A neuronal nicotinic acetylcholine receptor subunit (α7) is developmentally regulated and forms a homo-oligomeric channel blocked by α-BTX

Couturier-Goebel, Sabine; Bertrand, Daniel; Matter, Jean-Marc; Hernandez, Maria Clemencia; Bertrand, Sonia; Millar, Neil; Valera, Soledad; Barkas, Thomas; Ballivet, Marc

How to cite

COUTURIER-GOEBEL, Sabine et al. A neuronal nicotinic acetylcholine receptor subunit (α7) is developmentally regulated and forms a homo-oligomeric channel blocked by α-BTX. In: Neuron, 1990, vol. 5, n° 6, p. 847–856. doi: 10.1016/0896-6273(90)90344-F

This publication URL: https://archive-ouverte.unige.ch/unige:104931
Publication DOI: 10.1016/0896-6273(90)90344-F

Neuron, Vol. 5, 847–856, December, 1990, Copyright © 1990 by Cell Press

A Neuronal Nicotinic Acetylcholine Receptor Subunit (α7) Is Developmentally Regulated and Forms a Homo-Oligomeric Channel Blocked by α-BTX

Sabine Couturier,* Daniel Bertrand,† Jean-Marc Matter,* Maria-Clemencia Hernandez,* Sonia Bertrand,† Neil Millar,‡ Soledad Valera,* Thomas Barkas,‡ and Marc Ballivet*
*Department of Biochemistry
†Department of Physiology (Medical School)
University of Geneva
1211 Geneva 4
Switzerland
‡Department of Biochemistry and Genetics
University of Newcastle
Newcastle upon Tyne
England

Summary

cDNA and genomic clones encoding α7, a novel neuronal nicotinic acetylcholine receptor (nAChR) α subunit, were isolated and sequenced. The mature α7 protein (479 residues) has moderate homology with all other α and non-α nAChR subunits and probably assumes the same transmembrane topology. α7 transcripts transiently accumulate in the developing optic tectum between E5 and E16. They are present in both the deep and the superficial layers of E12 tectum. In Xenopus oocytes, the α7 protein assembles into a homo-oligomeric channel responding to acetylcholine and nicotine. The α7 channel desensitizes very rapidly, rectifies strongly above −20 mV, and is blocked by α-bungarotoxin. A bacterial fusion protein encompassing residues 124–239 of α7 binds labeled α-bungarotoxin. We conclude that α-bungarotoxin binding proteins in the vertebrate nervous system can function as nAChRs.

Introduction

The snake venom protein α-bungarotoxin (α-BTX) is a potent competitive blocker of the nicotinic acetylcholine receptor (nAChR) at the neuromuscular junction of most vertebrates, and it has played an essential role in the characterization of this neurotransmitter-gated ion channel (Raftery et al., 1980). There is a great deal of controversy, however, as to the nature and function of its widespread and lower affinity binding sites in the central and peripheral vertebrate nervous systems (reviewed in Lindstrom et al., 1987; Schmidt, 1988).

Recent work in our laboratory has led to the molecular cloning of 8 different genes and cDNAs encoding neuronal nAChR subunits from the chicken (α2–α6 and nα1–nα3; Nef et al., 1988; Couturier et al., 1990; Hernandez et al., unpublished data). Most of the corresponding rat subunits (α2–α5 and β2–β4) have also been isolated (Boulter et al., 1986, 1990; Goldman et al., 1987; Wada et al., 1988; Deneris et al., 1988, 1989; Duvoisin et al., 1989). Reconstitution studies in the Xenopus oocyte system have shown that α2, α3, and α4 can each lead to assembly of a functional nAChR in concert with either nα1 (β2) or nα3 (β4). These various receptor subtypes have different electrophysiological and pharmacological properties (Wada et al., 1988; Papke et al., 1989; Couturier et al., 1990), but all of them are totally insensitive to concentrations of α-BTX that completely and irreversibly block the classic nAChR at the neuromuscular junction.

In this paper, we describe the molecular cloning of the chicken neuronal nAChR protein α7. We show that the α7 protein has rather weak but definite homology with all other muscle and neuronal nAChR subunits and that the structure of its gene differs markedly from those of all known genes in the nAChR family.

We find that α7 mRNA accumulates transiently in the developing optic tectum at the time when tectal neurons form their connections with the retina and other regions of the brain. In situ hybridization reveals the presence of α7 transcripts in all layers of the developing tectum, including those of the deep layers, whose neurons make no connections with retinal axons.

Using an improved Xenopus oocyte system (Ballivet et al., 1988; Bertrand et al., 1990a, 1990b), we show that α7 alone, in the absence of any coinjected non-α subunit cDNA, leads to the assembly of a functional neuronal nAChR with unusual properties: it is more sensitive and responds with greater currents to nicotine than to ACh, it desensitizes very rapidly, and the voltage dependence of its ACh currents is pronounced at elevated membrane potentials. In addition, the α7 channel is totally blocked by low concentrations of α-BTX, a most interesting finding reinforced by the demonstration that the extracellular domain of α7 expressed as a bacterial fusion protein binds labeled α-BTX.

We conclude that α7 is a developmentally regulated protein capable of forming a functional homo-oligomeric nAChR in oocytes. Since it binds to and is inactivated by α-BTX, we argue that at least some and perhaps all of the abundant α-BTX binding activity of the vertebrate nervous system (Wang and Schmidt, 1976; Wang et al., 1978; Conti-Tronconi et al., 1985) consists of hitherto unrecognized ACh- and nicotine-gated ion channels.

Results

Molecular Cloning of the α7 cDNA and Gene

Independent recombinant clones (10^6) of an adult chicken brain cDNA library in the vector λgt10 were screened under conditions of low hybridization stringency with a ^{32}P-labeled probe of 333 bp encoding residues 85–196 of the chicken α3 subunit. Several faint positives were purified, and their insert cDNAs were analyzed by restriction mapping and DNA sequencing. Of these, phage 45 was found to contain a

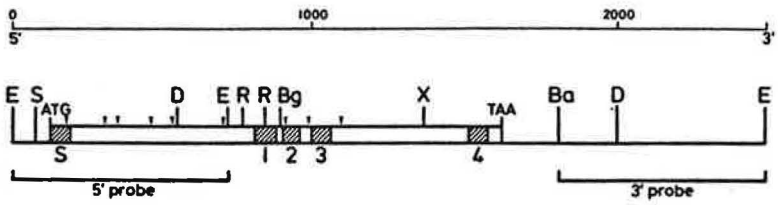

Figure 1. Structure of the Chicken α7 cDNA and Gene

After the α7 cDNA had been isolated and sequenced, the indicated 5′ and 3′ restriction fragments were radioactively labeled and used to probe a chicken genomic library. A set of five overlapping genomic clones was isolated, and the splice junctions (arrowheads) were determined after subcloning into plasmid vectors (exons 2 and 5–10) or by direct sequencing on phage DNA. Hatched segments represent signal peptide and transmembrane regions TM1–4. Restriction sites: E, EcoRI; S, SacII, D, DraI; R, EcoRV; Bg, BglII; X, XmnI; Ba, BamHI.

cDNA insert of 2500 bp (Figure 1) whose open reading frame encodes the nAChR-related mature α7 protein (479 residues) and its signal peptide (23 residues).

To isolate the α7 gene and its promoter sequences, 5′ coding and 3′ untranslated probes were prepared by radioactive labeling of the appropriate α7 cDNA restriction fragments (Figure 1). These were used under conditions of high hybridization stringency to screen 10^6 plaque-forming units of an amplified chicken genomic library constructed in the vector λL47. A set of five different but overlapping genomic inserts was obtained; two of them hybridized only with the 5′ probe and three hybridized with both probes. Appropriate restriction fragments were subcloned and sequenced from their ends or with a set of seven ad hoc oligonucleotide primers to determine intron locations and splice sites. In some instances exon boundary sequences were obtained directly from purified recombinant phage DNA. In the course of this work, most of the α7 open reading frame was resequenced: there were no differences between gene and cDNA.

The α7 gene is split into ten exons (Figure 1; Figure 2a), of which the first four exactly match the corresponding exons in all known nAChR subunit genes from vertebrates. To our surprise, however, we found that none of the remaining six exons in the α7 gene

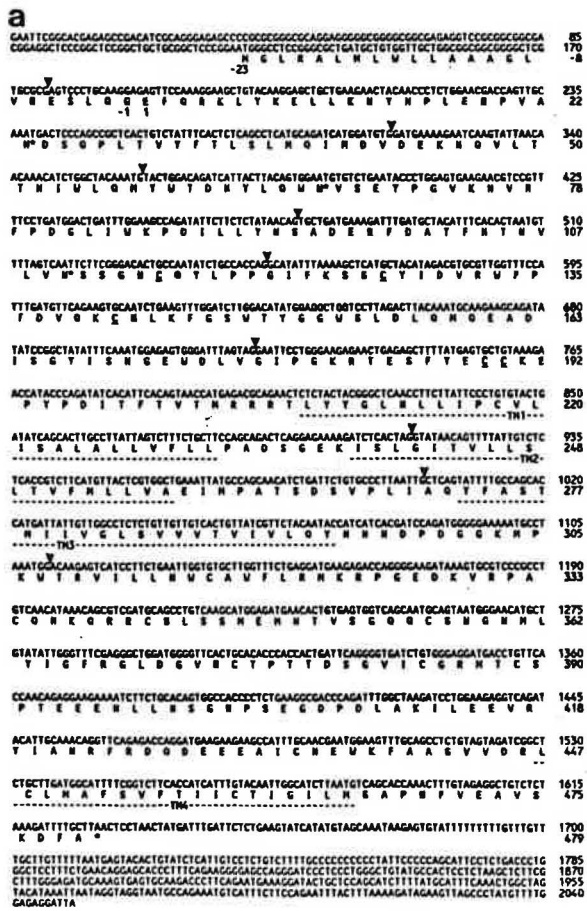

Figure 2. Primary Structure of the α7 Protein

(a) Nucleotide and deduced protein sequences of the chicken α7 cDNA. The open reading frame in α7 encodes a mature protein of 479 residues with a calculated, nonglycosylated molecular mass of 54500 daltons. There are three potential N-linked glycosylation sites in the extracellular domain of the protein (asterisks). Potential phosphorylation sites in the cytoplasmic domain are found at positions 342, 344, 390, 392, 404, 419, and 442. Arrowheads indicate splice sites; transmembrane regions TM1–4 and 5 cysteines in the extracellular domain are underlined.

(b) Alignment of the mature avian α7 and α1 proteins. Of the aligned residues, 38% are identical (vertical bars) and an additional 16% have similar chemical properties. Homology is highest in the extracellular and transmembrane TM1–3 domains. There is nearly perfect identity between the N-terminal sequence of α7 and the N-terminal sequence XEFETKLYKELL-KNYNPLEXPVAXD of an α-BTX binding protein purified from chick optic tectum (Conti-Tronconi et al., 1985).

Percent identity between α7 and other neuronal and muscle nAChR subunits (Nef et al., 1988; Bossy et al., 1988; Couturier et al., 1990) is as follows:

	α1	α2	α3	α4	α5	α6	nα1	nα2	nα3	γ	ALS
α7	38.4	39.7	38.8	41.3	38.4	38.2	35.1	34.8	38.1	32.2	39.7

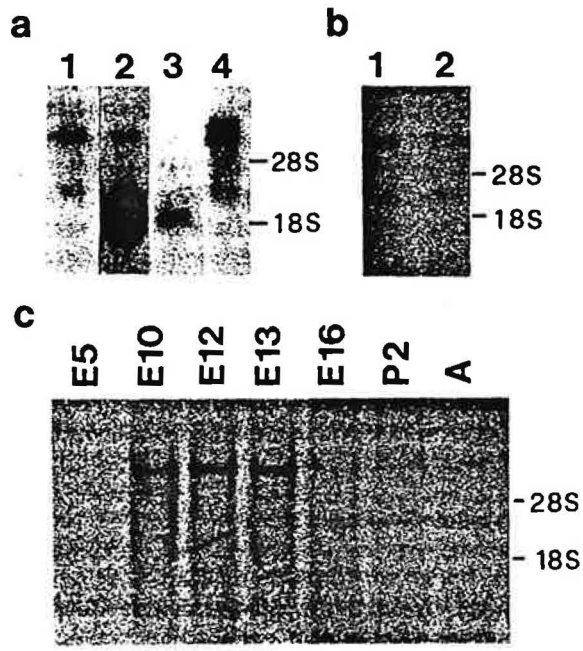

Figure 3. Northern Blot Analysis of α7 Transcripts in the Chick Optic Tectum

(a) poly(A)$^+$ RNA (500 ng per lane) isolated from optic tectum on E12 was fractionated by gel electrophoresis, blotted, and hybridized with different ^{32}P-labeled probes: lane 1, 5′ α7 cDNA; lane 2, 5′ α7 and nα1 cDNAs; lane 3, 5′ nα1 cDNA; lane 4, 3′ α7 cDNA. Exposure times for lanes 1–4 were 18, 10, 3, and 48 hr, respectively.

(b) Total RNA (2 μg per lane) isolated from the optic tectum of "eyeless" (lane 1) and normal (lane 2) embryos at E10 was hybridized with ^{32}P-labeled 5′ α7 cDNA and autoradiographed for 7 days.

(c) Total RNA (5 μg per lane) isolated from the optic tectum at five embryonic stages (E5, E10, E12, E13, and E16) and at two stages after hatching (P2 and adulthood) was hybridized with the 5′ α7 probe and autoradiographed for 3 days. After autoradiography, membranes were stained with methylene blue and analyzed by scanning densitometry to check that rRNA loads were similar in all lanes.

match any of the exons in muscle (Noda et al., 1983; Nef et al., 1984; Buonanno et al., 1989) or in neuronal nAChR genes (Nef et al., 1988), whereas all exon–intron boundaries in α2–α5 and nα1–nα3 are exactly conserved.

The α7 Protein

Although its overall identity score with other nAChR subunits from chicken or with the Drosophila ALS protein is rather low (Figure 2), the α7 protein has all the features expected of a nAChR subunit: it has a typical signal peptide, which we suppose is cleaved as shown (Figure 2a) to yield a mature protein of 479 residues containing four hydrophobic stretches of sufficient length to span the membrane as α-helices. Three of these are closely spaced in the middle of the sequence, and the fourth is near the C-terminus. This structure is a hallmark of all known ligand-gated ion channel subunits and suggests a common transmembrane topology for all of them (Grenningloh et al., 1987; Schofield et al., 1987; Hollmann et al., 1989).

The putative extracellular domain of the α7 protein (residues 1–206) has three potential N-linked glycosylation sites (at N23, N67, and N110), the first and second of which are conserved in many neuronal nAChR subunits. It also has a cysteine residue at position 115 in addition to the 4 conserved cysteines (at positions 128, 142, 192, and 193, α1 subunit numbering) that operationally define all previously isolated nAChR α subunits. Transmembrane regions TM1, TM2, and TM3 of α7 are the most highly conserved, as expected of a nAChR subunit in view of the critical role this region plays in channel formation and ion permeation (Giraudat et al., 1986; Imoto et al., 1988). The cytoplasmic domain (residues 295–446) and TM4 are poorly conserved, except for patches of high homology distal to TM4. The cytoplasmic domain contains several potential sites for serine, threonine, and tyrosine phosphorylation (Figure 2a), whose significance is currently unknown.

α7 mRNA in the Developing Optic Tectum

Total and polyadenylated RNA was isolated from the optic tectum and retina of chick embryos at intervals between E5 and hatching and at two stages after hatching (P2 and adulthood). Poly(A)$^+$ RNA was also isolated from the adult telencephalon and cerebellum. RNA was fractionated by gel electrophoresis, transferred to nylon membranes, and hybridized with ^{32}P-labeled probes from the α7 cDNA.

Very low levels of α7 mRNA were detected in the telencephalon, cerebellum, and retina (data not shown). In contrast, we observed relatively high levels of α7 mRNA during embryonic development of the optic tectum. Two α7 mRNAs of about 7 and 3 kb were detected in the poly(A)$^+$ RNA fraction of E12 tectum (Figure 3a). The largest of these was by far the most abundant and hybridized with probes from both the 5′ and the 3′ ends of α7 cDNA; the smaller transcript was sparse and could be detected only with the 5′ probe (Figure 3a). The 7 kb α7 mRNA was hardly detectable at E5, but its level increased sharply over the next several days to reach a maximum sometime around E12, when retinal axons are invading the tectum. α7 mRNA levels decreased abruptly between E12 and E16 and then slightly between E16 and adulthood (Figure 3c). In a previous study (Matter et al., 1990), we have shown that a marked increase in nα1 mRNA levels takes place at the same stages of development (Figure 3a, lane 2) and can be prevented by bilateral eye removal of E2. In contrast, we found that the levels of α7 mRNA remained quite similar in the tectum of "eyeless" and normal embryos (Figure 3b). Thus, the developmentally regulated modulation of α7 mRNA levels appears not to be dependent upon innervation of the tectum by retinal axons.

Topographic Distribution of α7 Transcripts in the Optic Tectum

Tectum sections prepared from E12 embryos were hy-

Table 1. Localization of α7 and nα1 nAChR Transcripts in the Optic Tectum at E12

Layers	nAChR Transcripts α7	nα1
XI–V (SGFS)	12 ± 4 (4)	92 ± 26 (4)
IV (SGC)	13 ± 7 (4)	4 ± 1 (4)
III (SAC)	6 ± 3 (4)	5 ± 2 (4)
II–I (SGP, SFP)	2 ± 1 (4)	0 (4)
NE	0 (4)	0 (4)
XI–I	32 ± 12 (4)	106 ± 25 (4)
XI–I	[0 (7)]	[0 (6)]

Tissue sections through E12 optic tectum were hybridized with ^{35}S-labeled antisense α7 and nα1 probes, and in situ hybridizations were quantified by counting radioactively labeled cells in sectors of about 1.5 mm^2. Values represent the mean ± standard deviation; the number of sections analyzed is indicated in parentheses. Values in square brackets represent the number of labeled cells in adjacent sections hybridized with the sense probes. Designation of the layers of the developing optic tectum conforms to that described by LaVail and Cowan (1971). SGFS, stratum griseum et fibrosum superficiale; SGC, stratum griseum centrale; SAC, stratum album centrale; SGP, stratum griseum periventriculare; SFP, stratum fibrosum periventriculare; NE, neuroepithelium.

bridized with an ^{35}S-labeled antisense probe from the 3′ untranslated end of α7 cDNA. Although labeled cells were observed throughout the different layers of the optic tectum (Table 1), about 41% of them were localized in layer IV, the future stratum griseum centrale. In this layer, α7 transcripts were mainly detected in large, horizontally disposed cells (Figures 4c and 4d). In the future stratum griseum et fibrosum superficiale, labeled cells were detected in the densely packed cell layers V and VI. Layer III, the future stratum album centrale, is the broadest plexiform layer of the tectum and only a few neurons are scattered between the large fascicles; we found that some of these neurons were strongly labeled. Nonetheless, in each of these layers the proportion of labeled cells was very small, suggesting that α7 expression is stimulated in individual cells only for brief periods. When adjacent sections were hybridized with ^{35}S-labeled antisense nα1 probe, about 87% of the labeled cells were localized in the superficial layers (V–VII) of the optic tectum (Figure 4a; Table 1; see also Matter et al., 1990). No labeled cells were found in tissue sections hybridized with α7 or nα1 sense probes (Table 1).

Electrophysiology of the α7 Channel

Two days after nuclear injection of 2 ng of expression plasmid flip α7 (or its derivative flip α7Δ; see Experimental Procedures), voltage-clamped Xenopus oocytes responded to ACh and nicotine, in the presence of atropine, with inward currents of up to several microamperes (of 89 cells clamped at −100 mV, 61 responded to 100 μM ACh with currents in the range 0.1–4.0 μA). In addition, when a cell was challenged with increasing concentrations of ACh and nicotine (Figure 5a), the sensitivity and amplitude of the responses were always greater with nicotine than with ACh. As illustrated in Figure 5b, the ACh sensitivity of α7 channels was much lower (EC_{50} = 115 μM, n = 1.4) than that of the α4/nα1 neuronal channel (EC_{50} = 0.77

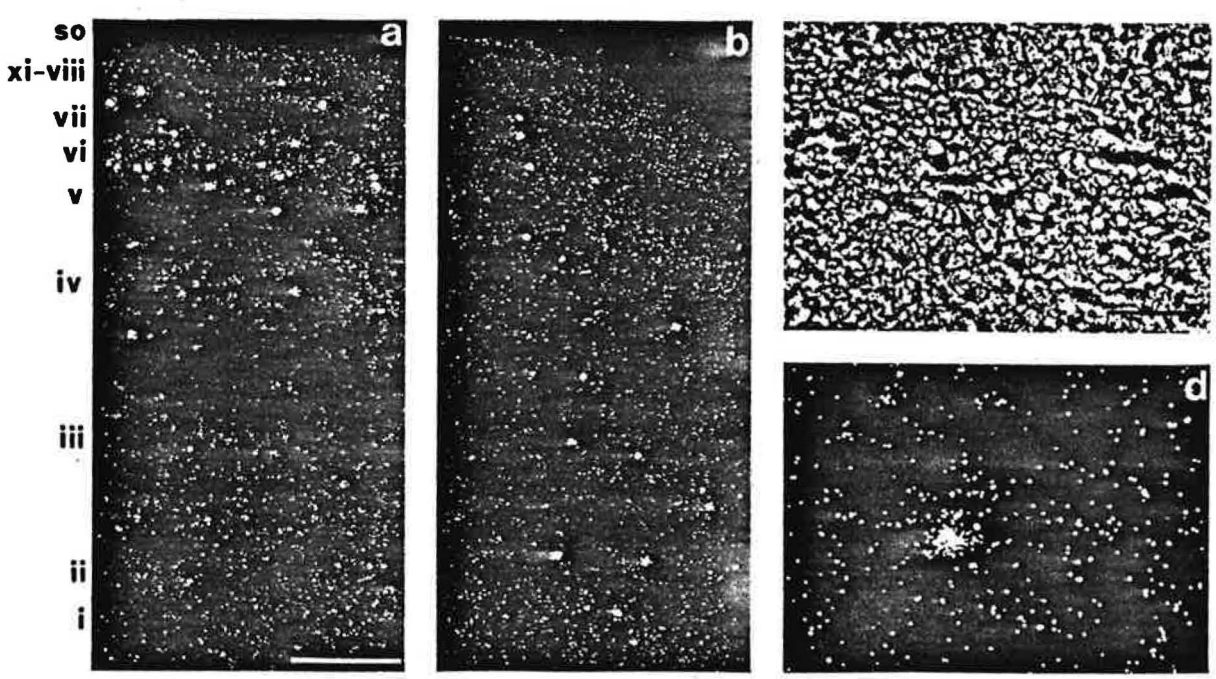

Figure 4. Topographic Distribution of α7 and nα1 Transcripts in the Optic Tectum

Tissue sections across E12 optic tectum were hybridized with ^{35}S-labeled nα1 (a) or 3′ α7 (b, c, and d) antisense probes. Bright-field (c) and dark-field (d) photomicrographs of cells in layer IV. Autoradiography was for 3 weeks. Bars, 200 μm (a and b); 40 μm (c and d).

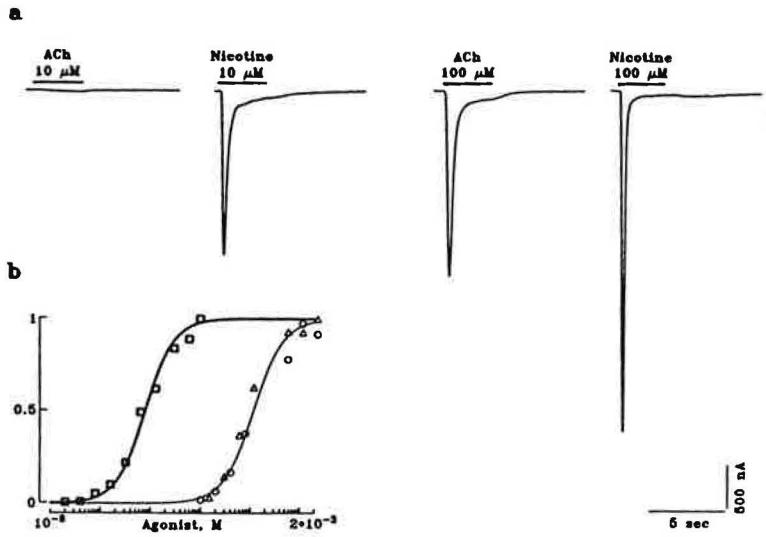

Figure 5. Responses of α7-Injected Oocytes to ACh and to Nicotine

(a) An oocyte injected 2 days earlier with 2 ng of α7 expression plasmid was voltage-clamped at −100 mV and challenged with increasing concentrations of ACh and nicotine. Time between applications was 1 min or longer, and full recovery had been determined to take less than 1 min for short (3 s) agonist applications.

(b) Mean dose-response curves to ACh of the reference neuronal α4/nα1 (squares, 20 cells; Bertrand et al., 1990a) and α7 channels (triangles, 3 cells from one ovary; circles, 9 cells from two ovaries). Responses from each cell were normalized to its maximum evoked current. Mean data points were fitted to Hill's equation (solid lines) to yield α4/nα1, EC_{50} = 0.77 μM, n = 1.5, and α7, EC_{50} = 115 μM, n = 1.4.

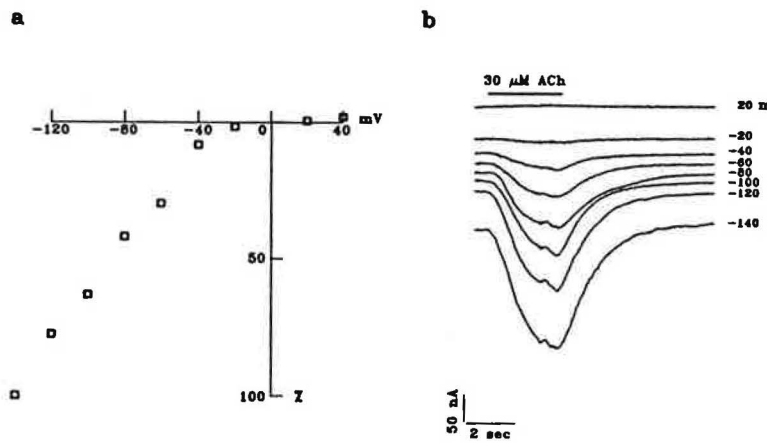

Figure 6. Voltage Dependence of ACh-Induced Currents in α7 Channels

(a) Current–voltage values (4 cells, mean of currents normalized at −140 mV) were obtained by consecutive 30 μM ACh applications at increasingly depolarized voltages.

(b) Individual α7 currents in a single oocyte challenged with 30 μM ACh between −140 and +20 mV.

μM, n = 1.5). The nicotine sensitivity of the α7 channel has not been fully documented, but it is certainly 5 times greater than the sensitivity for ACh, whereas the α4/nα1 channel is equally sensitive to ACh and to nicotine (Figure 5a; Bertrand et al., 1990a). Another obvious feature of the α7 responses shown in Figure 5a is their very fast and thorough desensitization at high agonist concentrations. In all cells we recorded from (n = 61), the time course of desensitization was similar and could be fitted by the sum of two exponentials (an adequate fit for 1 cell yielded T_f = 0.26 s and T_s = 13 s). In α7 receptors, the time constant of the fast component is much shorter than that in any other receptors we have analyzed (Couturier et al., 1990), and the ratio between peak and plateau (about 25 to 1) is the largest of all reconstituted nAChRs.

Coinjections of expression plasmids for α7 and for either nα1, nα2, nα3, or a mixture of the three muscle nAChR subunits β, γ, and ∂ all resulted in the assembly of functional ACh-gated channels. These channels, however, did not differ in their sensitivity to ligands or to toxins from those assembled from α7 alone. Thus, we have no reason to believe at present that α7 and any of the above non-α subunits can coassemble into functional hetero-oligomeric structures.

Figure 6a demonstrates that the ACh-induced currents (mean of 4 cells) through α7 channels are dramatically voltage sensitive at depolarized potentials: above −20 mV, the current–voltage curve rectifies so thoroughly that no reversal potential is detectable. In Figure 6b, we show the individual responses recorded from a single cell repeatedly challenged with the same low, nondesensitizing ACh concentration (30 μM), at eight different imposed potentials from −140 to +20 mV.

Figure 7a illustrates the full block resulting from a 30 min application of 100 nM α-BTX in an oocyte expressing the α7 receptor (5 of 5 cells responded in the same way). The same preparation of highly purified α-BTX has been applied for 30 min at 700 nM in oocytes expressing α4/nα1, α4/nα3, α3/nα1, and α3/nα3 channels (Couturier et al., 1990); responses to a sub-

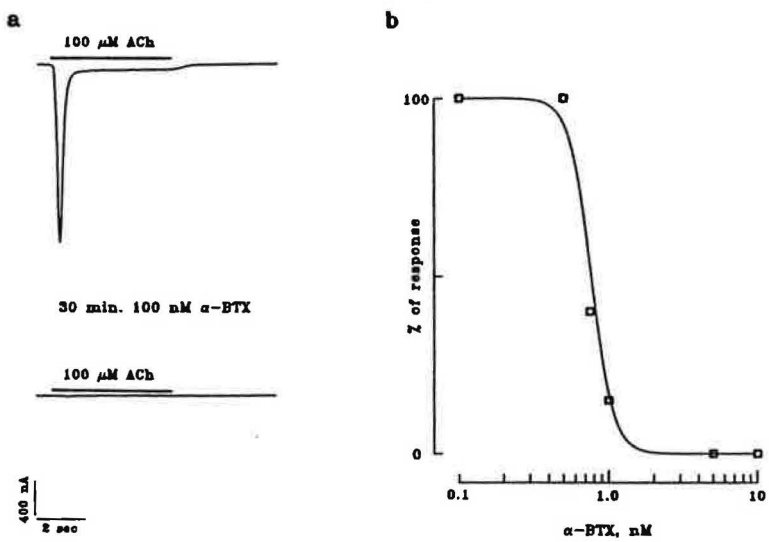

Figure 7. α7 Channels are Blocked by α-BTX

(a) In α7-injected oocytes, the response to 100 μM ACh was abolished by 30 min incubation in 100 nM α-BTX.

(b) Inhibition curve of α7 channels by α-BTX. Responses to 600 μM ACh in oocytes clamped at −100 mV were measured before and after 30 min incubation at the indicated toxin concentration in the presence of 100 μg/ml bovine serum albumin. Data points were fitted to Hill's inhibition equation (solid line) to yield IC_{50} = 0.73 nM, n = 6.

sequent application of ACh were totally unaffected. In contrast, currents of up to 15 μA evoked by 1 μM ACh in a chimeric muscle nAChR (chicken α, γ, and ∂ cDNAs, mouse β cDNA) expressed in oocytes were abolished by a 30 min incubation in 100 nM α-BTX (data not shown). In Figure 7b, we demonstrate that the α7 channel expressed in oocytes is exquisitely sensitive to α-BTX (IC_{50} = 0.73 nM) and that the inhibition curve is extremely steep (Hill coefficient of 6 or greater), an indication that the homo-oligomeric α7 channel may be blocked by only one or a very few α-BTX molecules. Two different, highly purified preparations of the neuronal toxin κ-BTX were also tested on oocytes injected with α7. ACh responses were fully blocked after a 30 min incubation at 100 nM, but there was no block at 10 or 1 nM.

To control for the possible interference of nAChR α-like subunits that might be endogenously produced in the oocytes (Buller and White, 1990; Hartman and Claudio, 1990), we tested series of oocytes injected with an equimolar mixture of the muscle subunit β, γ, and ∂ cDNAs or with nα1, nα2, or nα3 cDNAs. Of 57 such cells examined, none responded to 100 μM ACh, although most or all α/β/γ/∂- or α4/nα1-injected oocytes from the same batches gave responses in the microampere range when challenged with 1 μM ACh.

Labeled α-BTX Binding to Fusion Proteins

A restriction fragment encoding residues 124–239 of the mature α7 protein was spliced in phase downstream from the constitutive *lac* promoter in plasmid pUC18 to yield pCα7A. A culture of E. coli strain DH5 transformed with pCα7A was lysed, and the released proteins were tested for labeled α-BTX binding in dot (Figure 8a) or Western (Figure 8b) blots. Proteins released from DH5 cells transformed with pUC18 served as negative control. As positive control, we used lysates of DH5 cells transformed with pTαX1Ω (Barkas et al., 1988), a pUC8 derivative expressing high levels of a Torpedo nAChR α1 subunit fusion protein (residues −2 to +200). A simple dot blot assay (Figure 8a) was used to show that lysates of cells transformed with pCα7A and pTαX1Ω do contain an α-BTX binding activity absent from lysates of cells transformed with the vector alone. As seen in Figure 8b, Western blots of the cα7A and TαX1Ω lysates each contain a protein

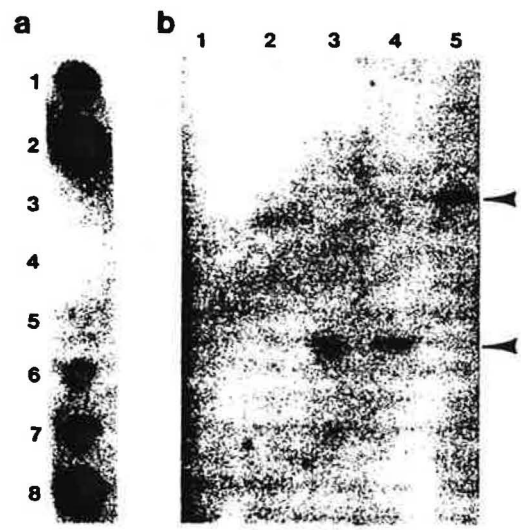

Figure 8. Autoradiographs of ^{125}I-labeled α-BTX Bound to Chicken α7 and to Torpedo α1 Fusion Proteins

(a) Samples of cell lysates from E. coli DH5 transformed with Torpedo αl plasmid TαX1Ω, chicken α7 plasmid Cα7A, and control pUC18 were deposited on nitrocellulose and incubated with labeled α-BTX: lanes 1 and 2, 1 and 5 μl of TαX1Ω lysate; lanes 3–5, 1, 5, and 10 μl of pUC18 lysate; lanes 6–8, 1, 5, and 10 μl of Cα7A lysate.

(b) Samples of cell lysates were separated by electrophoresis on a 20% SDS-polyacrylamide gel, transferred to nitrocellulose, and incubated with labeled α-BTX; lanes 1 and 2, 10 μl of pUC18 lysate; lanes 3 and 4, 10 μl of Cα7A lysate; lane 5, 1 μl of TαX1Ω lysate. Arrowheads point to 14 kd (lower) and 24 kd (upper) α-BTX binding fusion proteins.

of the expected molecular mass (14 and 24 kd, respectively) that binds ^{125}I-labeled α-BTX, whereas lysates of the negative control do not.

Discussion

The α7 protein belongs to the growing family of vertebrate nAChR-related subunits that have been definitely proven to assemble into functional ACh-gated channels. In addition to the five subunits of muscle nAChR (Mishina et al., 1986), this family includes the neuronal subunits α2, α3, α4, nα1 (β2), and nα3 (β4). Other subunits, α5 and nα2 (β3), have been cloned, but their function has not been determined because they fail to form functional channels in the Xenopus oocyte system (Boulter et al., 1990; Couturier et al., 1990). Structural and functional findings reported here indicate that α7 occupies a special place in the ensemble of neuronal nAChR subunits.

The α7 Gene Has a Unique Structure among nAChR Genes

The structure of the α7 gene departs from the invariant structure of the 7 neuronal subunit genes so far sequenced (Nef et al., 1988; Wada et al., 1988; Boulter et al., 1990; Couturier et al., 1990), and it also differs from the structures of the 5 muscle receptor subunit genes. Of the ten exons encompassing the coding sequence of α7, the 3′ proximal six have unique splice sites, whereas all other genes in the family use subsets of the 11 splice sites identified in the muscle β, γ, ∂, and ε genes (Nef et al., 1984; Buonanno et al., 1989). This, taken together with the relative low homology between the α7 protein and all other members of the family, suggests that the α7 gene diverged early from the main phylogeny that led to the other 12 muscle and neuronal subunit genes whose structures have been established.

α-BTX Binds to the α7 Protein

Our results (Figures 7 and 8) strongly suggest that α7 is one of the proteins that bind α-BTX in the nervous system of vertebrates (Wang et al., 1978; Meeker et al., 1986). This suggestion is reinforced by the quasi-identity between the N-terminus of α7 and that of the protein Conti-Tronconi et al. (1985) purified from neonate chick optic lobe by affinity chromatography on α-BTX. The two sequences differ at only 2 of 22 identified residues (see Figure 2b) and resemble each other much more than any other known nAChR subunit N-terminus. In view of the heroic nature of membrane protein microsequencing, it may perhaps be concluded that the two proteins are identical. Alternatively, α7 may correspond to another of the proteins coeluted in the purification scheme and found to react with bromo-acetylcholine, an affinity reagent specific for nAChR α subunits. The hetero-oligomeric structure of the chick optic lobe α-BTX binding protein was confirmed by Whiting and Lindstrom (1987) and extended by Gotti et al. (1989) to the α-BTX binding protein expressed in the human neuroblastoma cell line IMR32. Thus, as is the case for the muscle receptor and for the neuronal receptors incorporating α2, α3, and α4, it appears likely that the neuronal α-BTX binding proteins consist of several distinct subunits.

Regulation of α7 mRNA during Neural Development

During development of the optic tectum, the level of α7 mRNA transiently increased between E5 and E16 and reached a peak around E12. Among the neuronal nAChR genes already identified, only nα1 exhibits a similar pattern of expression (Matter et al., 1990). The nα1 transcripts are predominantly localized in the superficial layers of the tectum at the time when cells in these layers form their connections with retinal axons. In contrast, α7 transcripts are more abundant in the deeper layers of the optic tectum. Although there is some overlap in the hybridization patterns of nα1 and α7 probes, it appears that the corresponding mRNAs are mostly expressed in distinct cell populations. Furthermore, early eye enucleation does not influence the transient accumulation of α7 transcripts, but it prevents the 10-fold increase in nα1 mRNA taking place at the same stages of development (Matter et al., 1990).

Whereas transcriptional regulation of the nα1 gene possibly plays a role in the formation of retino-tectal synapses, the α7 nAChR gene may instead participate in neurogenesis of connections between tectal cells and the several brainstem nuclei with which the tectum is connected. The α7 gene is expressed in layer IV, the future stratum griseum centrale, and this layer contains axon terminals from the nucleus semilunaris and nucleus spiriformis lateralis (Reiner et al., 1982; Hunt and Brecha, 1984). Sorenson et al. (1989) have shown that the majority of neurons in the nucleus semilunaris are cholinergic. In agreement with our findings, nAChR-like molecules and ^{125}I-labeled α-BTX binding sites have been detected in the stratum griseum centrale and in the stratum griseum et fibrosum superficiale of adult chicken (Polz-Tejera et al., 1975; Swanson et al., 1983, 1987). Moreover, there is excellent correlation between the levels of α7 mRNA and of α-BTX binding protein in the developing optic tectum: toxin binding activity has been shown (Wang and Schmidt, 1976; Wang et al., 1978) to increase sharply between E10 and hatching and then to decrease to a low plateau in the neonate and adult. Wang and Schmidt (1976) have suggested that this decrease in toxin binding sites may coincide with the maturation of cholinergic synapses.

α7 Forms a Nicotinic Ion Channel Blocked by α-BTX

The results in Figures 5–7 prove that α7 forms functional nicotinic ion channels giving robust (up to 4 μA, a current corresponding to millions of open channels) and reproducible responses in the oocyte membrane.

Whether such homo-oligomeric channels assemble in neuronal membranes and what their function might be remain to be determined. It is indeed likely that hetero-oligomeric forms predominate, as is the case for the related strychnine-sensitive glycine receptor, whose α subunits do nevertheless efficiently assemble into homo-oligomeric glycine-gated chloride channels upon transformation into mammalian cells (Sontheimer et al., 1989) or in the oocyte system (Grenningloh et al., 1990). We note that α7 and all known glycine receptor α subunits have an odd number of cysteine residues in their extracellular domains, suggesting that efficient self-assembly of α subunits may depend on dimer formation.

The finding that α7 forms an ACh-gated channel (Figure 5) which is efficiently blocked by α-BTX (Figure 7) provides very strong evidence that the complete, hetero-oligomeric in vivo species also function as α-BTX-sensitive nAChRs. The close agreement between the IC_{50} we measure in oocytes (0.73 nM; Figure 7b) and the EC_{50} of the complex formed between α-BTX and toxin binding proteins in CNS membranes (0.35–0.61 nM; Wang and Schmidt, 1976) suggests that most or all of the high-affinity α-BTX binding proteins in chicken brain may contain the α7 protein. At present, we cannot say whether the related, neuron-specific κ-BTX also blocks α7 channels because this toxin is not available in sufficiently pure form. Chromatographically pure κ-BTX may contain as much as 2% α-BTX, a level that suffices to account for the full block we observe at 100 nM κ-BTX and for the absence of block at 10 nM κ-BTX.

ACh sensitivity of the homo-oligomeric α7 channel is low compared with that of α4/nα1, yet the complete, hetero-oligomeric structure found in vivo may be much more sensitive to ACh as a result of the influence of other subunits in the structure. Support for this view comes from the observation that α4- and α3-containing neuronal nAChRs have very different ACh sensitivities, depending on whether they assemble with the nα1 or with the nα3 structural subunit (Couturier et al., 1990). Unlike α4/nα1 or muscle receptors, α7 is more sensitive to nicotine than to ACh, and it may be of interest to determine whether it has a role in nicotine dependence.

The rectification properties of the α7 channels are so pronounced that exact measurement of their reversal potential will have to await single-channel analysis. Although the nature of the permeant ions remains to be determined, we note that significant currents are detected at −30 mV and below, a value compatible with cationic fluxes. The extremely fast desensitization of α7 homo-oligomers suggests that neuronal receptors containing this subunit may produce phasic responses to acute ACh release, whereas other neuronal nAChRs, such as the slowly desensitizing α4/nα1, may be implicated in tonic responses.

Experimental Procedures

Standard Molecular Biological Procedures

Construction of chicken brain cDNA and genomic libraries, radioactive probe synthesis, screening procedures, bacteriophage purification, subcloning, and sequencing protocols were as described by Nef et al. (1988) and by Couturier et al. (1990).

RNA Extraction and Northern Blot Analysis

Embryos, newborn chicks, and chickens of a White Leghorn strain were used in this work. Embryos were staged according to Hamburger and Hamilton (1951), and following dissection, optic tecta were immediately frozen in liquid nitrogen and stored at −70°C. Eyeless embryos were prepared as previously described (Matter et al., 1990). Total RNA was isolated according to Feramisco et al. (1982), and poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography (Aviv and Leder, 1972). Total RNA and poly(A)$^+$ RNA were fractionated by electrophoresis in 1% agarose gel containing formaldehyde and electrophoretically transferred to Dupont GeneScreen membrane (Khandjian, 1986). Ribosomal RNAs were visualized by staining the membrane with methylene blue. Blots were prehybridized for 6 hr in 1 M NaCl, 2× Denhardt's solution, 1% SDS, 50 μg/ml of salmon sperm DNA, 10% dextran sulphate, 50% formamide at 42°C. The hybridization buffer was the same except that it contained 5×10^5 cpm/ml heat-denatured probe. Blots were hybridized for 24 hr at 42°C and washed twice in 2× SSC, 0.05% SDS at 21°C and twice at 65°C. Blots were then exposed to X-ray film (Fuji RX) at −70°C with an intensifying screen.

In Situ Hybridization

Optic tecta dissected on E12 were fixed in 4% paraformaldehyde, dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Tissue sections (10 μm) mounted on glass slides were pretreated and hybridized with ^{35}S-labeled single-stranded (sense or antisense) probes (Matter et al., 1990). Hybridization was for 8 hr at 42°C in a buffer containing 50% formamide, 5× SSC, 2× Denhardt's solution, 0.2% sodium pyrophosphate, 600 μg/ml salmon sperm DNA, and 5×10^6 cpm/ml probe. Sections were then washed for 10 min in 4× SSC at 21°C, for 30 min in 2× SSC at 40°C, and for 30 min in 0.1× SSC at 50°C. Sections were dipped in Kodak NTB-2 photo emulsion (diluted 1:1 in water), exposed for 3 weeks, developed (Kodak D-19), and fixed.

Expression Plasmids for Nuclear Injection

The full-length α7 cDNA and a subclone from which 700 bp of 3′ untranslated sequence had been deleted (3′ BamHI–EcoRI fragment; see Figure 1) were spliced into the Xenopus oocyte expression plasmid flip cat (Bertrand et al., 1990a) to yield plasmids flip α7 and flip α7Δ, respectively. Full-length chicken α1, γ, and δ and mouse β cDNAs were spliced in the flip configuration to reconstitute a chimeric muscle nAChR. In flip vectors, the cDNA of interest is cloned in the proper orientation downstream from the SV40 early promoter and upstream from the bidirectional SV40 polyadenylation sequence (Gorman et al., 1982).

Nuclear Injection and Electrophysiological Procedures

A detailed technical paper on oocyte preparation, nuclear injection, and electrophysiological procedures will appear elsewhere (Bertrand et al., 1990b). Adequate descriptions of protocols have been provided by Ballivet et al. (1988) and by Bertrand et al. (1990a). All recordings were in the presence of 0.5 μM atropine to block endogenous muscarinic ACh responses.

Bacterial Fusion Protein Expression Vectors

A 348 bp DraI–BglII restriction fragment of plasmid flip α7 was ligated into SmaI- and BamHI-restricted plasmid vector pUC18 to give a plasmid designated pCα7A. The N- and C-terminal regions of pCα7A were sequenced to ascertain that the correct translation frames had been obtained. pCα7A encodes a fusion protein containing 116 amino acids of the α7 protein (residues 124–239) preceded by the first 11 amino acids of the modified β-galactosidase α-peptide (MTMITNSSSVP) and followed by a single L residue. The construction of plasmid pTαX1Ω has been published (Barkas et al., 1988).

^{125}I-Labeled α-BTX Binding

Isolates of E. coli strain DH5 transformed with pCα7A, pTαX1Ω,

or pUC18 were grown overnight in 1.5 ml of medium with antibiotic selection, pelleted by brief centrifugation, resuspended in 150 μl of SDS sample buffer, and boiled for 5 min. Samples of the lysates were transferred to nitrocellulose either directly, by means of a Bio-Rad Bio-Dot apparatus, or following electrophoresis on a 20% SDS-polyacrylamide gel. Nitrocellulose membranes were washed for 10 min in TBST (50 mM Tris-Cl [pH 7.9], 150 mM NaCl, 0.05% Tween-20), incubated for 2 hr at room temperature in TBST containing 5 mM ^{125}I-labeled α-BTX (Barkas and Fulpius, 1984), and washed twice for 10 min in TBST. Membranes were air-dried, covered in Saran Wrap, and exposed to Kodak X-OMAT film with intensifying screens at −70°C.

Neuronal Bungarotoxins

Highly purified κ-BTX (Chiappinelli, 1983) and bungarotoxin 3.1 (Ravdin and Berg, 1979) were shipped and stored freeze-dried and reconstituted in oocyte buffer containing 100 μg/ml bovine serum albumin (Sigma B-2518). κ-BTX and bungarotoxin 3.1 are the same protein and are also known as toxin F (Loring and Zigmund, 1988).

DNA and Protein Sequence Analysis

All sequence data were stored and analyzed using the PC/Gene program package, courtesy of Amos Bairoch, University of Geneva.

Acknowledgments

We thank Christine Alliod for excellent technical assistance, Lidia Matter-Sadzinski for comments on the manuscript, Duri Rungger and Vincent Chiappinelli for many helpful suggestions, Ann Kato for the generous loan of equipment, and the following for the kind gifts of toxins and clones: Vincent Chiappinelli (κ-BTX), Darwin Berg and Stanley Halvorsen (bungarotoxin 3.1), and Jim Boulter (mouse β subunit clone BMB49). This work was funded by the Swiss National Science Foundation and by the State of Geneva. N. M. and T. B. were supported by the Medical Research Council and the Muscular Dystrophy Group of Great Britain. S. C., M.-C. H., and S. V. are doctoral students supported in part by the Swiss NSF.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 USC Section 1734 solely to indicate this fact.

Received July 2, 1990; revised August 20, 1990.

References

Aviv, H., and Leder, P. (1972). Purification of biologically active globin messenger RNA by chromatography on oligothymidilic acid-cellulose. Proc. Natl. Acad. Sci. USA *69*, 1408–1412.

Ballivet, M., Nef, P., Couturier, S., Rungger, D., Bader, C. R., Bertrand, D., and Cooper, E. (1988). Electrophysiology of a chick neuronal acetylcholine receptor expressed in Xenopus oocytes after cDNA injection. Neuron *1*, 847–852.

Barkas, T., and Fulpius, B. (1984). Radiolabelling of alpha-bungarotoxin to high radioactivity with complete retention of biological activity. Biochem. Soc. Trans. *12*, 813–814.

Barkas, T., Gabriel, J.-M., Mauron, A., Hughes, G., Roth, B., Alliod, C., Tzartos, S., and Ballivet, M. (1988). Monoclonal antibodies to the main immunogenic regions of the nicotinic acetylcholine receptor bind to residues 61–76 of the alpha subunit. J. Biol. Chem. *263*, 5916–5920.

Bertrand, D., Ballivet, M., and Rungger, D. (1990a). Activation and blocking of neuronal nicotinic acetylcholine receptor reconstituted in Xenopus oocytes. Proc. Natl. Acad. Sci. USA *87*, 1993–1997.

Bertrand, D., Cooper, E., Valera, S., Rungger, D., and Ballivet, M. (1990b). Electrophysiology of neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes, following nuclear injection of genes or cDNAs. Meth. Neurosci., in press.

Bossy, B., Ballivet, M., and Spierer, P. (1988). Conservation of neuronal nicotinic acetylcholine receptors from Drosophila to vertebrate central nervous systems. EMBO J. *7*, 611–618.

Boulter, J., Evans, K., Goldman, D., Martin, G., Treco, D., Heinemann, S., and Patrick, J. (1986). Isolation of a cDNA clone coding for a possible neural nicotinic acetylcholine receptor alpha-subunit. Nature *319*, 368–374.

Boulter, J., O'Shea-Greenfield, A., Duvoisin, R., Connolly, J., Wada, E., Jensen, A., Gardner, P., Ballivet, M., Deneris, E., McKinnon, D., Heinemann, S., and Patrick, J. (1990). Alpha3, alpha5 and beta4: three members of the rat neuronal nicotinic acetylcholine receptor-related gene family form a gene cluster. J. Biol. Chem. *265*, 4472–4482.

Buller, A., and White, M. (1990). Functional acetylcholine receptors expressed in Xenopus oocytes after injection of Torpedo beta, gamma and delta subunit RNAs are a consequence of endogenous oocyte gene expression. Mol. Pharmacol. *37*, 423–428.

Buonanno, A., Mudd, J., and Merlie, J. (1989). Isolation and characterization of the beta and epsilon subunit genes of mouse muscle acetylcholine receptor. J. Biol. Chem. *264*, 7611–7616.

Chiappinelli, V. (1983). Kappa toxin: a probe for neuronal nicotinic receptors in the avian ciliary ganglion. Brain Res. *277*, 9–21.

Conti-Tronconi, B., Dunn, S., Barnard, E., Dolly, J., Lai, F., Ray, N., and Raftery, M. (1985). Brain and muscle nicotinic acetylcholine receptors are different but homologous proteins. Proc. Natl. Acad. Sci. USA *82*, 5208–5212.

Couturier, S., Erkman, L., Valera, S., Rungger, D., Bertrand, S., Boulter, J., Ballivet, M., and Bertand, D. (1990). Alpha5, alpha3, and non-alpha3: three clustered avian genes encoding nicotinic acetylcholine receptor-related subunits. J. Biol. Chem., in press.

Deneris, E. S., Connolly, J., Boulter, J., Wada, E., Wada, K., Swanson, L. W., Patrick, J., and Heinemann, S. (1988). Primary structure and expression of β2: a novel subunit of neuronal nicotinic acetylcholine receptors. Neuron *1*, 45–54.

Deneris, E. S., Boulter, J., Swanson, L., Patrick, J., and Heinemann, S. (1989). Beta3: a new member of nicotinic acetylcholine receptor gene family is expressed in brain. J. Biol. Chem. *264*, 6268–6272.

Duvoisin, R. M., Deneris, E. S., Patrick, J., and Heinemann, S. (1989). The functional diversity of the neuronal nicotinic acetylcholine receptors is increased by a novel subunit: β4. Neuron *3*, 487–496.

Feramisco, J., Smart, J., Burridge, K., Helfman, D., and Thomas, G. (1982). Co-existence of vinculin and a vinculin-like protein of higher molecular weight in smooth muscle. J. Biol. Chem. *257*, 11024–11031.

Giraudat, J., Dennis, M., Heidmann, T., Chang, J.-Y., and Changeux, J.-P. (1986). Structure of the high-affinity binding site for noncompetitive blockers of the acetylcholine receptor: serine-262 of the delta subunit is labeled by ^{3}H chlorpromazine. Proc. Natl. Acad. Sci. USA *83*, 2719–2723.

Goldman, D., Deneris, E., Luyten, W., Kochhar, A., Patrick, J., and Heinemann, S. (1987). Members of a nicotinic acetylcholine receptor gene family are expressed in different regions of the mammalian central nervous system. Cell *48*, 965–973.

Gorman, C., Moffat, L., and Howard, B. (1982). Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. *2*, 1044–1051.

Gotti, C., Ogando, A., and Clementi, F. (1989). The alpha-bungarotoxin receptor purified from a human neuroblastoma cell line: biochemical and immunological characterization. Neuroscience *32*, 759–767.

Grenningloh, G., Rienitz, A., Schmitt, B., Methfessel, C., Zensen, M., Beyreuther, K., Gundelfinger, E., and Betz, H. (1987). The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. Nature *328*, 215–220.

Grenningloh, G., Schmieden, V., Schofield, P., Seeburg, P., Siddique, T., Mohandas, T., Becker, C.-M., and Betz, H. (1990). Alpha subunit variants of the human glycine receptor: primary struc-

tures, functional expression and chromosomal localization of the corresponding gene. EMBO J. *9*, 771–776.

Hamburger, V., and Hamilton, H. (1951). A series of normal stages in the development of chick embryo. J. Morphol. *48*, 4949–4992.

Hartman, D., and Claudio, T. (1990). Coexpression of two distinct muscle acetylcholine receptor alpha-subunits during development. Nature *343*, 372–375.

Hollman, M., O'Shea-Greenfield, A., Rogers, S., and Heinemann, S. (1989). Cloning by functional expression of a member of the glutamate receptor family. Nature *342*, 643–648.

Hunt, S., and Brecha, N. (1984). The avian optic tectum: a synthesis of morphology and biochemistry. In Comparative Neurology of the Optic Tectum, H. Vanegas, ed. (New York: Plenum Publishing Corp.), pp. 619–648.

Imoto, K., Busch, C., Sakmann, B., Mishina, M., Konno, T., Nakai, J., Bujo, H., Mori, Y., Fukuda, K., and Numa, S. (1988). Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. Nature *335*, 645–648.

Khandjian, E. (1986). UV crosslinking of RNA to nylon membrane enhances hybridization signals. Mol. Biol. Rep. *11*, 107–115.

LaVail, J., and Cowan, W. M. (1971). The development of the chick optic tectum. I. Normal morphology and cytoarchitectonic development. Brain Res. *28*, 391–419.

Lindstrom, J., Schoepfer, R., and Whiting, P. (1987). Molecular studies of the neuronal nicotinic acetylcholine receptor family. Mol. Neurobiol. *1*, 281–337.

Loring, R., and Zigmond, R. (1988). Characterization of neuronal nicotinic receptors by snake venom neurotoxins. Trends Neurosci. *11*, 73–78.

Matter, J.-M., Matter-Sadzinski, L., and Ballivet, M. (1990). Expression of neuronal nicotinic acetylcholine receptor genes in the developing chick visual system. EMBO J. *9*, 1021–1026.

Meeker, R., Michels, K., Libber, M., and Hayward, J. (1986). Characteristics and distribution of high- and low-affinity alpha bungarotoxin binding sites in the rat hypothalamus. J. Neurosci. *6*, 1866–1875.

Mishina, M., Takai, T., Imoto, K., Noda, M., Takahashi, T., Numa, S., Methfessel, C., and Sakmann, B. (1986). Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. Nature *321*, 406–411.

Nef, P., Mauron, A., Stalder, R., Alliod, C., and Ballivet, M. (1984). Structure, linkage, and sequence of the two genes encoding the delta and gamma subunits of the nicotinic acetylcholine receptor. Proc. Natl. Acad. Sci. USA *81*, 7975–7979.

Nef, P., Oneyser, C., Alliod, C., Couturier, S., and Ballivet, M. (1988). Genes expressed in the brain define three distinct neuronal nicotinic acetylcholine receptors. EMBO J. *7*, 595–601.

Noda, M., Furutani, Y., Takahashi, H., Toyosato, M., Tanabe, T, Shimizu, S., Kiyotani, S., Kayano, T., Hirose, T., Inayama, S., and Numa, S. (1983). Cloning and sequence analysis of calf cDNA and human genomic DNA encoding alpha-subunit precursor of muscle acetylcholine receptor. Nature *305* 818–823.

Papke, R. L., Boulter, J., Patrick, J., and Heinemann, S. (1989). Single-channel currents of rat neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes. Neuron *3*, 589–596.

Polz-Tejera, G., Schmidt, J., and Karten, H. (1975). Autoradiographic localisation of alpha-bungarotoxin-binding sites in the central nervous system. Nature *258*, 349–351.

Raftery, M., Hunkapiller, M., Strader, C., and Hood, L. (1980). Acetylcholine receptor: complex of homologous subunits. Science *208*, 1454–1456.

Ravdin, P., and Berg, D. (1979). Inhibition of neuronal acetylcholine sensitivity of a toxin from Bungarus multicinctus venom. Proc. Natl. Acad. Sci. USA *75*, 2072–2076.

Reiner, A., Brecha, N., and Karten, H. (1982). Basal ganglia pathways to the tectum: the afferent and efferent connections of the lateral spiriform nucleus of pigeon. J. Comp. Neurol. *208*, 16–36.

Schmidt, J. (1988). Biochemistry of nicotinic acetylcholine receptors in the vertebrate brain. Int. Rev. Neurobiol. *30*, 1–38.

Schofield, P. R., Darlison, M. G., Fujita, N., Burt, D. R., Stephenson, F. A., Rodriguez, H., Rhee, L. M., Ramachandran, J., Reale, V., Glencorse, T. A., Seeburg, P. H., and Barnard, E. A. (1987). Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. Nature *328*, 221–227.

Sontheimer, H., Becker, C.-M., Pritchett, D. B., Schofield, P. R., Grenningloh, G., Kettenmann, H., Betz, H., and Seeburg, P. H. (1989). Functional chloride channels by mammalian cell expression of rat glycine receptor subunit. Neuron *2*, 1491–1497.

Sorenson, E., Parkinson, D., Dahl, J., and Chiappinelli, V. (1989). Immunohistochemical localization of choline acetyltransferase in the chicken mesencephalon. J. Comp. Neurol. *281*, 641–657.

Swanson, L., Lindstrom, J., Tzartos, S., Schmued, L., O'Leary, D., and Cowan, W. M. (1983). Immunohistochemical localization of monoclonal antibodies to the nicotinic acetylcholine receptor in chick midbrain. Proc. Natl. Acad. Sci. USA *80*, 4532–4536.

Swanson, L., Simmons, D., Whiting, P., and Lindstrom, J. (1987). Immunohistochemical localization of neuronal nicotinic acetylcholine receptors in rodent central nervous system. J. Neurosci. *7*, 3334–3342.

Wada, K., Ballivet, M., Boulter, J., Connolly, J., Wada, E., Deneris, E., Swanson, L., Heinemann, S., and Patrick, J. (1988). Functional expression of a new pharmacological subtype of brain nicotinic acetylcholine receptor. Science *240*, 330–334.

Wang, G.-K., and Schmidt, J. (1976). Receptors for alpha-bungarotoxin in the developing visual system of the chick. Brain Res. *114*, 524–529.

Wang, G.-K., Molinaro, S., and Schmidt, J. (1978). Ligand responses of alpha-bungarotoxin binding sites from skeletal muscle and optic lobe of the chick. J. Biol. Chem. *253*, 8507–8512.

Whiting, P., and Lindstrom, J. (1987). Purification and characterization of a nicotinic acetylcholine receptor from rat brain. Proc. Natl. Acad. Sci. USA *84*, 595–599.

Note Added in Proof

After submission of this paper, Schoepfer and co-workers described a cDNA from chicken brain (termed αBgtBp α1) whose sequence is identical to the α7 cDNA reported here (Schoepfer, R., Conroy, W. G., Whiting, P., Gore, M., and Lindstrom, J. 1990. Brain α-bungarotoxin binding protein cDNAs and MAbs reveal subtypes of this branch of the ligand-gated ion channel gene family. Neuron *5*, 35–48).