



**The Effect of Pyridostigmine
and Physostigmine
on the Neural Portion of the Visual System**

By

**Albert W. Kirby
Alfred T. Townsend**

Sensory Research Division

February 1990

Approved for public release; distribution unlimited.

**United States Army Aeromedical Research Laboratory
Fort Rucker, Alabama 36362-5292**

Notice

Qualified requesters

Qualified requesters may obtain copies from the Defense Technical Information Center (DTIC), Cameron Station, Alexandria, Virginia 22314. Orders will be expedited if placed through the librarian or other person designated to request documents from DTIC.

Change of address

Organizations receiving reports from the US Army Aeromedical Research Laboratory on automatic mailing lists should confirm correct address when corresponding about laboratory reports.

Animal use

In conducting the research described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals, as promulgated by the Committee on Care and Use of Laboratory Animals, National Academy of Sciences-National Research Council.

Disposition

Destroy this report when it is no longer needed. Do not return it to the originator.

Disclaimer

The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision unless so designated by other official documentation. Citation of trade names in this report does not constitute an official Department of the Army endorsement or approval of the use of such commercial items.

Reviewed:

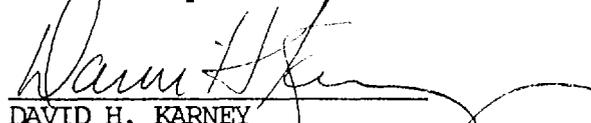


THOMAS L. FREZELL
LTC, MS
Director, Sensory Research
Division



J. D. LAMOTHE, Ph.D.
COL, MS
Chairman, Scientific
Review Committee

Released for publication:



DAVID H. KARNEY
Colonel, MC
Commanding

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED		1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited	
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE		4. PERFORMING ORGANIZATION REPORT NUMBER(S) USAARI. Report No 90-4	
6a. NAME OF PERFORMING ORGANIZATION U.S. Army Aeromedical Research Laboratory		6b. OFFICE SYMBOL (If applicable) SGRD-UAS-NS	7a. NAME OF MONITORING ORGANIZATION U.S. Army Medical Research and Development Command
6c. ADDRESS (City, State, and ZIP Code) Fort Rucker, AL 36362-5292		7b. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5012	
8a. NAME OF FUNDING / SPONSORING ORGANIZATION	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State, and ZIP Code)		10. SOURCE OF FUNDING NUMBERS	
		PROGRAM ELEMENT NO. 62787A	PROJECT NO. 3M1627 87A875
		TASK NO.	WORK UNIT ACCESSION NO. 383
11. TITLE (Include Security Classification) (U) The Effect of Pyridostigmine and Physostigmine on the Neural Portion of the Visual System			
12. PERSONAL AUTHOR(S) Albert W. Kirby and Alfred T. Townsend			
13a. TYPE OF REPORT Final	13b. TIME COVERED FROM _____ TO _____	14. DATE OF REPORT (Year, Month, Day) 1990 February	15. PAGE COUNT 12
16. SUPPLEMENTARY NOTATION Appeared in slightly different form in the "Proceedings of the 3rd International Symposium on Protection against Chemical Warfare Agents.			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	(U)carbamates, (U) pyridostigmine, (U) physostigmine, (U) evoked potentials, (U)Cats, (U)visual processing	
06	04		
06	11		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) Carbamates are currently the pretreatment drugs of choice for protection against possible nerve agent exposure. Pyridostigmine does not cross the blood-brain barrier easily, and therefore provides no central protection. Physostigmine readily enters the central nervous system, but as might be expected, has strong central effects. These experiments were done to access the role of pyridostigmine and physostigmine on visual processing in a mammalian animal model. The results show that physostigmine has strong central visual effects which operationally would not be acceptable. Pyridostigmine does not enter the central nervous system after acute administration until very high levels of cholinesterase inhibition are reached. Based upon our limited sample, central visual processing appears not to be affected until inhibition of blood cholinesterase approaches 80%. This should provide an adequate safety margin following pretreatment with pyridostigmine. We did not investigate the effect of chronic low dose pyridostigmine administration on sensory processing.			
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION	
22a. NAME OF RESPONSIBLE INDIVIDUAL Chief, Scientific Information Center		22b. TELEPHONE (Include Area Code) (205) 255-6907	22c. OFFICE SYMBOL SGRD-UAX-SI

Acknowledgment

The technical assistance of Ms. Carolyn Pope, Sgt. Bernard Lanoue, SPC Timothy Tapia, SPC Rafael Espejo, and SPC Jose Lopez, and the secretarial assistance of Ms. Jimmie Henderson and Ms. Cindy Hyatt is deeply appreciated.

This page intentionally left blank.

Table of contents

	<u>Page No.</u>
List of illustrations	3
List of tables	3
Introduction	5
Methods	6
Results	6
Discussion	12
References	14

List of illustrations

<u>Figure</u>		<u>Page No.</u>
1	VER amplitude following multiple doses of pyridostigmine	7
2	Low spatial frequency loss in VER follow- ing pyridostigmine	8
3	Atropine and methyl atropine effects on VER loss induced by pyridostigmine . . .	9
4	Average VER change following intraocular physostigmine	11

List of tables

<u>Table</u>		<u>Page No.</u>
1	Pyridostigmine administration to five cats	10

=====
This page intentionally left blank.
=====

Introduction

Chemical warfare agents, especially the organophosphorus containing nerve agents (OPs), have toxic effects on the central nervous system (CNS). These often are related to the action of OPs on neural transmission in the cholinergic system. OPs are irreversible inhibitors of cholinesterase, the enzyme or enzymes which degrade synaptically released acetylcholine (ACh), thereby terminating its activity. In the presence of cholinesterase inhibitors such as OPs, the degradative enzyme is inhibited and the synaptic activity of ACh is prolonged greatly. Cholinergic modification of the visual signal through the primary visual pathway is supported by a continuously increasing database, including results from biochemical, histochemical, and physiological studies on visual structures. So in addition to known cholinergic effects on the image forming apparatus (spasms of accommodation and miosis), any drug which enters the CNS and interferes with the normal functioning of the cholinergic pathway therefore might be expected to alter visual function.

Carbamates are currently the pretreatment drugs of choice for protection against possible nerve agent exposure. Carbamates are reversible inhibitors of cholinesterase, being able to protect it from nerve agent exposure by reversibly binding to the enzyme. The enzyme then spontaneously decarbamylates, making it available once again to degrade synaptically released ACh. The two carbamates of primary interest are pyridostigmine and physostigmine. Pyridostigmine, a quaternary carbamate, does not cross the blood brain barrier easily, and therefore provides essentially no central protection. Physostigmine, a tertiary carbamate, readily enters the CNS and is available to protect central cholinesterase. Although it has a demonstrated protective effect against nerve agent lethality and toxicity (Harris and Stitcher, 1984; Leadbeater Inns, and Rylands, 1985), it also has been demonstrated to have pronounced effects on central sensory processing (Harding, Wiley, and Kirby, 1983).

The experiments described here were designed to access the role of pyridostigmine and physostigmine on visual processing. Since anticholinesterases are known to cause image blurring, the experiments were designed to investigate only neural changes and by-pass the image forming apparatus. The visual evoked response (VER) was selected as our response measure, since it represents the sum of massed neural events in the visual system, and should indicate any changes occurring in or prior to the visual cortex.

Methods

Experiments were done on anesthetized and paralyzed adult cats. Surgical preparation and recording techniques have been described in detail previously (Kirby et al., 1986). To minimize the ocular effects of excess ACh (spasms of accommodation and miosis), atropine and phenylephrine hydrochloride were instilled into the conjunctival sacs. Contact lenses with 3 mm-diameter artificial pupils were fitted to each eye to keep the corneae moist and reduce optical aberrations that would normally degrade retinal images. One eye was focused onto a cathode ray tube on which vertical square wave luminance gratings were generated. The other eye was occluded. The gratings were phase alternated (light bars become dark and dark bars light) at 2 Hz. The number of light and dark bars per unit of visual angle defines the spatial frequency of the grating (coarse bars, low spatial frequency; fine bars, high spatial frequency). VERs were recorded from bone screws over visual and parietal cortex. Six spatial frequencies, each having the same mean luminance, were presented in quasirandom fashion under computer control. The computer also collected 2-minute response averages (12 10-second collection periods) for each spatial frequency. Because responses generate harmonics of their fundamental, we were able to select as our response measure the sum of the amplitudes of the first five even Fourier harmonics of the fundamental less the sum of the first five odd harmonics, which represent noise in the response.

Once baseline VERs and blood cholinesterase assays (Ellman et al., 1961) were obtained, pyridostigmine or physostigmine was administered i.v., and additional VERs and cholinesterase assays recorded periodically. Intraocular, rather than i.v. injections of physostigmine were made in several animals. Following the completion of an experiment, samples of visual cortex were removed for cholinesterase assay. Since a baseline cortex cholinesterase level was not available for each cat, we used a previously determined value based upon samples from 11 normal animals.

Results

VER changes following the i.v. administration of physostigmine have been reported previously (Harding Wiley, and Kirby, 1983; Kirby, Harding, and Wiley, 1986). Enough physostigmine to inhibit 35-55 percent of blood cholinesterase resulted in a preferential loss of responses to low spatial frequencies. Responses to the two lowest spatial frequencies often were abolished completely, while

the responses to the highest spatial frequencies essentially were unchanged. To be certain that the observed effect was due to cholinergic hyperactivity resulting from cholinesterase inhibition, atropine sulfate was given. Atropine competes with ACh for cholinergic receptor sites, thus reducing the effect of excess ACh. Following atropine, responses usually recovered to within baseline variation.

Figure 1 demonstrates the relative lack of effect on the VER to our lowest spatial frequency (0.1 cycle/deg) of enough i.v. pyridostigmine to inhibit blood cholinesterase by 73 percent. We selected and tracked the response to the lowest spatial frequency grating since it is always strongly affected following physostigmine or OP administration. The four different doses correspond to blood inhibition of 20, 23, 35, and 73 percent. There is very little change in the VER amplitude over this range. Similar cholinesterase inhibition levels following physostigmine or diisopropyl

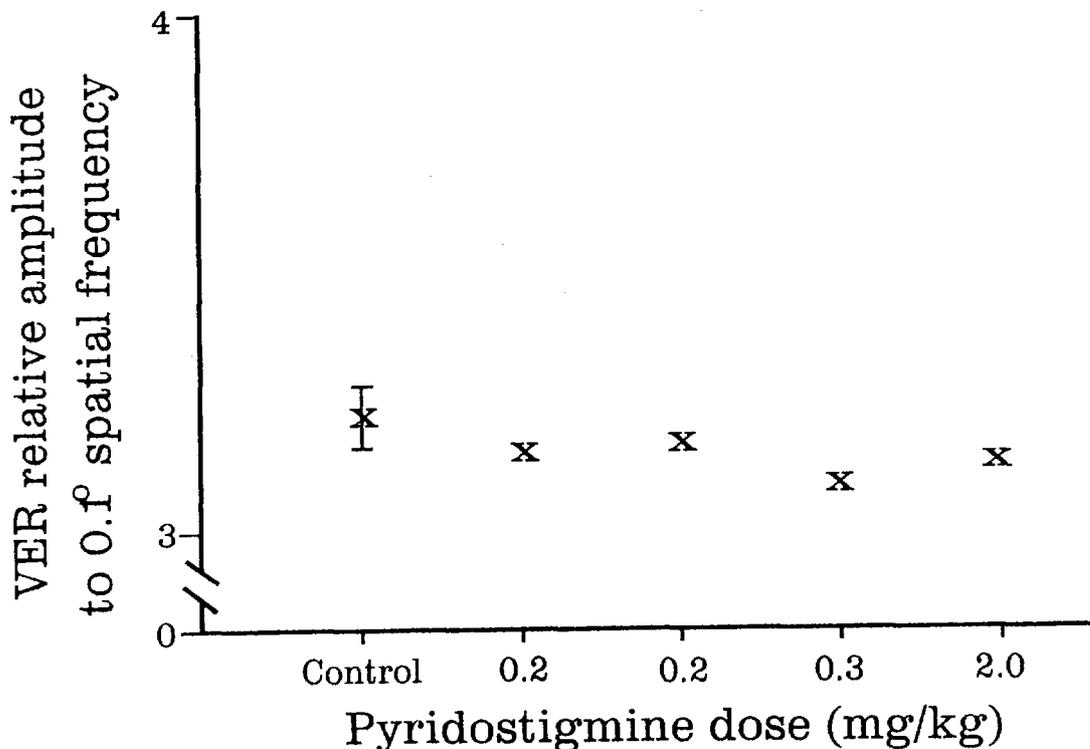


Figure 1. VER amplitude to a low spatial frequency grating during multiple doses of pyridostigmine. There is little change in the VER even though blood cholinesterase inhibition reaches 73 percent.

fluorophosphate (DFP - an OP) would result in very strong reduction or abolition of the VER to the same grating (Harding Wiley, and Kirby, 1983; Harding, Kirby, and Wiley, 1985).

Figure 2 shows a low spatial frequency loss following enough i.v. pyridostigmine to inhibit blood cholinesterase by 80 percent. The upper curve (plotted with *) is the average of five baseline determinations and also shows one standard deviation about each baseline VER. The lower curve (plotted with +) shows the VER change following pyridostigmine. The VER to the three lowest spatial frequencies is reduced, while the VER to the three highest spatial frequencies remains within baseline levels. Although not shown in the figure,

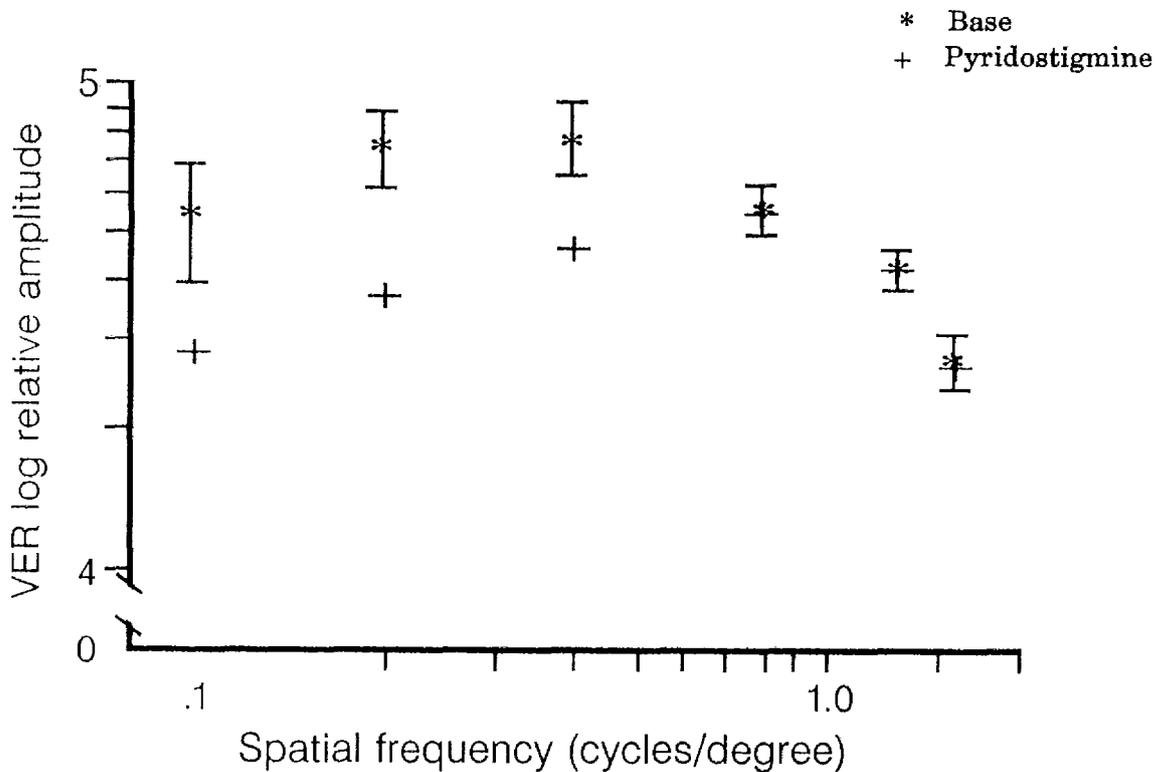


Figure 2. Low spatial frequency loss in VER following enough i.v. pyridostigmine to inhibit 80 percent of blood cholinesterase. Bars indicate standard deviation from baseline measures.

administration of 3.0 mg/kg atropine immediately returned the lowest spatial frequencies to within baseline conditions.

Since pyridostigmine does not cross the blood brain barrier easily and Figure 2 shows a definite low spatial frequency loss, we wanted to determine whether our low spatial frequency change could be due to some type of peripheral change, perhaps in blood flow. In Figure 3 we have plotted percent change in the VER for our six different spatial frequencies following enough pyridostigmine to inhibit 85 percent of the blood cholinesterase. There is a clear loss at the lower spatial frequencies and an enhancement at the higher spatial frequencies. If the low spatial frequency loss resulted from a peripheral change,

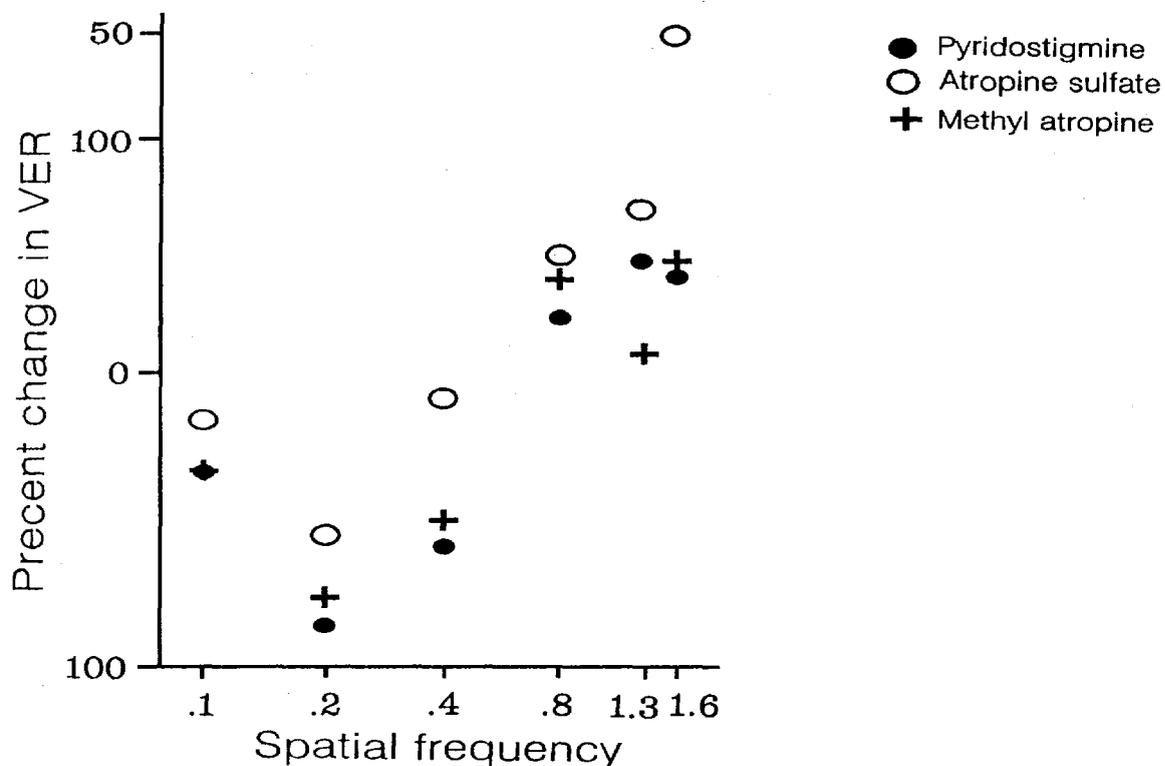


Figure 3. Loss in VER low spatial frequencies following pyridostigmine (blood cholinesterase reduced 85 percent). One mg/kg methyl atropine had little effect on the VER while 1.0 mg/kg atropine sulfate increased the response at all spatial frequencies, indicating a central rather than peripheral effect.

methyl atropine, which does not cross the blood brain barrier, should reverse it. Figure 3 (+s) shows the VER changes following the i.v. administration of 1.0 mg/kg methyl atropine. There is very little difference between the VER following pyridostigmine alone, and that following pyridostigmine and methyl atropine. We then administered 1.0 mg/kg atropine sulfate (open symbols), and the low spatial frequency information was increased (previously reduced by pyridostigmine). Although we don't understand the mechanism behind the enhancement of higher spatial frequency information following pyridostigmine, it was enhanced even further after atropine sulfate. Although this experiment has only been done on one animal, the results support the conclusion that pyridostigmine is in fact entering the CNS.

Table 1 compares blood and visual cortex acetyl- and butyrylcholinesterase activity and VER changes following pyridostigmine administration in five cats. VER changes are denoted either as no change (NC) or low spatial frequency loss (LSF), and are documented at the same time as the blood was drawn for the cholinesterase assay. Shortly thereafter, the animal was sacrificed and samples of visual cortex removed for assay. There is a lack of consistency in

Table 1.

Pyridostigmine administration
to five cats

	<u>#1</u>	<u>#2</u>	<u>#3</u>	<u>#4</u>	<u>#5</u>
Blood AChE	26%	38%	21%	15%	18%
Blood BuChE	43%	57%	45%	15%	95%
CNS AChE	100%	70%	70%	52%	--
CNS BuChE	100%	80%	60%	20%	--
VER	NC	LSF	LSF	NC	LSF

observed changes following pyridostigmine administration in different animals. Compare the results presented here for cats 1, 2, and 4. Animal number 1 had blood cholinesterase activity measuring 26 percent of control, but showed no inhibition of cortical cholinesterase, and as might be predicted, no VER loss. Animal 2 showed the least inhibition in blood cholinesterase activity (62 percent) of the three, and yet the VER showed a loss of low spatial frequency information. Animal number 4 demonstrated the greatest inhibition of cholinesterase activity in both blood (85 percent) and visual cortex (48 percent), and yet there was no change in the VER.

Since the VER represents the sum of massed neural events in the visual system, and it can be demonstrated that both physostigmine and pyridostigmine (in sufficient quantities) are able to reduce the VER, we wanted to obtain a better

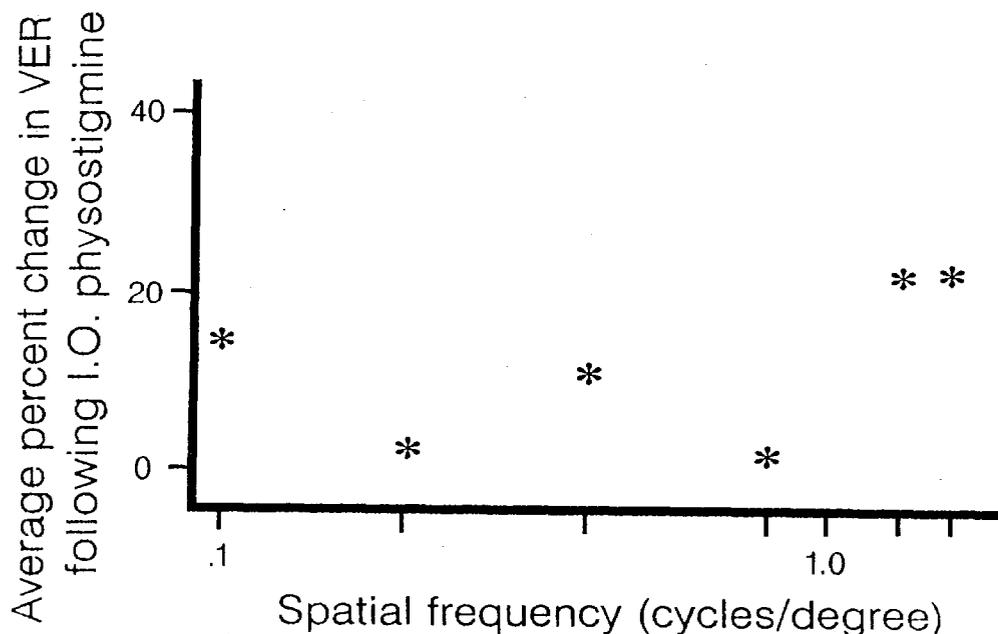


Figure 4. Changes in the VER (average from three cats) following intraocular injections of physostigmine. Note that there is either no change or a slight enhancement across all spatial frequencies instead of the preferential low spatial frequency loss normally seen following i.v. administration.

understanding of the location of cholinergic induced VER changes within the visual pathway. To obtain additional information, we made multiple intraocular injections of increasing amounts of physostigmine (from 2-100 g) in three different cats. Average percent VER change is plotted against spatial frequency in Figure 4 for our six different spatial frequency gratings following physostigmine. As the figure demonstrates, there is either no change or enhancement in the VER at all frequencies. Changes in blood cholinesterase activity in these studies indicated there was physostigmine spill over into the systemic circulation and data was no longer collected from that animal. Since we have seen previously that the i.v. administration of either carbamate results in a preferential loss of low spatial frequency information which is quite different from the results obtained here following retina exposure, we must conclude that the retina plays a minor role in the VER change.

Discussion

These experiments were undertaken to assess the effect of the carbamates pyridostigmine and physostigmine on visual processing, thereby estimating their usefulness as potential pretreatment compounds for protection against nerve agent exposure. Pyridostigmine, a quaternary compound, is currently the carbamate of choice as a pretreatment agent. At reasonable doses, it is thought not to enter the CNS, and therefore provides no central protection or degradation of sensory processing. Although our limited study involved the administration of pyridostigmine to only seven cats, there is little question that it was able to penetrate the CNS. Table 1 showed depression of cortical cholinesterase activity in three of the four cats in which it was measured, and Figure 3 showed the effects of pyridostigmine to be sensitive to atropine sulfate and not methyl atropine. It should be emphasized that in most cases we used doses of pyridostigmine sufficient to inhibit close to 80 percent or more of the blood cholinesterase activity. It probably could be argued that there is ample safety factor between levels of cholinesterase inhibition resulting from fielded pretreatment doses of pyridostigmine and those reported here. And yet, again in a limited study, animal 2 from Table 1 showed a low spatial frequency loss with only 62 percent inhibition of blood cholinesterase. This study did not address the effects of chronic administration of pyridostigmine. Since the blood brain barrier may become even more permeable after 6 to 8 days of administration, the effects of such treatment on sensory processing should probably be investigated.

Physostigmine, a tertiary carbamate, has been considered as a pretreatment compound which would cross the blood brain barrier and provide the necessary central protection. Unfortunately, because of its ready penetration into the brain, its effects upon CNS processing are widespread. We previously have described the loss of low spatial frequency information in the VER resulting from the administration of physostigmine at doses sufficient to inhibit 35 percent or more of blood cholinesterase activity (Harding Wiley, and Kirby, 1983; Kirby, Harding, and Wiley, 1986). More recent results suggest that similar losses occur at less than 25 percent inhibition of blood cholinesterase (Kirby, unpublished observations). Neurochemical changes in retina and visual cortex also have been reported following its administration (Kirby et al., 1988). Although it is possible to counter many of the unwanted central effects of physostigmine through the combined use of other drugs, pronounced deficits in sensory processing tremendously complicate its possible use as a pretreatment against nerve agent exposure.

References

- Ellman, G.L., Courtney, K.D., Andres, V., Jr., and Featherstone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology. 7: 88-95.
- Harding, T.H., Kirby, A.W., and Wiley, R.W. 1985. The effects of diisopropylfluorophosphate on spatial frequency responsivity in the cat visual system. Brain research. 325: 357-361.
- Harding, T.H., Wiley, R.W., and Kirby, A.W. 1983. A cholinergic-sensitive channel in the cat visual system tuned to low spatial frequencies. Science. 221:1076-1078.
- Harris, L.W., and D.L. Stitcher. 1984. Protection against diisopropylfluorophosphate intoxication by pyridostigmine and physostigmine in combination with atropine and mecamylamine. Naunyn-Schmiedeberg's archives of pharmacology. 327: 64-69.
- Kirby, A.W., Harding, T.H., and Wiley, R.W. 1986. Cholinergic effects on the visual evoked potential. Evoked potentials, (Eds.) Cracco, R.Q. and Bodis-Wollner, I., pp. 296-306, New York: Allan R. Liss, Inc.
- Kirby, A.W., Townsend, A.T., Stafford, R.G., and Harding, T.H. 1988. Neurochemical differences in the cat visual system after anticholinesterase agents. Society for neuroscience abstracts. 14: 1070.
- Leadbeater, L., Inns, R.H., and Rylands, J.M. 1985. Treatment of poisoning by soman. Fundamental applied toxicology. 5: S225-S231.