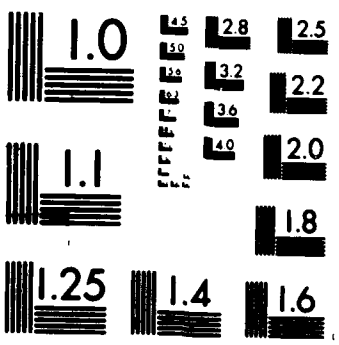
AD-A192 227 AN INVESTIGATION INTO THE EFFECTS OF PEPTIDE NEUROTRANSMITTERS AND INTRAC (U) AT AND T BELL LABS MURRAY HILL NJ J A COONOR 04 FEB 88 AFOSR-TR-86-0258 1/1

UNCLASSIFIED F49620-85-C-0009 F/G 6/1 NL


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3
7.1

SEC

AD-A192 227 ORT DOCUMENTATION PAGE

DTIC FILE COPY

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S): AFOSR-TR- 88-0258
6a. NAME OF PERFORMING ORGANIZATION: AT&T Bell Laboratories; 6b. OFFICE SYMBOL (If applicable):	7a. NAME OF MONITORING ORGANIZATION: Air Force Office of Scientific Research
6c. ADDRESS (City, State, and ZIP Code): 600 Mountain Avenue, Murray Hill, NJ 07974	7b. ADDRESS (City, State, and ZIP Code): Building 410, Bolling AFB, DC 20332-6448
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: AFOSR; 8b. OFFICE SYMBOL (If applicable): NL	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: F49620-85-C-0009
8c. ADDRESS (City, State, and ZIP Code): Building 410, Bolling AFB, DC 20332	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 61102F; PROJECT NO. 2312; TASK NO. K2; WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification) An investigation into the effects of peptide neurotransmitters and intracellular second messengers in rat central neurons in culture.

12. PERSONAL AUTHOR(S): John A. Connor

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 10-15-86 TO 10-15-87	2/4/88	

DTIC SELECTED MAR 10 1988 S D

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): intracellular calcium; second messengers; cerebellum; diencephalon; peptides; cAMP; GABA; glutamate; histamine; presynaptic inhibition; Inositol 1,4,5-triphosphate; Gamma Aminobutyric acid.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Research has been carried out in the three areas outlined below. Calcium Central Nervous System

I. Excitatory amino acid responses in adult mammalian hippocampal neurons. This topic has become the focus of extensive research due to its possible relationship to short term memory. We have made the first measurements of calcium ion changes in these cells using fluorescence imaging techniques, and have demonstrated a long lasting response involving Ca^{2+} flux induced after conditioning input to the cells. II. The developmental time course in culture of ion channels and transmitter sensitivity in cerebellar Purkinje neurons. We have demonstrated differential soma-dendritic responses to depolarization and the neurotransmitter glutamate in these neurons. Parallel immunocytochemical experiments using a number of antibodies have demonstrated the development of antigens in culture in comparison to in vivo development. III. Neuromodulation of synaptic efficacy in an invertebrate preparation that may be a useful model system for the actions of histamine in the CNS. Keywords:

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Dr. William O. Berry	22b. TELEPHONE (Include Area Code): (202) 767-5021 — 22c. OFFICE SYMBOL: NL

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE: Unclassified

SUMMARY

Significant progress has been made in the three areas outlined below.

1. Excitatory amino acid responses in adult mammalian hippocampal neurons that are a possible basis for short term memory. Measurements have incorporated fluorescence imaging for calcium ion changes and electrophysiological techniques.

2. The developmental time course in culture of ion channels and transmitter sensitivity in cerebellar Purkinje neurons.

3. Neuromodulation of synaptic efficacy in an invertebrate preparation that may be a useful model system for the actions of histamine in the CNS.

RESEARCH OBJECTIVES

The primary objective of this research is to elucidate how endogenoun neuroactive substances, neurotransmitters, neuromodulators, affect brain function. Nerve cells are the functional units of the brain, and changes in neuronal activity are ultimately expressed through modifications in membrane conductances and levels of intracellular messengers such as calcium ion. We have therefore focused our attention on examining the mechanisms by which neuroactive substances alter these conductances and messenger levels.

STATUS OF RESEARCH

A. Action of excitatory amino acids on Ca1 neurons from mammalian hippocampus.

Spatially resolved measurements of intracellular free calcium, [Ca^{2+}], and the changes produced by excitatory amino acids, have been made for the first time in neurons isolated from adult mammalian brain. We report the induction of extremely long lasting (minutes) gradients of Ca^{2+} in the apical dendrites of hippocampal CA1 neurons following brief (1-3 s), local application of either glutamate or NMDA. These gradients reflect the continuous flux of Ca^{2+} into the dendrite. The persisting gradients, but not the immediate, transient response to the agonists were abolished by pretreatment with the protein kinase-C inhibitor, sphingosine. Expression of the long lasting Ca^{2+} gradients generally required priming, or conditioning, exposures to the excitatory agonist. That is, the initial application triggered a transient Ca elevation that recovered within 1 minute; whereas subsequent, identical applications induced the longer responses. Our findings demonstrate that successive applications of glutamate and NMDA elicit prolonged rises in intracellular Ca^{2+} that persist beyond the period of direct agonist-receptor interaction, and possibly reflect the activation of dormant Ca channels by the stimulus paradigm.

This coupling between NMDA receptor activation and long-lasting intracellular Ca^{2+} elevation could underly certain use-dependent modifications of synaptic responses in hippocampal CA1 neurons. Most importantly, we believe that the events that we have described are at least part of the mechanism underlying associative long term potentiation in the hippocampus, a phenomenon widely studied as a model for short term memory. This work was a collaborative study with Robert Wong (Columbia U.) and Wytse Wadman (U. Amsterdam).

neurons.

Culture conditions have been worked out whereby cerebellar Purkinje neurons are maintained for periods of 60-80 days and develop extensive dendritic tree structures. Identity and morphology of these cells have been established by antibody staining techniques applied to both tissue cultures and to neurons isolated acutely from the brains of animals at various ages. Using electrical recording with whole-cell patch electrodes we have determined the developmental timing of the appearence of membrane Na and K channels as well as neurotransmitter activated channels. We have made the first thorough comparison of these timings between in vivo and in vitro development.

Using the imaging techniques developed in this lab in 1984, we have made measurements of internal Ca^{2+} changes in these cells during electrical and chemical stimulation. We have shown that the neurons, even in culture conditions, localize Ca channels on the dendrite membrane.

C. Invertebrate neuron model for studying synaptic plasticity.

Using the methods of Schacher et al. [1], we cultured identified neurons from the marine mollusc *Aplysia*, in which different forms of synaptic plasticity have been observed [2]. We reconstructed connections between neurons by co-culturing identified neurons. Using physiological criteria, we established that these connections were similar to those made by the neurons *in vivo*. We then constructed a hybrid circuit in order to study a particular form of synaptic plasticity in culture. Two neurons which are synaptically connected *in vivo* (C2 and C4) were co-cultured with a third neuron

(L10), which does not form synaptic connections with them *in vivo*. We predicted, however, that L10 would from connections with the other two neurons in culture because it shares two properties with a neuron (B18) that is synaptically connected to C2 and C4 *in vivo*: it is cholinergic, and is inhibited by histamine. since B18 and L10 are part of different circuits in which presynaptic inhibition is observed *in vivo* [3,4], we predicted that this form of synaptic plasticity would be observed in our cultured hybrid circuit.

We established that presynaptic inhibition occurred in the hybrid circuit, i.e., the connection from L10 to C4 could be inhibited by neuron C2's action on the presynaptic neuron, L10. Firing C2, or applying histamine, hyperpolarized L10 and reduced the size of the postsynaptic potential (PSP) which L10 induced in C4. Hyperpolarization of L10, which is known to reduce the release of its transmitter *in vivo*, also reduced the size of the PSP it induced in C4 in culture. Cimetidine, a drug which blocks histaminergic responses in *Aplysia* [5], blocked the hyperpolarization that C2 or histamine induced in L10, and also prevented histamine from reducing the PSP which L10 induces in C4.

Our studies have demonstrated that a form of synaptic plasticity, presynaptic inhibition, can be reconstructed *in vitro*. In addition, extensive work on L10 *in vivo* suggests that the presynaptic inhibitory actions of histamine are due to reductions in the intracellular levels of calcium in L10 [5]. Since one can directly visualize the processes of L10 in culture, we can use this model system and our techniques for observing intracellular calcium levels to study more directly the role of calcium in this form of synaptic plasticity.

[1] Schacher, S. and Proshansky, E. Neurite regeneration by *Aplysia* neurons in dissociated cell culture: Modulation by *Aplysia* hemolymph and the presence of the initial axonal segment. J. Neurosci. 3:2403-2413 (1983).

[2] Kretz, R., Shapiro, E., Connor, J. and Kandel, E. R. Posttetanic potentiation, presynaptic inhibition, and the modulation of the free calcium level in the presynaptic terminals. Exp. Br. Res. Suppl. 9:240-283 (1984).

[3] Chiel, H. J., Kupfermann, I., and Weiss, K. R. An identified histaminergic neuron can modulate the outputs of buccal-cerebral interneurons in *Aplysia* via presynaptic inhibition. J. Neurosci (in press).

[4] Kretz, R., Shapiro, E., and Kandel, E. R. Presynaptic inhibition produced by an identified presynaptic inhibitory neuron. I. Physiological mechanisms. J. Neurophysiol. 55:113-130 (1986).

[5] Kretz, R., Shapiro, E., Bailey, C. H., Chen, M. and Kandel, E. R. Presynaptic inhibition produced by an identified presynaptic inhibitory neuron. II. Presynaptic conductance changes caused by histamine. J. Neurophysiol. 55:131-146 (1986).

PUBLICATIONS IN PRINT, 1987

1. Hockberger, P.E., Tseng, Hsiu-Yu, and Connor, John A. Immunocytochemical and electrophysiological differentiation of rat cerebellar granule cells in explant cultures. J. Neurosci., 7:1370-1383, 1987.

2. Connor, John A., Tseng, Hsiu-Yu, and Hockberger, P.E. Depolarization and transmitter induced changes in intracellular Ca of rat cerebellar granule cells in explant cultures. J. Neurosci., :1384-1400, 1987.

3. Connor, John A. and Hockberger, Phillip E. Digital imaging of Ca levels in CNS neurons under conditions which induce facilitating increases in Ca levels and sustained Ca elevation. In: Cellular Mechanisms of Conditioning and Behavioral Plasticity. ed. C.M. Woody Plenum (in press) 1987.

4. Cohan, C.S., Connor, J.A., and Kater, S.B. Electrically and chemically mediated increases in intracellular calcium in neuronal growth cones. J. Neurosci., 7:3588-3599, 1987.

5. Ahmed, Z. & Connor, J.A., Calcium regulation by and buffer capacity of mulluscan (Archidoris monteryensis) neurons during calcium transients. Cell Calcium (in press).

PERSONNEL

John A. Connor, PhD (principal investigator)

Philip E. Hockberger, PhD

Hillel Chiel, PhD

Wytse Wadman, PhD (Univ. of Ansterdam)

Kenneth McMillan B.S.E.E.

END

DATE

FILMED

6-88

DTIC