Nicotinic Receptor Function in Schizophrenia

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Abstract

Schizophrenia can be partially characterized by deficits in sensory processing. Biochemical, molecular, and genetic studies of one such endophenotype, the P50 auditory-evoked potential gating deficit, suggest that one of the neuronal nicotinic receptors, the α7 nicotinic receptor, may function in an inhibitory neuronal pathway involved in this phenotype. The P50 deficit is normalized in nongating subjects by nicotine. Although most schizophrenia patients are heavy smokers, the effects of nicotine may be transient, as α7 receptors are known to desensitize rapidly. In an animal model of the P50 gating deficit, antagonists of the α7 nicotinic receptor block normal gating of the second of paired auditory stimuli. Regional localization of receptor expression includes areas known to function in sensory filtering. An inhibitory mechanism, in the hippocampus, may involve nicotinic stimulation of γ-aminobutyric acid (GABA)ergic interneurons, resulting in decreased response to repetitive stimuli. Expression of the α7 receptor is decreased in hippocampal brain tissue, dissected postmortem, from schizophrenia subjects. The P50 deficit is inherited in schizophrenia pedigrees, but it is not sufficient for disease development and thus represents a predisposition factor. Linkage analysis between the P50 deficit in multiplex schizophrenia pedigrees and deoxyribonucleic acid (DNA) markers throughout the genome yielded positive lod scores to DNA markers mapping to a region of chromosome 15 containing the α7 nicotinic receptor gene. Elucidation of possible interactions of the P50 with other factors, known to be important in the etiology of the disease, is important in determining an overall pathobiology of schizophrenia.

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Schizophrenia is a complex disease that has been difficult to characterize by either biochemical or classical genetic methods. Although a genetic component for the disease has been demonstrated from family and twin studies (Gottesman and Shields 1982; McGue and Gottesman 1989), there is not a Mendelian pattern of inheritance, and linkage of schizophrenia with a specific chromosomal locus has yet to be definitively established (Cloninger 1994). Biochemical measurements, candidate gene analysis, and environmental factor investigation have failed to find a single determinant that makes a major contribution to this mental illness (Sarkar et al. 1991; Catalano et al. 1992). In a broad sense, however, schizophrenia might be viewed as a disease consisting of a number of interactive phenotypes, each biochemically and genetically independent, which together contribute to clinical manifestation of the disease. In the early 1960s, P. E. Meehl suggested that a useful strategy would be to search for a neurophysiological "schizotaxic" factor, described by a single neuronal abnormality that would be inherited but would not necessarily be sufficient to cause schizophrenia by itself (Meehl 1962). Specific focus on such an endophenotypic factor might afford the investigator a single neuronal pathway for investigation and per-

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haps simplify the genetic component as well.

Schizophrenia patients suffer from several types of sensory deficiencies, some of which can be measured experimentally (Adler et al. 1982; Braff and Saccuzzo 1985; Braff et al. 1992). Patients have problems with the interpretation of both sound and sight, resulting in the commonly occurring auditory and visual hallucinations (Andreasen 1985; McGlashan and Fenton 1992; Tsuang 1993). It is as though the information is reaching sensory nuclei in the brain, but normal processing of that input and comparison with prior knowledge of a similar event do not take place. This article reviews the progress on the use of an auditory-evoked potential deficit as a model endophenotype in schizophrenia for biochemical, molecular, and genetic research.

Measurement of Sensory Processing Deficits in Schizophrenia

The schizophrenia patient can be described as hypervigilant; sensory input that a normal individual can filter out continues to stimulate the patient (Venables 1964). Repetitive sounds in the environment, such as traffic or construction sounds, are heard but ignored by normal subjects; the schizophrenia patient continues to respond, often interpreting the noise as threatening or directive. Auditory stimulation, such as sounds from a refrigerator or a furnace, often become overwhelmingly bothersome.

Several approaches to measuring this deficit in human subjects have been successful (Braff and Saccuzzo 1985; Freedman et al. 1987; Braff et al. 1992). All involve the delivery of a conditioning stimulus, followed after a set period of time by a test stimulus. The normal subject has the capacity to filter out or gate the response to the second input; the schizophrenia subject does not. In one paradigm, a soft sound precedes a louder test sound by 100 ms; normally there is a diminution of response to the second and louder sound. However, this diminution of response, referred to as prepulse inhibition, is less robust in the schizophrenia subject (Braff et al. 1992).

In the visual backward masking assay of visual processing, two different objects are presented within 100 ms, and the subject is asked to recall the first. In schizophrenia subjects, identification of the first object is obscured or masked by visual input from the second object. Normal subjects can easily identify both objects (Braff and Saccuzzo 1985). These types of analyses suggest that schizophrenia subjects cannot control or inhibit their responses to sensory stimuli.

Our laboratory uses yet another paradigm to study a neuronal pathway in the brain that regulates the processing of auditory stimuli. It was chosen not only because the phenotype can be studied by recording auditory-evoked potentials in patients and normal subjects, but also because it can be assayed in a laboratory animal in which invasive electroencephalogram (EEG) and pharmacological experiments are possible. The procedure, derived from a phenomenon originally reported by Davis et al. (1966), has been adapted here to measure inhibitory neuronal processes, or gating, of sensory auditory stimuli in the brain (Adler et al. 1982; Boutros et al. 1991; Freedman et al. 1991). An initial auditory stimulus activates or conditions an inhibitory response, and a second auditory stimulus then tests the extent of inhibition (Eccles 1969). The conditioning response is stimulatory because inhibitory mechanisms have not been activated; the test response normally is decreased by inhibitory circuits activated by the first stimulus. Although several waves can be measured, as a result of evoked potentials from the delivery of auditory stimuli (Simpson and Knight 1993), we have focused on the P50, a positive wave occurring 50 ms after the stimulus, for analysis. The response at 50 ms is decreased for several seconds following the test stimulus, defining the inhibition of extraneous auditory input, but the P50 does not exhibit longer-term habituation, and many pairs of stimuli can thus be examined in a given testing session (Adler et al. 1982; Freedman et al. 1983; Boutros et al. 1991; Judd et al. 1992).

This inhibitory gating effect is reported as the ratio of the amplitude of the test (T) P50 wave over the amplitude of the conditioning (C) wave or P50 ratio. In a study of 37 schizophrenia subjects and 43 normal subjects, 94 percent of normal subjects had P50 ratios ≤ 0.50; 91 percent of the schizophrenia subjects had ratios ≥ 0.50 ($\chi^2 = 55.5$, $df = 1$, $p < 0.001$) (Waldo et al. 1994). The mean for normal subjects was 0.18 ± 0.17 and for schizophrenia subjects, 1.0 ± 0.32. The schizophrenia subject is thus unable to filter or gate out repetitive auditory input—a measurable phenotype that may result in abnormal interpretation of extraneous sounds.

Gating of auditory-evoked potentials in control subjects can be overcome by directing attention to the test stimulus, which can be demonstrated by requiring an observation about the test stimulus, such as counting odd tones. Gating of the test response is then lost (Guterman et al.

1992). Gating is also lost in normal subjects by increased catecholamine release, introduced either by discomfort (Johnson and Adler 1993) or by pharmacological intervention (Adler et al. 1994). This capacity to override the inhibitory response when necessary, by increasing attention to all stimuli in the environment, allows us to respond when required, even if the stimulus would normally be ignored. Effects of selective attention and subject comfort, however, are important parameters in the comparison of auditory gating between schizophrenia subjects and controls. These and other procedural considerations affect the reliability and repeatability of the assay (Boutros et al. 1991; Jerger et al. 1992; Waldo et al. 1992).

Interestingly, the gating deficit is also found in about 50 percent of the schizophrenia patient's first-degree relatives, who do not have the illness. The deficit therefore probably has a genetic component, and indeed, it appears to be inherited from the side of the family carrying the schizophrenia phenotype (Siegel et al. 1984; Waldo et al. 1991). Other sensory endophenotypes, such as deficits in smooth-pursuit eye movements (Holzman et al. 1988) and in reaction time (De Amicis et al. 1986), may be inherited in a similar manner. Most such deficits, including the P50 deficit, have an apparent autosomal dominant transmission pattern, that is, the deficit is found in one parent, in the proband, in half the siblings, and in half of the siblings' and proband's descendents. The P50 deficit thus represents an endophenotype that may predispose the person who expresses it to schizophrenia.

Pharmacology of Auditory Gating in Schizophrenia Subjects and Controls

Neuroleptic Medication. Clinical drug treatments and the measurement of auditory gating deficits in other psychiatric illnesses such as mania suggest that the deficits seen in schizophrenia are not directly related to aberrant dopaminergic neurotransmission. Typical neuroleptic drugs such as haloperidol do not normalize the P50 deficit (Freedman et al. 1983; Adler et al. 1990). Although unmedicated schizophrenia subjects have smaller P50 waves than do normal controls and the size of the waves is normalized by neuroleptics, both the conditioning and the test waves are affected equally, resulting in retention of the abnormal T/C ratio (Straumanis et al. 1982; Freedman et al. 1983; Adler et al. 1990). In patients who respond clinically to the atypical neuroleptic clozapine, P50 ratios are normalized (Nagamoto et al., in press). Manic patients exhibit abnormal P50 gating ratios, but only during their manic phase; the abnormal P50 ratios normalize when the patients are euthymic. The response during mania is probably related to increased adrenergic activity, as the P50 ratio is correlated with levels of the norepinephrine metabolite 3-methoxy,4-hydroxyphenylglycol (Adler et al. 1990). Increased adrenergic response also has been implicated in altered P50 ratios in controls, resulting either from discomfort or from exposure to potentially dangerous situations (Waldo et al. 1992; Johnson and Adler 1993). However, in schizophrenia subjects, the P50 ratio remains abnormal in conditions of discomfort and following administration of conventional neuroleptic medication, suggesting that their P50 ratios are controlled by neurotransmitter systems other than dopamine.

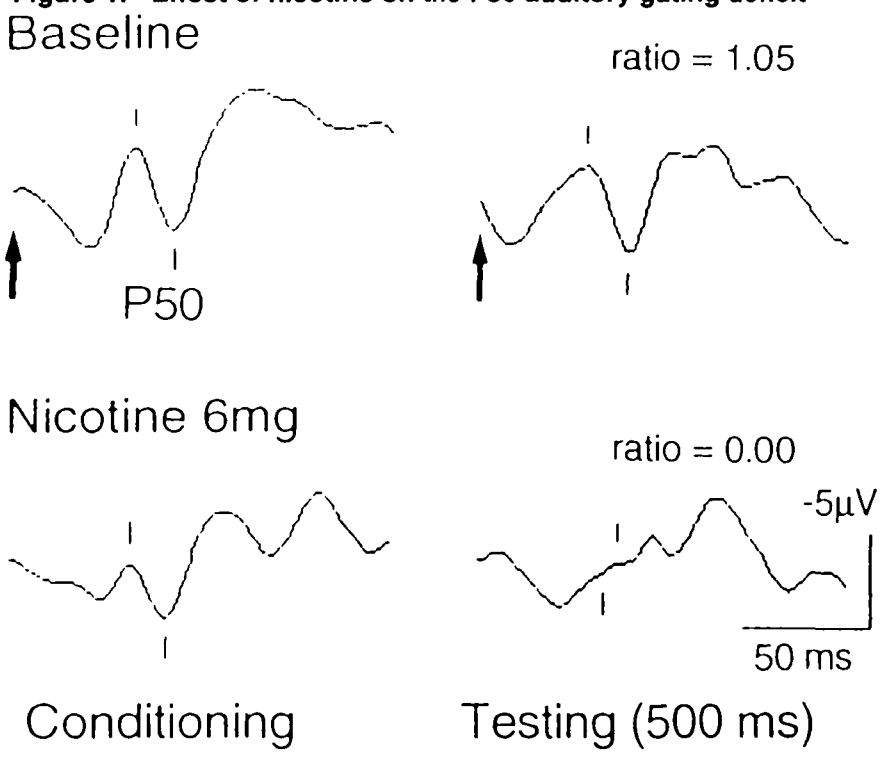
Regulation of the Auditory Gating Deficit by Nicotine. Nicotine is a self-administered drug that has received only limited interest in the field, probably due to the relative failure of cholinergic agonists and acetylcholinesterase inhibitors to significantly ameliorate the symptoms of schizophrenia (Pfeiffer and Jenny 1957; Rosenthal and Bigelow 1973; Berger et al. 1979); muscarinic anticholinergics, however, have exacerbated some symptoms of schizophrenia (Tandon and Greden 1989). The idea that a deficiency in nicotinic cholinergic neurotransmitter systems might underlie sensory deficits in schizophrenia patients and their first-degree relatives has only recently been considered (Hughes et al. 1986; Adler et al. 1992, 1993; Goff et al. 1992; Karson et al. 1993). Yet schizophrenia subjects may have discovered this long ago; it is clinically well recognized that smoking incidence in the mentally ill is much higher than normal and is inordinately increased in the population with schizophrenia (Greeman and McClellan 1991; Menza et al. 1991; Goff et al. 1992). Smoking in schizophrenia subjects can be pathologic; patients may smoke or chew tobacco to the point of nausea and have developed water intoxication from decreased secretion of antidiuretic hormone, a side effect of nicotine (Kirch et al. 1985).

Investigation of smoking effects on the auditory-evoked potential deficit, however, could be complicated by chronic exposure to nicotine. In initial experiments to investigate the effects of nicotine on the P50 deficit, we chose six nonschizophrenia, first-degree relatives of schizophrenia subjects who exhibited the P50 gating deficit but who were nonsmokers and who, because they had no psychiatric illness, were neuroleptic-free. In a double-blind, placebo-controlled crossover study, nicotine was administered in the form of nicotine-containing chewing gum (total of 6

mg). We found that nicotine transiently normalized the P50 deficit in all the subjects (figure 1), suggesting that the nicotinic acetylcholine receptor system might be active in a neuronal pathway regulating the filtering of auditory information (Adler et al. 1992). The P50 gating deficit also is transiently normalized by nicotine in schizophrenia subjects (Adler et al. 1993). Because these subjects were smokers, they were studied before their first cigarette of the day.

Nicotinic acetylcholine receptors are members of the super family of ligand-gated ion channels, including the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/ kainate, γ-amino-butyric acid (GABA), and glycine receptor families (Lukas and Bencherif 1992). The nicotinic receptor family is subdivided into receptors with high and low affinity for nicotine. The high-affinity nicotinic receptors, containing α subunits 2–5, require a β subunit for function; the low-affinity nicotinic receptors do not (Deneris et al. 1991). The low-affinity subunits, including α 7–9, also bind the snake toxin, α-bungarotoxin (α-BTX) (Schoepfer et al. 1990; Elgoyhen et al. 1994). Of these α-BTX-binding subunits, only α7 is found in the mammalian brain (Séguéla et al. 1993).

Figure 1. Effect of nicotine on the P50 auditory gating deficit



P50-evoked potential was recorded following conditioning and test stimuli in a nongating and nonsmoking subject, before and after chewing nicotine-containing gum. Results show the averaged response of 32 presentations of paired auditory stimuli, given 0.5 seconds apart. Arrows indicate when the stimulus was presented. The P50 wave is selected by a computer algorithm and measured relative to the preceding negative trough. P50 ratio is shown above the test response.

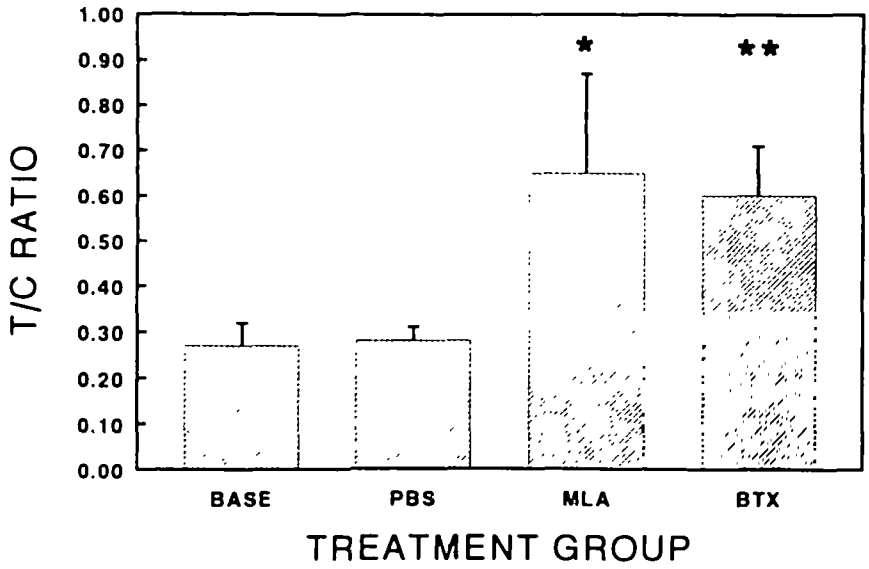
Pharmacology of Auditory Gating in an Animal Model

Characterization of Auditory Gating in the Laboratory Rat. To extend the study of sensory gating mechanisms, we developed an animal model of both the P50 gating paradigm and its deficit. This is not an animal model of schizophrenia, but rather an animal model of a simple sensory phenotype that can be manipulated pharmacologically to identify neuronal mechanisms that are potentially defective in the disease. Using evoked potential recordings from the surface of the skull of unanesthetized rats, we identified a negative wave, occurring 40 ms following the first stimulus (N40), which is diminished following a second stimulus; this corresponds to the gating of the P50 wave in humans (Adler et al. 1986, 1988; Simpson and Knight 1993). A principal source of this gated wave has been localized in the rat to the cornu ammonis (CA)3 and CA4 pyramidal neurons of the hippocampus (Bickford-Wimer et al. 1990). Ablation of this area nearly eliminates the N40 wave (Simpson and Knight 1993). Depth recordings in humans also indicate the hippocampus as a possible source of the P50 (Goff et al. 1980) and suggest that individual neurons in that location decrease their response to repeated sounds (Wilson et al. 1984). Thus, an inhibitory mechanism for regulation of auditory stimuli is extant in both humans and rats and may be localized in the hippocampus.

Of the two major inputs to the hippocampus, only one appears to be involved in the inhibitory function. The perforant path, carrying information from the lemniscal pathway and the entorhinal cortex, perforates or crosses the hippocam-

pal fissure and enters CA3–CA4. This pathway also synapses on granule cells of the dentate gyrus, whose mossy fibers project to the CA3–CA4. Neurons of the entorhinal cortex do not show a decreased response to auditory stimulation, as seen in the hippocampus (Stafekhina and Vinogradova 1975). The second major input to the hippocampus is through the fimbria-fornix, which separates the two lateral ventricles and carries afferent fibers from the septum and brainstem into most areas of the hippocampal formation. Lesion of the fimbria-fornix fibers results in a diminished response to sensory stimuli in neurons in this region of the hippocampus (Vinogradova 1975); administration of nicotine to the fimbria-fornix-lesioned animal normalized auditory gating (Bickford and Wear 1995). Cholinergic neurons of the septal region, in a response similar to that of neurons in the hippocampus, also exhibit gating of auditory stimuli (Miller and Freedman 1993). These results suggest that cholinergic input from the septal area may be critical for the inhibitory response to repeated auditory stimuli.

Figure 2. Effect of intracerebroventricular (ICV) administration of antagonists of the α7 nicotinic receptor on auditory gating in the rat



Skull screws and a cannula were surgically implanted in rats. Following a 2-week recovery, evoked potentials following paired auditory stimuli were recorded to establish a baseline (BASE). Three treatments were administered in the same rats on different days, with return to baseline levels required before a new treatment. Results are shown for test to conditioning (T/C) ratios averaged over the 20- to 40-minute interval following administration of the test substance. Phosphate-buffered saline (PBS) produced no change in the T/C ratio. Methyllycaconitine (MLA), 17 μg/kg ICV, produced a significant (*) increase in T/C ratio ($p < 0.05$, independent t-test, pooled variances) as did α-bungarotoxin (BTX; highly significant [**]) ($p < 0.01$).

Auditory Gating in the Rat Is Disrupted by Antagonists of a Specific Nicotinic Receptor. Cholinergic synapses in the hippocampus, as in most central nervous system synapses, express two classes of receptors—muscarinic and nicotinic. Administration of muscarinic antagonists such as scopolamine had no effect on the filtering of auditory input in the rat. Antagonists of the high-affinity nicotinic receptor subtypes, including mecamylamine, also had no effect. We found, however, that antagonists of the low-affinity nicotinic receptor, α7, specifically affected the capacity of the animal to gate the second auditory stimulus (Luntz-Leybman et al. 1992). Methyllycaconitine, found in delphinium seed, and α-BTX, derived from venom of the banded krait, are both potent blockers of the α7 nicotinic receptor (Chiappinelli 1985; Clarke et al. 1985; Wonnacott 1986). Both compounds also block the inhibitory response to the second tone in the awake behaving rat, inducing a significant increase in the test-to-conditioning ratio, shown in figure 2. In a more specific experiment, we injected antisense oligonucleotides, complementary to the translation start site of the rat α7 messenger ribonucleic acid (mRNA) into the lateral ventricles, over a 3-day period. These antisense oligonucleotides bind specifically to α7 mRNA, preventing translation into protein and also inducing destruction of the mRNA by nucleases. Injection of the α7 antisense oligonucleotides resulted in a 40 percent reduction in the binding of [^{125}I]-α-BTX and a loss in gating of the response to the second of two auditory

stimuli (Rollins et al. 1993).

More recently, nicotine has been found to normalize an amphetamine-induced P50 gating impairment in freely moving rats (Stevens et al., in press), and mice expressing low levels of α-BTX-binding nicotinic receptors have deficits in P50 auditory gating (Stevens et al. 1995).

In both humans and laboratory animals, normalization of gating deficits by nicotine, and introduction of the gating deficit in rats by specific α7 antagonists suggests that this nicotinic receptor may play an important role in the inhibition of extraneous noise in our environment.

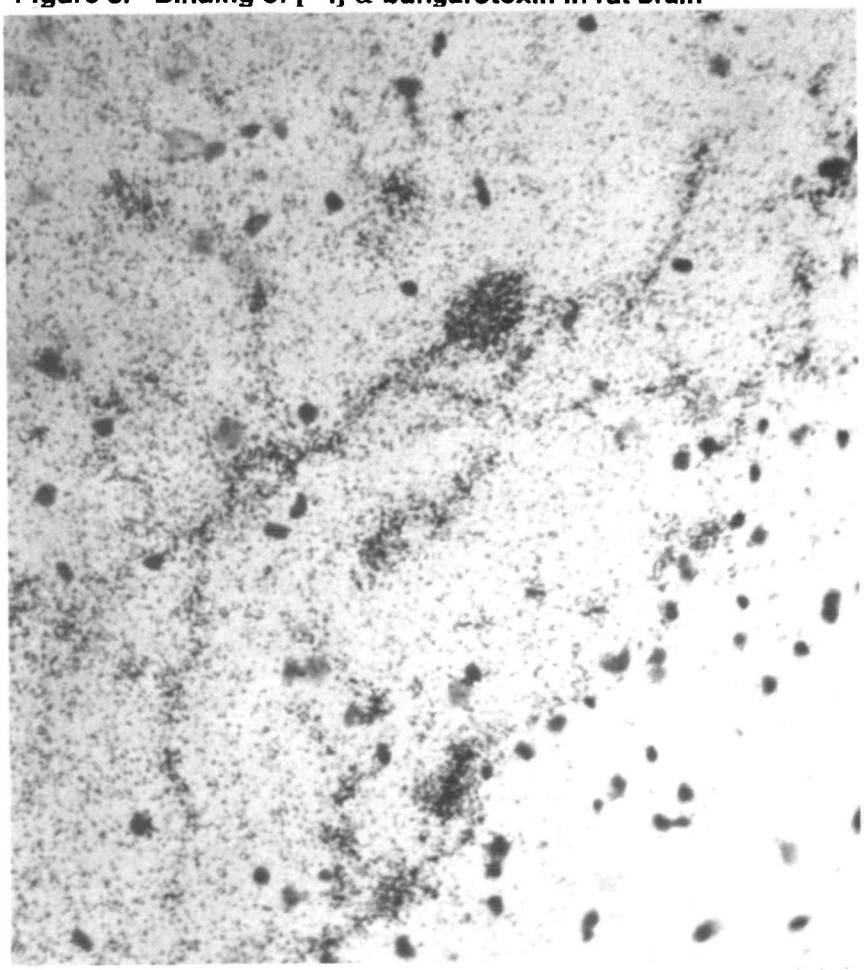
Expression of the α7 Nicotinic Acetylcholine Receptor in Human Brain

Presence of the α7 receptor in hippocampal neurons has been shown by receptor autoradiography, using [^{125}I]-α-BTX, at sites near the dentate gyrus and the CA3–CA4 region (Freedman et al. 1993). In a comparative study of α-BTX binding in the hippocampus of schizophrenia and control subjects, done by quantitative receptor autoradiography on human postmortem brain samples, we found that the level of binding in the schizophrenia samples was significantly decreased in both dentate gyrus and CA3 regions of the hippocampus as compared to matched controls. The mean reduction in expression of the receptor was 40 percent (Freedman et al. 1995).

We have recently completed a regional localization of the receptor protein in both rat and human brain, using [^{125}I]-α-BTX autoradiography. The α7 receptor was found in many areas of the brain, including areas known to be involved in sensory processing, such as hippocampus, medial and lateral geniculate, amygdala, and brainstem. In many regions, binding is present on both the cell bodies and on cellular processes, as shown on a cell located in the hilar region of the rat hippocampus (figure 3). Examples of binding in several regions of human postmortem brain are shown in figure 4. In the hippocampus (figure 4B), a cell with labeled processes extending into both the hilar region and molecular layers is shown. Not all the cells in the hippocampus exhibited labeled processes. In the diagonal band of Broca, near the septal nucleus (figure 4D), binding is also seen only on the cell bodies. Binding on cellular processes of these neurons cannot be ruled out, but it might be expressed at some distance from the cell; none were observed in the plane of the tissue section examined.

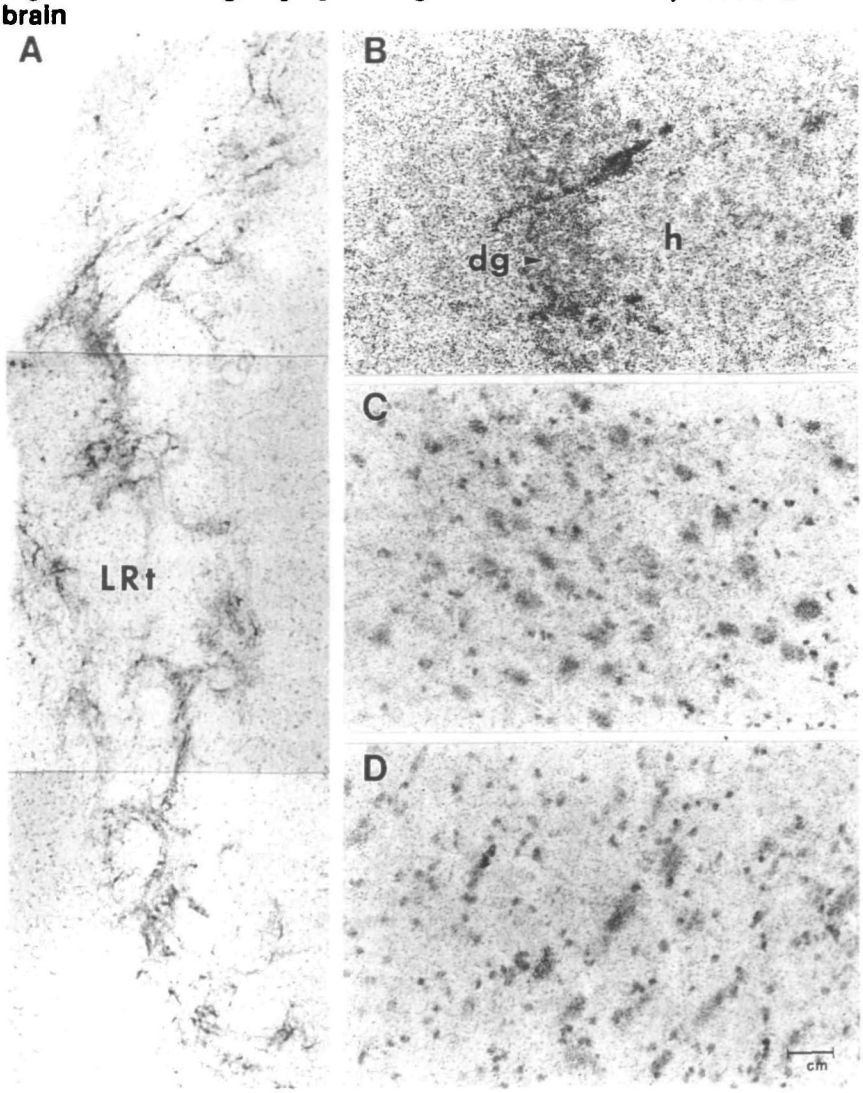
Of particular interest was the pattern of [^{125}I]-α-BTX binding in the

Figure 3. Binding of [^{125}I]-α-bungarotoxin in rat brain



In rat brain, receptor autoradiography using [^{125}I]-α-bungarotoxin shows binding on large nonprincipal cells and processes lying in and near the dentate gyrus. Section was counterstained with cresyl violet. The large labeled neuron with processes is 40 μm in size.

Figure 4. Binding of [^{125}I]-α-bungarotoxin in human postmortem brain



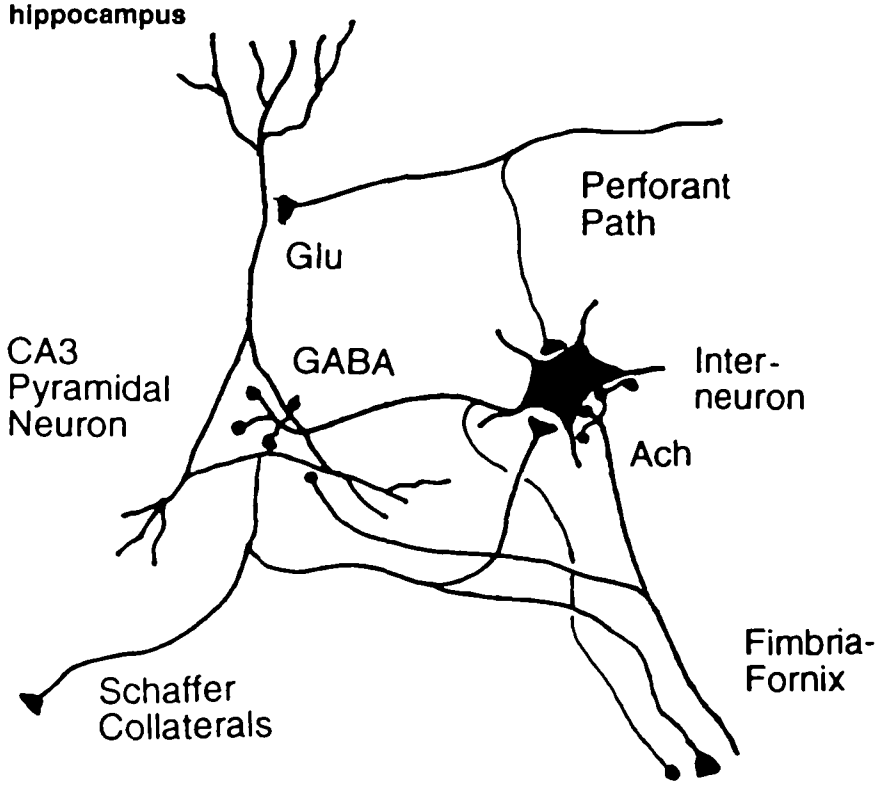
(A) Reticular thalamic nucleus showing binding to both cell bodies and a network of processes. LRt = lateral reticular thalamus. (B) A large cell, similar to those seen in rat hippocampus, positioned just outside the dentate gyrus with processes extending into the hilar region and molecular layers. dg = dentate gyrus; h = hilus. (C) Lateral geniculate showing [^{125}I]-α-bungarotoxin binding on cell bodies only. (D) Diagonal band of Broca with labeling on elongate cell bodies. Scale bar: (A) 200 μm; (B,C,D) 75 μm.

reticular thalamic nucleus (figure 4A). Neuronal cell bodies are heavily labeled by the ligand, as are processes. The latter form dense and intertwined bundles of fibers that run throughout this nucleus. The reticular thalamic nucleus (RTN) is thought to be a pacemaker in corticothalamic and thalamocortical connections (Crick 1984), since it provides the principal inhibitory input into the dorsal thalamus (Mitrofanis and Guillery 1993). Anatomically, RTN is a capsular structure that surrounds the dorsal and ventral thalamus. Neurons of the RTN are known to be uniformly GABAergic (Lozsádi 1994), and nerve-growth-factor receptor is highly expressed in this nucleus (Chen and Bentivoglio 1993), as it is in the hippocampus. The heavy concentration of α7 receptor expression in the RTN and its presence in GABAergic interneurons of the hippocampus (Freedman et al. 1993) suggest that α7 may also be localized on inhibitory neurons in this thalamic nucleus and might participate in the processing or filtering of sensory information in thalamocortical circuits as well as in the hippocampus.

A Neuronal Pathway for Processing of Auditory Information in the Brain

The animal model of auditory gating has been useful for elucidating a possible neuronal pathway in the brain for the processing of auditory input. A model pathway, based on our experimental evidence and on that of others, illustrates how such a mechanism might function in the hippocampus (figure 5). The major input for excitatory information comes from the perforant path to the dentate gyrus and CA3 pyramidal neurons. These neurons relay information through the Schaffer collaterals to CA1. The output from CA1 is mainly to the frontal lobe via the subiculum. A mechanism for regulation of the information received by the CA1 might be the inhibitory neurons of the CA3–CA4 regions. These neurons bear modulatory α7 nicotinic receptors, but they are activated primarily by glutamitergic synapses from the mossy fibers of the dentate gyrus and from the perforant path. Stimulation by auditory input would

Figure 5. Neuronal model for gating of auditory stimuli in the hippocampus



Auditory information reaches the hippocampus through the perforant path. Mossy fibers synapse on CA3 pyramidal cells and on CA3 interneurons as well. The interneuron inhibits the pyramidal cell by GABAergic and other inhibitory synapses. Additionally, the pyramidal cell and the interneuron are stimulated by cholinergic synapses coming from the septum through the fornix. Blockade of this septal cholinergic input removes the effect of the interneuron and permits the pyramidal cell to fire in response to the second stimulus. In this model, cholinergic stimulation is necessary for inhibition. Ach = acetylcholine; Glu = glutamate; GABA = γ-amino-butyric acid.

cause the interneuron to release GABA and other inhibitory neurotransmitters onto the pyramidal neuron, resulting in cell discharge (Miller and Freedman 1995).

There are two types of GABA receptors on pyramidal cells of the hippocampus: $GABA_A$ and $GABA_B$. The combined effect is an inhibition capable of lasting 300 ms (Dutar and Nicoll 1988). Additionally, $GABA_B$ and other G_i protein-linked receptors are found presynaptically (Manzone et al. 1994) and can depress glutamate release. Although the inhibitory neurotransmitters released by the interneuron mainly contact the cell body receptors of the CA3 neurons, diffusion can occur through the synaptic cleft to activate presynaptic receptors (Isaacson et al. 1993). This would result in inhibition of glutamate release from perforant path and mossy fiber terminals. Indeed, $GABA_B$ antagonists block auditory gating in the rat (Hershman et al. 1995). Because interneurons are activated for longer than 250 ms after the first tone is delivered, the combination of these events could result in the long-term inhibition seen when the test auditory stimulus is given 500 ms after the conditioning stimulus.

Input onto the interneuron from the perforant path and input from the pyramidal neurons probably are not sufficient to cause the interneurons to discharge the necessary burst of action potentials. The interneuron, however, also receives cholinergic input from the medial septal nucleus. Our data, showing that antagonists of the α7 neuronal nicotinic receptor block inhibition of the evoked response to paired auditory stimuli, suggest that this receptor may play an important role in the filtering of auditory information passing through the hippocampus. A defect in the expression or function of the α7 receptor could thus result in a phenotype of failed inhibitory responses to sensory input, which partially characterizes schizophrenia.

Cholinergic modulation of inhibitory responses may also function elsewhere in the brain. Acetylcholine modulates an inhibitory response to auditory stimuli in the dentate gyrus (Foster and Deadwyler 1992), and fibers extending from the CA3 pyramidal neurons back through the fornix excite the lateral septal nucleus. The lateral septum has a weak inhibitory input into the medial septal nucleus, and inhibitory fibers also project from the CA3 interneurons to the medial septum (Alonso and Kohler 1982). The principal input to the medial septal nucleus, however, comes from the brainstem reticular formation, which also exhibits a decreased response to the second of paired stimuli (Bickford et al. 1993). Reticular neurons express nicotinic receptors (Stevens et al. 1993) and are labeled by [^{125}I]-α-BTX, suggesting that they may also express the α7 receptor. Auditory information passes through the nucleus of the lat-

eral lemniscus into a group of neurons of the reticular formation that express auditory-induced startle, and it has been proposed that the hippocampus is responsible for the prepulse inhibition of this startle response (Swerdlow et al. 1994). Several of the inhibitory phenotypes measured in schizophrenia subjects may thus have common functional elements in their neuronal circuitry.

Processing of Auditory Information in the Human Brain

Although the animal model of auditory gating has been helpful in the pharmacological analysis of this phenotype, the neuronal pathway in the human brain is likely to be more complex because of increased development in the forebrain areas. The superior temporal gyrus is a major source of auditory input to the human limbic system; a dipole for the P50 response has been localized to this gyrus by magnetoencephalography (Reite et al. 1988). The superior temporal gyrus receives a cholinergic innervation from the nucleus basalis, which regulates an auditory response in the neocortex (Metherate and Ashe 1993), probably by a mechanism similar to the one we have found in the hippocampus. Abnormal P300 responses to auditory-evoked potentials have been recorded from surface electrodes over the superior temporal gyrus in schizophrenia subjects (McCarley et al. 1993), although it is not known whether this aberrancy is biochemical or is related to structural changes in the superior temporal gyrus.

In addition to the cholinergic neurotransmitter system, other inputs to inhibitory neurons are likely to be important in proper function. Noradrenergic and serotonergic inputs from the brainstem are known to decrease interneuron activity (Madison and Nicoll 1988; Freund 1992). This effect could be aberrant in mania, where remission of symptoms and normal P50 gating is seen when norepinephrine metabolite levels return to baseline levels (Adler et al. 1990). Clozapine, a serotonergic antagonist, may be effective in some schizophrenia subjects by blocking serotonin's normal inhibitory input to the interneurons (Schotte et al. 1993). This would have the effect of increasing the interneurons' capacity to gate sensory response and would account for the normalization of the P50 deficit seen in schizophrenia patients on clozapine therapy (Nagamoto et al., in press). Typical neuroleptics do not normalize auditory gating in schizophrenia patients, suggesting that these drugs may not have a direct effect on inhibitory responses in the hippocampus but instead may decrease the synaptic responses in the forebrain nuclei to sensory activity coming through the perforant pathway, resulting in a general decrease in excitability of the hippocampal region (Johnson et al. 1983).

Other neurotransmitter systems could interact with cholinergic innervation to enhance or inhibit nicotine-induced responses. Nicotinic receptors are found on many catecholaminergic neurons, and stimulation of these high-affinity receptors by nicotine is known to induce release of norepinephrine. These receptors are blocked by mecamylamine in the human, and the normalizing effect of nicotine on the abnormal P50 is enhanced by mecamylamine, perhaps because the decrease in interneuronal activity caused by norepinephrine has been removed.

The P50 Deficit as a Risk Factor for Schizophrenia

The P50 Deficit Is Inherited. The P50 deficit is one of several measurable phenotypes, including decreased P300 amplitude (Blackwood et al. 1991) and aberrant smooth-pursuit eye movement (Holzman et al. 1988), that are found in approximately 50 percent of the first-degree relatives of schizophrenia subjects, who do not have the disease. Usually, the deficit is observed in one parent and in half of the siblings, describing an autosomal dominant type of inheritance. Nine families with multiple-generational schizophrenia were studied for codistribution of schizophrenia and the P50 gating deficit. A total of 120 subjects were recorded, of whom 34 were diagnosed with schizophrenia. Subjects with P50 quotients below 0.40 were classified as normal, those with T/C ratios of 0.50 or more were classified as abnormal, and those with ratios in between were regarded as unknown. Based on these criteria, 32 of the 34 schizophrenia subjects had abnormal ratios and 2 were unknown. Schizophrenia was always transmitted through a parent with an abnormal P50 ratio, suggesting the phenotype came from only one side of the family. A mathematical model fitted to the pedigrees supports the hypothesis that presence of an abnormal P50 is directly related to the inheritance of risk for schizophrenia (Waldo et al. 1991).

A Secondary Factor Is Required for Expression of the Schizophrenia Syndrome. Presence of the gating deficit in nonschizophrenia relatives that have an abnormal P50 suggests that this deficit is not sufficient for development of the schizophrenia disease state. A study of siblings dis-

cordant for the P50 deficit and schizophrenia was undertaken. Magnetic resonance imaging was used to determine the hippocampal volume in three groups: siblings with schizophrenia and abnormal P50 ratios, nonschizophrenia siblings with abnormal P50 ratios, and normal siblings with normal ratios. We found that a reduction in volume of the hippocampus was related to the development of schizophrenia in siblings with abnormal P50 ratios (Waldo et al. 1994). Although there was variability in hippocampal size in normal subjects, the nonschizophrenia siblings with abnormal P50s had normal to large hippocampi; the schizophrenia subjects had both abnormal P50s and significantly smaller hippocampal volumes. Expressed alone, neither of these deficits was sufficient for disease development, as many of the siblings with normal P50 ratios had reduced hippocampal volumes. Interestingly, although the nonschizophrenia siblings with abnormal P50 gating deficits had no history of chronic psychiatric disease, the siblings with normal P50 ratios often were diagnosed with disorders including alcoholism and minor depression, suggesting that a second factor may be involved (Waldo et al. 1994).

Hippocampal pathology is well documented in schizophrenia (Jeste and Lohr 1989; Suddath et al. 1990). However, it is not clear whether its cause is genetic, developmental, or environmental. Correct temporal expression of growth factors and neurotransmitter systems both have been shown to be necessary for competent maturation of neuronal circuits in the central nervous system (Snider and Johnson 1989; Knusel et al. 1990; McDonald and Johnstone 1990; Crowley et al. 1994). The investigation of interactive biochemical systems such as the P50 deficit in established endophenotypes found in schizophrenia thus represents a logical approach to a disease of remarkable complexity.

Possible Linkage of the P50 Deficit to Specific Chromosomal Regions

Since the P50 deficit appeared to be inherited in the nine pedigrees examined, these families were used in a preliminary linkage study with more than 300 mapped markers, covering the entire genome. Linkage analysis represents an independent approach for analysis of a disease or phenotype because it makes no assumption about the identification of the genes involved. Linkage studies in schizophrenia, however, are difficult because of the low rates of reproduction in many patients and the possibility that multiple genes may be involved. As an exploratory approach, a 95-percent confidence level for lod (log to the base 10 of the odds ratio) scores was determined for markers that were not linked to the P50 or schizophrenia. The results showed positive lod scores ($p < 0.05$) for the P50 deficit, which were also nonnegative for schizophrenia at four sites in the genome (Coon et al. 1993). One of these regions, 15q14, on the long arm of chromosome 15, has subsequently been shown to be the locus of the α7 nicotinic receptor gene (Chini et al. 1994).

Although the study was preliminary and only suggests further exploration of a rather large region of chromosome 15, it is important that three independent lines of investigation—gene expression, pharmacological studies, and genetic-linkage analysis—have all suggested involvement of the α7 nicotinic receptor in gating of sensory stimuli and schizophrenia. Work is in progress to determine whether abnormalities in the α7 gene itself are linked to the P50 deficit and schizophrenia.

Two other disorders with psychotic elements are caused by genetic defects with loci near 15q14. Prader-Willi syndrome, usually the result of a deletion involving 15q11–14, has a high association with schizophrenia (Clarke 1993). Marfan's syndrome, linked to 15q15, also has been observed to cosegregate with schizophrenia (Sirota et al. 1990). It is possible that genes localized in the interim region between 15q11 and 15q22 are associated with schizophrenia and, in some families, are inherited with these disease alleles.

Conclusion

Concentrated study of endophenotypes in diseases of complex origin provides several advantages. Unlike the disease state, which is difficult to quantitate, endophenotypes such as the P50 deficit and aberrant smooth-pursuit eye movement can be examined in humans and limited pharmacological research can be accomplished. This information can then be used for linkage analysis, since a single endophenotype is more likely to have a simple, Mendelian form of inheritance. Additionally, several of the traits associated with schizophrenia can be examined in laboratory animals where specific neuroanatomical areas, such as the hippocampus, can be studied using pharmacological and electrophysiological methods. We have characterized one such trait—the auditory-evoked potential deficit—found in nearly all schizophrenia subjects and about half of their clinically unaffected first-degree relatives. The deficit is inherited, pos-

sibly as a necessary but not sufficient condition for schizophrenia, and thus represents a predisposition factor. Interaction with other biochemical and/or developmental defects or environmental factors may be necessary for full presentation of the illness. Although several neurotransmitter systems probably function in the neuronal pathway regulating gating of the P50, and have been demonstrated with state-dependent changes in P50 gating, we have examined the role of the neuronal nicotinic acetylcholine receptor family in this pathway.

Biological and pharmacological evidence suggests that a subtype of this receptor family—the α7 nicotinic receptor—is specifically involved in trait-related P50 gating deficits. Levels of α7 expression are decreased in schizophrenia subjects, and the genomic locus for this gene lies in a region of chromosome 15, identified in a genetic-linkage study of P50 as a region of interest. The α7 receptor is expressed in many sensory-filtering nuclei in the brain, including the hippocampus and RTN. The synaptic characteristics of auditory gating, which we have measured in the hippocampus, may also be extant in these other nuclei. It will be important to determine the expression and function of the receptor, wherever it is found, to understand possible pathogenic effects of dysfunction. Although evidence for involvement of the α7 receptor as a candidate gene is promising, interaction with other known pathologies in the disease must be investigated before an underlying model for the pathogenesis of schizophrenia can be determined.

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