



HAL
open science

Identification of a New Component of the Agonist Binding Site of the Nicotinic 7 Homooligomeric Receptor

Pierre-Jean Corringer, Jean-Luc Galzi, Jean-Luc Eiselé, Sonia Bertrand, Jean-Pierre Changeux, Daniel Bertrand

► **To cite this version:**

Pierre-Jean Corringer, Jean-Luc Galzi, Jean-Luc Eiselé, Sonia Bertrand, Jean-Pierre Changeux, et al.. Identification of a New Component of the Agonist Binding Site of the Nicotinic 7 Homooligomeric Receptor. *Journal of Biological Chemistry*, 1995, 270 (20), pp.11749 - 11752. 10.1074/jbc.270.20.11749 . pasteur-01714720

HAL Id: pasteur-01714720

<https://pasteur.hal.science/pasteur-01714720>

Submitted on 21 Feb 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Identification of a New Component of the Agonist Binding Site of the Nicotinic $\alpha 7$ Homooligomeric Receptor*

(Received for publication, February 6, 1995, and in revised form, March 27, 1995)

Pierre-Jean Corringer[‡], Jean-Luc Galzi[‡],
Jean-Luc Eiselé[‡], Sonia Bertrand[§],
Jean-Pierre Changeux^{‡¶}, and Daniel Bertrand[§]

From [‡]Neurobiologie Moléculaire, Unité de recherche associée D1284, CNRS, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France and the [§]Département de Physiologie, Centre Médical Universitaire (Faculté de Médecine), 1211 Geneva 4, Switzerland

Tryptophan 54 of the $\alpha 7$ neuronal nicotinic homooligomeric receptor is homologous to γ -Trp-55 and δ -Trp-57 of non- α subunits of *Torpedo* receptor labeled by *d*-tubocurarine. This residue was mutated on the $\alpha 7$ -V201-5-hydroxytryptamine (5HT)₃ homooligomeric chimera, which displays $\alpha 7$ nicotinic pharmacology, and for which both equilibrium binding studies and electrophysiological recordings could be carried out in parallel. Replacement of Trp-54 by a Phe, Ala, or His causes a progressive decrease both in binding affinity and in responses (EC₅₀ or IC₅₀) for acetylcholine, nicotine, and dihydro- β -erythroidine, without significant modification in α -Bgtx binding. Except for Gln-56, comparatively small effects are observed when the other residues of the 52–58 region are mutated into alanine. These data support the participation of Trp-54 to ligand binding, and provide evidence for a new “complementary component” of the $\alpha 7$ nicotinic binding site, distinct from its three-loop “principal component,” and homologous to the “non- α component” present on γ and δ subunits.

Binding of ACh¹ to nicotinic acetylcholine receptors causes channel opening and receptor desensitization. Affinity labeling and expression experiments support the notion that the two ACh binding sites of $\alpha 2\beta\gamma\delta$ muscle-type *Torpedo* receptor result from the association of specific domains of the α subunits (referred to as *principal component* of the site) with regions of the non- α subunits (referred to as *complementary component* of the site) (1–6). This notion has been extended to neuronal

nicotinic receptors, whose pharmacology varies with their α and β subunit composition (7–9).

The principal component of the nicotinic binding site of muscle and neuronal α subunits comprises three separate regions from the large N-terminal domain: loop A (Trp-86, Tyr-93), loop B (Trp-149), and loop C (Tyr-190, Cys-192, Cys-193, and Tyr-198) (numbering according to the *Torpedo* receptor) (10). Some evidence supports the participation of several residues of the γ and δ subunits of the *Torpedo* receptor to the complementary binding component (5, 11–13). In particular, sequencing experiments have shown that *d*-tubocurarine labeling of non- α *Torpedo* receptor subunits occurs on the homologous γ -Trp-55 and δ -Trp-57 residues (3, 11). Moreover, the γ W55L mutation results in a 8-fold decrease in ACh apparent affinity and *d*-tubocurarine inhibitory potency from electrophysiological recordings in the *Xenopus* oocytes (14).

Neuronal nicotinic receptor subunits $\alpha 7$, $\alpha 8$, and $\alpha 9$ assemble as functional homooligomers in the *Xenopus* oocytes (15–18). Under these conditions, the nicotinic binding sites form from neighboring identical subunits, implying that each subunit potentially contains both the principal component (which includes loops A, B, and C as demonstrated for $\alpha 7$ receptor; Ref. 19) and the complementary component. In this work, we investigate this second aspect of $\alpha 7$ contribution and mutate Trp-54, which is homologous to the γ -Trp-55 and δ -Trp-57 of *Torpedo* receptor.

To combine biochemical and electrophysiological approaches, we used the $\alpha 7$ -V201-5HT₃ chimera (referred to as wild-type) (20), which is efficiently transiently expressed in HEK 293 cells whereas $\alpha 7$ is not. While the chimera appears somewhat “artificial” receptor, it possesses the extracellular domain of $\alpha 7$. This confers to this construct a pharmacological profile of activation nearly identical to that of $\alpha 7$ in the *Xenopus* oocytes (20), supporting that the agonist binding site of both receptors are structurally homologous. Here, the pharmacological binding properties of the wild-type and mutant chimeras are characterized by electrophysiological recording in the *Xenopus* oocytes and parallel binding measurements in HEK 293 cells.

MATERIALS AND METHODS

Mutants were obtained as described previously (19) and subcloned in pMT₃ vector for expression (21). Electrophysiological recordings in the *Xenopus* oocytes were performed as described previously (22).

Chimeric cDNAs were transfected into HEK 293 cells by calcium phosphate precipitation (23). The level of expression was typically 5–20 fmol of ¹²⁵I- α -Bgtx (Amersham Corp.) binding sites/cm² of confluent cells. Receptor membranes were prepared as described previously (24).

¹²⁵I- α -Bgtx binding measurements were performed at 18 °C. For ¹²⁵I- α -Bgtx association experiments, receptor membranes (0.2–2 pmol of ¹²⁵I- α -Bgtx binding sites) were diluted in 250 μ l of HEPES buffer (10 mM HEPES, 2.5 mM CaCl₂, 2.5 mM MgCl₂, 82.5 mM NaCl, pH 7.2). ¹²⁵I- α -Bgtx was added, and, after a selected time, the sample was quickly diluted in 5 ml of phosphate-buffered saline buffer, filtered through GF-C filter (Whatman), rinsed with 5 ml of phosphate-buffered saline buffer, and counted. Nonspecific binding was determined in the presence of 1 mM L-nicotine. Association experiments in the presence of competitors were performed by preincubating the diluted membranes with the ligands for at least 10 min, and then measuring the initial rate of toxin binding after 5 min incubation with 2.5 nM ¹²⁵I- α -Bgtx. We verified that 3 μ M nicotine equilibrated with the wild-type chimera under the present conditions in less than 1 min (data not shown). ACh was always used in combination with 0.1 mM eserine. For ¹²⁵I- α -Bgtx dissociation experiments, 2 ml (1–5 pmol) of diluted receptor membranes were preincubated with 5 nM ¹²⁵I- α -Bgtx for 30 min. 1 μ M of

* This work was supported by research grants from the Collège de France, CNRS, and the Association Française contre les Myopathies (to J. P. C.), the Swiss National Science Foundation, OFES (to D. B.), Human Frontier Science Program, the EEC Biotech Program (to D. B. and J. P. C.), and the European Molecular Biology Organization (short term fellowship to J. L. G.). 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 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed. Tel.: 33-1-456-888-05; Fax: 33-1-456-888-36.

¹ The abbreviations used are: ACh, acetylcholine; 5HT, 5-hydroxytryptamine; HEK 293 cells, human embryonic kidney 293 cells; α -Bgtx, α -bungarotoxin; DH β E, dihydro- β -erythroidine; GABA, γ -aminobutyric acid.

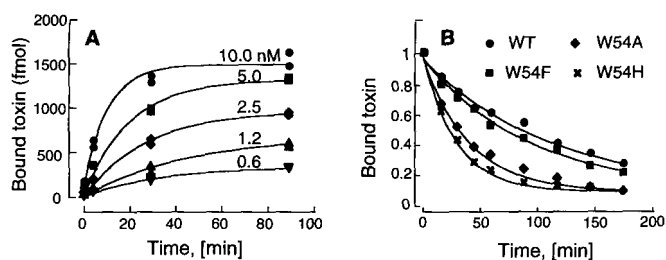


FIG. 1. A, time course of specific ^{125}I - α -Bgtx binding to the wild-type $\alpha 7$ -V201-5HT₃ chimera; the solid lines are the best fits obtained with the theoretical exponential. B, time course of dissociation of ^{125}I - α -Bgtx with the wild-type and mutant $\alpha 7$ -V201-5HT₃ chimeras. For each construct, data from three separate experiments were normalized to maximum values and then averaged. The solid lines are the best fits obtained with the theoretical single exponential.

unlabeled α -Bgtx was then added, and 200 μl of the mixture was filtered every 15 min to measure the decay of bound labeled ^{125}I - α -Bgtx. Nonspecific binding was measured by preincubating membranes with 1 μM unlabeled α -Bgtx.

RESULTS

Kinetics and Equilibrium Binding in Vitro of α -Bgtx and Nicotinic Ligands—Since the kinetics of α -Bgtx association and dissociation to the $\alpha 7$ receptor are slow, an accurate measurement of the dissociation constant of nicotinic ligands using ^{125}I - α -Bgtx as a labeled probe requires kinetic measurements. We thus used the methodology described by Weber and Changeux (25, 26) to analyze the binding of [^3H]- α -Bgtx to membranes from *Torpedo* electric organs. The time courses of association and dissociation of ^{125}I - α -Bgtx with the wild-type chimera expressed in HEK 293 cells can be fitted by single exponentials (Fig. 1). This is consistent with a bimolecular mechanism of binding to an homogeneous class of sites, giving the rate constants and K_d values of ^{125}I - α -Bgtx (Table I). As shown in Fig. 2, ACh, nicotine, and DH β E decrease the initial rates of ^{125}I - α -Bgtx binding on membranes expressing the wild-type chimera. Fitting the competition data by the Hill equation yielded protection constants K_p (with which *Torpedo* ACh receptor-rich membranes were shown to represent actual dissociation constants; Ref. 26) and Hill coefficients. The wild-type chimera displays high affinity (1.1 nM) for ^{125}I - α -Bgtx and micromolar affinities for ACh ($K_p = 83 \mu\text{M}$), nicotine ($K_p = 3.6 \mu\text{M}$) and DH β E ($K_p = 2.5 \mu\text{M}$) (Tables I and II). These values are close to the K_i values measured in binding experiments on $\alpha 7$ homomeric receptors expressed in the *Xenopus* oocytes for α -Bgtx (1.6 nM), ACh (25 μM), and nicotine (0.5 μM) (27) confirming that the wild-type chimera exhibits a pharmacology closely related, if not identical, to that of $\alpha 7$.

Effect of Mutations at Position 54 on the Pharmacological Response to Nicotinic Effectors and on Ligand Binding in Vitro—To investigate the role of the Trp side chain at position 54, we constructed mutants W54F, W54A and W54H. The time courses of the currents evoked by ACh and nicotine on oocytes expressing each mutants were similar to those observed for the wild-type chimera (Fig. 3) (20). However, although both W54F and W54A mutations yielded a 4–6-fold increase in ACh EC_{50} , introduction of a histidine at this position caused a 190-fold increase (Fig. 2, D–F). In contrast, W54F mutation had no significant effect on nicotine elicited currents, while the W54A and W54H mutations resulted, respectively, in 10- and 50-fold increases in nicotine EC_{50} . All mutations caused a comparable 10–20-fold increase in the IC_{50} of the competitive antagonist DH β E. Fig. 3 also shows that the currents elicited by saturating concentrations of ACh and nicotine were identical with W54A and W54F, whereas in W54H ACh-evoked currents were only about 30% of the nicotine-evoked currents.

TABLE I
Kinetic constants and calculated dissociation constants of ^{125}I - α -Bgtx for $\alpha 7$ -V201-5HT₃ wild-type and mutant chimeras
All values are shown as the mean and standard deviation from triplicate experiments.

	K_{on} $\text{min}^{-1}\text{nM}^{-1}$	K_{off} min^{-1}	K_d nM
Wild-type	$(8.2 \pm 3.0)10^{-3}$	$(7.5 \pm 1.6)10^{-3}$	1.1 ± 0.6
W54F	$(10.3 \pm 3.0)10^{-3}$	$(10.0 \pm 2.7)10^{-3}$	1.0 ± 0.5
W54A	$(14.3 \pm 3.5)10^{-3}$	$(20.0 \pm 7.0)10^{-3}$	1.5 ± 0.7
W54H	$(11.0 \pm 1.0)10^{-3}$	$(26.0 \pm 7.2)10^{-3}$	2.4 ± 0.9

All constructs yielded ^{125}I - α -Bgtx binding sites when expressed in HEK 293 cells. Mutations of Trp-54 did not change the k_{on} of ^{125}I - α -Bgtx, and only slightly increased the k_{off} of ^{125}I - α -Bgtx in the case of the W54A and W54F (2- and 3-fold increase, respectively) (Table I). The mutations caused increases in the K_p of ACh, nicotine, and DH β E, with no significant changes of Hill coefficients (Fig. 2, A–C). The increases in K_p values for the three ligands were close to the increases in apparent dissociation constants observed in electrophysiological recordings (Table II). For nicotine, the 1-, 10-, and 60-fold shifts in K_p are almost identical to the 1.5-, 10-, and 50-fold shifts in EC_{50} , for mutants W54F, W54A, and W54H, respectively. For ACh, the shifts in K_p (3.5-, 10-, and 44-fold) correlate reasonably well with the shifts in EC_{50} (4-, 6-, and 190-fold). All mutations caused similar increases in DH β E K_p (3–10-fold) and IC_{50} (10–20-fold). W54H mutation also resulted in a 10-fold increase in *d*-tubocurarine K_p (data not shown).

Involvement of Residues at Positions 52–59 in the Agonist Binding Site—We explored the possible contribution of amino acids neighboring Trp-54 by mutating those present from position 52 to 59 into alanines (Fig. 4). These mutants yielded ^{125}I - α -Bgtx binding sites, except in the case of the mutation of the canonical residue Trp-59, with which no toxin binding nor electrophysiological response were observed. This feature can result from either a poor expression of the receptor or a disruption of the nicotinic binding site. The N52A, I53A, and L55A mutants appeared identical to the wild-type chimera when tested for ACh and nicotine inhibition of ^{125}I - α -Bgtx binding (data not shown). The M57A and Y58A mutations caused small although significant effects on receptor pharmacology, and the Q56A mutation caused a 3- and 20-fold increase in K_p and a 8- and 7- fold increase in EC_{50} , for ACh and nicotine, respectively (Table II).

DISCUSSION

Mutation of Trp-54 causes parallel increases in binding affinity (K_p) and in apparent affinity for channel activation (EC_{50} or IC_{50}) for ACh, nicotine, and DH β E. This is compatible with the simple notion that mutations of Trp-54 specifically alter the nicotinic binding site. In this respect, the different efficacies observed for ACh and nicotine for the W54H mutant can be interpreted either by a channel block of ACh, since the recordings were performed in the presence of a high concentration of agonist, or by the fact that the mutation affects to a different extent the affinity for one of these agonists in the resting and active states of the receptor. In contrast, the kinetics and equilibrium dissociation constants of ^{125}I - α -Bgtx were not significantly altered, showing that modifying the side chain of Trp-54 did not disrupt the α -Bgtx binding site and thus the general tertiary and quaternary structure of the related part of the pentamer. Altogether, these mutagenesis results are consistent with a local alteration of the ACh binding domain. Moreover, the labeling of *Torpedo* γ -Trp-55 and δ -Trp-57 by *d*-tubocurarine supports the notion that Trp-54 contributes directly to ligand binding. Consistent with this view, we find

FIG. 2. A-C, effect of ACh, nicotine, and DH β E on the initial rate of 125 I- α -Bgtx binding to the $\alpha 7$ -V201-5HT $_3$ wild-type and mutant chimeras. Effect of these ligands are expressed as their capacity to slow down the initial rate of specific 125 I- α -Bgtx binding normalized to the maximum values. Each point represents the mean value of three separate experiments. D-F, ACh and nicotine dose-response relationship, and DH β E inhibition of ACh-evoked responses, of $\alpha 7$ -V201-5HT $_3$ wild-type and mutant chimeras. Responses evoked by 3-s application of increasing concentrations of ACh and nicotine were measured in 3-8 cells from several batches held at -100 mV. In DH β E experiments, several (3-7) oocytes were perfused with half-maximally effective dose of ACh and increasing concentrations of DH β E. Peak currents are normalized to the maximum values and averaged. In all cases, lines are the best fits obtained with the empirical Hill equation.

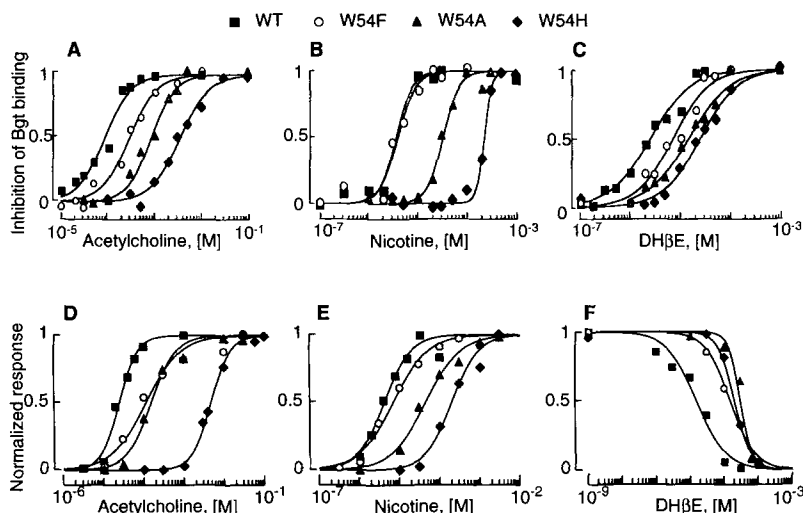


TABLE II
Affinity constants of $\alpha 7$ -V201-5HT $_3$ wild-type and mutant chimeras

The normalized currents and binding data are determined as described in Fig. 2. K_p , EC_{50} , and IC_{50} are presented as the mean and standard deviation. ND, not determined.

	ACh				Nicotine				DH β E			
	K_p	n_H	EC_{50}	n_H	K_p	n_H	EC_{50}	n_H	K_p	n_H	IC_{50}	n_H
	μM		μM		μM		μM		μM		μM	
WT	83 \pm 12	1.6	25 \pm 6.7	2	3.6 \pm 0.3	2.0	4.3 \pm 0.7	1.3	2.5 \pm 0.3	1.1	1.7 \pm 1.0	1
W54F	280 \pm 25	1.5	110 \pm 18	1	3.8 \pm 0.4	1.9	7.7 \pm 1.9	1	7.0 \pm 1.2	1.2	15 \pm 5.4	1.2
W54A	840 \pm 85	1.7	160 \pm 35	1.5	32 \pm 3.3	1.9	45 \pm 10	1	14 \pm 1.2	1.0	34 \pm 0.5	2.5
W54H	3500 \pm 350	1.4	4700 \pm 470	1.8	220 \pm 64	2.1	200 \pm 11	1.4	24 \pm 1.6	1.2	21 \pm 2.5	2
Q56A	230 \pm 42	3.4	200 \pm 10	1.4	72 \pm 3.5	1.8	30 \pm 0.6	1.4	ND	ND	ND	ND
M57A	27 \pm 7.2	2.4	6.4 \pm 0.3	2	4.5 \pm 0.8	1.8	2.1 \pm 0.3	1.5	ND	ND	ND	ND
Y58A	180 \pm 18	1.6	25 \pm 0.5	2	15 \pm 0.7	1.9	8.3 \pm 0.7	1.5	ND	ND	ND	ND

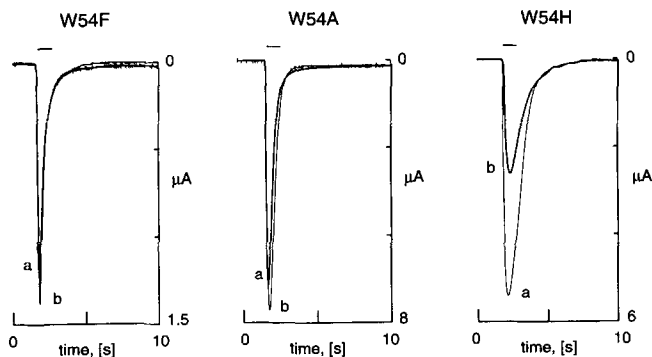


FIG. 3. Current evoked by saturating concentrations of nicotine (3 mM, trace a) and ACh (30 mM, trace b) in the *Xenopus* oocytes expressing $\alpha 7$ -V201-5HT $_3$, W54F, W54A, and W54H chimeras.

that the presence of electronegative aromatic residues (Trp and Phe as compared to Ala) favors agonist binding, while the introduction of a positive charge with a histidine causes a dramatic decrease in agonist affinity, suggesting a direct involvement of Trp-54 in the quaternary ammonium binding pocket of nicotinic ligands.

It is noteworthy that this residue is highly conserved in all subunits expected to contribute a complementary binding component: in muscle γ and δ subunits and neuronal $\beta 2$, $\beta 4$, $\alpha 8$, and $\alpha 9$ subunits. However, it is absent in the corresponding regions of muscle α and β subunits (Fig. 4). A tryptophan, however, is present at a homologous position in the neuronal $\alpha 2$ -4 subunits, which are not expected to contribute a complementary component. A similar feature is seen for residues composing loops A and B, which, while implicated in the prin-

		50	54	56	61
$\alpha 7$	chicken	T	T	N	I
$\alpha 1$	Torpedo	E	T	N	V
$\beta 1$	Torpedo	T	T	N	V
$\gamma 1$	Torpedo	T	T	N	V
$\delta 1$	Torpedo	T	S	N	V
$\alpha 2$	rat	T	T	N	V
$\alpha 3$	rat	E	T	N	L
$\alpha 4$	rat	T	T	N	V
$\beta 2$	rat	T	T	N	V
$\beta 4$	rat	T	T	S	I
$\alpha 1$ GABAA	rat	T	I	D	V
5HT $_3$	rat	T	T	Y	I
$\alpha 1$ Glycine	rat	R	V	N	I

FIG. 4. Comparison of the amino acid sequences of some nicotinic, GABA $_A$, 5HT $_3$, and glycine receptors (reviewed in Ref. 31) containing Trp-54 of chicken $\alpha 7$ nicotinic receptor (15).

cipal binding component of muscle and neuronal receptors, are also conserved in $\beta 2$ and $\beta 4$ subunits. This observation may point to a particular function of these neuronal subunits.

Mutations of residue Gln-56, and to a smaller extent that of residues Met-57 and Tyr-58, affect the apparent affinities of ACh and nicotine. These positions are occupied by different residues in *Torpedo* γ and δ subunits, and in β neuronal subunits (Fig. 4). This suggests that amino acids located at these positions could be involved in the non-equivalence of the two *Torpedo* receptor binding sites (13, 28) and/or in the pharmacological diversity of neuronal receptors distinct from the non- α subunits of muscle receptor.

Labeling and mutagenesis experiments have shown that residue Phe-65 of the $\alpha 1$ subunit of GABA $_A$ receptors contributes to the binding site of GABA (29, 30). This residue is homologous

to $\alpha 7$ Trp-54, and α subunits of GABA_A receptors appear to be functionally homologous to nicotinic non- α subunits (10). The notion that residues homologous to $\alpha 7$ Trp-54 contribute to a complementary component of the agonist binding site in addition to its principal one may thus be extended to other members of the superfamily of ligand-gated ion channels.

REFERENCES

- Oswald, R. E., and Changeux, J. P. (1982) *FEBS Lett.* **139**, 225–229
- Blount, P., and Merlie, J. P. (1989) *Neuron* **3**, 349–357
- Pedersen, S. E., and Cohen, J. B. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 2785–2789
- Galzi, J. L., Revah, F., Bouet, F., Ménez, A., Goeldner, M., Hirth, C., and Changeux, J. P. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 5051–5055
- Czajkowski, C., and Karlin, A. (1991) *J. Biol. Chem.* **266**, 22603–22612
- Kreienkamp, H. J., Maeda, R. K., Sine, S. M., and Taylor, P. (1995) *Neuron*, in press
- Couturier, S., Erkman, L., Valera, S., Rungger, D., Bertrand, S., Boulter, J., Ballivet, M., and Bertrand, D. (1990) *J. Biol. Chem.* **265**, 17560–17567
- Luetje, C. W., and Patrick, J. (1991) *J. Neurosci.* **11**, 837–845
- Hussy, N., Ballivet, M., and Bertrand, D. (1994) *J. Neurophysiol.* **72**, 1317–1326
- Galzi, J. L., and Changeux, J. P. (1994) *Curr. Opin. Struct. Biol.* **4**, 554–565
- Cohen, J. B., Blanton, M. P., Chiara, D. C., Sharp, S. D., and White, B. H. (1992) *J. Cell. Biochem.* **16E**, 217–T003
- Czajkowski, C., Kaufman, C., and Karlin, A. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 6285–6289
- Sine, S. M. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 9436–9440
- O'Leary, M. E., Filatov, G. N., and White, M. M. (1994) *Am. J. Physiol.* **266**, C648–C653
- Couturier, S., Bertrand, D., Matter, J. M., Hernandez, M. C., Bertrand, S., Millar, N., Valera, S., Barkas, T., and Ballivet, M. (1990) *Neuron* **5**, 845–856
- Schoepfer, R., Conroy, W. G., Whiting, P., Gore, M., and Lindstrom, J. (1990) *Neuron* **5**, 35–48
- Séguéla, P., Wadiche, J., Dineley-Miller, K., Dani, J. A., and Patrick, J. W. (1993) *J. Neurosci.* **13**, 596–604
- Elgoyhen, A. B., Johnson, D. S., Boulter, J., Vetter, D. E., and Heinemann, S. (1994) *Cell* **79**, 705–715
- Galzi, J. L., Bertrand, D., Devillers-Thiéry, A., Revah, F., Bertrand, S., and Changeux, J. P. (1991) *FEBS Lett.* **294**, 198–202
- Eiselé, J. L., Bertrand, S., Galzi, J. L., Devillers-Thiéry, A., Changeux, J. P., and Bertrand, D. (1993) *Nature* **366**, 479–483
- Swick, A. G., Janicot, M., Cheneval-Kastelic, T., McLenithan, J. C., and Lane, M. D. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 1812–1816
- Bertrand, D., Cooper, E., Valera, S., Rungger, D., and Ballivet, M. (1991) *Methods Neurosci.* **4**, 174–196
- Chen, C., and Okayama, H. (1987) *Mol. Cell. Biol.* **7**, 2745–2752
- Thom, D., Powell, A., Lloyd, C., and Rees, D. (1977) *Biochem. J.* **168**, 187–194
- Weber, M., and Changeux, J. P. (1974) *Mol. Pharmacol.* **10**, 1–14
- Weber, M., and Changeux, J. P. (1974) *Mol. Pharmacol.* **10**, 13–34
- Anand, R., Peng, X., and Lindstrom, J. (1993) *FEBS Lett.* **327**, 241–246
- Neubig, R. R., and Cohen, J. B. (1979) *Biochemistry* **18**, 5464–5475
- Smith, G. B., and Olsen, R. W. (1994) *J. Biol. Chem.* **269**, 20380–20387
- Sigel, E., Baur, R., Kellenberger, S., and Malherbe, P. (1992) *EMBO J.* **11**, 2017–2023
- Cockcroft, V. B., Osguthorpe, D. J., Barnard, E. A., Friday, A. E., and Lunt, G. G. (1992) *Mol. Neurobiol.* **4**, 129–169