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Symposium Overview: Mechanism of Action of Nicotine on Neuronal Acetylcholine Receptors, from Molecule to Behavior

T. Narahashi,*† C. P. Fenster,‡ M. W. Quick,† R. A. J. Lester,† W. Marszalec,* G. L. Aistrop,* D. B. Sattelle,‡ B. R. Martin,§ and E. D. Levin¶

*Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, Illinois 60611; †Department of Neurobiology, University of Alabama at Birmingham, Birmingham, Alabama 35294; ‡MRC Functional Genetics Unit, Department of Human Anatomy and Genetics, University of Oxford, Oxford OX1 3QX, United Kingdom; §Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, Virginia 23298; and ¶Departments of Psychiatry and Pharmacology, Duke University Medical Center, Durham, North Carolina 27710

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Regulation of Acetylcholine Receptor Desensitization at Low Concentrations of Nicotine

(C. P. Fenster, M. W. Quick, and R. A. J. Lester)

Neuronal nicotinic acetylcholine receptors (nAChRs) are localized to pre- and postsynaptic sites in the brain (Role and Berg, 1996). In a physiological context, e.g., synaptic stimulation, nAChRs are likely to be exposed to the neurotransmitter acetylcholine (ACh) for very brief periods, during which they will become transiently activated and may even become briefly desensitized. During more prolonged exposure to agonist, such as would occur with chronic nicotine use, these receptors may enter conformational states not normally encountered (Fig. 1). These receptor states may directly underlie and/or promote cellular processes that result in tolerance and dependence on nicotine (Dani and Heinemann, 1996).

Any assessment of the consequences of nicotine use at the cellular and molecular levels will require accurate knowledge of the concentration ranges over which nicotine can interact with the various conformational states of nAChRs. In the central nervous system (CNS), an analysis of this type is complicated because an unknown variety of nAChR subtypes exist, and their precise subunit compositions are uncertain. Current research indicates that there are at least 3 major subtypes of nAChRs in the brain, for which partial subunit structure and properties are known: (i) α-bungarotoxin (αBTX)-sensitive receptors containing α7 subunits; (ii) high affinity [3H]nicotine-labeled receptors containing α4 and β2 subunits; and (iii) low affinity nAChRs containing α3 and possibly β4 subunits (Colquhoun and Patrick, 1997; Lindstrom, 1997). Several studies have systematically evaluated the specific properties of receptors with these basic compositions. However, in addition, because the steady-state concentrations of nicotine in the CSF after cigarette smoke inhalation have been estimated as in the low to moderate nanomolar range, it is essential to inquire specifically about the long-term effects of these con-
The desensitization-upregulation hypothesis has, however, been difficult to prove, and recent results have suggested that it may be too simple (e.g., Peng et al., 1994; Whiteaker et al., 1998). The most direct way of testing this theory would be to assess both functional receptor desensitization and the upregulation of receptor number for the same population of receptors, provided that recovery from desensitization was not dependent on PKC. This could account for the findings that some types of nAChRs, including in some systems those containing α4β2 subunits, may be upregulated both in number and in function following prolonged nicotine exposure (Rowell and Wonnacott, 1990; Gopalakrishnan et al., 1996).

Thus, for α4β2 nAChRs expressed in oocytes, receptor desensitization may contribute to the processes that initiate both receptor upregulation and functional inactivation. It need not be the case that these two phenomena are always related, e.g., upregulation could occur independently of inactivation, provided that recovery from desensitization was not dependent on PKC. This could account for the findings that some types of nAChRs, including in some systems those containing α4 and β2 subunits, may be upregulated both in number and in function following prolonged nicotine exposure (Rowell and Wonnacott, 1990; Gopalakrishnan et al., 1996).

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Interactions of Nicotine and Alcohol at Neuronal Nicotinic Acetylcholine Receptors (nAChRa)
(Toshio Narahashi, William Marszalec, and Gary L. Aistrup)

A positive correlation between alcohol drinking and tobacco smoking is well-established (Bien and Burge, 1990; Collins, 1995; Bien and Burge, 1990; Peng et al., 1994). Normal recovery from desensitization, slow as it is (Fenster et al., 1997), does not appear to account for these apparently “permanently” inactive receptors (Lukas, 1991). Since the length of time a receptor spends in any one state is largely related to the rate constant out of that state, we reasoned that if recovery from desensitization became impaired after chronic nicotine exposure, receptors could become “trapped” in seemingly inactive conformations. Inhibition of protein kinase C (PKC) can slowly inactivate α4β2 nAChRs (Eilers et al., 1997), implying that inactivation of α4β2 receptors might be mediated by phosphorylation-dependent effects on the rate of recovery from desensitization. We found that recovery from desensitization could be enhanced by PMA, an activator of PKC, and slowed by calphostin C, a PKC inhibitor. Furthermore, these effects were restricted to a deep state of desensitization (see Boyd, 1987), which may only be reached after prolonged exposure to nicotine (Fenster et al., 1999). These data alone do not directly demonstrate that the inactivation of α4β2 nAChRs during chronic exposure to nicotine results from a loss of recovery from desensitization, but offer a plausible mechanism that may in part explain this phenomenon (Fig. 1).
1990; Zacny, 1990). Approximately 70% of alcoholics smoke more than one pack of cigarettes a day, compared with 10% of the general population. Alcohol seems to influence smoking more than smoking influences drinking. However, the mechanism underlying this correlation remains largely to be seen. One possible explanation is that ethanol potentiates the pleasurable or behavior-reinforcing effects associated with nicotine use. Behavioral reinforcement and locomotor stimulation evoked by either nicotine (Balfour et al., 1998; Benwell and Balfour, 1992; Benwell et al., 1995) or ethanol (Koob et al., 1998; Phillips and Shen, 1996; Samson et al., 1992) are both associated with the release of dopamine from mesolimbic dopaminergic terminals located in the nucleus accumbens. Furthermore, stimulation of cholinergic neurons is known to cause release of various neurotransmitters including dopamine, GABA, epinephrine, and glutamate (Wonnacott, 1997). Thus, interactions of nicotine and ethanol at neuronal nicotinic acetylcholine receptors (nAChRs) may explain the linkage between drinking and smoking. We performed patch clamp experiments using rat cortical neurons in long-term primary culture to prove this hypothesis (Marszalec et al., 1999).

At least 2 types of currents were generated in response to the application of ACh, a rapidly desensitizing (a7-like), a-bungarotoxin (a-BuTX)-sensitive current, and a slowly desensitizing (a4b2-like), a-BuTX-insensitive current. The a4b2-like current was potentiated by the application of 3–300 mM ethanol, while the a7-like current was slightly inhibited by 30–300 mM ethanol. Nicotine by itself, at a concentration of 300 nM or 1 μM, generated a4b2-like currents, albeit in much smaller amplitude than those evoked by ACh. As previously observed with muscle nicotinic AChRs (Wang and Narahashi, 1972) and more recently with nnAChRs (Bellwell et al., 1995; Fenster et al., 1997; Ochoa et al., 1989; Pidoplichko et al., 1997), nicotine at low concentrations desensitized the a4b2-like receptor of cortical neurons. Nicotine, at concentrations of 30, 100, and 300 nM, reversibly desensitized the ACh-induced current by 38, 54, and 62%, respectively. However, these concentrations of nicotine evoked only small currents by itself, with the EC50 concentration for nicotine-induced desensitization (~100 nM) being about 300-fold less than that required for current activation (~3 μM). It should be noted that blood nicotine levels transiently peak at 30 to 180 nM with each cigarette smoked (Benowitz et al., 1989; Henningfield et al., 1993; Russell, 1987). These peaks, however, are superimposed over continuously rising steady-state nicotine levels of 60 to 300 nM, which tend to persist between each cigarette smoked. Therefore, the nnAChRs are desensitized considerably during repeated cigarette smoking.

Ethanol potentiated a4b2-like currents, even in the presence of the desensitizing concentrations of nicotine. In neurons desensitized by 100 to 200 nM nicotine, 100 mM ethanol enhanced residual currents by 30 ± 4% compared with a 24 ± 5% enhancement of control currents (n = 6). Thus, ethanol potentiates the currents to approximately the same extent regardless of the degree of desensitization produced by nicotine perfusion.

An example of a crucial experiment is illustrated in Figure 2. Currents evoked by 300 μM ACh pulses were monitored over time (black circles). Perfusion of nicotine at a concentration of 30 nM (solid bar) desensitized ACh-induced currents by 43% (compare traces a with b). Following washout of nicotine, the bath perfusion of 100 mM ethanol (broken bar) potentiated the amplitude of ACh-induced currents by 37% (white circles, compare trace c with d). Now, a co-perfusion of 30 nM nicotine with 100 mM ethanol desensitized ACh-induced currents by 35% (white triangles, compare trace d with e). It should be noted that the nicotine desensitization relative to the initial control response (trace c) is only 6% when ethanol is present (compare traces c with e). Thus, ethanol partially offsets inhibition of the ACh-induced current due to nicotine desensitization.

Nicotine has been shown to release dopamine in the nucleus accumbens through activation of a4b2-like AChRs at concentrations comparable with those required to evoke currents in cortical neurons (Balfour et al., 1998; Benwell and Balfour, 1992; Benwell et al., 1995). It has also been demonstrated that ethanol-induced locomotion and dopamine release are blocked by the nicotinic channel blocker mecamylamine (Blomqvist et al., 1992, 1993, 1997). These data, when combined with the present results, suggest that ethanol, together with nicotine, enhances dopamine release, resulting in the behavioral reinforcement mediated by this transmitter to increase the incidence of tobacco smoking. If the smoking urge is prompted by the overall recovery of ACh-induced currents from nicotine desensitization, then the potentiation of these currents by ethanol intake might shorten the interval between each cigarette smoked. Ethanol potentiation and nicotine desensitization of ACh-induced currents may form the basis for the heavy drinking-heavy smoking correlation. It remains to be seen exactly how these in vitro interactions between ethanol and nicotine are reflected in each step of behavioral changes.

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**Identifying Members of the Nicotinic Acetylcholine Receptor Subunit Family Targeted by Anthelmintics and Insecticides (David B. Sattelle)**

The nematode worm *Caenorhabditis elegans*, and the fruitfly *Drosophila melanogaster* have proved to be excellent genetic models with which to investigate the nervous system and are now facilitating the identification of particular ionotropic receptor subunits that are targeted by pesticides. Investigations employing genetics, genomics, and functional studies on cloned, heterologously expressed receptor subunits have contributed to these advances. Here we review the recent developments in understanding of the nicotinic receptor (nAChR) subunit gene families of these organisms and the identification
FIG. 2. Ethanol opposes the effect of ACh-induced current desensitization due to nicotine perfusion. Current responses were evoked by 300 μM ACh pulses (black circles). A co-application of 30 nM nicotine (solid bar) and ACh (white circles) did not alter the test response until nicotine was introduced into the bath, during which time current desensitization developed (trace a versus b). The test current recovered after nicotine washout (trace c). Next, a co-application of 300 μM ACh and 100 mM ethanol (white squares) potentiated the ACh current above that of the control response (trace c versus d). During this period, 100 mM ethanol was also perfused into the bath (broken bar). When nicotine and ethanol were subsequently perfused together, the response once again underwent desensitization (white triangles, traces d versus e). However, the degree of current reduction observed with nicotine perfusion in the presence of ethanol was less than that observed with nicotine alone (compare traces a/b with traces c/e). From Marszalec et al. (1999).
of nACHR subunit family members targeted by anthelmintics and insecticides.

**The C. elegans nACHR Gene Family**

By offering access to a fully sequenced genome, an advanced “genetic toolkit”, and a wealth of neurobiological information, the nematode *Caenorhabditis elegans* is well suited to the functional analysis of genes encoding synaptic proteins. Cholinergic chemical synapses at which acetylcholine (ACH) is the neurotransmitter have been well studied. The *cha-1* gene encodes choline acetyltransferase and appears to be the only gene for this ACh synthesizing enzyme (Alfonso et al., 1994). The nACHRs (composed of combinations of α and non-α-type subunits) are ionotropic receptors, mediating the fast synaptic actions of ACh, yet for no organism is the full complement of nACHR subunits known. In the nematode *C. elegans*, nACHRs are present on nerve (Treinin and Chalfie, 1995) and muscle (Fleming et al., 1997), and subunits are encoded by a large gene family. Brenner (1974) first isolated mutants of this nematode resistant to tetramisole (the enantiomeric mixture in which levamisole, an anthelmintic drug, is one stereoisomer). Of the 11 genes found to be associated with resistance to levamisole, 3 encode nACHR subunits: lev-1, unc-29 (non-α) and unc-38 (α) (Fleming et al., 1997). In the same study, Fleming et al. showed that expression in Xenopus oocytes of combinations of these subunits that include the UNC-38 α subunit resulted in low-amplitude, inward currents in response to 100 μM levamisole. These currents were suppressed by nicotinic antagonists such as mecamylamine (100 μM). Mutant phenotypes show that UNC-38 and UNC-29 subunits are necessary for nACHR function, whereas the LEV-1 subunit is not. Two dominant mutant alleles of LEV-1, each with a single amino acid change in the second transmembrane (M2) region, which contributes most of the residues that line the channel, were found to be highly resistant to levamisole. A gain of function mutation of the *deg-3* α subunit-encoding gene, which leads to the degeneration of a small set of neurons, also alters a residue in the M2 region (Treinin and Chalfie, 1995). The identification of viable nicotinic receptor mutants in *C. elegans* permits manipulation of receptor expression and synaptic targeting in *vivo*.

At this time, 13 nicotinic receptor α and 7 non-α subunits have been shown to be transcribed in *C. elegans*, and other candidates are under investigation, making it the largest nACHR α subunit gene family and also the largest nACHR gene family overall currently known (Mongan et al., 1998). Sequence diversity is evident in the M2 channel-lining region and in regions that contribute to the ACh binding site (Mongan et al., 1998; Sattelle, 1998). At present, 4 genes are known to encode ACh, inactivating enzymes in *C. elegans*, the acetylcholinesterases (AChEs) (Arpagaus et al., 1994; Grauso et al., 1998). The genes *ace-1*, *ace-2*, *ace-x*, and *ace-y* were found to encode 4 pharmacologically distinct classes of AChE. Thus extensive and diverse gene families encode proteins mediating fast synaptic responses to the neurotransmitter ACh and the termination of its actions.

**nACHR Subunits Contributing to Levamisole Sensitivity**

So how does this wealth of new information assist in identifying the subunits contributing to nACHR subtypes that may be targeted by anthelmintics? In the case of genetic screens based on resistance to levamisole, a drug that activates nACHRs and at higher concentrations shows open channel block. It appears that this approach highlights, from an extensive family, a particular subset of nACHR subunits. We have generated functional heteromeric nACHRs by expressing in Xenopus oocytes, combinations of α and non-α subunits that are products of genes linked to levamisole resistance. This results in functional recombinant nACHRs that mimic several of the properties of native nematode muscle nACHRs, including activation by levamisole (Fleming et al., 1997). In this aspect of their pharmacology, they differ strikingly from the only other well characterized *C. elegans* recombinant nACHRs, which result from the expression of the ACR-16 (= Ce21) nACHR subunit. In the case of ACR-16 homomeric receptors, levamisole has no agonist action but is in fact an nACHR antagonist (Ballivet et al., 1996). Thus, pharmacologically distinct recombinant receptors have already been generated using only a small fraction of the known nACHR family members, and different actions of levamisole have been observed. Based on the observed hypercontraction that precedes the cessation of movement in levamisole-treated *C. elegans*, it appears that the responses of the heteromeric receptors formed from genes linked to levamisole resistance provide the best approximation to date of the native receptors targeted by levamisole. Undoubtedly the current picture may be further refined as more *C. elegans* nACHR family members are cloned and expressed.

**The D. melanogaster nACHR Gene Family**

The nACHRs in insects appear to be confined to the nervous system (Breer and Sattelle, 1987; Sattelle, 1980). Although, to date, the *D. melanogaster* nACHR gene family is less well-characterized than that of *C. elegans*, there are detailed studies on 5 nACHR subunits. The cDNAs encoding α subunits *sad* (= *Da2*), *als*, *da3* and the non-α subunits *ard* and *sbd* have all been sequenced (Gundelfinger, 1992) but attempts at heterologous expression of a functional nACHR from only *Drosophila* subunit combinations have so far failed to yield robust functional receptors. This is the case in experiments using either transient expression in Xenopus oocytes (Bertrand et al., 1994; Matsuda et al., 1998), or stable expression in *Drosophila* cell lines (Lansdell et al., 1997). Thus, it appears that other nACHR subunits remain to be discovered. The only known recombinant insect nACHR composed entirely of insect subunits is the

Nevertheless, robust functional expression can be obtained for nAChR hybrid receptors composed of a Drosophila α subunit and a vertebrate neuronal non-α subunit (β2). Thus SAD/β2 receptors and ALS/β2 receptors have been generated and aspects of their pharmacology compared (Bertrand et al., 1994). These authors show that, whereas the SAD-containing hybrid nAChR is insensitive to α-bungarotoxin, the ALS-containing hybrid receptor is highly sensitive to this toxin. It is of interest that earlier studies on native insect nAChRs identified nAChR subtypes that differed in their sensitivity to this snake neurotoxin (Breer and Sattelle, 1987; Sattelle, 1980; Sattelle et al., 1983). Differences in affinity for nicotinic ligands have been observed between hybrid SAD/β2 and vertebrate brain α4/β2-type receptors (Matsuda et al., 1998). With the genome sequence of Drosophila advancing rapidly and the pharmacological diversity observed to date in native and hybrid recombinant nAChRs containing different Drosophila subunits, it seems we can anticipate diverse and extensive nAChR gene families in insects as well as nematodes.

nAChR Subunits Contributing to Imidacloprid Sensitivity

The insecticide with the fastest growing sales worldwide is imidacloprid, a potent ligand at insect nAChRs (Bai et al., 1991). It is a member of the nitromethylene/nitroguanidine class of insecticides and other members of this class are active on insect nAChRs (Sattelle et al., 1989). The nitromethylene nithiazin was found to have agonist actions on native insect nAChRs and on the recombinant locust αL1 nAChRs expressed in Xenopus oocytes (Leech et al., 1991). Of particular interest has been the recent comparison of the SAD/β2 hybrid receptor and the vertebrate α4/β2 heteromer (Matsuda et al., 1998). These authors compared the actions of imidacloprid and other nAChR ligands (+)-epibatidine, (-)-nicotine and ACh) on both expressed recombinant receptors. Imidacloprid, alone of the 4 agonists, behaved as a partial agonist on the α4/β2 receptor; (+)-epibatidine, (-)-nicotine, and ACh were all full, or near-full agonists. Imidacloprid was a partial agonist on the hybrid Drosophila SAD/β2 receptor, as was the case for (-)-nicotine, whereas (+)-epibatidine and ACh were full agonists. The EC₅₀ for imidacloprid was reduced by replacing the vertebrate α4 subunit by Drosophila SAD. This substitution resulted in an increase in EC₅₀ for the 3 other ligands tested. Thus the SAD subunit contributes to the greater apparent affinity of imidacloprid for recombinant insect/vertebrate nAChRs.

In conclusion, genetic, genomic and functional studies are enhancing our understanding of molecular and functional diversity in nAChR gene families and are providing insights into subunits targeted by anthelmintic drugs and insecticides.

Pharmacological Properties of Central Nicotinic Receptors (Billy R. Martin)

The pharmacological effects of nicotine are extremely complex as a result of several factors. First, nicotine produces a wide range of effects on the central nervous system, including excitation, sedation, analgesia, cognitive alterations, hypothermia, seizures, etc., depending upon the dose. Secondly, tachyphylaxis develops readily to many of nicotine’s effects, which complicates efforts to systematically characterize this pharmacological profile (Damaj et al., 1996b; Damaj and Martin, 1996). The recognition that nicotine is one of the most highly addictive drugs has attracted considerable attention in recent times and has led to increased efforts to elucidate the mechanisms by which nicotine produces central effects. Moreover, understanding the actions of nicotine in the central nervous system provides a strategy for development of smoking cessation therapies devoid of serious nicotine side effects.

Most of nicotine’s effects have been attributed to its interaction with the cholinergic ion channels α4β2, α2β2, and α7. Nicotine either stimulates or depresses spontaneous activity under some circumstances, reduces body temperature, generates a discriminative stimulus, and produces antinociception, all of which are blocked by the ganglionic antagonist mecamylamine. Nicotine exhibits high affinity for a binding site in brain tissue that is thought to represent primarily the pentameric ion channel α4β2. Structure-activity relationship studies have established a reasonable correlation between affinity for ³H-nicotine-labeled binding sites and depression of spontaneous activity and analgesia, particularly when only those compounds that are antagonized by mecamylamine are considered (Damaj et al., 1996a). These structure-activity relationship studies have been aided by the discovery of the highly potent epibatidine that greatly extended the potency range under consideration (Damaj et al., 1994). More recently, the emphasis has been on development of agonists and antagonists with selective pharmacological profiles, in order to further characterize the effects that are mediated via α4β2 receptors. One such compound is metanicotine, which is active in a wide range of analgesic tests but is devoid of depressant properties and seizure activity, even at very high doses (Damaj et al., 1999). This profile contrasts that of nicotine, which is equiactive in depressing spontaneous activity and producing analgesia, and producing seizures at slightly higher doses. Metanicotine is almost 10 times more potent at α4β2 than α3β2 receptors expressed in Xenopus oocytes. The involvement of α4β2 receptors in analgesia remains to be fully characterized. However, the findings that nicotine was devoid of activity in the hot-plate test and considerably less potent in the tail-flick procedure in α4 and β2 “knockout” mice established that these receptor subunits are critical for nicotine analgesic activity (Marubio et al., 1999).

There have been suggestions that nicotine-induced seizures are mediated through the homologous α7 pentameric receptor.
subtype. Nicotinic agonists produce seizures to varying degrees, and their potency does not appear to be directly related to affinity for $^3$H-nicotine-labeled binding sites. Seizures are blocked by both mecamylamine and methyllycaconitine, the latter of which has some preference for $\alpha 7$ subtypes. However, nicotine-induced seizures are highly susceptible to agents that alter intracellular calcium levels, an observation that underscores the role of receptor and non-receptor mechanisms (Damaj and Martin, unpublished observations).

Presently, it is difficult to assign specific pharmacological effects to nicotine receptor subtypes. However, $\alpha 4\beta 2$ receptors are strongly implicated in nicotine-induced analgesia as well as in depression of the central nervous system. The $\alpha 7$ receptor is undoubtedly involved in nicotine-induced seizures. Presently, it is not possible to establish whether other receptor subtypes may also play a role in some of these actions. Further development of selective agonists and antagonists for all receptor subtypes, coupled with a better characterization of receptor subtypes in the central nervous system, will provide new strategies for elucidating the physiological role of nicotinic receptors and for developing pharmacotherapies for diseases of the cholinergic nervous system.

Chronic Nicotine Infusion Effects on Memory: A Ventral Hippocampal Mechanism (Edward D. Levin)

Nicotinic systems are involved in a wide variety of neurobehavioral functions. Some effects of nicotine such as cognitive enhancement hold promise for the development of therapeutic treatments for cognitive dysfunction (Arneric et al., 1995; Levin et al., 1993a; Warburton, 1992). For the development of nicotinic-based therapeutics, it is important to determine the critical mechanisms for the therapeutic effect in order to guide the development of novel nicotinic drugs that are effective with minimal side effects.

Nicotine has been widely found to improve cognitive performance in experimental animals (Levin and Simon, 1998). In an experimental rat model, we have found that chronic 4-week nicotine infusion with osmotic minipumps significantly improves working memory performance in the radial-arm maze (Levin et al., 1997) (Fig. 3). Important for the potential therapeutic use of nicotine is the finding that the memory enhancing effect does not diminish with chronic administration. With chronic infusion over a period of 4 weeks via osmotic minipumps, the memory enhancement caused by nicotine did not diminish (Levin et al., 1993a). In fact, with 3 weeks of high-dose infusion, there was a persistence of the memory improvement for at least 2 weeks after withdrawal (Levin et al., 1990). Chronic nicotine infusion selectively improved working memory without significantly affecting reference memory (Levin et al., 1996b).

In humans, nicotine administration via skin patch has been found in our studies to significantly improve attentional performance in patients with Alzheimer’s disease, schizophrenics, adults with attention deficit/hyperactivity disorder, and normal nonsmoking adults (Jones et al., 1992; Levin et al., 1996a,b, 1998; White and Levin, 1999). Since nicotine has been shown to have adverse effects in terms of cardiovascular effects and the development of dependence, it is important to determine the critical mechanisms for the potential therapeutic effects, so that the side effects can be avoided and the potential therapeutic effects enhanced.

Work with the experimental rat model has revealed that the ventral hippocampus is critical to nicotinic involvement in memory function. We found local infusions of nicotinic antagonists into the ventral hippocampus to cause significant working memory impairments. Mecamylamine, as well as the specific $\alpha 4\beta 2$ antagonist DH$eta$E and the specific $\alpha 7$ antagonist MLA caused significant impairments in working memory performance on the radial-arm maze (Fig. 4) (Felix and Levin, 1997; Kim and Levin, 1996). In a recent study using the 16-arm maze, we have replicated these effects at lower doses.
and have found more pronounced effects with working vs. reference memory (Levin et al., 1999b). Given these effects, we hypothesized that this area is a critical locus for chronic nicotine infusion-induced memory improvements.

Knife cut lesions of the fimbria-fornix did not impair the memory improvements caused by chronic nicotine administration (Levin et al., 1993b). In contrast, small ibotenic acid lesions in the ventral hippocampus blocked the nicotine effect (Levin et al., 1999c). Half of the rats with each lesion treatment were given subcutaneous infusions of nicotine with osmotic minipumps (5 mg/kg/day) or saline. As seen previously, chronic nicotine infusion significantly ($p < 0.05$) improved working memory performance in the sham-lesioned group. The ventral hippocampal lesion blocked this effect even though by itself this small lesion did not impair working memory performance (Fig. 5). These data show that the ventral hippocampus is critical for chronic nicotine-induced memory improvements. Recently, we found that the selective $\alpha 7$ nicotinic agonist AR-R 17779 also reversed working memory deficits caused by fimbria-fornix knife cut lesions in rats (Levin et al., 1999a). This mechanism further demonstrates the importance of hippocampal nicotinic systems for cognitive function and specifies the relevance of $\alpha 7$ receptors.

These data show that the ventral hippocampus is critical for nicotinic involvement in memory function and that the memory improvement caused by chronic nicotine infusion is blocked by small lesions within the hippocampus (Levin et al., 1999c.

Given that lesions of the septohippocampal fiber bundles did not block this effect, it seems that nicotinic receptors located presynaptic to this connection were not critical for the chronic nicotine effect. Intrahippocampal, ibotenic-acid lesions did block the effect, suggesting that nicotinic receptors postsynaptic to the septohippocampal pathway were critical for the chronic nicotine-induced memory improvement. These findings indicate that both $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptors within the hippocampus are critically important for memory.

**REFERENCES**


**FIG. 4.** Acute ventral hippocampal infusions of the $\alpha 7$ nicotinic antagonist MLA and the $\alpha 4\beta 2$ nicotinic antagonist DHβE caused significant ($p < 0.025$) dose-related impairments in choice accuracy (entries to repeat) in the 8-arm radial maze (mean ± standard error of the mean).

**FIG. 5.** Chronic nicotine infusion (5 mg/kg/day) caused a significant improvement in 8-arm radial maze choice accuracy (entries to repeat) in sham-operated rats. This effect was abolished in the rats with small ibotenic acid lesions to the ventral hippocampus (mean ± standard error of the mean). The small lesions themselves did not impair accuracy.


