green eggs but not white eggs; crows at control sites showed no such discrimination. The responses of treatment site crows to the conditions of experiment 2 were markedly different from those of crows at control sites. Treatment site crows avoided nontoxic green eggs as well as toxic ones but continued to eat white eggs. The green eggs were from 400 m to 2 km from the sites where the crows first acquired the aversion to green eggs and frequently were replaced in new random patterns. These birds moved elsewhere when central sites no longer contained alternative food.

Taste-aversion conditioning appears to be an adaptation to the presence of toxic foods in the environment since aversions develop after one or two illnesses and are associated with a taste even with a delay of hours between feeding and illness (*17*). External conditions not readily associated with food by a consumer do not effect the acquisition of a conditioned taste aversion nor its subsequent expression as avoidance (*18*). This conditioning routinely reverses otherwise robust food preferences and has been shown to influence food selection for at least 8 months under field conditions of varying deprivation and, presumably, physiological state (*19*).

Our findings indicate that a Batesian mimic relying on a toxic model capable of producing taste aversions in its predators may have a powerful adaptive advantage. In addition, a properly applied taste-aversion process may provide wildlife ecologists with a nonlethal means of controlling egg predation.

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9. Union Carbide 27867 is 2,3,5- and 3,4,5-trimethylphenyl methyl carbamate, an aversion agent undetectable by taste at effective doses in eggs.
10. We arranged the ten sites in a ring with a mean distance between sites of 7.5 km (ranges 6.5 to 9.0 km) enclosing 375 km^2 of farmland north of Fargo, N.D., and Moorhead, Minn. Each site was placed as near as possible to an isolated roost.
11. Krylon green spray paint (John Deere) was used. Green color appears to have no special significance: comparable results were obtained with brown spotted eggs as toxic models and green eggs as safe alternate food (L. K. Nicolaus, unpublished observations).
12. The UC 27867 was suspended in egg yolk and injected into the eggs, which were then scrambled in their shells with a rapidly rotating bent steel wire.
13. Predation was attributed to crows if we saw eggs eaten by crows, or we saw crows at the site and only evidence of crow predation was recorded anywhere at the site. Only eggs left intact for > 24 hours were listed as survivors [J. D. Rearden, *J. Wildl. Manage.* **15**, 4 (1951)]. Sightings of crows at and between sites were made as we moved along a prescribed route to replace eggs every morning. Additional counts were made in the same manner at midday and at sunset (N = 12 days) and supplemented with time-lapse photography at nest sites (each of the ten sites at least three times). We concluded that each site had a separate, apparently stable group of crows since (i) 80 percent of all crow sightings were made within 1 km of a nest site; (ii) we repeatedly saw crows approaching from the same roosts each morning and different individuals at the next site, often more than 8 km away; (iii) the number of crows sighted at each site was characteristic of the site as were movements back to roosts after feeding; and (iv) crows became increasingly brazen, approaching within a few meters to feed before we finished putting out the eggs.
14. The three-way design for the first experiment used two colors, two treatments, and five locations. That for the second experiment was the same, with the addition of an analysis of two colors, two treatments, and clumping of nests. Consumption at the central nests was also analyzed separately by two-way analysis of variance [B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962), pp. 258–263].
15. Taste aversions appear to become established rapidly in free-ranging populations of predators when baiting procedures are adequate, and they do not appear to be extinguished quickly. Thus, the rate of bait consumption should be rapid at first and then decline to persistent low levels after aversions are established.
16. Although mammalian predators ate fewer eggs at treatment sites (7.2 per site) than at control sites (14.8 per site), they did not discriminate by color. Since neither the identity nor abundance of the predators was adequately known, no further analysis was attempted.
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20. We thank W. Bleier, J. Grier, and E. Devlin for critically reading the manuscript; J. C. Nicolaus, J. T. Nicolaus, and B. M. Nicolaus for sharing the work and the strain; and Union Carbide Corporation for supplying UC 27867. Supported by a grant from Mr. and Mrs. George C. Nicolaus.
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2 March 1982; revised 27 October 1982

Nicotinic Cholinergic Receptor Binding Sites in the Brain: Regulation in vivo

Abstract. *Tritiated acetylcholine was used to measure binding sites with characteristics of nicotinic cholinergic receptors in rat brain. Regulation of the binding sites in vivo was examined by administering two drugs that stimulate nicotinic receptors directly or indirectly. After 10 days of exposure to the cholinesterase inhibitor diisopropyl fluorophosphate, binding of tritiated acetylcholine in the cerebral cortex was decreased. However, after repeated administration of nicotine for 10 days, binding of tritiated acetylcholine in the cortex was increased. Saturation analysis of tritiated acetylcholine binding in the cortices of rats treated with diisopropyl fluorophosphate or nicotine indicated that the number of binding sites decreased and increased, respectively, while the affinity of the sites was unaltered.*

Nicotinic cholinergic receptor binding sites in the rat brain can be measured by using [^{3}H]acetylcholine as a binding ligand (*1*). In the presence of atropine to block muscarinic cholinergic receptors, [^{3}H]acetylcholine binds to a single class of sites that have the characteristics of neuronal membrane recognition sites for acetylcholine in the brain (*1*). These sites have high affinity and selectivity for nicotinic cholinergic agonists. For example, cytisine, (-)-nicotine, acetylcholine, and carbachol have apparent affinities of 1 to 13 nM for the binding site, while most nicotinic cholinergic antagonists have apparent affinities of 20 to 800 μM (*1, 2*).

We examined the in vivo regulation of this [^{3}H]acetylcholine recognition site by measuring the effects of drugs that increase stimulation of nicotinic cholinergic receptors in the brain. To increase stimulation, we repeatedly administered diisopropyl fluorophosphate (DFP), which inhibits cholinesterases (thereby prolonging cholinergic receptor stimulation by acetylcholine released at synapses), or nicotine, which stimulates nicotinic cholinergic receptors directly. Our results indicate that these two treatments produce opposite effects on [^{3}H]acetylcholine binding sites in the rat brain.

Male Sprague-Dawley rats (250 to 300 g) were housed in groups in a light- and temperature-controlled room (lights on from 0700 to 1900 hours; 23°C) and given unlimited food and water. DFP was injected subcutaneously at a dose of 1 mg/kg on day 1, 0.4 mg/kg on day 2, and 0.2 mg/kg on days 4 to 10. Control rats received injections of vehicle (sterile water) on the same schedule. Nicotine tar-

trate (2 mg/kg; 6.4 μmole/kg) was injected subcutaneously twice each day at 0800 and 1700 hours for 10 days. Control rats received sterile water on the same schedule.

The rats were killed 24 hours after the last injection of DFP and 18 hours after the last injection of nicotine. Their brains were removed, dissected on ice, and stored at −80°C until being assayed. [^{3}H]Acetylcholine binding sites were measured as described by Schwartz *et al.* (*1*). The cerebral cortex was suspended in tris-HCl containing 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, and 1.5 μ*M* atropine (*p*H 7.4, 0°C). The tissue was homogenized with a Brinkmann Polytron and washed twice by centrifugation in fresh buffer at 49,000*g* for 10 minutes. DFP (100 μ*M*) was added to the final homogenate to inhibit cholinesterases. Portions of tissue equivalent to 10 mg wet weight (approximately 620 μg of protein) were added in sextuplicate to assay tubes containing [^{3}H]acetylcholine and incubated for 40 minutes at 0°C. Half of the tubes contained 100 μ*M* carbachol for the determination of nonspecific binding. The reaction was stopped by addition of 4 ml of cold buffer to each tube and rapid filtration over Whatman GF/C filters treated with 0.05 percent polyethyleneimine to eliminate displaceable filter binding. The filters were washed three times with 4-ml portions of cold buffer and then counted by liquid scintillation spectrometry. [^{3}H]Acetylcholine of high specific activity (80 Ci/mmole) was synthesized by acetylation of [^{3}H]choline (80 Ci/mmole; New England Nuclear) (*1*). Muscarinic cholinergic receptor binding sites were measured with [^{3}H]quinuclidinyl benzilate (*3*). In all experiments, tissues from control and treated rats were assayed in parallel.

The schedule of DFP treatments used inhibits brain acetylcholinesterase activity 80 to 90 percent (*4*) and thereby increases the stimulation of cholinergic receptors by retarding the hydrolysis of acetylcholine. Repeated administration of DFP decreased [^{3}H]acetylcholine binding in the cerebral cortex approximately 23 percent (Table 1). Scatchard analysis indicates that the affinity of the sites for [^{3}H]acetylcholine was unchanged but that the density of the sites decreased (Fig. 1A). This down-regulation of nicotinic cholinergic receptor binding sites is similar to the down-regulation of muscarinic cholinergic receptor binding sites that follows DFP treatment (*4*). It appears to be a response to increased receptor stimulation by acetylcholine.

Table 1. Effects of repeated administration of DFP or nicotine over 10 days on the binding of [^{3}H]acetylcholine (10 n*M*) and [^{3}H]quinuclidinyl benzilate (0.2 n*M*) in rat cerebral cortex. Each value is the means ± standard error for the number of animals shown in parentheses.

Treatment	Ligand bound (pmole/g tissue)	
	[^{3}H]Acetylcholine	[^{3}H]Quinuclidinyl benzilate
Control	2.2 ± 0.06 (13)	
DFP	1.7 ± 0.08* (11)	
Percent difference	− 23	
Control	1.9 ± 0.05 (13)	42.0 ± 0.9 (7)
Nicotine	2.4 ± 0.07* (13)	37.3 ± 2.5 (7)
Percent difference	26	−11

*$P < .001$ (Student's *t*-test).

In contrast to the effects of indirect stimulation by DFP, repeated administration of nicotine increased [^{3}H]acetylcholine binding in the cerebral cortex approximately 25 percent (Table 1). Scatchard analysis indicates that the increase in binding was due entirely to an increase in the density of [^{3}H]acetylcholine binding sites (Fig. 1B). A single injection of nicotine did not affect [^{3}H]acetylcholine binding (data not shown), and muscarinic cholinergic binding sites labeled by [^{3}H]quinuclidinyl benzilate were not significantly affected by repeated nicotine treatment (Table 1).

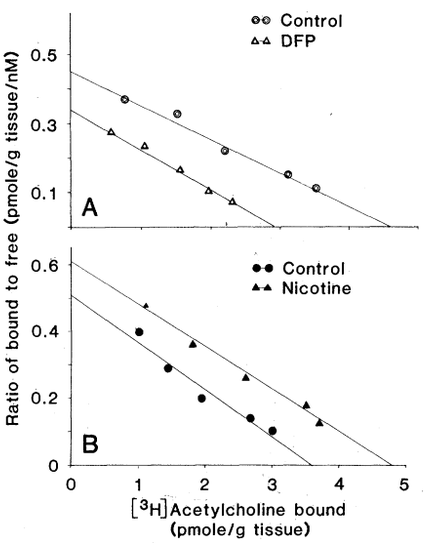

Fig. 1. Scatchard plots derived from saturation analysis of specific [^{3}H]acetylcholine binding to cerebral cortex homogenates from rats treated for 10 days with DFP (A) or nicotine (B). Binding of [^{3}H]acetylcholine (2.5 to 30 n*M*) to cortical homogenates was measured as described in text. (A) and (B) represent four separate analyses each. The maximum number of binding sites (B_{max}) and dissociation constant (K_d) were determined by least-squares linear regression. The mean B_{max} values (± standard errors) for control and DFP-treated rats were 4.4 ± 0.2 and 3.0 ± 0.1 pmole per gram of tissue, respectively ($P < .01$). The B_{max} values for control and nicotine-treated rats were 3.4 ± 0.1 and 4.4 ± 0.2 pmole/g, respectively ($P < .01$). The K_d values were 11.2 ± 1.1 and 11.4 ± 1.4 n*M* for control rats and DFP-treated rats, respectively, and 6.9 ± 0.1 and 6.9 ± 0.8 n*M* for control rats and nicotine-treated rats, respectively. The differences in K_d and B_{max} values between the two control groups are probably due to the use of different batches of [^{3}H]acetylcholine. In all cases, however, tissues from treated animals were assayed in parallel with the appropriate control tissues.

There are many examples of receptor changes in the brain following repeated administration of drugs that increase or decrease receptor stimulation either directly or indirectly (*5*). These changes in receptors are usually reciprocal to the changes in stimulation, resulting in a pattern consistent with compensatory adaptation to the change in stimulation. The decrease in [^{3}H]acetylcholine recognition sites after repeated administration of DFP indicates that these sites are responsive to changes in the acetylcholine concentration in the synapse in vivo. Furthermore, the down-regulation of [^{3}H]acetylcholine recognition sites by DFP suggests that the regulation of these sites by agonists is similar to that of other neurotransmitter and hormone receptor binding sites.

In contrast to the effects of DFP, repeated administration of nicotine increases the number of [^{3}H]acetylcholine recognition sites. Up-regulation of receptors following in vitro exposure to directly acting agonists has been reported (*6*), and in some cases may be a prelude to subsequent agonist-induced down-regulation in vitro (*7*). In vivo, however, repeated administration of drugs that increase receptor stimulation usually results in down-regulation of receptor binding sites (*5*). It is possible that the up-regulation of nicotinic cholinergic receptor binding sites observed after 10 days of nicotine administration is a preliminary step toward eventual down-regulation, but in studies in which nicotine was administered for 21 days, [^{3}H]acetylcholine binding in the cortex was still increased.

Nicotine is considered a classical cholinergic agonist at synapses in autonomic

ganglia and striated muscle. At these peripheral synapses, nicotine so clearly mimics acetylcholine, the endogenous neurotransmitter, that cholinergic receptors in ganglia and striated muscle are designated nicotinic cholinergic receptors (*8*). In the brain, too, nicotine appears to act as a cholinergic agonist (*9*). However, the pharmacological characteristics of brain nicotinic cholinergic receptor sites labeled by [^{3}H]acetylcholine (*1*) or [^{3}H]nicotine (*10*) suggest that they differ from ganglia and striated muscle receptors.

We suggest two possible explanations for the increase in nicotinic cholinergic receptor binding sites following repeated administration of nicotine:

1) Nicotine may interfere with the metabolism or release of acetylcholine, resulting in decreased availability of the transmitter at cholinergic synapses in the brain and subsequent up-regulation of the receptors. However, this would imply that muscarinic receptors labeled by [^{3}H]quinuclidinyl benzilate are less sensitive than nicotinic receptors labeled by [^{3}H]acetylcholine to decreases in acetylcholine availability, since no significant change in [^{3}H]quinuclidinyl benzilate binding is found after repeated nicotine administration.

2) At the dose used in this study, nicotine or a metabolite of nicotine may antagonize receptors in the brain. Although no adverse effects of nicotine were obvious in these experiments, high concentrations of nicotine decrease activity in autonomic ganglia and at the neuromuscular junction by depolarization blockade (*11*). And cotinine, a major metabolite of nicotine, competes for [^{3}H]acetylcholine binding sites in the brain (*1*). Although the affinity of cotinine for [^{3}H]acetylcholine binding sites is low (175 μM), it is comparable to that of several centrally acting drugs that are classified as nicotinic cholinergic antagonists (*1*). In addition, one or more metabolites of nicotine (possibly hydroxynicotine) confers protection against the lethal effects of nicotine in mice (*12*).

If a metabolite of nicotine were acting as a nicotinic cholinergic antagonist in the brain, it could explain the up-regulation of nicotinic cholinergic binding sites. In addition, it could provide a model for and insight into neurochemical processes underlying nicotine addiction and withdrawal.

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9 September 1982; revised 9 December 1982

Formaldehyde Damage to DNA and Inhibition of DNA Repair in Human Bronchial Cells

Abstract. *Cultured bronchial epithelial and fibroblastic cells from humans were used to study DNA damage and toxicity caused by formaldehyde. Formaldehyde caused the formation of cross-links between DNA and proteins, caused single-strand breaks in DNA, and inhibited the resealing of single-strand breaks produced by ionizing radiation. Formaldehyde also inhibited the unscheduled DNA synthesis that occurs after exposure of cells to ultraviolet irradiation or to benzo*[a]*pyrene diolexpoxide but at doses substantially higher than those required to inhibit the resealing of x-ray–induced single-strand breaks. Therefore, formaldehyde could exert its mutagenic and carcinogenic effects by both damaging DNA and inhibiting DNA repair.*

Formaldehyde (HCHO) is a highly reactive chemical (*1*) that is genotoxic in several species (*2*) including the rat, in which it is a respiratory carcinogen (*3*). The chemical is a common environmental pollutant occurring in tobacco smoke, consumer products, many occupational settings, and gasoline and diesel exhaust (*4*). It is also formed endogenously in the cell during demethylation reactions and during the metabolism of *N*-nitrosodimethylamine (*5*) and certain drugs (*6*). Exposure to HCHO has a variety of pathobiological consequences [for reviews, see (*2, 4*)].

The potential carcinogenic hazard of HCHO in the human respiratory tract is currently being debated (*7*). Since its effects on the respiratory tract are essentially unknown, we evaluated its ability to kill and to damage the DNA of human bronchial cells grown as clones in a serum-free medium as described (*8*). We compared the effects of HCHO on epithelial cells and fibroblasts that originated from the same bronchial specimen, and determined whether HCHO could interfere with the repair of DNA that had been damaged by other genotoxic agents. The repair mechanisms studied were (i) the rejoining of x-ray–induced DNA single-strand breaks and (ii) the unscheduled DNA synthesis induced by ultraviolet radiation or (+)-(7β,8α)-dihydroxy - (9α,10α) - epoxy - 7,8,9,10 -tetrahydrobenzo[*a*]pyrene (BPDE). We also investigated the direct effects of HCHO on DNA including the formation of DNA protein cross-links and DNA single-strand breaks.

Cells exposed to HCHO developed both DNA protein cross-links and single-strand breaks. Using methods previously described (*9*), we found that the number of DNA protein cross-links induced by 100 μM HCHO in epithelial cells and fibroblasts was similar (0.65 and 0.83 unit, respectively), and that the frequency of these cross-links was proportional to the concentration of HCHO. The rate at which these cross-links were removed was also similar in the two cell types; the half-removal time was approximately 2 hours (data not shown). This is consistent with results obtained in mouse L1210 cells (*10*). Formaldehyde-induced DNA single-strand breaks were also detected after removal of the cross-links with proteinase K. The frequency of DNA single-strand breaks induced by 500 μM HCHO was 4.2 per 10^{10} daltons in epithelial and 3.5 per 10^{10} daltons in fibroblastic cells