To determine the fate of post-DFP visual function in the absence of atropine, nine cats were given a single 4 mg/kg injection of DFP and the VERs were monitored periodically. Fig. 3 shows the relative amplitude of the response to the 0.1 cycle/deg grating and the relative activity of whole blood AChE during a 24 hr period for one of these animals. The 0.1 cycle/deg grating data were selected for presentation because, as discussed earlier, the VERs to lower spatial frequencies are more sensitive to cholinergic manipulation. Similar results were obtained with the other spatial frequency stimuli. Note the precipitous reduction in both the VER and blood AChE activity following administration of DFP. However, 2 hr later, the VER reduction reversed and the amplitude of the VER slowly increased. Within 15 hr the VER had recovered to within a standard deviation of the base line (dashed horizontal line), determined from six repetitions prior to DFP administration. This is distinctly

different from the blood AChE activity which rapidly fell to 15% of normal and showed little recovery tendency during the time course of the experiment.

TABLE 1

Status of VER and AChE in Cats Following 4.0 mg/kg DFP

Animal	Time of maximum reduction (hrs)	Time of visual recovery (hrs)	Whole Blood AChE activity at recovery	Cortex AChE activity	Retina AChE activity	Time of retina and cortex removal after VER recovery
16	Immediate	1.4	4%	---	---	---
23	1.9	18.5	4%	---	---	---
27	1.1	5.2	8%	30%	8%	17 hr
37	1.9	15.0	23%	11%	7%	7 hr
38	2.0	18.0	2%	26%	10%	2.5 hr
56	2.5	10.0	0	27%	13%	10.5 hr
58	2.7	>10.0	7%	1%	---	Immediate
65	6.0	>18.0	23%	30%	---	Immediate
81	1.0	17.0	0	37%	---	1.75 hr

Information obtained from the nine cats receiving a single administration of 4 mg/kg DFP is listed in Table 1. The VER amplitude diminished slowly over time in all animals except one (#16). For that animal, the VER had recovered to within a standard deviation of base line by 1.4 hr after DFP, when VER amplitude still was decreasing in most animals. In comparison, cat 65 demonstrated the greatest time to maximum VER reduction, and at 18 hr showed little sign of recovery. Whole blood AChE activity ranged from no measurable activity (animals 56 and 81) to 23% of base line activity (animal 37) at a time when the VER had recovered.

Reversal of the VER reduction with atropine (Fig. 2) suggests that the VER changes may be due to a simple accumulation of ACh following inactivation of AChE. However, recovery of the VER without recovery of AChE activity indicates that the process is more complex. Of course, recovery of AChE activity in central visual structures prior to recovery of AChE activity in blood also could explain our results. In the experiment of Fig. 3, we administered a second dose of DFP after visual recovery from the first dose. If central AChE activity had recovered, additional DFP should once again reduce the VER. As shown in Fig. 3, the VER amplitude remained within one standard deviation of the base line amplitude following an additional 4 mg/kg DFP at 18 hr. This experiment, in which a second dose of DFP was given following visual recovery, has been done on only one animal and certainly should be confirmed.

In several cats we compared retinal and cortical AChE activity following DFP with normal AChE values obtained in other cats. Listed in Table 1 are retinal and cortical enzyme activity expressed as a percent of normal (mean

retinal AChE:  $3.38 \times 10^{-6}$  moles substrate hydrolyzed/min/g tissue, S.D.  $\pm 1.37 \times 10^{-6}$ ,  $n = 13$ ); mean cortical AChE:  $2.47 \times 10^{-6}$  moles substrate hydrolyzed/min/g tissue, S.D.  $\pm 1.02 \times 10^{-6}$ ,  $n = 17$ ). Because we did not have control values from these animals before DFP, it is possible that they originally differed from our "normal" population and the values for cortical and retinal AChE activity in Table 1 should be adjusted. Some of the animals in Table 1 were studied beyond visual recovery, and cortical and retinal samples were obtained at a later time. This could account for the lack of strong correlation between blood and CNS cholinesterase activity. There was a much better correlation in the two animals sacrificed at the time of blood assay (58 and 65). The physiological condition of cats 58 and 64 was deteriorating, so retinal and cortical samples were removed without significant visual recovery being observed. Cat 37 received a second dose of DFP (see Fig. 3). Since all retinal AChE values are quite low, even though they were obtained up to 17 hr after VER recovery, the data certainly support VER recovery without recovery of retinal AChE. However, while there was more AChE activity in the cortex, AChE inhibition still was pronounced suggesting that mechanisms in addition to recovery of AChE activity must be involved in VER recovery.

As a more direct indication of AChE activity following DFP, two animals were prepared in the usual manner except they were used only for assay; VERs were not recorded. Both animals were anesthetized, one retina and a sample of visual cortex removed, 4 mg/kg DFP administered, and the second retina and additional visual cortex removed at 15 min in one animal and 9 hr after DFP in the other. Whole blood, retinal, and visual cortical AChE activity was reduced to 17%, 3%, and 8% respectively in the 15-min animal and 2%, 4%, and 5% in the 9-hr animal. These additional data argue against early recovery of central AChE.

### Discussion

Our previous studies have shown that following intravenous administration of the carbamate physostigmine in cats, the VER to different spatial frequency gratings shows preferential reduction at the lower frequencies and reversal of reduction after atropine (7). Similar results are obtained following administration of DFP (6,10). These suggest that excess cholinergic stimulation, resulting from inactivation of AChE, mediates at least the initial reduction of the VER. In comparison, our current studies demonstrate that: (a) following DFP (an irreversible inhibitor of AChE), the VER recovers over time without recovery of AChE activity; (b) atropine is able to reverse a complete abolition of the VER following several administrations of DFP over several hours (see Fig. 2). We are unable to say from our studies whether the observed VER reduction and recovery results from direct action of ACh or whether ACh affects or is affected by the release of another neurotransmitter. However, it does appear that both VER reduction and recovery occur in the presence of continually elevated levels of ACh.

If we assume continued excess of ACh at synaptic sites due to the lack of functional AChE, recovery of the VER could be related to a decreased sensitivity of ACh-receptors. Earlier work has shown that postsynaptic membranes can become desensitized to elevated levels of the depolarizing agent (11). Functionally, sensitivity may be altered through changes in either receptor number or their affinity. If either decreased receptor number or affinity occurred in the visual system following DFP, the continued high concentration of ACh at synaptic sites presumably would not produce the same degree of membrane depolarization, and this would result in a gradual return of the VER toward base line amplitudes.

Alternatively, an overall enhancement of gamma-aminobutyric acid function

recently was demonstrated in the rat striatum following DFP (12). This elevation in GABA activity could serve as a compensatory mechanism to counteract the cholinergic hyperactivity. We have preliminary evidence from the administration of picrotoxin (13) and neurochemical analysis of visual cortex (14) that GABA may be involved in VER recovery as well. However, there are many putative neurotransmitters in the visual pathway and any number of them could be increased or decreased by excessive cholinergic stimulation.

Based on the present results (Table 1), central recovery of function is not dependent upon recovery of AChE. This has been demonstrated also in rats (15), where hyperglycemia, depression of spontaneous motor activity, and other toxic symptoms following soman intoxication disappeared while cholinesterase activity remained inhibited. Of course, functional recovery could occur also if ACh returned to base line levels in central structures independent of AChE activity. Return of cortical ACh to base line levels within about 24 hr of OP exposure has been demonstrated in the rabbit (16) and rat (17), although AChE activity remains depressed. Although this may be a factor in VER recovery after DFP, our preliminary neurochemical results, implicating changes in other putative neurotransmitter systems during visual recovery (14), lead us to believe that the recovery process is probably more complex.

Earlier work (6), coupled with our present data, demonstrates that the maximum effects of DFP do not occur until about 2 hr after administration (Fig. 3 and Table 1). Maximum reduction of blood cholinesterase occurs rapidly, while VER reduction develops slowly over time. These results are in agreement with other studies. For example, the b-wave of the cat ERG was increased following sarin (an irreversible OP), reaching a maximum about 2 hr after administration (3). An analysis of six specific brain areas in rats revealed that the greatest elevation of ACh occurs in the cerebral cortex about 2 hr following OP exposure (17). Although one might attempt to explain these findings based upon the slow penetration of the blood-brain barrier by DFP, the animal we sacrificed 15 min after DFP showed only 3% of base line AChE activity in retina and 8% in cortex.

Although it is tempting to suggest a specific site as the origin of the VER reduction following DFP, it is more likely to be caused by the cumulative effects occurring at various central visual locations (2). Recovery of visual function in the absence of AChE recovery is likely complex as well. Although we have some preliminary evidence for the involvement of the GABA system, decreased sensitivity of ACh receptors as well as decreased cortical ACh activity could also be involved in the recovery process. These additional mechanisms need to be investigated directly.

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