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Long-Term Neurochemical and Behavioral Effects Induced by Acute Chlorpyrifos Treatment

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POPE, C. N., T. K. CHAKRABORTI, M. L. CHAPMAN AND J. D. FARRAR. *Long-term neurochemical and behavioral effects induced by acute chlorpyrifos treatment.* PHARMACOL BIOCHEM BEHAV **42**(2) 251–256, 1992.—A single dose of the organophosphate insecticide *O,O'*-diethyl-*O*-3,5,6-trichloro-2-pyridylphosphorothioate [chlorpyrifos (CPF), 279 mg/kg, SC] caused extensive inhibition of cortical and striatal cholinesterase (ChE) activity in adult rats at 2 (94–96%), 4 (82–83%), and 6 (58–60%) weeks after treatment. These persistent changes in ChE activity were concomitant with reductions in muscarinic receptor binding sites in cortex (34, 33, and 18% reduction in B_{max}) and striatum (48, 40, and 23% reduction in B_{max}) at 2, 4, and 6 weeks after exposure. Neither ChE activities nor muscarinic receptor densities were different from control levels at 12 weeks after exposure. CPF treatment caused a reduction in locomotor activity for the first 2 days after treatment, after which basal activity levels were not different from controls. CPF-treated rats showed higher activity relative to controls, however, following challenge with scopolamine (1 mg/kg, IP) at 2, 4, 6, 8, and 12 weeks after treatment. These data indicate that acute exposure to CPF in adult rats can cause long-term neurobehavioral changes that may persist following the recovery of neurochemical parameters associated with exposure and tolerance to cholinesterase inhibitors.

Cholinesterase inhibition Chlorpyrifos Organophosphate Motor activity
Muscarinic receptors Scopolamine

O,O'-DIETHYL-*O*-3,5,6-trichloro-2-pyridylphosphorothioate [chlorpyrifos (CPF)] is an organophosphorus (OP) pesticide used extensively in the United States and throughout the rest of the world (16). As with other OP pesticides, it is generally agreed that CPF exerts acute toxicity through inhibition of acetylcholinesterase, with subsequent accumulation of acetylcholine at synaptic terminals and hyperstimulation of postsynaptic cells (11). We previously observed (18) that while subcutaneous administration of a maximal tolerated dose of CPF (279 mg/kg) caused extensive inhibition of whole brain and blood cholinesterase (ChE) activity in adult rats for up to 7 days after treatment, such treatment failed to produce the "cholinergic crisis" typically associated with high doses of cholinesterase inhibitors.

This study was performed to determine the duration of ChE inhibition in the cortex and striatum following a single maximal tolerated dose of chlorpyrifos and the effects of such enzyme inhibition on muscarinic receptor ([^{3}H]quinuclidinyl benzilate, QNB) binding parameters. As the cortex and striatum are thought to be intimately involved in locomotor control, neurobehavioral consequences of CPF treatment were assessed by examining motor activity at various times after treatment. In addition, the effects of chlorpyrifos on sensitivity to scopolamine, an antimuscarinic agent known to induce hyperactivity, were also determined at various times after chlorpyrifos treatment as a functional indicator of the status of the cholinergic system.

METHOD

Animals and Treatments

Adult, male Sprague-Dawley rats (3 months of age, average body weight about 375 g) were used throughout. Chlorpyrifos (>98% purity, Chem Service, Lenexa, KS) was administered subcutaneously in peanut oil in an injection volume of 2 ml/kg. Previous studies (18) established a maximal tolerated dose for chlorpyrifos of 279 mg/kg and all treated animals were given this dose in the present study. Control animals received peanut oil only (2 ml/kg). Animals were maintained on a 12 L:12 D light illumination cycle.

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Biochemical Assays

Two, 4, 6, and 12 weeks after treatment, rats (n = 5/treatment group/time) were decapitated and trunk blood was collected into centrifuge tubes containing a small amount of heparin (20 IU), followed by gentle mixing and centrifugation to separate the plasma. The cerebral cortex and corpus striatum were dissected essentially as described by Glowinski and Iverson (12). Tissue samples were frozen at −55°C until time of assay (less than 3 weeks). For each analysis, one group of samples (i.e., one brain region from control and treated tissues at one time point) were thawed and analyzed. Homogenates (1:30, w:v) of thawed brain samples were prepared on ice with a Polytron PT 3000 homogenizer (32,000 rpm for 20 s, Brinkmann Instruments, Westbury, NY) in 50 mM Tris(hydroxymethyl)amino methane buffer, pH 7.4 (25°C) containing the following salts: NaCl, 120 mM; KCl, 5mM; $CaCl_2$, 2mM; $MgCl_2$, 1 mM (Tris-salts buffer).

Samples of the cortical or striatal homogenates were centrifuged at 48,000× g for 10 min to prepare a particulate fraction. The membranous pellets were washed twice by rehomogenization in 30 vol fresh Tris-salts buffer followed by centrifugation as before. Muscarinic receptor QNB binding was measured in washed membranes essentially by the method of Yamamura and Snyder (31) using approximately 0.1 mg protein/reaction. Tissues were incubated for 60 min at 37°C with QNB (seven final concentrations from 30–1,500 pM) and rapidly filtered and washed (3 ml, 3×) over Whatman GF/C paper using a receptor binding harvestor (Brandel Model M-24, Gaithersburg, MD). Specificity was determined by the inclusion of atropine (10 μM final concentration) in paired samples and calculated as the difference in binding between tissues incubated in the presence and absence of atropine. Binding data were calculated relative to protein concentration (15) and binding constants (i.e., K_d and B_{max}) were estimated by Scatchard analysis on a personal computer using a BASIC pharmacological calculations program (27).

ChE activity was assayed radiometrically essentially by the method of Johnson and Russell (14) using a final concentration of 0.12 mM [^{3}H]acetylcholine iodide (73.7 mCi/mmol, New England Nuclear, Wilmington, DE). Preliminary experiments delineated conditions of both incubation time and tissue concentration necessary for linear rates of substrate hydrolysis. For plasma, frozen samples were thawed and diluted 1:5 (v:v) in the above Tris-salts buffer before assay and enzyme activity was calculated relative to plasma volume. For brain regions, the washed membrane fractions from receptor binding studies were utilized for assay and enzyme activity was related to protein concentration (15).

Measurement of Locomotor Activity

The effects of chlorpyrifos treatment on motor activity were assessed essentially as described by Finn and coworkers (10) with five two-channel Electronic Activity Monitors (No. 31404, Stoelting Co., Wood Dale, IL). Each rat was placed into a polycarbonate cage (20 × 22 × 44 cm) that was centered on a sensor platform (No. SA 1566, Stoelting Co.). The sensitivity of each sensor was calibrated daily with a metronome. Activity counts were recorded for 30 min (following a 5-min acclimation period) between 1000 and 1300 h, 5 days a week. Activity was measured during the light phase of the light/dark cycle.

All rats were habituated to the activity boxes for 4 consecutive weekdays (Tuesday–Friday) prior to OP treatment. On the following Monday, rats were individually placed in the boxes and activity recorded as before prior to treatment with either peanut oil (2 ml/kg) or chlorpyrifos (279 mg/kg, SC) in peanut oil. Activity was measured on the subsequent 4 days and throughout the following week to examine the "acute" effects of chlorpyrifos on locomotor activity.

Pharmacological Challenges

Preliminary dose-response studies determined that 1 mg/kg scopolamine (IP) was the lowest dose that reproducibly elevated locomotor activity in the apparatus being used and this dose was therefore utilized to examine the effects of chlorpyrifos on scopolamine sensitivity. Beginning 2 weeks after chlorpyrifos treatment, animals were individually placed in the boxes and activity was measured as before. On the following day, half the rats received scopolamine (1.0 mg/kg, IP in saline, 1 ml/kg) while the other half received saline only and locomotor activity over a 30-min period was measured (beginning 30 min after treatment). On the next day, rats previously receiving scopolamine were given saline whereas those previously receiving saline were administered scopolamine. Each animal thus served as its own control, with the effect of scopolamine being calculated as the number of counts recorded for 30 min following scopolamine administration minus the number of counts recorded for 30 min following saline injection. These biweekly scopolamine challenges were continued through 12 weeks after chlorpyrifos treatment (n = 19/treatment group).

Statistical Analyses

Changes in cortical and striatal ChE activity and muscarinic receptor binding parameters were analyzed for significance by two-way analysis of variance (ANOVA) (main effects of treatment vs. time after treatment) using the SAS General Linear Model (GLM) procedure (22). Posthoc analyses were performed by least-square means (in cases of significant interactions between the class variables) or multiple t-tests using a

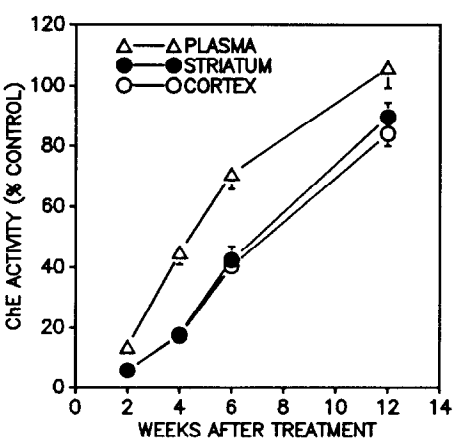

FIG. 1. Effects of CPF treatment on CHE activity. Rats were treated with either vehicle or CPF (279 mg/kg, SC), sacrificed at either 2, 4, 6, or 12 weeks after treatment, and CHE activity measured in cortex (○-○), striatum (●-●), and plasma (△-△). All values from 2–6 weeks after treatment are significantly different between treatment groups. Control levels (mean ± SE) of cholinesterase activity throughout the study were 26.2 ± 1.7 and 214.1 ± 10.1 nmol/min/mg protein for cortex and striatum, respectively, and 170.9. ± 9.0 nmol/min/ml for plasma.

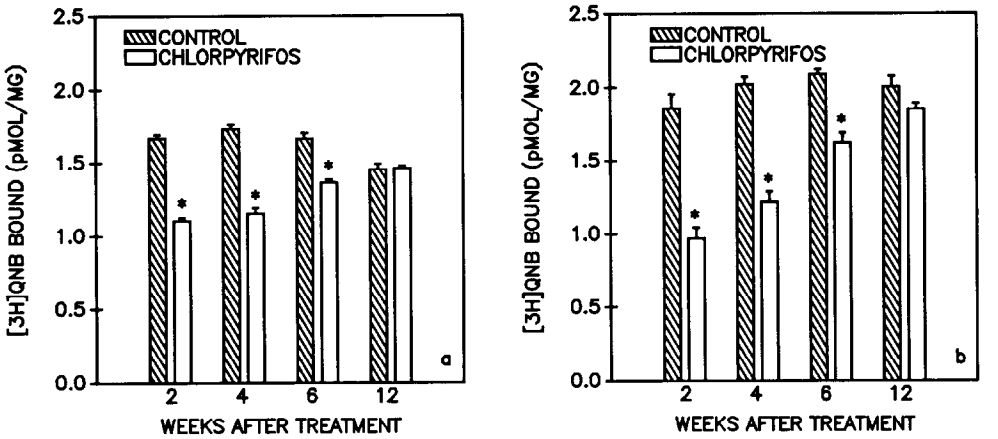

FIG. 2. Effects of CPF treatment on muscarinic receptor densities in (a) cortex and (b) striatum. Rats were treated as in the methods section and a particulate fraction was prepared from cerebral cortex and corpus striatum. Tissue samples were incubated with [^{3}H] QNB (30–1500 pM) and maximal binding density (B_{max}) values (mean ± SE) were determined as described in the methods section. Asterisks indicate significant differences in CPF-treated rats relative to respective controls at given time points.

Bonferonni correction (22). Differences in motor activity were analyzed for significance by repeated-measures ANOVA using the SAS GLM procedure (22). Except in cases where Bonferonni corrections were used, probability levels of 0.05 were considered significant.

RESULTS

As we reported previously (18), a relatively large, single dose (279 mg/kg, SC, maximal tolerated dose) of the organophosphorus insecticide chlorpyrifos caused few signs of overt acute toxicity in adult rats: Diarrhea was the only relatively consistent sign of toxicity. Interestingly, during the course of these studies two rats did exhibit a delayed but typical cholinergic "crisis" (i.e., fasciculations, extensive tremors, excessive salivation, diarrhea) after being asymptomatic for over 3 weeks. The reason for such isolated, delayed responses to CPF is unknown but in both cases unintentional food restriction was a common factor (i.e., both animals were without food for some time before the onset of signs).

Previous experiments indicated that the dose of chlorpyrifos used in the present study (i.e., 279 mg/kg, SC) caused extensive inhibition (>90%) of cholinesterase activity in whole brain for up to 7 days after treatment (18). Figure 1 shows the inhibition of cholinesterase activity in the cerebral cortex, corpus striatum, and plasma from 2–12 weeks after treatment. Virtually identical degrees of cholinesterase inhibition were noted in cortex and striatum throughout the study. Maximal inhibition (94–96%) was seen at 2 weeks, after which ChE activity recovered to 17–18 and 40–42% of control by 4 and 6 weeks after chlorpyrifos treatment, respectively. ChE inhibition in plasma was not as extensive as in either cortex or striatum at any time point during the observation period, but roughly equivalent rates of recovery of enzyme activity were noted between plasma and the brain regions. ChE activities were not significantly different between treatment groups 12 weeks after treatment in either plasma, cortex, or striatum.

Figure 2 shows the effects of a single dose of chlorpyrifos on muscarinic receptor densities (B_{max}) in cortex and striatum from 2–12 weeks after treatment. The striatum appeared to be more extensively affected, with reduction of approximately one half of the total muscarinic binding sites at 2 weeks after treatment. Each tissue exhibited a slow recovery in receptor density from 2–12 weeks after treatment. Table 1 shows the estimates of dissociation constants (K_d values) for cortical and striatal muscarinic receptors as affected by prior chlorpyrifos treatment. While considerable within-group fluctuation was noted in the apparent affinity constants (K_d values) as a function of time, chlorpyrifos treatment caused a significant reduction in K_d values in both cortex and striatum at several time points.

Figure 3 shows the acute effects of chlorpyrifos treatment on motor activity. Chlorpyrifos caused hypoactivity for the first 2 days after treatment, after which activity levels returned to normal. Figure 4 shows the effects of scopolamine (1 mg/kg, SC) on motor activity in control and chlorpyrifos-treated rats between 2–12 weeks after treatment. A significant in-

TABLE 1

DISSOCIATION CONSTANTS (K_d VALUES) IN CORTEX AND STRIATUM AFTER CHLORPYRIFOS TREATMENT

	Weeks After Treatment			
	2	4	6	12
Cortex				
Control	74 ± 2*	64 ± 2	67 ± 2	92 ± 2
Chlorpyrifos	66 ± 1†	52 ± 1†	61 ± 2†	95 ± 1
Striatum				
Control	74 ± 1	89 ± 2	74 ± 3	86 ± 2
Chlorpyrifos	67 ± 1†	82 ± 2	67 ± 1	86 ± 1

Rats were treated subcutaneously with peanut oil or chlorpyrifos (279 mg/kg) in peanut oil in an injection of 2 ml/kg.

*Affinity constant (K_d) values were estimated by Scatchard analysis of saturation isotherms using a range of final concentrations from 30–1500 pM [^{3}H]QNB and represent mean concentration (pM QNB) ± SE.

†Significant differences in CPF-treated groups compared to respective control values.

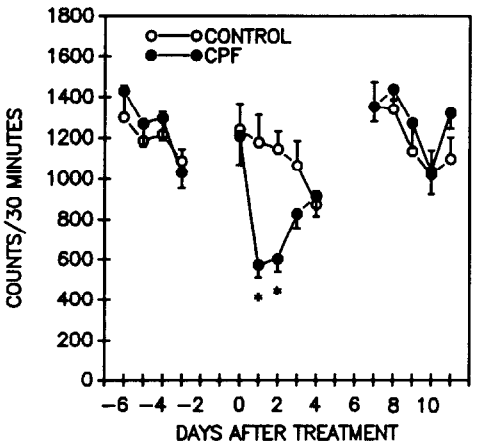

FIG. 3. Acute effects of CPF treatment on locomotor activity. Rats were placed individually into capacitance boxes and motor activity measured as described in the methods section for 4 consecutive weekdays to examine pretreatment activity levels. Following measurement of locomotor activity on the following Monday, rats (n = 19/treatment group) were either treated with peanut oil (2 ml/kg) or with CPF (279 mg/kg in peanut oil) and activity was measured as before on the following 9 weekdays. Data represent mean(±SE) counts recorded during 30-min period. Asterisks indicate significant differences in locomotor activity between treatment groups at the given times.

crease in scopolamine-induced hyperactivity was noted in the chlorpyrifos-treated animals compared to controls at all time points examined except 10 weeks after treatment. At no time between 2–12 weeks after treatment were any differences between treatment groups noted in activity levels following vehicle (saline) injections (data not shown).

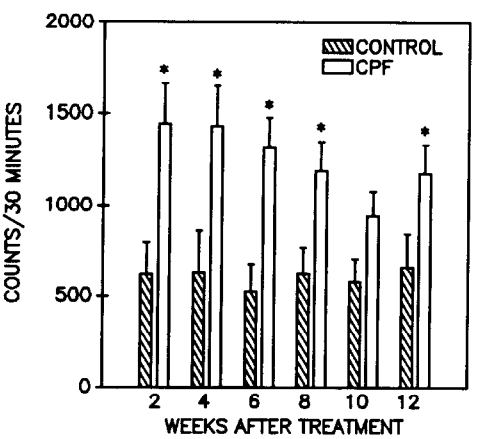

FIG. 4. Motor responses to scopolamine after CPF treatment. At biweekly intervals starting 2 weeks after treatment, rats were challenged on alternate days with either saline or scopolamine (1 mg/kg, IP) in saline and motor activity measured 30 min later as described in the methods section. The effect of scopolamine was calculated as the number of counts recorded after scopolamine challenge less the number of counts recorded after saline challenge. Values represent mean (± SE) counts and asterisks indicate significant differences between treatment groups.

DISCUSSION

Acute exposure to a maximal tolerated dose of chlorpyrifos (279 mg/kg, SC) caused extensive inhibition (94–96%) of cortical and striatal ChE activity up to 2 weeks after treatment. As we noted previously (18) following extensive inhibition of whole brain ChE activity by chlorpyrifos, few signs of acute cholinergic toxicity were evident at any time following treatment. Cortical and striatal ChE activities remained significantly inhibited for up to 6 weeks, indicating very persistent cholinesterase inhibition was produced by the acute treatment protocol.

As expected from the extensive cholinesterase inhibition, cortical and striatal muscarinic receptor ([^{3}H]quinuclidinyl benzilate) densities were also significantly reduced for up to 6 weeks after treatment. Cholinesterase inhibitors have previously been shown to cause a reduction in the density (downregulation) of muscarinic receptors, but such neurochemical modifications have typically followed repeated exposures (7,9,23,24). To our knowledge, such extensive long-term neurochemical changes noted in the present study have not been previously associated with acute exposures to organophosphorus cholinesterase inhibitors.

The significant main effect of chlorpyrifos on the apparent affinity constants of both cortical and striatal muscarinic receptors may have implications for the receptor subtypes involved in the observed receptor modifications. It is currently thought that at least five different muscarinic receptor subtypes exist in the rodent brain, denoted m_1, m_2, m_3, m_4, and m_5 (3,4) and all five of these subtypes are labeled with [^{3}H]QNB. The high-affinity m_1 muscarinic receptors are the prevalent subtype in the cortex and striatum (17,29). A reduction in the apparent dissociation constant following chlorpyrifos treatment (implying a net increase in receptor affinity) may therefore indicate that a relatively greater reduction in the lower-affinity subtypes (e.g., m_2) occurred during chlorpyrifos-induced downregulation.

Recent evidence suggests that the oxygen analog of the organophosphorus insecticide parathion (i.e., paraoxon, the oxidative metabolite of parathion) binds with high affinity to m_2 receptors (1,13). Considering the structural similarities between parathion and chlorpyrifos, the analogous metabolite of chlorpyrifos (i.e., chlorpyrifos oxon) may also bind to the m_2 receptor. If chlorpyrifos indeed directly binds to muscarinic receptors, the extensive downregulation of receptors noted in the present study may be mediated through two distinct mechanisms, that is, directly through binding to the receptors themselves and indirectly through cholinesterase inhibition. Direct interaction with m_2 receptors could also help explain the lack of typical cholinergic-mediated toxicity noted in the presence of extensive nervous system cholinesterase inhibition: m_2 muscarinic receptor subtypes are putative presynaptic autoreceptors involved in the modulation of acetylcholine release from presynaptic terminals (21,30).

The downregulation of cholinergic receptors, while being a compensatory response to elevated acetylcholine levels and a mechanism for tolerance to OP-induced cholinesterase inhibition, can actually increase sensitivity to cholinergic antagonists (6,20). Muscarinic cholinergic antagonists are known to cause an increase in locomotor activity in numerous behavioral settings (2,5,25). We therefore hypothesized (due to the extensive reduction in muscarinic receptor density from 2 to 6 weeks following CPF treatment) that CPF-treated animals would be supersensitive to scopolamine-induced hyperactivity. Indeed, a significant increase in the hyperactivity induced by

scopolamine was noted at these time points (2, 4, and 6 weeks after CPF treatment). Unexpectedly, however, CPF-treated rats continued to show a significantly higher level of locomotor activity in response to scopolamine challenge following recovery of muscarinic receptor density in both forebrain regions.

One possible explanation for the persistent differences in response to scopolamine is that some other region of the nervous system involved in regulation of locomotor activity showed more persistent neurochemical changes following chlorpyrifos administration than was observed in either cortex or striatum. This possibility seems remote given the extremely long-term neurochemical changes noted in the regions examined and the very similar responses noted between these two brain regions (Figs. 1 and 2).

Another possible explanation is that the persistent differences in response to scopolamine noted between control and CPF-treated rats were not mediated through cholinergic mechanisms. It has long been known that cholinergic and dopaminergic interactions play a prominent role in mediation of some motor behaviors and several investigations have reported that anticholinergics may modulate motor activity through either direct or indirect interaction with dopaminergic systems (19,26,28). Bushnell (5) provided evidence that catecholaminergic and cholinergic agents, while initially interacting with different neurotransmitter receptor systems, could increase locomotor activity through some common secondary process (e.g., reduction in metabolic rate). Interestingly, a recent study using the Flinders-Sensitive Line of rats (known to exhibit supersensitivity to muscarinic agonists and elevated forebrain muscarinic receptor density in adulthood) reported a lack of a relationship during postnatal development between muscarinic agonist-induced physiological responses and levels of muscarinic receptors in the nervous system (8). As should be expected, therefore, a direct, simple relationship between changes in receptor density and sensitivity to either agonists or antagonists need not apply under all experimental conditions.

Regardless of the mechanism(s) involved, the persistent differences in sensitivity to scopolamine following a single exposure to chlorpyrifos suggest that subtle, long-term changes in behavior may be induced by such acute treatments. Future studies should focus on the mechanism for these persistent changes, in particular regarding the possible role of noncholinergic processes in long-term alterations in nervous system following persistent cholinesterase inhibition.

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REFERENCES

1. Bakry, N.; El-Rashedy, A.; Eldefrawi, A.; Eldefrawi, M. Direct actions of organophosphate anticholinesterases on nicotinic and muscarinic acetylcholine receptors. J. Biochem. Toxicol. 3:235–244; 1988.
2. Bauer, R. H. Age-dependent effects of scopolamine on avoidance, locomotor activity, and rearing. Behav. Brain Res. 5:261–279; 1982.
3. Bonner, T. I.; Buckley, N. J.; Young, A. C.; Brann, M. R. Identification of a family of muscarinic acetylcholine receptor genes. Science 237:527–532; 1987.
4. Bonner, T. I.; Young, A. C.; Brann, M. R.; Buckley, N. J. Cloning and expression of the human and rat m5 muscarinic receptor genes. Neuron 1:403–410; 1988.
5. Bushnell, P. J. Effects of scopolamine on locomotor activity and metabolic rate in mice. Pharmacol. Biochem. Behav. 26:195–198; 1987.
6. Bushnell, P. J.; Padilla, S. S.; Ward, T.; Pope, C. N.; Olszyk, V. B. Behavioral and neurochemical changes in rats dosed repeatedly with diisopropylfluorophosphate. J. Pharmacol. Exp. Ther. 256: 741–750; 1991.
7. Costa, L. G.; Schwab, B. W.; Murphy, S. D. Tolerance to anticholinesterase compounds in mammals. Toxicology 25:79–97; 1982.
8. Daws, L. C.; Schiller, G. D.; Overstreet, D. H.; Orbach, J. Early development of muscarinic supersensitivity in a genetic animal model of depression. Neuropsychopharmacology 4:207–217; 1991.
9. Ehlert, F. J.; Kokka, N.; Fairhurst, D. S. Altered [^{3}H]quinuclidinyl benzilate binding in the striatum of rats following chronic cholinesterase inhibition with diisopropylfluorophosphate. Mol. Pharmacol. 17:24–30; 1980.
10. Finn, I. B.; Iuvone, P. M.; Holtzman, S. G. Depletion of catecholamines in the brain of rats differentially affects stimulation of locomotor activity by caffeine, d-amphetamine, and methylphenidate. Neuropharmacology 29:625–631; 1990.
11. Gallo, M. A.; Lawryk, N. J. Organic phosphorus pesticides. In: Hayes, W. J.; Laws, E. R., eds. Handbook of pesticide toxicology. vol. 2. Classes of pesticides. San Diego, CA: Academic Press; 1991:917–1123.
12. Glowinski, J.; Iversen, L. L. Regional studies of catecholamines in the rat brain—I. J. Neurochem. 13:655–669; 1966.
13. Jett, D. A.; Abdallah, E. A. M.; El-Fakahany, E. E.; Eldefrawi, E. M.; Eldefrawi, A. T. High-affinity activation by paraoxon of a muscarinic receptor subtype in rat brain striatum. Pest. Biochem. Physiol. 39:149–157; 1991.
14. Johnson, C. D.; Russell, R. L. A rapid, simple radiometric assay of cholinesterase, suitable for multiple determinations. Anal. Biochem. 64:229–238; 1975.
15. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
16. Marquis, J. K. Contemporary issues in pesticide toxicology and pharmacology. Basel: Karger; 1986.
17. Mash, D. C.; Potter, L. T. Autoradiographic localization of M1 and M2 muscarine receptors in the rat brain. Neuroscience 19: 551–564; 1986.
18. Pope, C. N.; Chakraborti, T. K.; Chapman, M. L.; Farrar, J. D.; Arthun, D. Comparison of *in vivo* cholinesterase inhibition in neonatal and adult rats by three organophosphorothioate insecticides. Toxicology 68:51–61; 1991.
19. Pycock, C.; Milson, J.; Tarsy, D.; Marsden, C. D. The effect of manipulation of cholinergic mechanisms on turning behavior in mice with unilateral destruction of the nigro-neostriatal dopaminergic system. Neuropharmacology 17:175–183; 1978.
20. Raffaele, K.; Olton, D.; Annau, Z. Repeated exposure to diisopropylfluorophosphate (DFP) produces increased sensitivity to cholinergic antagonists in discrimination retention and reversal. Psychopharmacology 100:267–274; 1990.
21. Richards, M. H. Relative potencies of agonists and differential sensitivity to *N*-ethylmaleimide on muscarinic autoreceptors and postsynaptic receptors in rat hippocampus. J. Pharmacol. Exp. Ther. 255:83–89; 1990.
22. SAS. Sas/stat user's guide. version 6.03 ed. Cary, NC: SAS Institute; 1988.

23. Schiller, G. D. Reduced binding of ^{3}H-quinuclidinyl benzilate binding associated with chronically low acetylcholinesterase activity. Life Sci. 24:1149–1154; 1979.
24. Schwab, B.; Hand, H.; Costa, L. G.; Murphy, S. D. Reduced muscarinic receptor binding in tissues of rats tolerant to the insecticide disulfoton. Neurotoxicology 2:635–647; 1981.
25. Seiden, L. S.; Dykstra, L. A. Acetylcholine and behavior. In: Psychopharmacology: A biochemical and behavioral approach. New York: van Nostrand Reinhold; 1977; 213–242.
26. Shannon, H. E.; Peters, S. C. A comparison of the effects of cholinergic and dopaminergic agents on scopolamine-induced hyperactivity in mice. J. Pharmacol. Exp. Ther. 255:549–553; 1990.
27. Tallarida, R. J.; Murray, R. B. Manual of pharmacologic calculations. New York: Springer; 1981.
28. Thornburg, J. E.; Moore, K. E. Inhibition of anticholinergic drug-induced locomotor stimulation in mice by alpha-methyltyrosine. Neuropharmacology 12:1179–1185; 1973.
29. Wamsley, J. K.; Gehlert, D. R.; Roeske, W. R.; Yamamura, H. I. Muscarinic antagonist binding site heterogeneity as evidenced by autoradiography after directly labeling with ^{3}H-QNB and ^{3}H-pirenzapine. Life Sci. 34:1395–1402; 1984.
30. Weiler, M. H. Muscarinic modulation of endogenous acetylcholine release in rat neostriatal slices. J. Pharmacol. Exp. Ther. 250: 617–623; 1989.
31. Yamamura, H. I.; Snyder, S. H. Postsynaptic localization of cholinergic receptor binding in rat hippocampus. Brain Res. 78: 320–326; 1974.