



Molecular Neurobiology: A Practical Course

INSTITUTO DE INVESTIGACIONES EN
INGENIERIA GENETICA Y
BIOLOGIA MOLECULAR
CONICET - UBA

Buenos Aires, Argentina
16 February - 1 March, 1998

Molecular Neurobiology: A Practical Course is designed to introduce neuroscientists at all levels to the basic concepts of molecular biology. With the recent emphasis on molecular approaches to fundamental questions in neurobiology, many graduate students, post-doctoral fellows and established scientists have expressed a desire to incorporate such methodologies in their ongoing research programs.

The course curriculum will include an extensive and up-to-date set of laboratory exercises, daily lectures on theoretical and practical aspects of molecular biological techniques, and a series of evening lectures given by leading experimentalists in the field of molecular neuroscience. These evening talks will feature the molecular neurobiology of both invertebrate and vertebrate systems and will cover a broad range of subjects including neurotransmitter receptors and ion channels, synaptic physiology, nerve cell growth and differentiation, and development of the central and peripheral nervous systems.

The laboratory exercises will emphasize fundamental techniques and will be presented as modules consisting of several individual but related protocols. Each laboratory exercise will be preceded by a lecture covering the purpose of the experiment, an in-depth presentation of the concepts, background and theory which underlie the experiment, as well as provide the participants with a step-by-step analysis of the actual procedure. In this two week course, the laboratory portion will include the following: isolation and characterization of plasmid, phagemid and viral DNAs; plating and screening of expression libraries; gel electrophoresis of nucleic acids; use of DNA modification and restriction enzymes; Southern and



northern blotting; hybridization of RNA and DNA using radiolabeled DNA probes; ligations, transformations and subcloning; oligonucleotide design and utilization; DNA sequencing, computer-assisted sequence analysis and database searches; polymerase chain reaction methods and applications; *in vitro* transcription for RNAase protection, *in situ* hybridization and *Xenopus* oocyte injections; evaluation of subtractive hybridization and site-directed mutagenesis protocols; genetic engineering of fusion proteins; expression of cloned genes in bacteria and eucaryotic cells.

Instructors

Belén Elgoyhen, Ph.D.

Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, HHMI
CONICET - UBA
Buenos Aires, Argentina

Marcelo Rubinstein, Ph.D.

Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, HHMI
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Buenos Aires, Argentina

Cary Lai, Ph.D.

Department of Neuropharmacology
The Scripps Research Institute
La Jolla, California

Anne-Marie Quinn

Department of Epidemiology and Public Health
Yale University Medical School
New Haven, Connecticut

Haeyoung Kong, Ph.D.

The Skirball Institute
New York University Medical Center
New York, New York

Jim Boulter, Ph.D.

Department of Psychiatry and Biobehavioral Sciences
University of California
Los Angeles, California

Invited Speakers (partial list)



Bernhard Bettler, Ph.D.
Novartis Pharma, Inc.
Basel, Switzerland


Moses Chao, Ph.D.
The Skirball Institute
New York University Medical Center
New York, New York


Gabriel Corfas, Ph.D.
Division of Neuroscience
Children's Hospital and Harvard Medical School
Boston, Massachusetts

David Julius, Ph.D.
Department of Pharmacology
University of California
San Francisco, California

Greg Lemke, Ph.D., Director
Molecular Neurobiology Laboratory
The Salk Institute for Biological Studies
La Jolla, California

Gerry Weinmaster, Ph.D.
Department of Biological Chemistry
University of California
Los Angeles, California


Dr. A. B. Elgayhen


Dr. H. Rubinstein



Neurobiología Molecular

Curso teórico-práctico sobre conceptos básicos de biología molecular enfocado a científicos en el área de la Neurociencia.

El curso comprenderá una serie de técnicas de laboratorio, clases teóricas y seminarios dictados por investigadores de renombre internacional en el área de la Neurobiología Molecular.

Las clases teóricas y prácticas incluirán lo siguiente:

Purificación y caracterización de ADN de plásmidos, fagos y ADN genómico.

Electroforesis de ácidos nucleicos

Uso de enzimas de restricción y modificación

Southern y Northern blot

Hibridización de ARN y ADN con sondas marcadas

Ligación, transformación y subclonado

Plaqueo y screening de bibliotecas genómicas y de ADN copia

Diseño y utilización de oligonucleótidos

Secuenciamiento de ADN

Análisis de secuencias por computación y búsqueda en bases de datos

PCR y sus aplicaciones

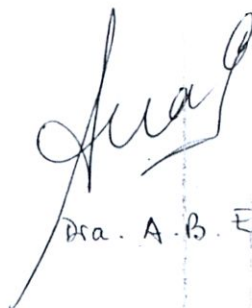
Transcripción in vitro para hibridización in situ e inyección en ovocitos

Evaluación de protocolos de mutagénesis dirigida

Construcción de proteínas de fusión

Expresión de genes clonados en bacterias y células eucariotes

Los seminarios incluirán temas relacionados con el uso de la biología molecular en el estudio de receptores para neurotransmisores, canales iónicos, fisiología sináptica, crecimiento y diferenciación neuronal y desarrollo del sistema nervioso central y periférico.


Dra. A.B. Elgayhen


Dr. M. Rubinshtein

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.

Photocopy this page or follow this format for each person.



NAME James R. Boulter, Ph.D.		POSITION TITLE Associate Professor	
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
UC Santa Barbara, CA.	B.A.	1969	Biology
UC Santa Barbara, CA.	M.A.	1971	Biology
UC Santa Barbara, CA.	Ph.D.	1974	Molecular Biology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Professional Experience

- 1974 - 1976 Postdoctoral Fellow, Dept of Biochemistry, Dartmouth Medical School, Hanover, N.H.
- 1976 - 1978 Research Associate, Dept of Neurobiology, The Salk Institute, La Jolla, Ca.
- 1979 - 1982 Staff Research Associate, Depts of Pathology & Chemistry, UCSD, La Jolla, Ca.
- 1982 - 1983 Research Scientist, Dept of Pathology & Lab. Medicine, Univ. of Texas Medical School, Houston, Tx.
- 1983 - 1990 Senior Research Scientist, Molecular Neurobiology Laboratory, The Salk Institute, La Jolla., Ca.
- 1990 - 1995 Senior Staff Scientist, Molecular Neurobiology Laboratory, The Salk Institute, La Jolla., Ca.
- 1995- Associate Professor, Department of Psychiatry and Bibehavioral Sciences, UCLA, Los Angeles, Ca.

Honors and Awards

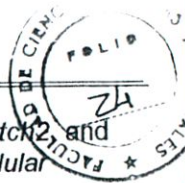
- 1974-1977 Individual NRSA, NINCDS, NIH

Selected Publications (from 1984 on)

- Goldman, D., Boulter, J., Heinemann, S. and Patrick, J. (1984) Muscle denervation increases the levels of mRNA coding for the acetylcholine receptor alpha-subunit. *J. Neurosci.* 5:2553-2558.
- Boulter, J., Evans, K., Ballivet, M., Goldman, D., Luyten, W., Martin, G., Mason, P., Stegelin, S., Ueno, S., Heinemann, S., and Patrick, J. (1984) Isolation of a clone coding for the alpha subunit of a mouse acetylcholine receptor. *J. Neurosci* 5:2545-2552.
- Boulter, J., Evans, K., Martin, G., Mason, P., Stengel, S., Goldman, D., Heinemann, S., and Patrick J. (1985) Isolation and sequence of cDNA clones coding for the precursor to the gamma subunit of mouse muscle acetylcholine receptor. *J Neurosci Res* 16:37-49.
- 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., Evans, K., Evans, S., Gardner, P., Goldman, D., Heinemann, S., Luyten, W., Martin, G., Mason, P., Patrick, J. and Treco, D. (1986) Molecular cloning of receptors for acetylcholine. In: *Molecular Aspects of Neurobiology* (eds. R. Levi Montalcini, et al.). Springer-Verlag, Berlin, p. 60-65.
- Heinemann, S. Asouline, G., Ballivet, M., Boulter, J., Connolly, J., Deneris, E., Evans, K., Evans, S., Forrest, J., Gardner, P., Goldman, D., Kochhar, A., Luyten, W., Mason, P., Treco, D., Wada, K. and Patrick J. (1986) Molecular biology of the neural and muscle nicotinic acetylcholine receptors. In: *Molecular Neurobiology: Recombinant DNA Approaches* (eds. J. Patrick & S. Heinemann) Plenum Press, New York, p 45-96.
- Heinemann, S., Goldman, D., Boulter, J., and Patrick, J. (1986) Molecular biology of the muscle and neural acetylcholine receptors. In: *Nicotinic Acetylcholine Receptor Structure and Function*. NATO Advanced Science Inst. Series (ed. A. Maelicke), Springer-Verlag Publishers, Berlin, p 359-388.



- Goldman, D., Evans, S., Boulter, J., Patrick J. and Heinemann, S. (1986) Neural regulation of acetylcholine receptor gene expression. In: *Myasthenia Gravis: Biology and Treatment*. Annals of the New York Academy of Science, Vo. 505 (ed. D. B. Drachman), New York, p. 286-300.
- Patrick, J., Boulter, J., Goldman, D., Gardner, P. and Heinemann, S. (1986) Molecular biology of nicotinic acetylcholine receptors. In: *Myasthenia Gravis: Biology and Treatment*. Ann N Y Acad Sci, Vo. 505 (ed. D. B. Drachman), New York, p. 194-207.
- Boulter, J., Connolly, J., Deneris, E., Goldman, D., Heinemann, S., and Patrick, J. (1987) Functional expression of two neuronal nicotinic acetylcholine receptors from cDNA clones identifies a gene family. *Proc Natl Acad Sci U.S.A.* 84:7763-7767.
- Deneris, E.S., Connolly, J., Boulter, J., Patrick, J., and Heinemann, S. (1988) Primary structure and expression of beta2: A novel subunit of neuronal nicotinic acetylcholine receptors. *Neuron* 1: 45-54.
- Wada, K., Ballivet, M., Boulter, J., Connolly, J., Wada, E., Deneris, E., Swanson, L.W., Heinemann, S., and Patrick, J. (1988) Functional expression from cDNA clones of a new pharmacological subtype of brain nicotinic acetylcholine receptor. *Science* 240:330-334.
- Deneris, E.S., Boulter, J., Swanson, L.W., 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.
- Deneris, E.S., Boulter, J., Connolly, J., Wada, E., Wada, K., Goldman, D., Swanson, L., Patrick J., and Heinemann, S. (1989) Genes encoding neuronal nicotinic acetylcholine receptors. *Clinical Chemistry* 35:731-737.
- Wada, E., Wada, K., Boulter, J., Deneris, E., Heinemann, S., Patrick J., and Swanson, L. (1989) Distribution of alpha2, alpha3, alpha4, and beta2 neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat. *J Comp Neurol* 284:314-335.
- Tappe, R.L., Boulter, J., Patrick J., and Heinemann, S. (1989) Single channel currents of rat neuronal nicotinic acetylcholine receptors expressed in *Xenopus laevis* oocytes. *Neuron* 3:589-596.
- Boulter, J and Gardner, P.D. (1989) Practical approaches to molecular cloning of nicotinic acetylcholine receptor genes. In: *Methods in Neurosciences*, Vol 1: Genetic Probes (ed. P.M. Conn), Academic Press, Orlando, FL., p 328-363.
- Boulter, J., O'Shea-Greenfield, A., Duvoisin, R.M., Connolly, J.G., Wada, E., Jensen, A., Gardner, P.D., Ballivet, M., Deneris, E.S., McKinnon, D., Heinemann, S., and Patrick, J. (1990) Alpha3, alpha5 and beta 4: Three members of the rat neuronal nicotinic acetylcholine receptor-related gene family form a gene cluster. *J. Biol Chem* 365:4472-4482.
- Boulter, J., Hollman, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C., and Heinemann, S. (1990) Molecular cloning and functional expression of glutamate receptor subunit genes. *Science* 249:1033-1037.
- Couturier, S., Erkman, L., Valera, S., Rungger, D., Bertrand, S.D., Boulter, J., Ballivet, M., and Bertrand, D. (1990) Alpha5, alpha3, and non-alpha3: Three clustered avian genes encoding neuronal nicotinic acetylcholine receptor-related subunits. *J. Biol Chem* 265:17560-17567.
- Bettler, B., Boulter, J., Jermans-Borgmeyer, I., O'Shea-Greenfield, A., Deneris, E.S., Moll, C., Borgmeyer, U., Hollmann, M. and Heinemann, S. (1990) Cloning of a new glutamate receptor subunit, GluR5: Expression in the nervous system during development. *Neuron* 5:583-595.
- Heinemann, S., Bettler, B., Boulter, J., Deneris, E., Gasic, G., Hartley, M., Hollman, M., Hughes, T., O'Shea-Greenfield, A., and Rogers, S. (1991) The glutamate receptors: genes, structure and expression. In: *Glutamate, Cell Death and Memory*, Research and Perspectives in Neurosciences (eds. P. Ascher, D.W., Choi and Y. Christen), Springer-Verlag Publishers, New York, P. 12-29.
- Boulter, J., Bettler, B., Dingledine, R., Duvoisin, R., Egebjerg, J., Gasic, G., Hartley, M., Hermans-Borgmeyer, I., Hollman, M., Hughes, T., Hume, R., Moll, C., Rogers, S., and Heinemann, S. (1992) Molecular biology of the glutamate receptors. In: *Excitatory Amino Acids*, Fidia Research Fdn. Symposium Series, Vol. 9 (ed. R. Simon), Thieme Medical Publishing, Inc., New York, p. 9-14.
- Connolly, J., Boulter, J. and Heinemann, S. (1992) Alpha4-2/beta2 and other nicotinic acetylcholine receptor subtypes as targets of psychoactive and addictive drgs. *British J. Pharmacology* 105:657-666.
- Hollmann, M., Boulter, J., Maron, C., Beasley, L., Sullivan, J., Pecht, G. and Heinemann, S. (1993) Zinc potentiates agonist-induced currents at certain splice variants of the NMDA receptor. *Neuron* 10:943-954.
- Elgoyhen, A.B., Johnson, D.S., Boulter, J., Vetter, D.E., and Heinemann, S. (1994) Alpha9: An acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. *Cell* 79: 705-715.
- Lindsell, C.E., Shawber, C.J., Boulter, J. and Weinmaster, G.A. (1994) *Jagged*: A mammalian ligand that activates *Notch*. *Cell* 80: 909-917.



- Lindsell, C.E., Boulter, J., di Sibio, G., and Weinmaster, G. (1996) Expression of *Jagged*, *Delta1*, *Notch1*, *Notch2*, and *Notch3* identified ligand-receptor pairs that may function in vertebrate neural development. *Molecular & Cellular Neuroscience*, 8:14-27.
- Shawber, C.J., Boulter, J., Lindsell, C.E., and Weinmaster, G. (1996) *Jagged2*: A *Serrate*-like gene expressed during rat embryogenesis. *Developmental Biology*, 180:370-376.
- Boulter, J., and Boyer, C. (1997) Expression cloning using oocytes from *Xenopus laevis*. In: Current protocols in neuroscience (eds. D. Sibley and V. Chanda), John Wiley & Sons, Inc., New York, N.Y., *in press*.



BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME Cary H.C. Lai	POSITION TITLE Assistant Professor
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EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
California Institute of Technology, Pasadena, CA	B.S.	1976	Biology
University of California, San Diego, CA	Ph.D.	1988	Biology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Research Experience:

August 1992-present: Assistant professor, Department of Neuropharmacology
The Scripps Research Institute

January 1989-August 1992: Postdoctoral fellow in the laboratory of Dr. Greg E. Lemke,
The Salk Institute.

Teaching Experience:

July 1991-1992 assistant instructor, Neural Genes Course, Cold Spring Harbor Laboratory

November 1992 instructor, Cloning of Neural Genes Course, UNAM, Mexico City

July 1993-1997 co-instructor, Neural Genes Course, Cold Spring Harbor Laboratory

Awards and Scholarships:

1977-1983 NIH Fellowship

1989-1991 National Multiple Sclerosis Society Postdoctoral Fellowship

Selected Publications:

Klein, W.H., Thomas, T.L., Lai, C., Scheller, R.H., Britten, R.J., and Davidson, E.H. (1978). Characteristics of individual repetitive sequence families in the sea urchin genome studied with cloned repeats. *Cell* 14, 889-900.

Milner, R.J., Bloom, F.E., Lai, C., Lerner, R.A., and Sutcliffe, J.G. (1984). Brain specific genes have ID sequences in their introns. *Proc. Natl. Acad. Sci. (USA)* 81, 713-717.

Milner, R.J., Lai, C., Nave, K.-A., Lenoir, D., Ogata, J., Sutcliffe, J.G., (1985). Nucleotide sequences of two mRNAs for rat brain proteolipid protein. *Cell* 42, 931-939.



- Nave, K.-A., Lai, C., Bloom, F.E., and Milner, R.J. (1986). Jimpy mutant mouse: A 74 base deletion in the mRNA for myelin proteolipid protein (PLP) and evidence for a primary defect in RNA splicing. *Proc. Natl. Acad. Sci. (USA)* 83, 9264-9268.
- Nave, K.-A., Kiel, M., Lai, C., Bloom, F.E., and Milner, R.J. (1987). The DM-20 protein of CNS myelin is generated by alternative splicing of proteolipid protein (PLP) gene transcripts. *Proc. Natl. Acad. Sci. (USA)* 84, 5665-5669.
- Lai, C., Brow, M.A., Nave, K.-A., Noronha, A.B., Quarles, R.H., Bloom, F.E., Milner, R.J., and Sutcliffe, J.G. (1987). Two forms of 1B236/Myelin-Associated Glycoprotein (MAG), a cell adhesion molecule for postnatal neural development, are produced by alternative splicing. *Proc. Natl. Acad. Sci. (USA)* 84, 4337-4341.
- Lai, C. and Lemke, G. (1991). An extended family of protein-tyrosine kinase genes differentially expressed in the vertebrate nervous system. *Neuron* 6, 691-704.
- Polvi, A., Armstrong, E., Lai, C., Lemke, G., Huebner, K., Spritz, R.A., Guida, L.C., Nicholls, R.D., and Alitalo, K. (1993). The human TYRO3 gene and pseudogene are located in chromosome 15q14-q25. *Gene* 134, 289-293.
- Easty, D.J., Ganz, S.E., Farr, C.J., Lai, C., Herlyn, M., and Bennett, D.C. (1993). Novel and known protein-tyrosine kinases and their abnormal expression in human melanoma. *Journal of Investigative Dermatology*, 101, 679-684.
- Lai, C. and Lemke, G. (1994). Structure and expression of the Tyro-10 receptor tyrosine kinase. *Oncogene* 9, 877-883.
- Lai, C., Gore, M., and Lemke, G. (1994). Structure, expression, and activity of Tyro 3, a neural adhesion-related receptor tyrosine kinase. *Oncogene* 9, 2567-2578.
- Edelhoff, S., Lai, C., and Disteche, C.M. (1995). Mapping of the receptor protein-tyrosine kinase 10 to human chromosome 1q21-q23 and mouse chromosome 1H1-5 by fluorescence in situ hybridization. *Genomics* 25, 337-339.
- Stitt, T.N., Conn, G., Gore, M., Lai, C., Bruno, J., Radziejewski, C., Mattsson, K., Fisher, J., Gies, D.R., Jones, P.F., Masiakowski, P., Ryan, T.E., Tobkes, N.J., Chen, D.H., DiStefano, P.S., Long, G.L., Basilico, C., Goldfarb, M.P., Lemke, G., Glass, D.J., and Yancopoulos, G.D. (1995). The anticoagulation factor protein S and its relative, gas6, are ligands for the tyro-3/axl family of receptor tyrosine kinases. *Cell* 80, 661-670.
- Gassmann, M., Casagrande, F., Orioli, D., Simon, H., Lai, C., Klein, R., and Lemke, G. (1995). Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature* 378, 390-394.
- Zhu, X., Lai, C., Thomas, S., and Burden, S.J. (1995). Neuregulin receptors, erbB3 and erbB4, are localized at neuromuscular synapses. *EMBO J.* 14, 5842-5848.
- Kilpatrick, T.J., Brown, A., Lai, C., Gassmann, M., Goulding, M., and Lemke, G. (1996). Expression of the Tyro-4/Mek4/Cek4 gene specifically marks a subset of embryonic motor neurons and their muscle targets. *Mol. Cell. Neurosci.* 7, 62-74.



Carraway III, K. L. , Weber, J. L., Unger, M. J., Ledesma, J., Yu, N., Gassmann, M. and Lai, C. (1997). Neuregulin-2, a new ligand of ErbB3/ErbB4-receptor tyrosine kinases. Nature 387, 512-516.