

DTIC FILE COPY

1

AD-A203 321

DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

2a. SECURITY CLASSIFICATION AUTHORITY NA		1b. RESTRICTIVE MARKINGS NA	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE NA		3. DISTRIBUTION/AVAILABILITY OF REPORT Distribution Unlimited	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Loyola University of Chicago		5. MONITORING ORGANIZATION REPORT NUMBER(S) NA	
6a. NAME OF PERFORMING ORGANIZATION Loyola Medical Center	6b. OFFICE SYMBOL (If applicable) NA	7a. NAME OF MONITORING ORGANIZATION Office of Naval Research	
6c. ADDRESS (City, State, and ZIP Code) Dept. of Anatomy Loyola Medical Center 2160 S. First Avenue, Maywood, IL 60153		7b. ADDRESS (City, State, and ZIP Code) 800 N. Quincy St.	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research	8b. OFFICE SYMBOL (If applicable) ONR	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-88-K-0010	
8c. ADDRESS (City, State, and ZIP Code) 800 N. Quincy St. Arlington, VA 22217-5000		10. SOURCE OF FUNDING NUMBERS	
		PROGRAM ELEMENT NO 61153N	PROJECT NO. RR04108
		TASK NO. 4414809-01	WORK UNIT ACCESSION NO.
11. TITLE (Include Security Classification) Amygdalo-hypothalamic Pathways Mediate Stress-induced ACTH Release (U)			
12. PERSONAL AUTHOR(S) Thackery S. Gray			
13a. TYPE OF REPORT Annual	13b. TIME COVERED FROM 01/1/87 TO 11/30/88	14. DATE OF REPORT (Year, Month, Day) 12-22-88	15. PAGE COUNT 8
16. SUPPLEMENTARY NOTATION			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	Stress	
08		Corticotropin Releasing Factor	
		Brain	
		Adrenocorticotropin Hormone	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The goals of this project are to characterize the anatomical pathways and neurotransmitters that participate in the brain neuroendocrine stress response. Studies have focused on the amygdala, a part of the limbic system. Specifically, studies are designed to 1) determine if and which (ACTH) releasing cells of the hypothalamus are innervated by the amygdala; 2) determine the neurotransmitter used by cells within the amygdala to activate the release of ACTH; and 3) determine the role of the amygdala in release of ACTH and related "stress" hormones to different psychological and physical stressors. During the first year, light microscopic studies on the amygdala-hypothalamic pathways have been completed. The amygdala topographically innervates subregions of the hypothalamic paraventricular nucleus. Amygdaloid terminals appear to contact CRF, vasopressin and oxytocin immunoreactive cells. Studies are in progress to determine if destruction of the amygdala will affect neuroendocrine responses to immobilization or cold stress. Studies on the identification of amygdaloid (cont.)			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION (U)	
22a. NAME OF RESPONSIBLE INDIVIDUAL Dr. J.A. Majde		22b. TELEPHONE (Include Area Code) 202-696-4055	22c. OFFICE SYMBOL ONR

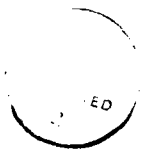
DTIC ELECTRONIC DEC 3 0 1988

19. (cont.)

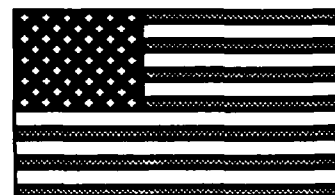
Corticotropin Releasing Factor

neurotransmitters that activate (CRE) neurons within the hypothalamus are also in progress. Thyrotropin releasing factor has been identified as a possible neurotransmitter or neuromodulator candidate. Second year studies will be a continuation of first year studies and electron microscopic studies of the amygdala-hypothalamic pathway. (11) X

Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	



FB



Date: December 1, 1988

R & T Code: 4414809-01

ANNUAL REPORT ON CONTRACT ONR N00014-88-K-0010

PRINCIPAL INVESTIGATOR: Thackery S. Gray

CO-INVESTIGATOR: Louis D. Van De Kar

CONTRACT TITLE: *Amygdalo-hypothalamic Pathways Mediate Stress-induced ACTH Release.*

Contract Period: Dec. 1, 1987 to Nov. 30, 1988

RESEARCH OBJECTIVES: To characterize the anatomical pathways and neurotransmitters that participate in the brain neuroendocrine stress response. We have focused our study on the amygdala which is composed of a heterogeneous group of nuclei and is part of the limbic system. Our studies are designed to determine which ACTH releasing cells of the hypothalamus are innervated by the amygdala, to determine the neurotransmitter used by cells within the amygdala to activate the release of ACTH, and to determine the role of the amygdala in release of ACTH and related "stress" hormones to different psychological and physical stressors.

PROGRESS REPORT: A series of inter-related studies on the anatomy, physiology and neurotransmitters of the amygdalo-hypothalamic pathway have been initiated. **First**, we have completed one study that characterizes the anatomical organization of the pathway from the amygdala to the paraventricular nucleus of the hypothalamus. The results of this study demonstrate that the amygdala directly innervates the posterior and anterior parvocellular regions of the paraventricular hypothalamic nucleus. It is the central amygdaloid nucleus that projects to the posterior parvocellular subregions of the paraventricular nucleus. The posterior parvocellular regions of the paraventricular nucleus

contain the corticotropin releasing cells of the hypothalamus (i.e., corticotropin releasing factor, vasopressin and oxytocin). The data was presented in preliminary form at the Molecular Biology of Stress Meetings in Keystone, Colorado, April 10-17 (see abstract). A manuscript was submitted to Neuroendocrinology and has been accepted pending revision. **Second**, during the summer we have developed a double labeling technique that will allow us to determine which ACTH releasing cells are being innervated by the amygdala. Our initial data using this technique shows that the central amygdaloid axon terminals directly contact parvocellular corticotropin releasing factor, vasopressin and oxytocin cells. Magnocellular CRF, vasopressin and oxytocin cells were not strongly innervated by the amygdala. Thus, this amygdalo-hypothalamic pathway is selectively linked to cells that have established ACTH releasing properties. This data was presented at the Society For Neuroscience Meetings at Toronto, Canada in November. **Third**, in February and March of this year the principal investigator visited Dr. Marvin Brown's lab at the University of California at San Diego. There we conducted studies to determine if thyrotropin releasing factor (TRF), a central nervous system stress-related peptide "neurotransmitter", could effect the release of ACTH. We suspected that it may have ACTH releasing properties, because it has potent stress-related cardiovascular activating effects when injected into the amygdala or cerebral ventricles. The data that we have collected so far demonstrate that TRF injected into lateral ventricle of the brain does indeed increase plasma levels of ACTH. This is the first evidence demonstrating ACTH releasing properties of thyrotropin releasing factor, although its effect upon pituitary release of other hormones is well established. The TRF-induced ACTH response was not blocked by intracerebroventricular injection of CRF antagonist alpha-helical CRF 9-41. However, the ACTH response was totally blocked by IV injections CRF antisera (passive immunization). Thus, TRF probably effects the release of ACTH from the pituitary by activating hypothalamic CRF neurons that project to the median eminence (portal release). The data was presented at the Society For Neuroscience Meetings at Toronto, Canada in November. Future studies will be conducted to determine if the amygdala is one of the sites of action for TRF-induced ACTH release. **Fourth**, experiments on the effects of amygdaloid lesions on neuroendocrine responses to cold stress, hemorrhagic stress and psychological (i.e., immobilization or tone signaling shock) stress are underway in Dr. Van de Kar's laboratory. In the initial control experiments, ibotenic acid induced-lesions were placed dorsal, ventral and lateral to the amygdala. These lesions failed to have any effect upon cold water-

and forced swimming-induced increases in plasma corticosterone and renin concentrations. Additional experiments are in progress to determine if lesions of the central amygdala will block the corticosterone and renin response to this stress. In another initial experiment, the catecholamine neurotoxin, 6-hydroxydopamine, was injected into the central amygdala of rats. The rats were pretreated with desipramine to protect noradrenergic terminals, but allowing the dopaminergic terminals to be selectively lesioned. Lesions of the dopaminergic terminals within the amygdala blocked the effect of "psychological" stress-induced increases in plasma renin and corticosterone. The psychological stress was exposure to a cage in which the animals had previously been exposed to mild foot shock. Placement in the cage caused significantly elevated levels of the plasma renin and corticosterone in sham-lesioned controls. Thus, these preliminary results implicate an important role for the dopamine in amygdaloid mediated ACTH and renin responses to stress. These experiments are being conducted by Peter Rittenhouse who is receiving his Ph.D. training in Dr. Van de Kar's laboratory.

EXPENDITURES:

Supplies: expenditures normal

Equipment: ONR Funds were used to purchase an Olympus Vanox microscope.

Travel: see below.

1. The PI (Thackery S. Gray) was funded to engage in collaborative research in Dr. Marvin R. Brown's laboratory at the University of California at San Diego from February 21 to March 19, 1988.
2. Partial funding of the PI's trip to Molecular Biology of Stress Meetings in Keystone Colorado April 10-17, 1988.
3. Full funding of the PI's and CO-PI's trip (Louis Van De Kar) to the Society For Neuroscience Meeting in Toronto Canada November 12-18, 1988.

Other: expenditures normal.

Personnel: Rashel Piechowski: Research Assistant I (100% support from ONR)

Joseph Costello: Research Assistant I (approx. 50% support from ONR)

Training Activities: Peter Rittenhouse is currently receiving his Ph.D training in Dr Van De Kar's lab. The present ONR contract only supports the portion of his research that is directly related to the goals of the project. The support is in the form of paying for supplies and animal care.

Women or minorities - 0; Non-citizens - 0

PUBLICATIONS AND REPORTS:

Abstracts

Gray, T.S. and M. Carney Demonstration of direct projections from the amygdala to the paraventricular hypothalamic nucleus: Possible role in stress induced ACTH release. *J. Cellular Biochemistry*, 12D (1988) Q302.

Brown, M.R., C. Rivier and T.S. Gray Thyrotropin-releasing factor (TRF) effects on pituitary ACTH release and autonomic function, *Soc. Neurosci. Abstr.* 14 (1988) 1287.

Magnuson, D.J. and T.S. Gray Amygdala directly innervates parvocellular paraventricular hypothalamic CRF, vasopressin and oxytocin. *Soc. Neurosci. Abstr.* 14 (1988) 1288.

Manuscripts

Gray, T.S., M.E. Carney and D.J. Magnuson Direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular nucleus: Possible role in stress-induced ACTH release. *Neuroendocrinology*, accepted pending revisions.

2ND YEAR WORK PLAN: The objectives of Year 2 are 1). to complete the above studies and submit them as publications; 2). to begin electron microscopic studies on the amygdala-hypothalamic pathway. Electron microscopic techniques are necessary to prove that the terminals we have observed on hypothalamic "CRF" cells

form synaptic contacts; 3). to determine which "neurotransmitter" candidates are contained with the amygdaloid cells that directly innervate hypothalamic CRF cells. This will be accomplished via combined anatomical retrograde tracing and immunohistochemical experiments, and through continued collaborative efforts with Dr. Marvin R. Brown; 4). to continue and complete studies on the effects of neurotoxic lesions of the amygdala on the expression of ACTH responses to stress. Other stress models such as immobilization and hemorrhagic stressors will be examined.

NOTE TO SCIENTIFIC OFFICER:

1). The expenditures for year 1 correspond to original expectations except for our purchase of an Olympus AH2 Vanox microscope. The purchase of this microscope was necessary for processing of the two color immunocytochemical histological and fluorescent anatomical tracing material collected for completion of the ONR contracted experiments. The Olympus AH2 (which we compared and found superior to our present and other microscopes) cost \$35,865.. The ONR budgeted \$19,000 for the microscope. We still were able to purchase the microscope by using the \$19,000, plus \$9,000 from the ONR personnel budget and \$7,865 from the PI's NIH grant. Funds became available in the personnel part of the budget for two reasons: 1) there was a delay in funding of the contract [start date Dec. 1, 1987 and funding actually started the last week of January, 1988]; 2) there was delay in hiring a part time research assistant because we had difficulty finding a qualified person to fill the position. I would like to note that the Olympus AH2 microscope has been a tremendous boost to the anatomical studies of the ONR contract. The histological material would have been difficult to process and publish without this instrument.

2). I will be on sabbatical from January 15 to June 15, 1988. During my absence Dr. Van de Kar will still be on site to conduct studies and provide guidance for the personnel of the ONR project. Dr. Anthony Castro (Dept. Anat., adjacent laboratory) has consented to also check in on my laboratory regularly.

My mailing address will be: Thackery S. Gray
C/O Marvin R. Brown
Dept. Medicine & Surgery, T-017
UCSD Medical Center
225 Dickinson St.
San Diego, CA 92103-9981
619-543-5464

DISTRIBUTION LIST

Investigators:

Dr. H. Elliot Albers
Lab. Neuroendocrin. & Beh.
Depts. Biology and Psychology
Georgia State University
Atlanta, GA 30303

Dr. Gwen V. Childs
Dept. Anat. & Neuroscience
Univ. Texas Medical Branch
Galveston, TX 77550

Dr. Carl E. Creutz
Dept. Pharmacology
University of Virginia
Charlottesville, VA 22908

Dr. Mary F. Dallman
Dept. Physiology
Univ. California, Box 0444
San Francisco, CA 94143-0444

Dr. Caleb E. Finch
Dept. of Neurobiology
Univ. Southern California
Los Angeles, CA 90089-0191

Dr. Richard F. Ochillo
College of Pharmacy
Xavier Univ. of Louisiana
7325 Palmetto St.
New Orleans, LA 70125

Dr. Terry Reisine
Dept. Pharmacology
Univ. Penn. Sch. Med.
36th and Hamilton Walk
Philadelphia, PA 19104

Dr. C. Frank Starmer
P.O. Box 3181
Duke Univ. Med. Ctr.
Durham, NC 27710

Administrators:

Scientific Officer, Physiology
Code 1141SB
Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

Administrator
Defense Technical Information Center
Building 5, Cameron Station
Alexandria, VA 22314

Jeanine Majde
Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

Program Manager, Code 1213
Human Factors Biosciences Division
Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

Program Manager, Code 223
Support Technology Directorate
Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

DoD Activities:

Commanding Officer
Naval Medical Center
Washington, D.C. 20372

Commanding Officer, Code 404
Naval Medical Research & Development
Command
National Naval Medical Center
Bethesda, MA 20814

Commander
Chemical and Biological Sciences Division
Army Res. Office, P.O. Box 12211
Research Triangle Park, NC 27709

Directorate of Life Sciences
Air Force Office of Scientific Research
Bolling Air Force Base
Washington, D.C. 20332