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Dissociation of Locomotor Depression and ChE Activity After DFP, Soman and Sarin[1]

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LYNCH, M R., M A RICE AND S E ROBINSON *Dissociation of locomotor depression and ChE activity after DFP, soman and sarin* PHARMACOL BIOCHEM BEHAV 24(4) 941–947, 1986 —The effect of direct intrastriatal injection of three organophosphate cholinesterase inhibitors, DFP (diisopropylphosphorofluoridate), soman (pinacolyl methylphosphonofluoridate) and sarin (isopropyl methylphosphonofluoridate) has been studied on locomotor activity in the rat. The degree of ChE inhibition has been monitored in the striatum, as well as in surrounding brain areas and blood, in order to verify the selectivity of the treatment and rule out effects attributable to actions in these areas and/or the periphery. It has been determined that while enzyme activity is inhibited in the striatum by all three compounds, only DFP significantly reduces locomotor activity at doses that produce no other observable behavioral deficits, or significant leakage into the periphery Behavioral recovery occurs before enzyme activity returns to control levels Possible contributions of DFP's action on other neurotransmitters and on ChE in other brain areas to the inhibition of locomotor activity are discussed

Locomotor activity Striatum Cholinesterase DFP Soman Sarin

MANIPULATIONS of brain cholinergic systems typically induce changes in locomotor behavior, with ACh and cholinomimetics decreasing activity [19, 26, 27, 50] and anticholinergics and cholinergic neurotoxins increasing unconditioned behaviors [42, 46, 51, 53, 55, 61, 65]. This latter effect has been termed 'disinhibition' [45] and may result from release of a tonic inhibitory influence on behavior [9, 10, 58], possibly through interference with a striatonigral feedback loop [37, 39, 41, 49].

Several investigations suggest that cholinergic response inhibition may be localized to the neostriatum [13, 29, 38, 46], a basal ganglia structure intimately involved in motor function [71] Cholinergic manipulations of the striatum may interfere with behavioral activity through a direct effect on this inhibitory output or indirectly by affecting dopamine (DA) functioning [29] For example, the bradykinesia associated with Parkinson's disease can be alleviated with anticholinergic treatment [45], possibly by increasing striatal DA turnover [11]

In light of this role for ACh in behavioral activity, the present study has been undertaken to compare locomotor changes induced by intrastriatal infusion of several cholinesterase-inhibitors (ChE-Is), which have been shown to increase brain ACh levels [7, 21, 57, 60] Although these compounds typically reduce locomotor behavior [1, 25, 54], only one study has examined behavioral effects of direct infusion into the striatum (to verify a central site of action) with regard to motor function [66]. Moreover, only a few studies have compared behavioral data across several different ChE-Is [1, 24, 52, 56], especially for the organophosphate compounds [20,70]. Therefore, the present study has examined the effects of intrastriatal organophosphate administration on locomotor activity in the rat. The degree of selectivity of this treatment for inhibition of ChE in the striatum has been determined by measuring ChE activity at sites throughout the brain and in the peripheral circulation.

METHOD

Subjects

One hundred twenty-six male Sprague-Dawley rats (Charles River, Wilmington, MA) served as subjects. They weighed 105–245 g at the time of surgery and were given food and tap water ad lib throughout the experiments with the

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exception of during dosing and behavioral testing Housing was on a 12-hr light/dark cycle (0700–1900 hr), under conditions of controlled temperature and humidity

Drugs

Soman (pinacolyl methylphosphonofluoridate) and sarin (isopropyl methylphosphonofluoridate) were supplied by the U S. Army Medical Research and Development Command DFP was obtained from Aldrich (lot No. 0210DK, purity >97%). DFP and soman were warmed to room temperature for injection, whereas sarin was kept on ice and syringes loaded fresh for each injection

Apparatus

Bilateral central drug or vehicle infusion was accomplished with two Hamilton syringes, mounted on an electric infusion pump (Razel Scientific). Syringes were connected to internal cannulae (28 gauge, Plastic Products, Roanoke, VA) by PE-20 tubing. Pump speed was calibrated to deliver solutions at a rate of 1 μl/1 min for studies involving DFP and at a rate of 1 μl/2 min for studies with soman and sarin

Behavioral activities were measured with animals in clean, opaque plastic cages, fitted with wire tops Cages were placed on Automex capacitance-coupled platforms (Columbus Instruments), which measure horizontal and vertical movement and which were housed in darkened wooden chambers with white noise provided

Procedure

Rats were anesthetized with equithesin (0 2 cc/50 g of body weight) and stereotaxically implanted with 4 0 mm stainless steel guide cannulae (22 gauge, Plastic Products, Roanoke, VA) aimed at the bilateral striata (AP +7 9, L ±3 0, and DV determined so that internal cannulae would terminate at −0 6 when inserted, according to the atlas of Konig and Klippel [30]). After 72 hr of recovery in the home cage, rats were infused simultaneously into the bilateral striata with organophosphate or an equal volume of the appropriate vehicle Eight rats per group were tested in each behavioral study and six per group for each biochemical determination Infusions were made in unanesthetized rats which were gently wrapped in a towel to prohibit movement The internal cannulae were left in place for one min after each injection The ChE-Is administered were 81 5 nmol DFP/0 5 μl 1 9 emulphor (polyoxyethylated vegetable oil, GAF Corp., New York, NY) CSF vehicle, 14 85 nmol soman/1 5 μl sterile saline and 24 2 nmol sarin/2 μl sterile saline Volumes were adjusted so that the spread of ChE inhibition to other brain areas was minimized while maximum inhibition of striatal ChE was achieved Doses that inhibited striatal ChE activity by 40% or more were chosen, as earlier studies have found that reductions in enzyme activity to 40% of control values produce concomitant behavioral changes [4, 22, 59] At this level, the enzyme appears to lose control of its substrate [2], and a sharp drop in neural conduction has been reported [67] Previous testing has indicated that larger doses produced less selective inhibition of striatal ChE relative to other brain areas (unpublished observations)

For behavioral testing, rats remained housed in groups of four until individually tested on the activity platforms. Tests were conducted between 1100 and 1600 hr at 20 min, 1 hr and 24 hr post-injection, using separate groups of drug and vehicle rats for each time point. Observations were made over 15 min, with the number of activity counts recorded for each

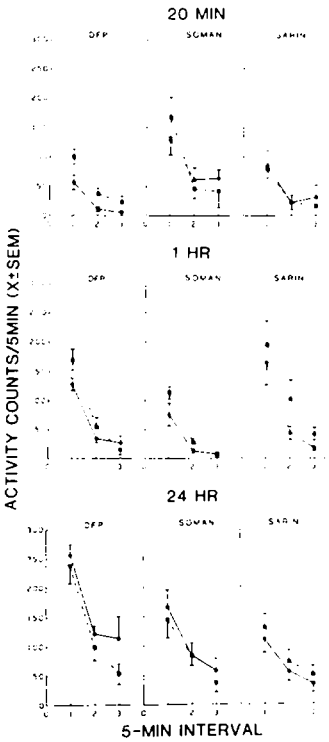

FIG 1 Mean number of activity counts per 5-min interval at 20 min, 1 hr and 24 hr post-DFP (81 5 nmoles), -soman (14 85 nmoles), and -sarin (24 2 nmoles) where drug is ● and vehicle ■ A significant difference between vehicle and organophosphate groups was found only for DFP at 20 min post-injection ($p>0$ 046) A $p>0$ 05 was accepted as significance Eight subjects were used for each data point

5-min period. After the first 15 min of the test session, the vehicle-infused group had habituated sufficiently so that it would not be possible to measure any decreases in activity The number of drug and vehicle rats tested on each platform was counterbalanced for each study Following this procedure, all rats were anesthetized and infused with dye through the guide cannulae Brains were removed and frozen for visual inspection of cannulae placement through coronal sections of the striatum

For determination of ChE activity, rats were placed in holding cages until decapitation at 20 min, 1 hr or 24 hr post-injection Trunk blood was collected in heparinized tubes and 10 μl was immediately diluted to a total volume of 6 ml in pH 8.0 0 1 M phosphate buffer This dilution remained on ice and was warmed to room temperature just prior to the assay of cholinesterase activity Brains were rapidly removed and dissected into striata, parietal cortices, hippocampi, amygdaloid complexes, hypothalamus and medulla/pons The amygdala was removed as a wedge of tissue made by placing a 2 mm deep cut 3 mm lateral to the hypothalamus, a cut 1 mm anterior to the posterior aspect of the mamillary bodies and a cut at the level of the optic chiasm This piece of tissue essentially contained all of the amygdaloid nuclei as described in the atlas of Konig and Klippel [32] The parietal cortex was taken as the area described by Krieg [34] The complete hippocampi and striata (caudate-putamen) were then removed The cerebellum was

TABLE 1

CHOLINESTERASE ACTIVITY AT VARIOUS TIMES AFTER BILATERAL INTRASTRIATAL INJECTION OF 81 5 nmol DFP

Treatment	(N)	Blood	Striatum	Medulla/Pons	Hypothalamus	Parietal Cortex	Hippocampus	Amygdala
		Acetylthiocholine hydrolyzed per min						
		μmol/ml	μmol/g					
20-min								
Vehicle	(5)	1 4 ± 0 1	36 9 ± 2 2	12 7 ± 0 7	7 6 ± 0 6	5 0 ± 0 3	8 0 ± 0 5	12 5 ± 1 1
DFP	(6)	1 1 ± 0 1	14 2 ± 2 0†	8 8 ± 0 6†	6 0 ± 0 5	2 7 ± 0 3†	6 2 ± 0 4	9 1 ± 1.0
1-Hr								
Vehicle	(5)	1 3 ± 0 1	43 8 ± 2 2	15 5 ± 0 7	10 3 ± 0 6	6 6 ± 0 3	10 7 ± 0 5	16 3 ± 1.1
DFP	(6)	1 4 ± 0 1	18 4 ± 2 0†	10 9 ± 0 6†	7 0 ± 0 5†	3 9 ± 0 3†	7 6 ± 0 4†	12 5 ± 1 0
24-hr								
Vehicle	(6)	1 8 ± 0 1	37 7 ± 2 0	14 6 ± 0 8	9 9 ± 0 6	5 4 ± 0 3	8 9 ± 0 5	18.5 ± 1 1
DFP	(6)	0 9 ± 0 1†	20 0 ± 2 0†	10 7 ± 0 6†	6 8 ± 0 5†	3 9 ± 0 3*	6 2 ± 0 4†	10 8 ± 1 0†

*$p<0.0002$, as compared to control, determined by univariate ANOVA
†$p<0.0001$, as compared to control, determined by univariate ANOVA

TABLE 2

CHOLINESTERASE ACTIVITY AT VARIOUS TIMES AFTER BILATERAL INTRASTRIATAL INJECTION OF 14 85 nmol SOMAN

Treatment	(N)	Blood	Striatum	Medulla/Pons	Hypothalamus	Parietal Cortex	Hippocampus	Amygdala
		Acetylthiocholine hydrolyzed per min						
		μmol/ml	μmol/g					
20-min								
Vehicle	(6)	2 1 ± 0 1	39 1 ± 2 0	15 3 ± 0 6	8 0 ± 0 5	5 5 ± 0 3	9 1 ± 0 4	12 2 ± 1 1
Soman	(6)	0 6 ± 0 1‡	13 4 ± 2 0‡	14 0 ± 0 6	9 0 ± 0 6	4 4 ± 0 3	8 7 ± 0 4	10 7 ± 1 0
1-hr								
Vehicle	(6)	1 7 ± 0 1	38 5 ± 2 2	11 4 ± 0 7	7 3 ± 0 6	4 2 ± 0 3	7 0 ± 0 5	5 0 ± 1 1
Soman	(6)	1 0 ± 0 1†	10 9 ± 2 0‡	9 6 ± 0 6	5 0 ± 0 5*	2 6 ± 0 3†	5 5 ± 0 4	5 7 ± 1 0
24-hr								
Vehicle	(6)	1 1 ± 0 1	36 2 ± 2 0	11 3 ± 0 6	8 7 ± 0 5	3 9 ± 0 3	6 9 ± 0 4	10 9 ± 1 0
Soman	(6)	0 5 ± 0 1*	12 0 ± 2 0‡	12 3 ± 0 6	6 2 ± 0 5*	2 9 ± 0 3	6 0 ± 0 4	8.2 ± 1 0

*$p<0.005$, as compared to control, determined by univariate ANOVA
†$p<0.0005$, as compared to control, determined by univariate ANOVA
‡$p<0.0001$, as compared to control, determined by univariate ANOVA

discarded and the medulla/pons separated from the remainder of the brain by a cut extending from posterior to the superior colliculi down to the junction of the midbrain and pons. The hypothalamus was removed according to Glowinski and Iversen [23]

Bilateral parts were pooled and all areas were weighed wet They were then kept frozen at −70°C until assayed, at which time they were diluted in 19 parts pH 8 0 0 1 M phosphate buffer (except for the medulla/pons, which was diluted in 39 volumes) and homogenized Aliquots of these homogenates (100 μl for cortex, amygdala and hypothalamus; 50 μl for striatum and hippocampus, 75 μl for medulla/pons) were added to a volume of pH 8.0 0 1 M phosphate buffer which resulted in a total of 3 ml After addition of 100 μl of 0.01 M dithionitrobenzoic acid and 30 μl of 0 075 M acetylthiocholine iodide, samples were assayed with a Beckman Model 34 spectrophotometer according to the method of Ellman *et al.* [15]. The rate of increase in absorbance at 412 nm was recorded and activity expressed as μmol substrate hydrolyzed/min per ml of dilute blood or per g wet weight. Although this technique does not distinguish between acetylcholinesterase and pseudocholinesterase, it has been demonstrated that the latter contributes little to total enzyme activity when acetylthiocholine is used as the substrate [28]. In addition, practically all brain cholinesterase is acetylcholinesterase [3] In the blood samples, the possibility

TABLE 3

CHOLINESTERASE ACTIVITY AT VARIOUS TIMES AFTER BILATERAL INTRASTRIATAL INJECTION OF 24 2 nmol SARIN

Treatment	(N)	Blood	Striatum	Medulla/Pons	Hypothalamus	Parietal Cortex	Hippocampus	Amygdala
		Acetylthiocholine hydrolyzed per min						
		μmol/ml	μmol/g					
20-min								
Vehicle	(6)	1 0 ± 0 1	41 8 ± 2 0	13 8 ± 0 6	7 9 ± 0 5	4 3 ± 0 3	8 1 ± 0 4	12 0 ± 1 0
Sarin	(6)	0 3 ± 0 1‡	12 3 ± 2 2‡	13 2 ± 0 7	7 1 ± 0 6	3 4 ± 0 3	7 2 ± 0 5	13 0 ± 1 2
1-hr								
Vehicle	(6)	1 0 ± 0 1	33 9 ± 2 0	13 1 ± 0 6	7 6 ± 0 5	4 2 ± 0 3	8 2 ± 0 4	7 0 ± 1 0
Sarin	(6)	0 6 ± 0 1*	20 9 ± 2 0‡	13 0 ± 0 6	7 1 ± 0 5	3 4 ± 0 3	7 4 ± 0 4	10 6 ± 1 0
24-hr								
Vehicle	(6)	1 2 ± 0 1	34 1 ± 2 2	11 8 ± 0 7	7 3 ± 0 6	4 0 ± 0 3	7 2 ± 0 5	11 3 ± 1 0
Sarin	(6)	0 6 ± 0 1†	23 3 ± 2 0‡	10 4 ± 0 6	6 3 ± 0 5	3 7 ± 0 3	7 4 ± 0 4	11 5 ± 1 0

*$p<0$ 006, as compared to control, determined by univariate ANOVA
†$p<0$ 002, as compared to control, determined by univariate ANOVA
‡$p<0$ 0001, as compared to control, determined by univariate ANOVA

exists that some of the inhibition of acetylthiocholine hydrolysis was due to inhibition of pseudocholinesterase

RESULTS

Gross inspection of brains from rats in the behavioral studies revealed guide cannula placements at the specified L and DV corrdinates, with AP location averaging 7470 to 8380 μ over the nine studies Informal observation of drug- or vehicle-injected rats revealed no noticeable changes in behavior following DFP treatment, whereas soman-injected rats often reared and sniffed in the air, sometimes displaying chewing movements and mild ataxia Sarin-treated rats also showed these first two symptoms on occasion, but there were no signs of peripheral toxicity for any of the three compounds (e g , salivation, diarrhea)

The mean number of activity counts for each organophosphate at each time point are presented for vehicle and drug groups in Fig 1 Multivariate analysis of variance was used to simultaneously compare the effects of vehicle and organophosphate treatment for each group and time point [44] Only the activity counts for the 20-min post-DFP injection were found to be significantly different from control (Hotelling T^2, $p<0$ 046)

Enzyme activities for each time point are presented in Table 1 for DFP, Table 2 for soman and Table 3 for sarin (values are expressed as mean±standard error of estimate) Multivariate analysis of variance indicated significant effects between treatment and vehicle simultaneously for all brain areas and blood ($p<0$ 0001) Univariate analyses of ChE activities were made comparing each drug treatment to vehicle Data were expressed as mean±standard error of estimate

Using Bonferroni's correction, $p<0$ 0071 was required for statistical significance (Bonferroni's correction requires $p<0$ 05/7, 7 being the number of response variables [44]) As is apparent from the data, all three organophosphates produced inhibition of striatal ChE at all three time points studied With the exception of DFP, intrastriatal injection of organophosphates produced a rather selective inhibition of

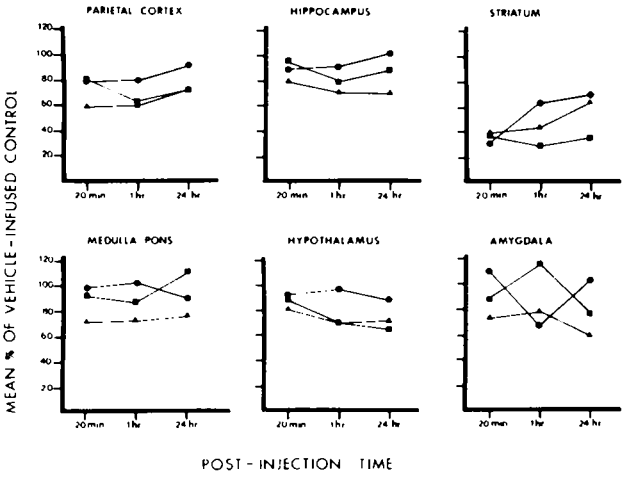

FIG 2 Effect of organophosphates on cholinesterase activity expressed as mean percent of control acetylthiocholine hydrolysis (μmol/g/min) as a function of time after drug infusion ▲ 81 5 nmoles DFP, ■ 14 85 nmoles soman and ● 24 2 nmoles sarin

striatal ChE at 20 min (≤38%, while all other areas remained at ≥69%) DFP reduced ChE activity in the parietal cortex to 59% of control at this time point Although striatal enzyme activity seems to have recovered by 1 and 24 hr for DFP and sarin, having increased from 38% to 62% of control for DFP and 30% to 69% for sarin, this structure continued to be more inhibited than any other area tested, and ChE remained significantly less than vehicle values Although soman and sarin appeared to diffuse less readily into surrounding brain parenchyma, as compared to DFP, they both appeared to leak into the periphery with little difficulty That is, trunk blood was inhibited by 41%–73% at all time points for both of these ChE-Is. This is interesting, given the lack of peripheral signs of toxicity in these groups, indicating that these com-

pounds remained bound in the blood. In fact, the enzyme inhibition for sarin and soman at 20 min exceeded inhibition produced by DFP in the blood at any time point tested, but did appear to recover over successive 1 hr and 24 hr measures for these two compounds. It is uncertain whether this recovery could represent synthesis of new enzyme at these early time points. Changes in synthesis of various isoenzymes of ChE have been reported to occur no earlier than 18 hr after enzyme inhibition [6]

The raw values for drug groups were converted to percent of vehicle control means and are presented in Fig 2. Separate 3×3 ANOVAs were performed for each brain area in order to compare organophosphate effects on ChE activity and examine the rate of recovery They revealed significant main effects for the drug variable in the parietal cortex, $F(2,45)=12.90$, $p<0.001$, striatum, $F(2,45)=11.83$, $p<0.001$, and medulla/pons, $F(2,45)=18.44$, $p<0.001$. Post-hoc tests with the Tukey hsd indicated that DFP produced greater inhibition than sarin in the parietal cortex ($p<0.05$) and than both soman and sarin in the medulla/pons ($p<0.01$) Soman was more potent than sarin in inhibiting striatal ChE ($p<0.05$) Significant interactions were noted for the striatum and medulla/pons, $F(4,45)=4.65$, $p<0.05$ and $F(4,45)=3.06$, $p<0.05$, respectively Post-hoc comparisons within drug groups over the three time points revealed significant enzyme recovery only for sarin in the striatum (20 min less than 1 hr and 24 hr, $p<0.01$) and soman in the medulla/pons (1 hr less than 24 hr, $p<0.01$)

DISCUSSION

Previous studies have reported either correspondence between behavioral deficits produced by ChE-Is and brain ChE activity (locomotor behavior [19, 35, 52], conditioned avoidance [5, 25, 56, 57], and intracranial self-stimulation [14]) or a lack of correlation [24,36] In the case of conditioned avoidance, the deficits appear to result from stimulation of cholinergic neurotransmission, as (−)hyoscyamine prevents the impairment of responding produced by physostigmine without affecting reductions in ChE activity [57]. Results of the present study support an association between behavior and enzyme activity only for DFP at 20-min postinjection, at which time striatal ChE activity was reduced to 38% of control, leakage into the periphery was not significant (blood ChE was 85% of control) and there were no observable signs of enhanced peripheral cholinergic activity A similar relationship between ChE inhibition in the caudate and forced circling has been reported after local DFP injection in the rabbit, as this motor effect was manifested when ipsilateral striatal enzyme activity decreased to 37% of control values [66]

That locomotor behavior was not significantly different from control at 1 hr and 24 hr post-DFP injection parallels earlier reports of behavioral recovery preceding enzyme recovery This is true for conditioned avoidance [5, 25, 35, 56, 57], single-alternation operant performance [33] and spontaneous motor activity with peripheral administration [30] Even with respect to general behavioral toxicity, symptoms are correlated with enzyme activity for only a short period of time after ChE-I treatment [5,30]. Better correlations exist for ACh levels, which can return to normal or near-normal levels within a few hours, possibly reflecting decreased ACh turnover [5,40]

That soman and sarin produced a similar inhibition of ChE in the striatum at 20 min post-injection as did DFP, but no significant change in locomotor behavior, suggests that either the observed behavioral depression may not be a result of enzyme inhibition per se, or that the change produced by DFP results from a greater inhibition in some other brain area, such as in the parietal cortex (59% of control, as compared to 80% and 79% for soman and sarin, respectively) Concerning the first of these possibilities, Wolthuis and VanWersch [70] also reported different behavioral profiles for several ChE-Is, with soman, physostigmine and pyridostigmine affecting behavioral activity while sarin and TEPP (in doses as high as 30% of the LD50) did not Different profiles for neostigmine and physostigmine in conditioned avoidance procedures [56] have often been attributed to peripheral versus central mechanisms of action, respectively A similar explanation was offered by Wolthuis and VanWersch for their results; TEPP and sarin were noted to have a predominantly peripheral mode of action [68], as contrasted with soman which has its predominant action in the CNS [69] The discrepancy between these reported effects of soman and the lack of effect in the present study may be due to differences in behavioral measurement procedures [51] or differences in the route of administration. A third possibility is that DFP, soman and sarin may differentially affect various isoenzymes of ChE which may vary in function with respect to control of neurotransmission [43]. A final possibility is that the rearing behavior produced by soman and sarin obscured any decrease in locomotor activity.

Central biochemical differences have been demonstrated as well. For example, the time-course of ChE recovery differs with organophosphates such as DFP and paraoxon [5], and even different brain areas have different recovery rates after peripheral administration of a single ChE-I [33]. These rate differences have been observed in the present study, with recovery in the striatum and medulla/pons, but only for sarin and soman, respectively Additionally, differential effects on other neurotransmitter systems as well as compensatory receptor alterations produced by acute administration of these different organophosphates must be considered in evaluating their behavioral effects These changes could result from direct effects of the inhibitors themselves on an enzyme other than ChE [47,62] or from compensatory changes in response to ACh elevation produced by ChE inhibition Acute treatment with carbamates or organophosphates has been found to affect the neurotransmitters norepinephrine, dopamine, serotonin, GABA and glutamate in several species [16–18, 21, 63] Compensatory changes in neurotransmitter systems in response to ChE-Is may also take the form of receptor alterations. Binding studies after acute organophosphate administration have demonstrated changes in either Bmax or K_I values for (^{3}H)QNB binding, as well as changes in Bmax for GABA and dopamine [12,64] In summary, either differential receptor changes or neurotransmitter alterations may underlie the discrepant behavioral results obtained in the present study The former may also be responsible for the behavioral recovery seen with DFP, as has been previously suggested [12, 48, 64]

In conclusion, reductions in locomotor behavior were observed in the present study, following intrastriatal doses of DFP that did not produce observable deficits in gross motor behavior or peripheral toxicity. Although a similar selective inhibition of ChE activity in the striatum was produced by soman or sarin infusion, significant changes in behavioral activity were not detected in spite of the slight motor disturbances observed following injection. Further examination of the role of the parietal cortex in this behavioral depres-

sion, (i e , local ChE-I infusion into that area), and inspection of neurotransmitter levels, turnover rates, receptor-binding data, etc , will be necessary in order to determine the exact nature of these differential effects

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