

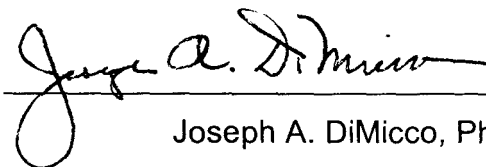
*This document only includes an excerpt of the corresponding thesis or dissertation. To request a digital scan of the full text, please contact the Ruth Lilly Medical Library's Interlibrary Loan Department (rlmlill@iu.edu).*

FUNCTIONAL, PHARMACOLOGICAL, AND ELECTROPHYSIOLOGICAL  
CHARACTERIZATION OF DORSOMEDIAL HYPOTHALAMIC NEURONS

Timothy W. Bailey

Submitted to the faculty of the University Graduate School  
in partial fulfillment of the requirements  
for the degree  
Doctor of Philosophy  
in the Department of Pharmacology  
Indiana University  
October, 2001

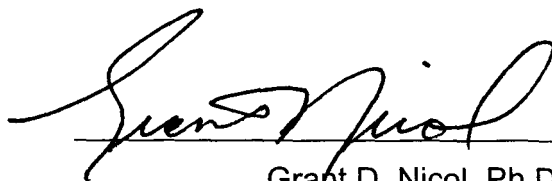
Accepted by the Graduate Faculty, Indiana University, in partial fulfillment  
of the requirements for the degree of Doctor of Philosophy.



---

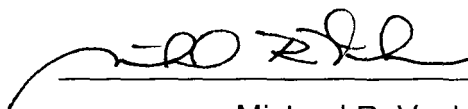
Joseph A. DiMicco, Ph.D.

Doctoral  
Committee



---

Grant D. Nicol, Ph.D.



---

Michael R. Vasko, Ph.D.



---

Zao C. Xu, M.D., Ph.D.

March 19, 2001

## ABSTRACT

Timothy W. Bailey

### Functional, Pharmacological, and Electrophysiological Characterization of Dorsomedial Hypothalamic Neurons

The dorsomedial hypothalamus (DMH) is thought to play a key role in initiating the multi-system response to neurogenic stress. The hallmark neuroendocrine response to stress is increased plasma adrenocorticotrophic hormone (ACTH) resulting from stimulation of the paraventricular hypothalamic nucleus (PVN). Accordingly, stimulation of the DMH should mimic behavioral, autonomic, and neuroendocrine responses to stress whereas similar treatment of the PVN should increase plasma ACTH alone. I tested the effect of microinjection of N-Methyl-d-aspartate, kainate (KA), or bicuculline into the DMH or the PVN on heart rate (HR), mean arterial pressure (MAP), locomotor activity (LA), and plasma ACTH. The effects of air-stress were also similarly assessed. Microinjection of KA or bicuculline into the DMH produced a pattern of changes that resembled that seen in air-stress including increased HR, MAP, and plasma ACTH. Similar treatment of the PVN evoked little or no change in these parameters and failed to replicate the response to air stress. Thus, these results support the notion that neurons in the DMH integrate the multi-system response to stress and may activate the hypothalamic-pituitary-adrenal axis through direct projections to PVN. Although little is known about the individual neurons that make up the DMH, the results of this and other studies in intact rats suggest that their activity is modulated by

GABA<sub>A</sub> receptors and ionotropic glutamate receptors. I employed whole cell patch clamp to characterize the membrane properties and spontaneous post-synaptic currents (PSCs) in individual neurons in the DMH, including those that project to PVN, in a brain slice preparation. Compared to neurons of unknown projection, PVN-projecting neurons (identified by the presence of retrograde tracer injected earlier into PVN) had higher action potential (AP) thresholds and fired fewer evoked APs. Spontaneous PSCs were observed in all neurons. One population of PSCs decayed rapidly (1.5-2.0 ms) and was blocked by non-NMDA receptor antagonists. Remaining PSCs decayed more slowly (4.5-6.0 ms), reversed near  $E_{Cl}$ , and were blocked by GABA<sub>A</sub> receptor antagonists. Thus, as suggested by studies in intact animals, individual neurons in the DMH, including those that project to the PVN, are regulated by GABA<sub>A</sub> and non-NMDA glutamate receptor-mediated synaptic transmission.

# TABLE OF CONTENTS

<b>ACKNOWLEDGEMENTS</b>	v
<b>ABSTRACT</b>	iv
<b>LIST OF TABLES AND FIGURES</b>	viii
<b>LIST OF ABBREVIATIONS</b>	xii
<b>I. INTRODUCTION</b>	1
<b>A. Stress and the Stress Response</b>	1
1. A Definition of Stress	1
2. The Multi-System Response to Neurogenic Stress	2
<b>B. Hypothalamic Mechanisms and the Stress Response:</b>	4
Integrative Studies in the Intact Organism	
1. The Hypothalamus and the Defense Reaction	4
2. The Paraventricular Hypothalamus and the Hypothalamic- Pituitary-Adrenal Axis.	6
3. The Role of the Dorsomedial Hypothalamus in the Multi- System Response to Neurogenic Stress	10
a. Cardiovascular Changes	10
b. Projections from the DMH to autonomic centers in the Central Nervous System.	12
c. Neurogenic stress-Induced Activation of the HPA axis	14
d. Summary: Role the DMH in both neurogenic stress-	18

induced cardiovascular changes and activation of the HPA axis.

<b>C. Hypothalamic Neurons and Stress Circuitry: Reductive Studies at the Level of Individual Neurons.</b>	<b>21</b>
1. Strategies used to identify and study individual neurons in brain slice preparations.	21
a. Whole-cell patch clamp electrophysiology.	21
b. Brain slice preparations for electrophysiology	22
c. Use of fluorescence microscopy to identify neurons of specific connectivity in brain slice preparations.	24
2. Amino Acid Neurotransmission in the Hypothalamus	26
a. Gamma-amino-butyric acid-mediated neurotransmission in the hypothalamus	26
b. Glutamic acid-mediated neurotransmission in the hypothalamus	29
3. From the whole animal to the single cell: The PVN as a Model	33
4. The Model Neuron: Characteristics of individual neurons in the DMH suggested by studies conducted in intact animals.	37
5. Rationale for studying individual neurons in DMH within a brain slice preparation using whole-cell electrophysiology	40
<b>D. Thesis Goals</b>	<b>45</b>
1. Integrative studies conducted in whole animals.	45

2. Reductive studies conducted in brain slice preparations.	47
<b>II. MATERIALS AND METHODS</b>	<b>48</b>
<b>A. Experiments Conducted in Whole Animals</b>	<b>48</b>
1. Animals	48
2. Surgical Procedures	48
3. Experimental Procedures	50
a. Microinjection Studies	50
b. Air-Stress Studies	52
4. Blood Collection and ACTH Radioimmunoassay	52
5. Histology	52
6. Statistics	53
<b>B. Experiments Conducted in Brain Slice Preparations</b>	<b>54</b>
1. Animals	54
2. Retrograde Labeling of Neurons in the DMH that Project to the PVN	54
a. Histological Studies	56
b. Brain Slice Electrophysiology	57
3. Preparation and Storage of Hypothalamic Brain Slices	57
4. Whole-Cell Recording Techniques, Solutions, and Materials	59
a. Patch Pipettes and Intracellular Solutions	59
b. Extracellular Bathing Solutions	60
c. Perfusion System	60

d. Bath-Applied Drugs	63
e. Recording Chamber Design	64
f. Brain Slice Visualization and Optics	66
g. Whole-Cell Recording Techniques	69
h. Data Acquisition and Storage	70
i. Recording Protocols	70
j. Data Analysis	71
k. Statistics	78
l. Intracellular Biocytin Labeling and Cell Morphology	79
<b>III. RESULTS</b>	<b>82</b>
<b>A. Results from Experiments Conducted in Whole Animals</b>	<b>82</b>
1. Microinjection Studies	82
2. Air-Stress Studies	86
<b>B. Experiments Conducted in Hypothalamic Brain Slices</b>	<b>104</b>
1. Identification of neurons that project to the PVN with Dil: fixed tissue prepared for histology.	105
2. Characteristics of spontaneous action potentials and membrane characteristics in Dil labeled and unlabeled neurons in the DMH region.	111
3. Comparison of the number of action potentials produced by injection of positive current in Dil+ and Dil- neurons in the DMH.	114

4. Pharmacological Analysis of Spontaneous Synaptic Currents.	117
a. Studies Employing KCl-based Pipette Solution:	120
Characterization of spontaneous excitatory post-synaptic currents observed in voltage clamp recording mode.	
-1. EPSCs are reduced by GABA <sub>A</sub> receptor antagonist and further reduced by non-NMDA glutamate receptor antagonist.	120
-2. EPSCs are reduced by glutamate receptor antagonists and further reduced by addition of GABA <sub>A</sub> receptor antagonist.	126
-3. Reversal Potential of GABA <sub>A</sub> receptor-mediated EPSCs	133
-4. The decay time constant of GABA <sub>A</sub> receptor-mediated synaptic currents is modulated by pentobarbital.	138
-5. EPSCs observed in neurons in the DMH are not dependent upon evoked synaptic release mediate by voltage-gated sodium channels.	143
b. Studies Employing Potassium Gluconate-Based Pipette Solution: Characterization of spontaneous EPSCs and inhibitory post-synaptic currents (IPSCs) recorded in	150

voltage clamp mode.

-1. Excitatory post-synaptic currents recorded at negative holding potentials are blocked by non-NMDA glutamate receptor antagonist.	153
-2. Decay kinetics of EPSCs recorded at negative holding potentials.	157
-3. EPSCs recorded at negative holding potentials (-90 to -60mV) and IPSCs observed at more positive holding potentials (-20 to -10mV) are observed in the same neurons.	164
-4. EPSCs are blocked by CNQX while IPSCs recorded in the same neurons are blocked by picrotoxin.	168
-5. EPSCs recorded at negative holding potentials (-90 to -70 mV) are dependent upon evoked release mediated by voltage-gated sodium channels.	172
5. Morphology of neurons in the region of the DMH	176
<b>IV. DISCUSSION</b>	181
<b>A. Integrative studies conducted in whole animals.</b>	181

1. Cardiovascular responses to microinjections of KA, NMDA, or BMI, into the DMH or the PVN.	181
2. Effects of microinjection of KA, BMI, or NMDA, into the DMH or the PVN on plasma levels of ACTH.	184
3. Summary and Conclusions	191
<b>B. Reductive Studies Conducted in Hypothalamic Brain Slices.</b>	<b>193</b>
1. Advantages and limitations of the use of Dil as a retrograde tracer.	193
2. Membrane properties of neurons in the region of the DMH.	196
3. Morphology of neurons in the region of the DMH.	200
4. Action potential waveform and firing characteristics of neurons in the region of the DMH.	220
5. Pharmacological characterization of glutamatergic and GABAergic synaptic currents in neurons in the region of the DMH.	203
6. Decay kinetics of glutamatergic and GABAergic synaptic currents in neurons in the region of the DMH.	206
7. Reversal potential of GABA <sub>A</sub> receptor-mediated synaptic currents.	208
8. Modulation of GABA <sub>A</sub> receptor-mediated synaptic currents by pentobarbital.	209
9. Dependence of GABAergic synaptic transmission in the DMH on evoked synaptic release mediated by sodium channels.	209

10. Summary and conclusions	213
C. Integration: From observations made in intact animals to those of individual cells.	216
<b>IV. BIBLIOGRAPHY</b>	<b>219</b>