INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
ANATOMICAL AND PHYSIOLOGICAL STUDIES ON THE CATECHOLAMINES
NOREPINEPHRINE AND DOPAMINE IN THE CEREBELLUM

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Thomas Ernest Nelson, B.S.

* * * * *

The Ohio State University

1995

Dissertation Committee:

G.A. Bishop
J.S. King
R.C. Rogers

Approved by

G.A. Bishop
Adviser
Neuroscience Graduate Studies Program
To My Parents
ACKNOWLEDGMENTS

I sincerely thank my co-advisors Dr. Georgia A. Bishop and Dr. James S. King for their patience and guidance during my four years in their laboratories. My experiences under their tutelage will benefit me for many years to come. The technical assistance of Katharine Dillingham and Barbara Diener-Phelan is greatly appreciated. I also thank Karl Rubin for his photographic assistance. Finally, I thank all of my family and friends for the incredible support they have provided me over the past five years.
VITA

June 2, 1968 . . . . . . . . . . . Born - Bayonne, New Jersey

1988-1990 . . . . . . . . . . . Research Assistant, Dr. David L. Quinn, Muskingum College, New Concord, Ohio

1990 . . . . . . . . . . . . . . . . . B.S., Biology, Muskingum College, New Concord, Ohio

1990-present . . . . . . . . . Neuroscience Graduate Research Associate, The Ohio State University, Columbus, Ohio

PUBLICATIONS


FIELDS OF STUDY

Major Field: Neuroscience

Studies in neuroanatomy and neurophysiology of catecholamines in the adult mammalian cerebellum—Dr. James S. King and Dr. Georgia A. Bishop.
TABLE OF CONTENTS

DEDICATION ......................................................... ii

ACKNOWLEDGMENTS ........................................... iii

VITA ................................................................. iv

LIST OF TABLES .................................................. vii

LIST OF FIGURES ............................................... viii

LIST OF ABBREVIATIONS ................................. xii

CHAPTER PAGE

I. THE DISTRIBUTION OF TYROSINE HYDROXYLASE-
IMMUNOREACTIVE AFFERENTS TO THE CEREBELLUM
DIFFERS BETWEEN SPECIES: A STUDY IN THE CAT,
OPOSSUM, AND MOUSE ............................... 1

   Introduction .............................................. 1
   Materials and Methods .......................... 3
   Results .................................................. 4
   Discussion .......................................... 10

II. ORIGINS OF TH-IMMUNOFLUORESCENT AXONS TO THE
ADULT MOUSE CEREBELLUM: EVIDENCE FOR A
DOPAMINERGIC HYPOTHALAMO-CEREBELLAR
PROJECTION ................................................... 30

   Introduction .......................................... 30
   Materials and Methods ........................ 33
   Results ................................................ 35
   Discussion ........................................... 39
III. THE EFFECTS OF THE CATECHOLAMINES NOREPINEPHRINE AND DOPAMINE ON PURKINJE CELLS IN CEREBELLAR SLICES OF ADULT MICE

Introduction ......................................... 69
Materials and Methods ............................. 71
Results ................................................... 73
Discussion .............................................. 77

LIST OF REFERENCES ................................. 110
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Summary of injection sites within the mouse cerebellum</td>
<td>44</td>
</tr>
<tr>
<td>2. Summary of TH double-labeling from the mouse cerebellum</td>
<td>45</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Varicose tyrosine hydroxylase-immunoreactive fibers in the cerebellar cortex (A, C, E) and nuclei (B, D, F) of the cat (A, B), opossum (C, D), and mouse (E, F). (A) Granule cell layer of lobule V of the cat cerebellum. (C) Granule cell layer of lobule IV of the opossum cerebellum. (E) Molecular layer of lobulus simplex of the mouse cerebellum. (B, D, F) Nucleus interpositus of the cat, opossum, and mouse, respectively. Calibration bar = 20 μm.</td>
</tr>
<tr>
<td>2.</td>
<td>(A-C) Camera lucida drawings of sagittal sections through the cat cerebellum showing the laminar and lobular distribution of TH-immunoreactive fibers. Calibration bar = 2 mm. (D) Darkfield photomicrograph showing the laminar distribution of TH fibers in lobule V of the cat cerebellum. Calibration bar = 50 μm.</td>
</tr>
<tr>
<td>3.</td>
<td>(A-C) Camera lucida drawings of sagittal sections through the opossum cerebellum showing the laminar and lobular distribution of TH-immunoreactive fibers. Calibration bar = 2 mm. (D) Darkfield photomicrograph showing the laminar distribution of TH fibers in lobule V of the opossum cerebellum. Calibration bar = 50 μm.</td>
</tr>
<tr>
<td>4.</td>
<td>(A-C) Camera lucida drawings of sagittal sections through the mouse cerebellum showing the laminar and lobular distribution of TH-immunoreactive fibers. Calibration bar = 500 μm. (D) Darkfield photomicrograph showing the laminar distribution of TH fibers in lobule VI of the mouse cerebellum. Calibration bar = 50 μm.</td>
</tr>
<tr>
<td>5.</td>
<td>(A-C) Camera lucida drawings of sagittal sections through the mouse cerebellum showing the distribution of TH-positive Purkinje cells. Arrows indicate regions containing numerous labeled Purkinje cells. Calibration bar = 500 μm. (D) Photomicrograph of TH-positive Purkinje cells in vermal lobule VIII of the mouse cerebellum. Calibration bar = 50 μm.</td>
</tr>
</tbody>
</table>
6. (A-J) Distribution of retrogradely-labeled (open squares), TH-immunofluorescent (open circles), and double-labeled (filled/starred circles) neurons in the brainstem of the adult mouse from case ca24. See text for details of individual figures. ..................................................... 46


8. Double-labeled neuron in the A5 cell group. (A) TH-immunofluorescence. (B) Retrograde labeling. Calibration bar = 25μm. ........................................ 57

9. Double-labeled neuron in the A7 cell group. (A) TH-immunofluorescence. (B) Retrograde labeling. Calibration bar = 25μm. ................................. 59

10. Double-labeled neuron in the A11 cell group. (A) TH-immunofluorescence. (B) Retrograde labeling. Calibration bar = 25μm. ........................................ 61

11. Double-labeled neuron in the A13 cell group. (A) TH-immunofluorescence. (B) Retrograde labeling. Calibration bar = 25μm. ........................................ 63

12. (A) TH-immunofluorescent neurons in the substantia nigra. (B) No retrograde labeling is present in the substantia nigra. Calibration bar = 100μm. ........................................ 65

13. (A) TH-immunofluorescent neurons in the VTA. (B) No retrograde labeling is present in the VTA. Calibration bar = 100μm. ........................................ 67

14. (Top) Chart recording demonstrating that norepinephrine (NE) and dopamine (DA) have no effect, whereas GABA has an inhibitory effect, on the spontaneous activity of Purkinje cells. (Bottom) Chart recording showing biphasic effects of DA on GABAergic inhibition and a suppression of GABA effects by NE. ......................................................... 84

15. (A) Chart recording showing the suppression of glutamate (GLUT)-induced activity by both DA and NE. When applied together NE and DA have a partially additive effect in suppressing GLUT. (B) Oscilloscope traces during the application of 1) GLUT alone; 2) GLUT + DA; 3) GLUT + NE; and 4) GLUT + NE + DA. Time = 200 msec; spike amplitude = 60-75 mV. 86
16. (A) Chart recording illustrating the dose-dependent suppression of GABAergic inhibition by both DA and NE. (B) Oscilloscope traces during spontaneous activity (1 and 5) and during the application of GABA alone (2 and 6); DA alone (3); DA + GABA (4); NE alone (7); and NE + GABA (8). Time = 200 msec; spike amplitude = 75-100 mV. 88

17. (A) Chart recording showing the suppression of GABA by both DA and NE including a partial additivity of NE and DA effects on GABA. (B) Oscilloscope traces during the application of GABA alone (1 and 3); DA + GABA; NE + GABA; NE + DA + GABA; and spontaneous activity. Time = 200 msec; spike amplitude = 100-175 mV. 91

18. Chart recording depicting the dose-dependent suppression of GABA by NE and biphasic effects of DA on GABA, particularly at higher application currents (eg. +70 nA). 94

19. Chart recording showing an augmentation of GABA by NE while having no effect on spontaneous activity. 96

20. (A) Chart recording illustrating an enhancement of GABA effects by NE and biphasic effects of DA, particularly at higher application currents (eg. +30 nA). (B) Oscilloscope traces during 1) GABA alone before NE application; 2) NE + GABA; 3) GABA alone after NE application; 4) GABA alone before DA application; 5) DA + GABA, showing an initial augmentation of GABA inhibition; and 6) DA + GABA, showing a secondary suppression of GABA. Time = 200 msec; spike amplitude = 35-50 mV. 98

21. Chart recording illustrating a suppression of GABAergic inhibition by DA and an enhanced effect of GABA by NE. The effects negate each other when DA and NE are applied simultaneously. 100

22. Chart recording illustrating no effect of either DA or NE on spontaneous activity, a reduction of GABA inhibition by DA, and variable effects of NE on GABA. Lower application currents (eg. +20 nA) of NE result in enhanced GABA effects whereas higher currents (eg. +80) result in a suppression of GABA. 102
23. (A) Chart recording showing suppression of GABA by DA and NE at higher current levels (e.g., +70 nA and +80 nA, respectively) and biphasic effects at lower levels. (B) Oscilloscope traces during the application of 1) GABA alone; 2) GABA + NE (at +35 nA), showing an initial enhancement of GABA effects; 3) GABA + NE (at +35 nA), showing a secondary suppression of GABA inhibition; 4) GABA alone; 5) GABA + NE (at +80 nA). Time = 200 msec; spike amplitude = 60 mV.

24. Chart recording illustrating a dose-dependent enhancement of GABA at lower current levels of NE (< +35 nA) and biphasic effects of NE at higher application currents (> +35 nA). Increasing the application current of GABA accentuates the biphasic response elicited at higher currents of NE (e.g., +50 and +70 nA). DA also has biphasic effects on GABA inhibition, particularly at higher currents (e.g., +70 nA).

25. Chart recording showing no effect of DA on spontaneous activity and variable effects on GABA inhibition. At lower current levels (e.g., +10 nA) of DA GABAergic inhibition is enhanced whereas at higher current levels (e.g., +80 nA) GABA is suppressed.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>serotonin</td>
</tr>
<tr>
<td>A1</td>
<td>A1 noradrenergic cell group</td>
</tr>
<tr>
<td>A2</td>
<td>A2 noradrenergic cell group</td>
</tr>
<tr>
<td>A5</td>
<td>A5 noradrenergic cell group</td>
</tr>
<tr>
<td>A6</td>
<td>A6 noradrenergic cell group</td>
</tr>
<tr>
<td>A7</td>
<td>A7 noradrenergic cell group</td>
</tr>
<tr>
<td>A9</td>
<td>A9 dopaminergic cell group</td>
</tr>
<tr>
<td>A10</td>
<td>A10 dopaminergic cell group</td>
</tr>
<tr>
<td>A11</td>
<td>A11 dopaminergic cell group</td>
</tr>
<tr>
<td>A12</td>
<td>A12 dopaminergic cell group</td>
</tr>
<tr>
<td>A13</td>
<td>A13 dopaminergic cell group</td>
</tr>
<tr>
<td>AP</td>
<td>area postrema</td>
</tr>
<tr>
<td>Aq</td>
<td>cerebral aqueduct</td>
</tr>
<tr>
<td>BC</td>
<td>brachium conjunctivum</td>
</tr>
<tr>
<td>BP</td>
<td>brachium pontis</td>
</tr>
<tr>
<td>CAI</td>
<td>capsula interna</td>
</tr>
<tr>
<td>CBL</td>
<td>cerebellum</td>
</tr>
<tr>
<td>CC</td>
<td>crus cerebri</td>
</tr>
</tbody>
</table>

xii
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI</td>
<td>colliculus inferior</td>
</tr>
<tr>
<td>CP</td>
<td>commissura posterior</td>
</tr>
<tr>
<td>CRI</td>
<td>crus I</td>
</tr>
<tr>
<td>CRII</td>
<td>crus II</td>
</tr>
<tr>
<td>CS</td>
<td>colliculus superior</td>
</tr>
<tr>
<td>cul</td>
<td>nucleus cuneatus lateralis</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DPCS</td>
<td>decussatio pedunculi cerebellarium superiorum</td>
</tr>
<tr>
<td>ep</td>
<td>nucleus entopeduncularis</td>
</tr>
<tr>
<td>FLM</td>
<td>fasciculus longitudinalis medialis</td>
</tr>
<tr>
<td>FMT</td>
<td>fasciculus mammilothalamicus</td>
</tr>
<tr>
<td>FR</td>
<td>fasciculus retroflexus</td>
</tr>
<tr>
<td>GL</td>
<td>granule cell layer</td>
</tr>
<tr>
<td>hl</td>
<td>nucleus habenulae lateralis</td>
</tr>
<tr>
<td>hm</td>
<td>nucleus habenulae medialis</td>
</tr>
<tr>
<td>IA</td>
<td>interpositus anterior</td>
</tr>
<tr>
<td>IP</td>
<td>interpositus posterior</td>
</tr>
<tr>
<td>ip</td>
<td>nucleus interpeduncularis</td>
</tr>
<tr>
<td>IVv</td>
<td>fourth ventricle</td>
</tr>
<tr>
<td>LA</td>
<td>lobus anterior</td>
</tr>
<tr>
<td>LC</td>
<td>locus coeruleus</td>
</tr>
<tr>
<td>LM</td>
<td>leminiscus medialis</td>
</tr>
</tbody>
</table>

xiii
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>LN</td>
<td>lateral nucleus</td>
</tr>
<tr>
<td>LS</td>
<td>lobulus simplex</td>
</tr>
<tr>
<td>ML</td>
<td>molecular layer</td>
</tr>
<tr>
<td>mml</td>
<td>nucleus mamillaris medialis, pars lateralis</td>
</tr>
<tr>
<td>mmm</td>
<td>nucleus mamillaris medialis, pars medialis</td>
</tr>
<tr>
<td>MN</td>
<td>medial nucleus</td>
</tr>
<tr>
<td>NE</td>
<td>norepinephrine</td>
</tr>
<tr>
<td>ncu</td>
<td>nucleus cuneiformis</td>
</tr>
<tr>
<td>ndm</td>
<td>nucleus dorsomedialis hypothalami</td>
</tr>
<tr>
<td>nlh</td>
<td>nucleus lateralis hypothalami</td>
</tr>
<tr>
<td>np</td>
<td>nuclei pontis</td>
</tr>
<tr>
<td>nts</td>
<td>nucleus tractus solitarius</td>
</tr>
<tr>
<td>nV</td>
<td>nucleus originis nervi trigemini</td>
</tr>
<tr>
<td>nvm</td>
<td>nucleus ventromedialis hypothalami</td>
</tr>
<tr>
<td>nX</td>
<td>nucleus originis nervi vagi</td>
</tr>
<tr>
<td>nXII</td>
<td>nucleus originis nervi hypoglossi</td>
</tr>
<tr>
<td>oad</td>
<td>nucleus olivaris accessorius dorsalis</td>
</tr>
<tr>
<td>oam</td>
<td>nucleus olivaris accessorius medialis</td>
</tr>
<tr>
<td>oi</td>
<td>nucleus olivaris inferior</td>
</tr>
<tr>
<td>P</td>
<td>tractus corticospinalis</td>
</tr>
<tr>
<td>PAP</td>
<td>peroxidase-antiperoxidase</td>
</tr>
<tr>
<td>PCI</td>
<td>pedunculus cerebellaris inferior</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>PCM</td>
<td>pedunculus cerebellaris medialis</td>
</tr>
<tr>
<td>PCS</td>
<td>pedunculus cerebellaris superior</td>
</tr>
<tr>
<td>PFL</td>
<td>paraflocculus</td>
</tr>
<tr>
<td>PK</td>
<td>purkinje cell layer</td>
</tr>
<tr>
<td>PML</td>
<td>paramedian lobule</td>
</tr>
<tr>
<td>RB</td>
<td>restiform body</td>
</tr>
<tr>
<td>rd</td>
<td>nucleus raphe dorsalis</td>
</tr>
<tr>
<td>rl</td>
<td>nucleus reticularis lateralis</td>
</tr>
<tr>
<td>rli</td>
<td>nucleus raphe linearis</td>
</tr>
<tr>
<td>rtp</td>
<td>nucleus reticularis tegmenti pontis</td>
</tr>
<tr>
<td>snc</td>
<td>substantia nigra, pars compacta</td>
</tr>
<tr>
<td>snl</td>
<td>substantia nigra, pars lateralis</td>
</tr>
<tr>
<td>snr</td>
<td>substantia nigra, pars reticulata</td>
</tr>
<tr>
<td>sub</td>
<td>nucleus subcoeruleus</td>
</tr>
<tr>
<td>TH</td>
<td>tyrosine hydroxylase</td>
</tr>
<tr>
<td>TO</td>
<td>tractus opticus</td>
</tr>
<tr>
<td>vl</td>
<td>nucleus vestibularis lateralis</td>
</tr>
<tr>
<td>vm</td>
<td>nucleus vestibularis medialis</td>
</tr>
<tr>
<td>vs</td>
<td>nucleus vestibularis spinalis</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
</tr>
<tr>
<td>XII</td>
<td>nervus hypoglossus</td>
</tr>
<tr>
<td>ZI</td>
<td>zona incerta</td>
</tr>
<tr>
<td>xiv</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER I

THE DISTRIBUTION OF TYROSINE HYDROXYLASE IMMUNOREACTIVE AFFERENTS TO THE CEREBELLUM DIFFERS BETWEEN SPECIES: A STUDY IN THE CAT, OPOSSUM AND MOUSE

INTRODUCTION

The presence of catecholaminergic afferents to the cerebellum was first described in the rat (Fuxe, 1965; Hökfelt and Fuxe, 1969; Bloom et al., 1971; Yamamoto et al., 1977; Kimoto et al., 1981) and mouse (Landis et al., 1975; Felten et al., 1986) using fluorescence histochemistry, and has since been substantiated in both species by immunohistochemical techniques utilizing antibodies directed against tyrosine hydroxylase (TH), the first enzymatic step in catecholamine synthesis (Austin et al., 1992; Ikai et al., 1992; Takada et al., 1993b). Catecholaminergic afferents form a plexus of thin, varicose fibers that are present in all lobules and laminae of the cerebellar cortex as well as in the cerebellar nuclei. Catecholaminergic labeling may indicate the presence of either norepinephrine or dopamine, or both. This plexus was originally thought to be solely noradrenergic in nature since selective depletion of norepinephrine-containing terminals abolished all fluorescent labeling in the cerebellum (Fuxe, 1965; Hökfelt and Fuxe, 1969; Bloom et al., 1971) and, using biochemical techniques, very little dopamine was found within the cerebellum (Carlsson,
1959; Glowinski and Iversen, 1966; Landis et al., 1975). In addition, an antibody against dopamine-β-hydroxylase, the enzyme responsible for synthesizing norepinephrine, labels a plexus in the rat cerebellum similar to that seen with histofluorescence (Verney et al., 1982). However, a more recent immunocytochemical study using an antibody against dopamine has suggested the presence of a dopaminergic innervation of the rat cerebellum as well (Panagopoulos et al., 1991).

Although numerous studies have described in detail the catecholaminergic input to the cerebella of rodents, little has been done to characterize the laminar and lobular distribution of this system in other species. In contrast, a number of immunohistochemical studies have been conducted that describe the distribution of another monoamine, serotonin. When taken together, the data demonstrate a differential laminar and lobular distribution of serotoninergic fibers within the cerebella of several species including the mouse, rat, cat, and opossum (Bishop et al., 1985; Bishop and Ho, 1985; Kerr and Bishop, 1991; Crivellato et al., 1992; Nelson et al., 1993). Interspecies variation in the density and distribution of catecholaminergic and serotoninergic plexuses within the cerebellum might suggest that each neurochemical acts upon a different neuronal population in each species. This information, in conjunction with studies on the physiological effects of these neuromodulators on cerebellar neurons, will be important in determining the overall effect of each neurochemical on cerebellar output and, thus, its effect on motor function. Therefore, in the present study we have used an antibody against TH in conjunction with the indirect peroxidase-antiperoxidase (PAP) technique (Sternberger, 1979) in order to compare the laminar and
lobular distribution of catecholaminergic afferents within the cerebellar cortex and nuclei of the adult cat, opossum, and mouse. These data are then compared to that derived from previous studies on the cerebellar distribution of serotonin in the same species.

**MATERIALS AND METHODS**

Three adult cats, five adult opossums (*Didelphis marsupialis virginiana*), and six adult mice (C57BL/6J) were anesthetized with sodium pentobarbital (Nembutal) and perfused transcardially with a 2% paraformaldehyde in picric acid (Zamboni's) fixative (Stephanini et al., 1967). The brains were removed and postfixed for up to six hours in Zamboni's fixative. They were then placed in Sorensen's phosphate buffer + 15% or 22% sucrose overnight. Frozen sections of 60 μm thickness were cut in either the sagittal or transverse plane. The sections were collected and rinsed in phosphate buffered saline (PBS) to remove residual fixative from the tissue.

After rinsing, the sections were incubated in a mouse monoclonal antibody (Chemicon) against the enzyme tyrosine hydroxylase (TH) and continuously agitated for 2-4 days at 4°C. The primary antibody was made up in a solution of phosphate buffered saline, 0.3% triton X-100 (PBT) plus 0.1% bovine serum albumin (BSA) at a concentration of 1:400, 1:1000, or 1:2000. Consistent results were obtained even at lower concentrations. As a control, sections were also placed in PBT + 0.1% BSA without the primary antibody. In all cases, no immunolabeling was observed above background levels after removal of the TH antibody.
All of the sections were then placed in a secondary mouse IgG antibody (1:300-1:500 in PBT) followed by mouse PAP (1:300-1:500 in PBT) for one hour each at room temperature. These steps were then repeated to double-link the secondary antibodies to the primary antibody. The sections were rinsed with PBS between each step.

The sections were exposed to the peroxidase substrate 3,3'-diaminobenzidine tetrahydrochloride (DAB; 50 mg/100 ml PBS) + 20 μl 30% H₂O₂ in a series of five minute intervals until a reaction product was visible under a light microscope. The DAB reaction was halted in PBS after each interval.

The tissue was then mounted onto slides in a 1% gelatin solution and dried in an oven at 38°C. The slides were then dehydrated in a series of alcohols, cleared in xylene and coverslipped with Permount.

Two cat, four opossum, and three mouse cases were used for critical analysis of the data. The sections were examined under both bright and dark field light microscopy. Camera lucida drawings of representative sagittal sections were done using dark field optics at magnifications of 4X, 6.3X, and 16X. Photomicrographs were taken under dark or bright field illumination at magnifications of 25X and 100X.

RESULTS

Morphological characteristics of TH-positive fibers

In all three species TH-immunoreactive fibers form a diffuse plexus in the cerebellar cortex and nuclei. Within the plexus, the morphology of the TH-immunoreactive fibers is
identical for each species. The plexus consists of fine axons (<1 µm in diameter) that exhibit numerous varicosities measuring approximately 1-2 µm in diameter (Fig. 1A-F). None of the TH-immunoreactive fibers have the typical morphology of either climbing fibers or mossy fibers in any of the three species. In the white matter (Figs. 2B,C; 3B,C; 4A-C), and particularly within the brachium conjunctivum (BC; mouse: Fig. 4A,B; cat and opossum: not shown), numerous immunolabeled afferents are visible entering the cerebellum. In certain sections, many of these fibers extend between the locus coeruleus and the cerebellar white matter (mouse: Fig. 4A,B; cat and opossum: not shown). In the white matter immunoreactive fibers have a greater diameter (approximately 1 µm) compared to those in the cerebellar cortex and nuclei and usually do not have varicosities (not shown). TH-positive fibers also are sparsely scattered within both the brachium pontis and restiform body of each species (Figs. 2C, 3C, 4C).

**Distribution of TH-positive fibers in the cat cerebellum**

In the cat, TH-positive fibers have a heterogeneous distribution in the cerebellar cortex (Fig. 2A-C). In the vermis (Fig. 2A), only sparse immunolabeling is present except for an area of greater density localized to the ventral folia of lobules V and VI. More anterior and posterior lobules, and the dorsal folia of lobules V and VI, contain only scattered positively stained fibers. The lowest densities are found in the most rostral (I-III) and caudal (VIII-X) lobules. Although it is not as pronounced as in the vermal lobules this general pattern is maintained in more lateral regions of the cerebellum (Fig. 2B,C). In transverse
sections (not shown) labeling is greatest within the ventral folia of lobules V and VI medially as well as lobulus simplex and crus I and II laterally, in agreement with the observations from sagittal sections.

With respect to laminar distribution there is a slightly higher density of fibers found in the granule cell layer and around the Purkinje cells (Fig. 2D). However, the molecular layer also contains a moderate number of TH-positive fibers. This is particularly evident in lobules V and VI where many of the fibers in the molecular layer have a perpendicular orientation relative to the Purkinje cell layer. These fibers often extend radially to the pial surface of the cerebellar cortex. The orientation in the granule cell layer is more random, but there is a tendency for fibers near to or within the Purkinje cell layer to course parallel to the monolayer of Purkinje cells (Fig. 2D).

There is a moderate density of TH-positive fibers and varicosities in all of the cerebellar nuclei relative to the surrounding white matter (2A-C). No TH-positive cell bodies are found in either the cerebellar cortex or nuclei of the adult cat.

**Distribution of TH-positive fibers in the opossum cerebellum**

The overall density of TH-immunoreactive fibers in the opossum is much higher than in the cat (Fig. 3A-C). Similar to the cat, there is a heterogeneous distribution of these fibers. Vermal lobules III-VIII contain an abundance of TH-positive fibers, while lobules I-II and IX-X are relatively sparse. In particular, lobules V and VI contain the highest concentration of fibers, whereas lobules I and X contain the fewest. This pattern maintains itself within the
intermediate cortex (Fig. 3B) but not in the hemispheres, which contain a more uniform lobular distribution of TH-positive fibers (Fig. 3C). Also, in transverse sections (not shown) there is a slight decrease in the density of labeled fibers laterally in the flocculus, paraflocculus, and paramedian lobule relative to more medial sections.

The laminar distribution of TH-immunoreactive fibers is organized such that the granule and Purkinje cell layers exhibit a high density whereas the molecular layer shows a relatively low density (Fig. 3D). A majority of the fibers in the molecular layer tend to be oriented perpendicular to the Purkinje cell layer and extend radially to the pial surface. As in the cat, the positively stained fibers in the granule cell layer are randomly organized while those that surround the Purkinje cells tend to be oriented parallel to the plane of the Purkinje cell layer (Fig. 3D).

Each of the cerebellar nuclei contains TH-positive varicose fibers, though not nearly as dense as in the cortex (Fig. 3A-C). The density in the cerebellar nuclei is only slightly higher than that of the surrounding white matter. As in the cat, no cell bodies in either the cerebellar cortex or nuclei are positive for tyrosine hydroxylase in the adult opossum.

Distribution of TH-positive fibers in the mouse cerebellum

The mouse cerebellum contains the greatest density of TH-labeled fibers when compared to the other two species (Fig. 4A-C). These fibers form a profuse plexus which is relatively uniform in its distribution among lobules and within layers (Fig. 4A-C). Vermal lobules V and VI are only slightly denser than more anterior or posterior lobules and lobule
X contains the fewest amount of fibers compared to other lobules (Fig. 4A). No differences in fiber density between medial and lateral regions of the mouse cerebellum are apparent in either sagittal or transverse sections.

Unlike the cat and opossum, the mouse cerebellum exhibits no marked difference in the relative densities of TH-immunoreactive fibers in the molecular and granule cell layers (Fig. 4D). The density of labeled fibers in the granule cell layer is only slightly higher than in the molecular layer. As in the previous two species, the molecular layer contains numerous fibers that orient perpendicular to the Purkinje cell layer and often extend radially to the pial surface whereas the granule cell layer has a random organization of fibers (Fig. 4D). Most of the stained fibers near to or within the Purkinje cell layer are oriented such that they course along the row of Purkinje cell bodies (Fig. 4D).

Each of the cerebellar nuclei also contains a dense plexus of TH-positive varicose fibers (Fig. 4A-C).

**TH-immunoreactive Purkinje cells in the mouse cerebellum**

In sharp contrast to the cat and opossum, the mouse cerebellum contains numerous Purkinje cells that exhibit TH-immunoreactivity (Fig. 5A-D). The somata and proximal dendrites of these Purkinje cells exhibit a reaction product well above background levels, though slightly less intense than the TH-positive cell bodies of the locus coeruleus and substantia nigra (not shown). The TH-immunoreactive Purkinje cells are primarily located in lobules VI-X (Fig. 5A) of the posterior vermis, and laterally in lobules IX-X (Fig. 5B-C),
paraflocculus, and flocculus (not shown). Most of the Purkinje cells in these regions are positive for TH. A few stained Purkinje cells are occasionally found scattered within the anterior lobe as well (Fig. 5B).
DISCUSSION

Catecholaminergic afferents to the cerebellum

In the present study, we have provided the first detailed description of the laminar and lobular distribution of TH-immunoreactive afferents in the cerebellum of three species: cat, opossum, and mouse. Previous studies by other investigators have reported the existence of TH-positive fibers in both the rat (Ikai et al., 1992; Takada et al., 1993b) and mouse (Austin et al., 1992) cerebellum but no detailed account of their lobular and laminar distribution nor any mention of the relative density of these fibers within the layers of the cerebellar cortex was provided.

It has generally been accepted that the cerebellum contains appreciable amounts of norepinephrine but very little, if any, dopamine. Original fluorescence histochemical studies, in which few dopamine fibers were observable (Fuxe, 1965; Hökfelt and Fuxe, 1969; Bloom et al., 1971), coupled with biochemical studies that reported that dopamine makes up only a small fraction of the cerebellar catecholamines (Carlsson, 1959; Glowinski and Iversen, 1966; Landis et al., 1975), had led to the conclusion that dopamine exists solely as a precursor for norepinephrine in this plexus. The present results indicate that, in each of the three species, a considerable number of TH-positive fibers course through the BC, presumably originating in the locus coeruleus—the primary noradrenergic cell group of the brainstem (Dahlström and Fuxe, 1964). Nonetheless, because TH is the key enzyme in the synthesis of both dopamine and norepinephrine we cannot rule out the possibility of a dopaminergic component of this plexus. Dopamine-immunoreactive fibers have been
identified in the rat cerebellum (Panagopoulos et al., 1991) and recent findings from double-label experiments (Ikai et al., 1992) have identified TH-immunoreactive neurons within the ventral tegmental area (VTA) that project to the cerebellar cortex of the rat. The VTA contains the dopaminergic A10 cell group (Dahlström and Fuxe, 1964). Moreover, this projection was shown to enter the cerebellum via the BC (Ikai et al., 1992). Thus, if such a projection exists in the cat, opossum, or mouse, it would likely have been obscured by the presumptive coeruleo-cerebellar TH fibers observed in the BC in the present data. Further studies utilizing an antibody to either TH or dopamine in conjunction with retrograde tracing techniques would be required to determine the existence of a projection from the VTA to the cerebellum in these species.

TH-immunoreactive Purkinje cells

Our results verify earlier findings of TH-positive Purkinje cells in both the rat (Takada et al., 1993b) and mouse (Hess and Wilson, 1991; Austin et al., 1992). TH-immunoreactive Purkinje cells have been reported in crus I and II, paraflocculus, and lobules I and X of the adult rat (Takada et al., 1993b). Both TH mRNA and TH immunoreactivity have been found in numerous Purkinje cells of the posterior vermis and the hemispheres of the mutant mice tottering and leaner (Hess and Wilson, 1991; Austin et al., 1992). However, Hess and Wilson (1991) have suggested that Purkinje cells of wild-type C57BL/6J mice only transiently express TH mRNA postnatally from P21 to P35 and that adult normal mice do not possess TH-immunoreactive Purkinje cells. Austin et al. (1992) have described only a
few TH-immunofluorescent or TH mRNA-positive Purkinje cells in the adult cerebellum of this strain. In contrast to both of these studies, our data demonstrate an abundance of TH-immunoreactive Purkinje cells in normal mice of the C57BL/6J strain between two and three months of age. In addition, Fujii et al. (1994) have recently shown a large number of TH-immunoreactive Purkinje cells at 11 months of age that follows a period of relatively decreased number during the fourth week of life in the ddy strain of mouse.

Several studies have attempted to define which neurochemical, if any, is synthesized by TH-positive Purkinje cells. Treatment with MPTP, a compound known to be neurotoxic to many monoaminergic cell types—particularly dopaminergic cells, also destroys Purkinje cells in crus I and II, and paraflocculus of the mouse cerebellum (Takada et al., 1993a). Furthermore, in rats TH-positive Purkinje cells do not stain for dopamine-β-hydroxylase (Takada et al., 1993b), the enzyme that converts dopamine into norepinephrine. Taken together, these results imply that, in rodents, a population of Purkinje cells, which are classically defined as being GABAergic (Ito, 1984), also contain either dopamine or L-DOPA, a precursor to dopamine. However, Panagopoulos et al. (1991) did not find dopamine-immunoreactive Purkinje cells in rat cerebellum although they did observe dopaminergic fibers. L-DOPA has more recently been ascribed a potential neurotransmitter role in several regions of the central nervous system (Kitahama et al., 1988; Okamura et al., 1988; Meisler et al., 1988; Komori et al., 1991, Komori et al., 1993). Additional support for the production of L-DOPA comes from the observation in leaner and tottering mutant mice
that Purkinje cells do not immunolabel for aromatic amino acid decarboxylase, the enzyme responsible for the conversion of L-DOPA to dopamine (Hess and Wilson, 1991).

Despite the evidence suggesting the production of catecholamines by rodent Purkinje cells several other studies have indicated that TH can be expressed in vivo by central neurons that do not contain catecholamines or their synthesizing enzymes, particularly during development (Jaeger and Joh, 1983; Berger et al., 1985; Verney et al., 1988). In addition, cultured Purkinje cells from Swiss-Webster mice exhibit TH-immunoreactivity by the twelfth day in vitro, but do not demonstrate catecholamine histofluorescence at this time (Seil et al., 1992). Whether TH-positive Purkinje cells observed in the cerebella of the adult rat and mouse synthesize catecholamines has yet to be established.

Comparison of catecholaminergic and serotoninergic afferents to the cerebellum

Another monoamine present within the cerebellum of several species is the indoleamine serotonin (5-HT) (for a review see: Bishop and Kerr, 1992). Like the catecholamines, 5-HT is found in a plexus of thin, varicose fibers that distributes diffusely to the cerebellar cortex and nuclei (Takeuchi et al., 1982; Bishop and Ho, 1985; Bishop et al., 1985; Kerr and Bishop, 1991; Crivellato et al., 1992; Nelson et al., 1993). Based on the present data, as well as numerous other studies (Hökfelt and Fuxe, 1969; Bloom et al., 1971; Landis et al., 1975; Yamamoto et al., 1977; Kimoto et al., 1981; Takeuchi et al., 1982; Verney et al., 1982; Bishop and Ho, 1985; Bishop et al., 1985; Felten et al., 1986; Kerr and Bishop, 1991; Panagopoulos et al., 1991; Crivellato et al., 1992; Nelson et al., 1993), it is
evident that all of the monoamines share a common orientation of fibers within each cerebellar lamina: there is a random orientation of fibers within the granule cell layer; the fibers surrounding the Purkinje cells tend to course parallel to the respective monolayer; and many of the fibers within the molecular layer maintain a radial orientation as they extend toward the pial surface of the cerebellum.

Comparison of the distributions of TH and 5-HT in the cat, opossum, mouse, and rat cerebella reveals several differences. First of all, in each species there are differences in the relative densities of the axons that contain these two neuromodulators. In the cat, 5-HT fibers are very dense (Takeuchi et al., 1982; Kerr and Bishop, 1991) while TH fibers are scant. Conversely, in the opossum the density of TH fibers is moderate to high while 5-HT fibers are relatively sparse (Bishop et al., 1985). Similarly, the TH plexus of the mouse is extremely dense while 5-HT fibers are very scarce (Crivellato et al., 1992; Nelson et al., 1993). The rat has been described as having a sparse distribution of noradrenergic fibers (Fuxe, 1965; Hökfelt and Fuxe, 1969; Bloom et al., 1971) and a moderate number of 5-HT fibers (Bishop and Ho, 1985).

A second difference is seen with respect to the laminar distribution of TH and 5-HT immunoreactive axons in each species. 5-HT fibers are present almost exclusively in the Purkinje cell and granule cell layers of the cat (Kerr and Bishop, 1991) and opossum (Bishop et al., 1985), whereas they are nonuniformly localized to all laminae of the rat (Bishop and Ho, 1985) and predominantly to the Purkinje cell layer of the mouse (Crivellato et al., 1992; Nelson et al., 1993). TH fibers distribute equally to all laminae of the mouse cerebellum, are
found predominantly in the Purkinje and granule cell layers of the opossum, and are present (at low levels) in all laminae of the cat cerebellum. Noradrenergic fibers are densest, having a patchy distribution, in the molecular layer of the rat (Hökfelt and Fuxe, 1969; Bloom et al., 1971; Yamamoto et al., 1977; Kimoto et al., 1981).

Finally, serotonergic inputs to the cerebellum arise from several brainstem reticular (Kerr and Bishop, 1991) and raphe nuclei (Bishop and Ho, 1985; Walker et al., 1988; Kitzman and Bishop, 1994) whereas TH-containing afferents are presumed to originate from neurons in the locus coeruleus, the A5 and A7 cell groups (noradrenergic) (Olson and Fuxe, 1971; Pasquier et al., 1980) and, potentially, the VTA (dopaminergic) (Ikai et al., 1992). However, in the cat a large amount of the noradrenergic innervation of the cerebellum comes from the A4 cell group, and the Kölliker-Fuse and parabrachial nuclei, in addition to the locus coeruleus (Chu and Bloom, 1974; Dietrichs, 1988). Thus, there appears to be interspecies variation in the origins of catecholaminergic fibers as well as their cerebellar distribution.

Another characteristic of the brainstem origins of serotonergic projections to the cat cerebellum is that they are segregated such that those fibers that terminate in the cerebellar cortex originate in more caudal brainstem nuclei (Kerr and Bishop, 1991) than those that project to the cerebellar nuclei (Kitzman and Bishop, 1994). Thus, it is evident that the cerebellar cortex and nuclei, at least in terms of their serotonergic inputs, do not necessarily process information in parallel (Kitzman and Bishop, 1994). Similarly, the fibers of the TH-immunoreactive projection from the VTA distribute to the cerebellar cortex, but
not the cerebellar nuclei, of the rat (Ikai et al., 1992). Thus, at least in the case of the
 dopaminergic input from the VTA, the potential exists for a segregation of catecholaminergic
 inputs to the cerebellar cortex and nuclei as well.

Functional considerations

The monoamines 5-HT, dopamine, and norepinephrine have been shown to function
as neuromodulators within the central nervous system (for reviews see: Moore and Bloom,
1979; Woodward et al., 1979; Grace and Bunney, 1985; Rogawski, 1985; Vandermaelen,
1985). Neuromodulation can be defined as "the ability of neurons to alter their electrical
properties in response to intracellular biochemical changes resulting from synaptic or
hormonal stimulation" (Kaczmarek and Levitan, 1987). By activating receptors that are
linked to second messenger systems within a neuron, a neuromodulator sets the "tone" or
"gain" of neuronal activity, and thus regulates spontaneous activity and/or responsiveness
to other synaptic inputs for potentially sustained periods of time (Barchas et al., 1978).
Neuromodulation is thus considered to be a form of synaptic plasticity which ultimately
enables an organism to respond to an ever-changing external and internal environment
(Kaczmarek and Levitan, 1987).

Numerous electrophysiological studies in the cerebellum have shown that both
norepinephrine (Hoffer et al., 1971; Freedman et al., 1976; Freedman, 1977; Moises et al.,
1979; Basile and Dunwiddie, 1984; McElligott et al., 1986; Mori-Okamoto and Tatsuno,
1988; Dennett and Hubbard, 1988; Woodward et al., 1991) and 5-HT (Strahlendorf et al.,
1784; Strahlendorf et al., 1986; Lee et al., 1986; Hicks et al., 1989; Strahlendorf et al., 1989; Darrow et al., 1990; Kerr and Bishop, 1992; Wang et al., 1992; Li et al., 1993) modulate Purkinje cell activity. The effects of these neuromodulators on Purkinje cells are very complex in that each can either enhance or suppress activity in ways that are not yet completely understood. Regardless of the effect(s) on Purkinje cells, by modulating their activity norepinephrine and 5-HT must certainly influence the flow of output from the cerebellar nuclei and thus, alter the gain of cerebellar modification of motor activity.

5-HT also has been shown to modulate granule cell activity (Armstrong et al., 1987) and nuclear cell activity (Gardette et al., 1987; Kitzman and Bishop, 1993) in the cerebellum, but no reports on the effects of norepinephrine on other cerebellar neurons exist to date. Yet, noradrenergic varicosities are present in all cerebellar laminae and nuclei of every species that has been studied (present data; Hökfelt and Fuxe, 1969; Bloom et al., 1971; Landis et al., 1975; Verney et al., 1982; Felten et al., 1986), and several subtypes of adrenergic receptors have been reported at low to moderate levels in all laminae of the cerebellar cortex and in the cerebellar nuclei of several species (Rainbow et al., 1984; Lorton and Davis, 1987; Palacios et al., 1987; Wilson and Minneman, 1989; Zeng and Lynch, 1991; Nicholas et al., 1993a,b). Clearly, the net effect of norepinephrine on cerebellar output would depend not only on its effect(s) on Purkinje cells, but also on its effect(s) on the other neurons of the cerebellar cortex and nuclei that express adrenergic receptors.

The physiological effect of dopamine on the activity of Purkinje cells, or any other cerebellar neuron, has not yet been determined. However, dopaminergic fibers are present
within the rat cerebellum (Panagopoulos et al., 1991). These thin, varicose, dopamine-immunoreactive axons are uniformly distributed to all cerebellar lobules with a slightly higher density within the molecular layer. In addition, dopaminergic binding has been reported in both mouse and rat cerebellar homogenates (Panagopoulos et al., 1988, 1991, 1993; Panagopoulos and Matsokis, 1994) and there have been a number of reports in several species of both $D_1$- and $D_2$-like dopamine receptors and mRNA within each lamina of the cerebellum (Martres et al., 1985; Bouthenet et al., 1987; Dawson et al., 1988; Mengod et al., 1989; Wamsley et al., 1989; Camps et al., 1990; Bouthenet et al., 1991; Mansour et al., 1992; Gehlert et al., 1993; Levant et al., 1993). Therefore, it seems probable that dopamine also acts at multiple sites within the cerebellar cortex to modulate cerebellar function.

In summary, monoamines are present within the cerebellum of every species studied to date as a plexus of thin, varicose fibers that is distinct from both the climbing and mossy fiber systems of afferents. Although all monoaminergic fibers have a similar morphology and orientation within the cortical laminae their relative density and distribution patterns vary in a species-dependent manner. This suggests that each monoamine acts upon a dissimilar neuronal population within the cerebellum and, in turn, has a unique net effect upon cerebellar output. Thus, monoamines likely assume distinctive roles in cerebellar function which vary from species to species relative to their anatomical differences.

Doubtless, other differences between the monoamines not related to the anatomical distribution of their fibers are also involved in distinguishing their effects on cerebellar
function. Such differences include the distribution and variable expression of specific receptor subtypes by neurons within the cerebellum, the intracellular biochemical mechanisms of their actions, and their interactions with other neuroactive compounds. Moreover, because each component of monoaminergic input to the cerebellum has distinct brainstem origins, it is likely that the behavioral or physiological conditions during which each is most active differ as well.
Fig. 1. Varicose tyrosine hydroxylase-immunoreactive fibers in the cerebellar cortex (A, C, E) and nuclei (B, D, F) of the cat (A, B), opossum (C, D), and mouse (E, F). (A) Granule cell layer of lobule V of the cat cerebellum. (C) Granule cell layer of lobule IV of the opossum cerebellum. (E) Molecular layer of lobulus simplex of the mouse cerebellum. (B, D, F) Nucleus interpositus of the cat, opossum, and mouse, respectively. Calibration bar = 20 μm.
Fig. 2. (A-C) Camera lucida drawings of sagittal sections through the cat cerebellum showing the laminar and lobular distribution of TH-immunoreactive fibers. Calibration bar = 2 mm. (D) Darkfield photomicrograph showing the laminar distribution of TH fibers in lobule V of the cat cerebellum. Calibration bar = 50 μm.
Fig. 2
Fig. 3. (A-C) Camera lucida drawings of sagittal sections through the opossum cerebellum showing the laminar and lobular distribution of TH-immunoreactive fibers. Calibration bar = 2 mm. (D) Darkfield photomicrograph showing the laminar distribution of TH fibers in lobule V of the opossum cerebellum. Calibration bar = 50 μm.
Fig. 3
Fig. 4. (A-C) Camera lucida drawings of sagittal sections through the mouse cerebellum showing the laminar and lobular distribution of TH-immunoreactive fibers. Calibration bar = 500 μm. (D) Darkfield photomicrograph showing the laminar distribution of TH fibers in lobule VI of the mouse cerebellum. Calibration bar = 50 μm.
Fig. 4
Fig. 5  (A-C) Camera lucida drawings of sagittal sections through the mouse cerebellum showing the distribution of TH-positive Purkinje cells. Arrows indicate regions containing numerous labeled Purkinje cells. Calibration bar = 500 μm. (D) Photomicrograph of TH-positive Purkinje cells in vermal lobule VIII of the mouse cerebellum. Calibration bar = 50 μm.
CHAPTER II

ORIGINS OF TH-IMMUNOFLOURESCENT AXONS TO THE ADULT MOUSE CEREBELLUM: EVIDENCE FOR A DOPAMINERGIC HYPOTHALAMO-CEREBELLAR PROJECTION

INTRODUCTION

The presence of a noradrenergic input to the cerebellum has been well established in a number of studies using fluorescence histochemistry (Fuxe, 1965; Hokfelt and Fuxe, 1969; Bloom et al., 1971; Yamamoto et al., 1977; Kimoto et al., 1981; Landis et al., 1975; Felten et al., 1986) as well as immunohistochemically by utilizing antibodies against the enzyme dopamine-β-hydroxylase (Verney et al., 1982). Norepinephrine is found in a plexus of varicose fibers that are morphologically distinct from both mossy and climbing fibers and that distribute sparsely to all lobules and laminae of the rat cerebellar cortex as well as the cerebellar nuclei. The primary brainstem origin of cerebellar noradrenergic fibers has been shown to be the locus coeruleus in both rats and cats with additional contributions from the A4-A7 cell groups of Dahlström and Fuxe (1964) in both species and the parabrachial and Kölliker-Fuse nuclei in the cat (Dietrichs, 1988; Pasquier et al., 1980; Chu and Bloom, 1974). Previous studies in our laboratory (Nelson et al., 1993; see Ch. 1), using an antibody directed against the enzyme tyrosine hydroxylase (TH), have confirmed a catecholaminergic...
input to the cerebellum of the mouse, opossum, and cat that closely resembles the noradrenergic plexus. However, because TH is the initial enzymatic step in the synthesis of both norepinephrine and dopamine we cannot rule out a dopaminergic component of this plexus.

Until recently, the existence of a dopaminergic input to the cerebellum had not been recognized. It had generally been accepted that the cerebellum contains very little dopamine and that all the dopamine that is present exists only as a precursor to norepinephrine. However, several lines of evidence have suggested a dopaminergic input to the rat cerebellum. Biochemical studies have demonstrated that cerebellar dopamine levels drop after lesions to the midbrain A8-A10 cell groups (Geffard et al., 1984) and that cerebellar norepinephrine levels drop independent of dopamine levels following lesions of the locus coeruleus (Kizer et al., 1976). In addition, dopamine release has recently been detected within the cerebellum of the rat (Chrapusta et al., 1994) as well as dopamine uptake into mouse cerebellar synaptosomes via a distinct transport system (Efthimiopoulos et al., 1991). Dopamine receptors (Panagopoulos et al., 1991; Mansour et al., 1992; Gehlert, 1993; Bouthenet et al., 1987; Levant et al., 1993; Camps et al., 1990; Wamsley et al., 1989; Dawson et al., 1988; Martres et al., 1985) and their mRNA (Mansour et al., 1992; Mengod et al., 1989; Bouthenet et al., 1991; Levent et al., 1993; Gehlert, 1993; Wamsley et al., 1989; Mengod et al., 1989; Bouthenet et al., 1987; Camps et al., 1990). The most direct evidence supporting a dopaminergic input to the cerebellum has
come from the use of an antibody against dopamine. Panagopoulos et al. (1991) have found dopamine-immunoreactive fibers with morphological characteristics similar to those containing norepinephrine throughout the rat cerebellum. However, the dopamine antibody used in their study faintly stains noradrenergic cell bodies in the locus coeruleus as well as fibers within known dopaminergic projection areas throughout the brain (Sidman et al., 1971). Thus, it is possible that the immunoreactivity within the cerebellum was at least in part due to binding to precursor dopamine within noradrenergic fibers (Panagopoulos et al., 1991). One way to avoid this problem is to use an antibody against tyrosine hydroxylase. Using a double-label paradigm, Ikai et al. (1992) have identified TH-immunoreactive cell bodies within the ventral tegmental area (VTA)—a dopaminergic region (A10 of Dahlström and Fuxe) within the midbrain—that project to the cerebellum of the rat.

In the present study we have employed a double-label paradigm in order to determine the brainstem origin(s) of the TH-immunoreactive afferents to the cerebellum of the adult mouse, a species that contains an abundance of TH-positive fibers (Nelson et al., 1993) as well as a population of dopaminergic binding sites within the cerebellum (Panagopoulos and Matsokis, 1994; Panagopoulos et al., 1993; Panagopoulos et al., 1988). The results of this study will allow us to 1) compare the origin(s) of noradrenergic afferents to the mouse cerebellum with those of the rat and cat, and 2) search for potential dopaminergic origins of TH-immunoreactive fibers in the mouse cerebellum as was found previously in the rat (Ikai et al., 1992).
MATERIALS AND METHODS

Seven adult mice (C57BL/6J) were anesthetized with Avertin (tribromoethanol; 0.20 cc/10g, intraperitoneal) anesthesia and placed in a stereotaxic frame. An incision was made and a small area of occipital bone overlying the cerebellar vermis was removed. Using a Hamilton microsyringe, a total volume of $1.5-4 \mu l$ of rhodamine- or fluorescein-tagged latex microspheres (LumaFluor Inc.) was then injected into the vermis at depths of 1.5 to 3.5 mm over a period of about 5 minutes. After completion of the injection the mice were allowed to survive for 24-30 hours to allow for sufficient uptake and retrograde transport of the microspheres from the axon terminals within the cerebellum to the cell bodies of origin within the brainstem.

The mice were anesthetized with sodium pentobarbital (Nembutal, diluted 1:10; 0.4-0.6 cc, intraperitoneal) and the brains were fixed by transcardial perfusion of a 2% paraformaldehyde in picric acid (Zamboni’s) fixative (Stephanini et al., 1967). The brains were then removed and placed in Sorensen’s phosphate buffer + 20% sucrose overnight. Frozen sections of the entire brain were cut in the transverse plane at a thickness of 60 \( \mu m \). Cerebellar sections were also saved for subsequent mapping of the injection site of each case.

After sectioning, the tissue was rinsed in phosphate buffered saline (PBS) to remove residual fixative and processed for immunohistochemistry. The sections were allowed to incubate for three days in a mouse monoclonal antibody (Chemicon) generated against the enzyme tyrosine hydroxylase (TH) while under continuous agitation at 4°C. The primary TH antibody was made up at a final dilution of 1:2000 in a 0.3% solution of triton X-100 in...
PBS (PBT). After the incubation period the primary antibody was rinsed from the tissue and the sections were exposed for 2 hours to an anti-mouse IgG secondary antibody (1:500 in PBT) conjugated to either fluorescein or rhodamine. Fluorescein-conjugated secondary antibody was used when rhodamine-tagged microspheres were injected and vice versa. The secondary antibody was then rinsed from the tissue and the sections were mounted onto microscope slides in a 1% gelatin solution. After air-drying the slides were cleared for 30 seconds in xylene and coverslipped with Krystalon mounting medium.

The sections were examined under fluorescence microscopy to visualize both retrograde labeling and TH-immunofluorescence. A filter of wavelength 515-560 nm was used to observe rhodamine fluorescence (microspheres or immunolabeling) and a filter of wavelength 450-490 nm was used to reveal fluorescein labeling (immunolabeling or microspheres). Three types of labeled neurons were observed: 1. Retrogradely-labeled alone--characterized by the presence of rhodamine or fluorescein microspheres in the cytoplasm. 2. TH-immunofluorescent only--characterized by a uniform fluorescein or rhodamine immunolabeling of the cell body. 3. Double-labeled cells--which contained fluorescent microspheres and were also TH-immunofluorescent. Plots of representative transverse serial sections of the mouse brainstem showing the locations of retrogradely-labeled, TH-immunofluorescent, and double-labeled cell bodies were done with a Leitz Orthoplan 2 fluorescent microscope equipped with a position transducer (Boeckeler) using the Neurolucida software package (v. 1.42; MicroBrightField, Inc.). Catecholaminergic nuclei were identified according to the nomenclature of Dahlström and Fuxe (1964). Non-
catecholaminergic nuclei were identified according to a mouse brain atlas (Sidman et al., 1971). In addition, photomicrographs were taken of labeled neurons at a magnification of 16 or 40X using a Zeiss epifluorescence microscope.

RESULTS

Distribution of TH-immunofluorescent neurons

The distribution of TH-immunofluorescent neurons in the mouse is in agreement with previous studies describing catecholaminergic neurons in the rat brain (Dahlstrom and Fuxe, 1964). Populations of fluorescent cell bodies are located throughout the mouse brainstem and are identifiable according to the nomenclature of Dahlström and Fuxe (1964). TH-immunofluorescent cell bodies are represented by open circles on the right half of Figure 6. As in the rat, TH-positive cells are found in the lateral tegmental groups of the medulla (A1-A2; Fig. 6A-C) and pons (A5, or 'subcoeruleus'; Fig. 6D-E; and A7; Fig. 6F); the locus coerulear groups in the medulla (A4; not shown) and pons (A6; Fig. 6D-E); the mesencephalic groups (A8-A10), which include the substantia nigra pars compacta, lateralis, and reticulata (A9; Fig. 6G-H) and the ventral tegmental area (VTA; A10; Fig. 6G); and the diencephalic groups (A11-A14), which include the caudal diencephalic group (A11; Fig. 6H-I), the tuberal group (A12; Fig. 6I-J), the dorsal hypothalamic group (A13; Fig. 6J), and the rostral periventricular group (A14; not shown).
Analysis of injection sites and labeled afferent fibers projecting into the cerebellum

Table 1 summarizes the locations of the injection sites made in each case of this study. All seven of the cases involve injections of fluorescent beads into the posterior cerebellum. In six of the cases the injection site involves only the posterior lobe of the cerebellum; in one case (ca25) the injection site is localized mainly within the anterior lobe (lobules III-V) but also includes lobules IX and X of the posterior lobe. In all but one of the cases the injection site includes lobule IX; one case involves only lobule IX (ca29). In four cases (ca24-27) the injection site includes lobule X. In the cerebellar nuclei, six out of the seven cases exhibit an injection of beads into the nucleus medialis and two of the cases show an injection into nucleus interpositus as well. In only one case (ca29) do the cerebellar nuclei not contain any beads at all and in all seven cases the nucleus lateralis contains no beads.

In three cases (ca24, 25 and 27) bundles of axons within the pedunculus cerebellaris superior are heavily labeled by retrogradely transported microspheres. In certain sections this labeled fiber bundle passes by or through the caudal portion of the locus coeruleus (shaded area, Fig. 6D), at times partially or fully obscuring the nucleus, and appears to terminate abruptly in the vestibular nuclei. In each of these cases the injection site includes lobule X (see Table 1), the termination site of axons originating in the vestibular nuclei.

Distribution of retrogradely-labeled neurons

Due to the fact that the injections were placed near to or at the midline within the cerebellar vermis of each mouse, and since lateral spread of the beads toward each of the
hemispheres is presumed to be equal, retrogradely-labeled neurons are bilaterally distributed throughout the brainstem in every case. Figure 6 is a drawing of case ca24. This case was chosen as a representation of the entire study since its retrograde and double-label profiles are inclusive of all of the other cases. Retrogradely-labeled cells are represented by open squares on the left half of Figure 6.

Labeled cells in the caudal brainstem are found in the nuclei cornu commissuralis ventralis and dorsalis of the rostral spinal cord (not shown), the nucleus cornu commissuralis medulla oblongatae, nucleus reticularis lateralis medulla oblongatae, nucleus cuneatus lateralis, nucleus prepositus hypoglossi, all regions of the inferior olivary complex, nuclei vestibularis spinalis, medialis, superior and lateralis, nuclei locus coeruleus and subcoeruleus, nuclei raphe magnus and dorsalis, nuclei pontis, nuclei paragigantocellularis, gigantocellularis, and parvocellularis, nucleus centralis caudalis pontis, and nuclei reticularis paramedianus and tegmenti pontis. In the midbrain, a number of retrogradely-labeled cells are found in the nucleus raphe linearis and nucleus cuneiformis, as well as a few scattered within and dorsal to the substantia nigra pars compacta and lateralis. In the diencephalon, numerous labeled cells are found in the lateral, dorsomedial, ventromedial, and paraventricular regions of the hypothalamus as well as nucleus entopeduncularis.

**Distribution of double-labeled cells**

The distribution of double-labeled cells (filled-in or starred circles) is illustrated in the insets taken from the right half of Fig. 6 (also from ca24). In every case the primary
source of TH-immunofluorescent, or catecholaminergic, projections to the mouse cerebellum is the locus coeruleus, or A6 cell group (Fig. 6D-E; Fig. 7). Over 80% of all double-labeled cells are found in this region in each case. Smaller contributions from two other noradrenergic cell groups are also observed. In cases where widespread injections were made in the cerebellum (ca24-28, 30; see Table 1) the A5 (Fig. 6D-E; Fig. 8) and A7 (Fig. 6F; Fig. 9) pontine regions make up between 3-6% and 4-11% of the total of double-labeled neurons, respectively. In case 29, in which a very localized injection was made in lobule IX alone, 99% of the double-labeled cells are located within the locus coeruleus. One additional double-labeled cell is located in the A5 cell group. In the four cases (ca24-27) that include lobule X in the injection site, double-labeled cell bodies are located within the diencephalon in the A11 (Fig. 6G-I; Fig. 10) and A13 (Fig. 6J; Fig. 11) cell groups. These hypothalamic regions contain only 1-3% of the total of double-labeled cells in each case. In each of these cases the injection site includes at least lobule X (see Table 1). The remaining three cases (ca28-30) exhibit neither the spread of beads into lobule X nor any retrograde labeling in the diencephalon. None of the seven cases exhibit any double-labeled cells within the mesencephalic dopaminergic cell groups (A8-A10), including the VTA and substantia nigra (Fig. 6G-H; Figs. 7 and 8).
DISCUSSION

Noradrenergic afferents to the cerebellum

The present double-label study confirms previous reports in the rat (Pasquier et al., 1980) and cat (Chu and Bloom, 1974; Dietrichs, 1988) that the locus coeruleus represents the main source of noradrenergic fibers to the cerebellum and that smaller projections from the A7 and A5 groups also contribute to the cerebellar noradrenergic plexus. A previous study in our laboratory has described the plexus of TH-immunoreactive fibers within the mouse cerebellum that differs in both its pattern of distribution and density from similar plexa in the cat and opossum (see Ch. 1) as well as the noradrenergic input to the cerebellum of the rat (Hokfelt and Fuxe, 1969; Bloom et al., 1971; Yamamoto et al., 1977; Kimoto et al., 1981; Verney et al., 1982; Fuxe, , 1965). Thus, if cerebellar TH-immunoreactive fibers represent a predominantly noradrenergic input in the mouse it is likely that the species differences in fiber density within the cerebellum are due to either a greater number of coerulear cells that project to the mouse cerebellum or a higher frequency of branching of coerulear-cerebellar afferents rather than contributions from other noradrenergic areas. In addition, the smaller total volume of the mouse cerebellum relative to the rat, cat, and opossum may contribute to the greater TH fiber density even if the number of coerulear cells that project to the cerebellum is uniform across these species. Although the number of coerulear cells projecting to the cerebellum appears to be much higher in the mouse than was reported in retrograde label studies in the cat (Dietrichs, 1988) and rat (Pasquier et al., 1980) it is not possible to make direct comparisons due to the methodological differences in the
injection of tracer molecules into the cerebellum. Unlike the widespread injections done in our study which encompass rather large regions of the cerebellar cortex and nuclei previous studies in these larger species used a number of more localized injections.

**Dopaminergic afferents to the cerebellum**

The present study is the first to report the existence of projections from the dopaminergic A11 and A13 cell groups of the dorsal and caudal hypothalamus to the cerebellum. Each of these cell groups has been previously reported in the rat (Bjorklund and Lindvall, 1979; Hokfelt et al., 1979; Skagerberg et al., 1982) and rabbit (Blessing and Chalmers, 1979), respectively, to send axons that descend to all levels of the spinal cord. Although a projection from dorsal and posterior regions of the hypothalamus to the cerebellum has previously been described in several species using retrograde and anterograde tracing techniques (Dietrichs and Haines, 1989) the neurochemical associated with this hypothalamo-cerebellar projection has not been determined. Nevertheless, the terminals of hypothalamo-cerebellar axons are morphologically similar to the varicose fibers of the monoaminergic system that distributes throughout the cerebellum of many species, suggesting that they might contain at least one of the monoamines (Dietrichs and Haines, 1985). The cerebellar projection from the hypothalamus is regarded to be separate from the hypothalamo-spinal projection although a few cells were reported to be double-labeled following injections of retrograde tracers into both the cerebellum and spinal cord (Dietrichs and Zheng, 1984). Taken together these studies, in concert with the present data, suggest
the existence of a descending dopaminergic system that projects to the cerebellum as well as the spinal cord either by collateralization or through distinct projections. It should be noted that non-TH-immunofluorescent, retrogradely-labeled neurons were also found within the dorsal and posterior hypothalamic areas in the present study. Thus, the hypothalamo-cerebellar projection is not a solely dopaminergic input.

The current finding that dopaminergic cell groups in the hypothalamus contribute to the TH-positive innervation of the cerebellum of the mouse is consistent with an earlier study in the rat that reported dopaminergic fibers within the cerebellar cortex (Panagopoulos et al., 1991). However, our data does not substantiate the findings of Ikai et al. (1992) in the rat that the source of these fibers is the VTA, or A10 cell group, in the midbrain. Although we cannot rule out the possibility of species differences between mice and rats one reason for this discrepancy could be the difference in injection sites in the two studies. Their cerebellar injections were done in a more lateral position than ours although some overlap might be expected due to spread of the injected tracers in each study. Also, in their investigation anterograde tracing of VTA projections showed a lateral distribution of terminals within the crus I and II ansiform lobules and paraflocculus of the cerebellar hemispheres, as well as labeling of terminals in the lateral and interposed nuclei. Thus, if a similar VTA-cerebellar projection exists in the mouse our injection sites might not have been lateral enough to detect it. Another possible reason for this discrepancy is that their experimental approach was only concerned with projections originating in the midbrain. Apparently, no attempt was made to uncover other potential dopaminergic origins in their study.
Dopaminergic hypothalamo-cerebellar connections terminate in lobule X

By analyzing the injection sites of each of the cases in our study it can be concluded that the dopaminergic projection from groups A11 and A13 terminates primarily in lobule X of the cerebellar cortex. Only those cases (ca24-27) in which the injection site included lobule X showed any double-labeling in the diencephalon (see Tables 1 and 2). Exclusion of lobules VI-VIII (ca25) or lobule IX (ca26) did not eliminate this pattern. However, it is only with exclusion of lobule X in the posterior lobe (ca28 and 30) that double-labeling does not occur.

Functional considerations

At present it is not known what the functional significance of the hypothalamo-cerebellar projection might be. In light of the low numbers of double-labeled neurons found in the diencephalon in the current study it could be argued that such a projection is relatively insignificant, particularly when compared to the large number of coerulear projections to the cerebellum. However, when using a double-label paradigm one must consider the number of double-labeled cells found as an underestimation of the total. This is primarily due to the fact that not all neurons in a given region that project to the cerebellum are likely to terminate in the region of the injection site. Thus, the number of retrogradely-labeled neurons represents a subset of cerebellar projections.

Termination of dopaminergic afferents within lobule X implies that these projections, which originate in the hypothalamus, might be involved in vestibular function, particularly concerning the modulation of eye movements and the maintenance of equilibrium via
connections to the vestibular nuclei. The primary function of the hypothalamus is to regulate the internal physiological environment of the organism (Brooks, 1988). By its connections to the cerebellum the hypothalamus might then influence somatomotor activity relative to the physiological state of the organism. More specifically, the hypothalamic areas that give rise to cerebellar projections might influence motor activity that is directly linked to the particular physiological function(s) that is (are) being regulated. For instance, the posterior hypothalamic area, which contains the A11 dopaminergic cell group, is involved in responses of the sympathetic nervous system (Ranson, 1934). Thus, the cerebellar projection originating in this region might be important in modulating motor activity related to the activation of the sympathetic nervous system.

To date, little is known about the physiological effects of dopamine on cerebellar neurons (see Chapter 3). Further studies will need to be done in this area to elucidate the potential role of this projection in modulating cerebellar function.
Table 1. Summary of injection sites within the mouse cerebellum.

<table>
<thead>
<tr>
<th>case #</th>
<th>lobule</th>
<th>nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I-II</td>
<td>III</td>
</tr>
<tr>
<td>ca24</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>ca25</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>ca26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ca27</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>ca28</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>ca29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ca30</td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>
Table 2. Summary of TH double-labeling from the mouse cerebellum.

<table>
<thead>
<tr>
<th>case #</th>
<th>A1-4</th>
<th>A5</th>
<th>A6</th>
<th>A7</th>
<th>A8-10</th>
<th>A11</th>
<th>A12</th>
<th>A13</th>
<th>A14</th>
</tr>
</thead>
<tbody>
<tr>
<td>ca24</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ca25</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>ca26</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ca27</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>ca28</td>
<td></td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ca29</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ca30</td>
<td></td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 6. (A-J) Distribution of retrogradely-labeled (open squares), TH-immunofluorescent (open circles), and double-labeled (filled/starred circles) neurons in the brainstem of the adult mouse from case ca24. See text for details of individual figures.
Fig. 6 (continued)
Fig. 6 (continued)
Fig. 7. Double-labeled neurons in the locus coeruleus. (A,C) TH-immunofluorescence. (B,D) Retrograde labeling. (A,B) Calibration bar = 100\,\mu m. (C,D) Calibration bar = 40\,\mu m.
Fig. 7
Fig. 8. Double-labeled neuron in the A5 cell group. (A) TH-immunofluorescence. (B) Retrograde labeling. Calibration bar = 40μm.
Fig. 9. Double-labeled neuron in the A7 cell group. (A) TH-immunofluorescence. (B) Retrograde labeling. Calibration bar = 40μm.
Fig. 10. Double-labeled neuron in the A11 cell group. (A) TH-immunofluorescence. (B) Retrograde labeling. Calibration bar = 40μm.
Fig. 11. Double-labeled neuron in the A13 cell group. (A) TH-immunofluorescence. (B) Retrograde labeling. Calibration bar = 40μm.
Fig. 12. (A) TH-immunofluorescent neurons in the substantia nigra. (B) No retrograde labeling is present in the substantia nigra. Calibration bar = 100μm.
Fig. 13. (A) TH-immunofluorescent neurons in the VTA. (B) No retrograde labeling is present in the VTA. Calibration bar = 100\(\mu\)m.
CHAPTER III

THE EFFECTS OF THE CATECHOLAMINES NOREPINEPHRINE AND DOPAMINE ON PURKINJE CELLS IN CEREBELLAR SLICES OF ADULT MICE

INTRODUCTION

It has been well established from studies in various species that the cerebellum receives both a noradrenergic and a serotonergic input from several brainstem nuclei (Fuxe, 1965; Hokfelt and Fuxe, 1969; Bloom et al., 1971; Yamamoto et al., 1977; Kimoto et al., 1981; Landis et al., 1975; Felten et al., 1986; Verney et al., 1982; Kerr and Bishop, 1991; Crivellato et al., 1992; Bishop et al., 1985; Bishop and Ho, 1985). Each of these monoamines is present in a plexus of varicose axons that are morphologically distinct from mossy and climbing fibers and are distributed throughout the cerebellar cortex and nuclei. In addition, numerous studies have described the physiological effects of both norepinephrine and serotonin on cerebellar neurons, particularly Purkinje cells (Wang et al., 1992; Hicks et al., 1989; Darrow et al., 1990; Strahlendorf et al., 1986; Lee et al., 1986; Strahlendorf et al., 1989; Strahlendorf et al., 1991; Armstrong et al., 1987; Mitoma et al., 1994; Kerr and Bishop, 1992; Li et al., 1993; Strahlendorf et al., 1984; Lee et al., 1987; Moises et al., 1979; Freedman et al., 1976; Hoffer et al., 1971; Granholm and Palmer, 1988; Mori-Okamoto and Tatsuno, 1988; Parfitt et al., 1990; Freedman, 1977; Dennett and
Hubbard, 1988; Basile and Dunwiddie, 1984; Bickford-Wimer et al., 1991). Based on these studies both of these monoamines have been classified as neuromodulators in that each one alters the responsiveness of neurons to other inputs rather than directly modifying neuronal activity on its own.

Recently, it has become evident that the cerebellum also receives a dopaminergic input (Panagopoulos et al., 1991). Dopamine-immunoreactive fibers (Panagopoulos et al., 1991) that are similar in morphology to serotonergic and noradrenergic fibers, and dopaminergic receptors (Panagopoulos et al., 1991; Mansour et al., 1992; Gehlert, 1993; Pourquié et al., 1992; Levant et al., 1993; Camps et al., 1990; Wamsley et al., 1989; Dawson et al., 1988; Martres et al., 1985; Mengod et al., 1989; Bouthenet et al., 1991; Bouthenet et al., 1987) have both been reported within the rat cerebellum. These inputs arise from the ventral tegmental area (VTA; Ikai et al., 1991)—the A10 dopaminergic cell group of Dahlström and Fuxe (1964). Similarly, the mouse cerebellum receives a rich supply of varicose fibers that are immunoreactive for tyrosine hydroxylase (TH; Nelson et al., 1993), the initial enzymatic step in the synthesis of catecholamines, and contains a population of dopaminergic binding sites (Panagopoulos and Matsokis, 1994; Panagopoulos et al., 1993; Panagopoulos et al., 1988). It is likely that a subpopulation of these TH-immunoreactive fibers are dopaminergic since a recent double-label study in our laboratory has revealed cerebellar inputs from the caudal diencephalic (A11) and dorsal hypothalamic (A13) dopaminergic cell groups (unpublished data; see Ch. 2).
Although several lines of anatomical data have suggested a dopaminergic input to the cerebellum the physiological effects of dopamine on Purkinje cells, or any other cerebellar neuron, have yet to be determined. Therefore, in the present study we provide the first report of the physiological effects of dopamine on Purkinje cells. We have used extracellular recording techniques in conjunction with iontophoretic application of neurotransmitters to investigate the effects of dopamine on spontaneous firing rate and its interactions with glutamate and γ-aminobutyric acid (GABA) in cerebellar slices from adult mice. In addition, these effects will be compared with the actions of another catecholamine, norepinephrine, whose effect(s) on Purkinje cells have not yet been reported in this species.

MATERIALS AND METHODS

Eight adult mice (C57BL/6J) were used in this study. Each mouse was quickly decapitated and its cerebellum was removed and submerged in ice cold (0-5°C) artificial cerebrospinal fluid (ACSF). The cerebellum was blocked off and the cut surface glued with cyanoacrylate to the stage of a vibrating microtome (Vibroslice; Campen Instruments Ltd.). Parasagittal slices of the cerebellar vermis were then cut at a thickness of 350μm in ice cold ACSF and transferred to an incubation chamber where they were stored for at least 60 minutes before recording. Each slice was individually transferred to the recording chamber and was allowed to incubate for at least 10 minutes prior to recording. The recording chamber was a gas/liquid interface perfusion chamber (Haas top; Medical Systems Corp.) maintained at a flow rate of approximately 2 ml/min. Both chambers were kept at 35-37°C and were bubbled with a 95% O₂/5% CO₂ mixture.
The ACSF (pH 7.3-7.4; 295-305 mOsm) was modified from Llinas and Sugimori (1980) and consisted of 124 mM NaCl, 2.5 mM KCl, 1.25 mM KH$_2$PO$_4$, 28 mM NaHCO$_3$, 1.15 mM MgSO$_4$·(7H$_2$O), 2.5 mM CaCl$_2$·(2H$_2$O) and 12 mM dextrose. The CaCl$_2$ concentration was routinely lowered to 2.0 mM in the incubation chamber ACSF to help maintain slice viability.

Multi-barreled (5 barrels) iontophoretic electrodes were constructed from glass micropipettes (WPI), twisted and pulled on a vertical pipette puller (Narishige), and the tips broken back to an outer diameter of 3-5 μm. The center barrel was filled with dH$_2$O as a control barrel and the remaining outer barrels were filled with the following neurotransmitters: 0.2 M dopamine HCl (RBI), 0.25 M norepinephrine HCl (Sigma), 0.02, 0.1, or 0.2 M L-glutamate (Sigma), and 0.1 or 0.2 M GABA (Sigma). All drugs were dissolved in dH$_2$O and were brought to a final pH optimal for iontophoretic ejection (pH 7.5-9.0 for glutamate and pH 4.0-4.5 for all others). The multi-barreled electrode was used as a remote iontophoretic electrode placed in the molecular layer within the dendritic field of an isolated unit. A separate single-barreled electrode (1-2 μm tip) was filled with 4 M NaCl (2-4 MΩ) and used for recording from the Purkinje cell layer.

Drugs were ejected and retained by electrical currents applied to the barrels using an iontophoretic unit (Fintronics, Inc.). Automatic current balancing was not maintained while using remote iontophoretic electrodes. Negative currents of 20-30 nA were used to retain the drugs in the barrels with the exception of glutamate which was retained with equivalent amounts of positive current. Drugs were ejected from the barrels by reversing or,
occasionally, by reducing the polarity of the retain current. Typically, several different ejection current levels were used in order to establish the concentration-dependency of drug effects.

Extracellular recording of Purkinje cell activity was done with an Axoclamp 200A amplifier (Axon Instruments). Spikes were counted and converted to firing rate (spikes/second) using a window discriminator/timed counter (Frederick Haer and Company) and the data was stored on a chart recorder as a second-by-second update of firing rate. In addition, all drug ejection periods were indicated on the chart paper. Using an analog-to-digital converter (Medical Systems Corp.) data was also stored on VHS magnetic tape for subsequent viewing and data analysis. Taped data was analyzed using the Spike2 for Windows (version 1.64; Cambridge Electronic Design Ltd.) software package.

RESULTS

In all, the effects of norepinephrine and/or dopamine were tested on 36 Purkinje cells. Each neurochemical was applied alone as well as in combination with other neurotransmitters in order to assess its effects on spontaneous firing rate, glutamate-induced excitatory activity, and GABA-mediated inhibition.

Effects of norepinephrine on Purkinje cells

When applied alone, norepinephrine exhibited no effect on the spontaneous firing rate of 18 out of 19 neurons (Fig. 14, top). The remaining neuron showed a slight increase of spontaneous activity in response to application of norepinephrine.
The effect of norepinephrine on glutamate-induced firing was tested on 17 Purkinje cells. In 15 cells the application of norepinephrine resulted in a suppression of glutamate-induced activity (Fig. 15). Norepinephrine never completely inhibited the effect of glutamate, and it often only attenuated the rate of rise in neuronal firing rate during glutamate application. In addition, this effect of norepinephrine appeared to be dose-dependent because higher ejection currents resulted in greater suppression of glutamate activity. One cell initially responded with an elevation of glutamate-induced excitation but within 6 minutes showed a reduction of this activity in response to norepinephrine. The remaining cell exhibited no effect of norepinephrine on glutamatergic activity.

The responses were more varied when norepinephrine and GABA were applied simultaneously. Out of a total of 18 Purkinje cells tested, 9 cells showed a reduction of GABA-mediated inhibition (Figs. 14, 16-18) while 6 cells showed an augmentation of GABA effects in response to norepinephrine (Figs. 19-21). Both of these effects appeared to be to be dose-dependent as well; greater suppression or augmentation of GABA was observed as the ejection current of dopamine was increased. The three remaining cells responded differently depending on the amount of current at which norepinephrine was being applied. The first cell showed an increase in GABA effects at low current levels and a decrease in GABA effects at high levels (Fig. 22). The second cell, like the first, exhibited a reduction of GABA effectiveness at higher ejection currents (Fig. 23). However, it responded in a biphasic manner to low ejection currents of norepinephrine. That is, the initial response to norepinephrine was an increase of the effectiveness of GABA on the cell
(ie. a decrease in firing rate), followed by a lasting reduction in GABA efficacy (ie. an increased firing rate). The third cell, like the first, showed an enhanced GABA effect at lower ejection currents. However, it responded in a biphasic manner at higher levels of GABA (Fig. 24). Like the second cell, the response was characterized by an initial increase, followed by a suppression, of GABAergic inhibition.

**Effects of dopamine on Purkinje cells**

Dopamine had no effect on 20 out of 22 spontaneously active Purkinje cells (Fig. 14, top). Of the two remaining neurons, one cell showed a slight increase, and one a slight decrease, in spontaneous activity.

Out of 23 cells in which dopamine and glutamate were applied together, 18 showed a dose-dependent suppression of glutamate-induced excitatory activity (Fig. 15), 2 showed an increase in activity, and 2 exhibited no effect. The remaining neuron responded in a biphasic manner to the application of dopamine. That is, after an initial brief reduction in glutamatergic excitation dopamine enhanced glutamate activity. Although dopamine suppressed glutamate activity in a majority of cells it never completely blocked it, and often it only attenuated the rate of rise in the firing rate of Purkinje cells during glutamate application (Fig. 15).

As was the case for norepinephrine, dopamine produced variable results when paired with GABA. Eight cells out of 20 exhibited a reduction of GABA-mediated inhibition in response to dopamine (Figs. 16, 21-22). In three cells dopamine caused an enhancement of inhibitory responses to GABA. However, one of these cells later (about 10 minutes) showed
a decrease in GABA effectiveness in response to dopamine and another cell later (within 14 minutes) exhibited biphasic responses. Five of the 20 cells responded in a biphasic manner to dopamine when the catecholamine was applied during continuous ejection of GABA (Fig. 14, bottom; Fig. 20). One of these five neurons later (within 3 minutes) showed only a suppression of GABA effects in response to dopamine. The responses of the remaining five cells were dependent on the level of dopamine ejection: In three cells GABA became more effective at lower ejection currents of dopamine and less effective at higher currents (Fig. 25). Another cell that displayed an augmentation of GABA when dopamine was applied at lower currents responded in a biphasic manner at higher levels (Fig. 18). Finally, one cell responded to high levels of dopamine with a reduction in GABA efficacy and responded to low levels of dopamine in a biphasic manner (Fig. 23). In each case, regardless of the level of dopamine ejection, the biphasic response was characterized by a brief enhancement of GABA effects, followed by a longer-lasting suppression of its effects.

Comparison of norepinephrine and dopamine effects on Purkinje cells

In 25 Purkinje cells the effects of both dopamine and norepinephrine were tested on the same cell. As was mentioned above, neither catecholamine had any effect on spontaneous Purkinje cell activity (Fig. 14, top); 16 out of 16 cases showed no effect when each was tested separately on the same neuron. In 13 of 15 cases, when both norepinephrine and dopamine were tested separately on glutamate-induced excitatory activity, each catecholamine caused a reduction in firing rate (Fig. 15). Further, the suppression of
glutamatergic activity by dopamine and norepinephrine were about equal in magnitude in a majority of cases (12 of 15).

Only 6 out of 15 cells, in which the effects of both norepinephrine and dopamine on GABA-mediated inhibition were examined separately, showed similar effects of these two neurochemicals. Five of these cells exhibited a reduction of GABAergic inhibition by both dopamine and norepinephrine (Fig. 16); the remaining neuron showed a decreased effect of GABA at high ejection currents and a biphasic response at low currents (Fig. 23). In most cases (5 out of 7) the magnitude of the suppressive effect of each of the catecholamines on GABA was about the same.

Norepinephrine and dopamine were simultaneously applied to five Purkinje cells in this study. In 3 out of 3 cases the suppressive effects of dopamine and norepinephrine on glutamate-induced excitatory activity were partially additive (Fig. 15). In one neuron the suppressive effects of dopamine and norepinephrine on GABA-mediated inhibition were also additive (Fig. 17). In the remaining cell, an augmentation of GABA by norepinephrine and a suppression of GABA by dopamine were observed to negate each other (Fig 21).

DISCUSSION

Norepinephrine effects

This study represents the first report of the effects of norepinephrine on Purkinje cells within the adult mouse cerebellum. The present data is consistent with a number of previous in vivo and in vitro studies in other species that have shown that norepinephrine can suppress the firing rate of Purkinje cells driven by glutamate as well as augment the actions of GABA.
(Yeh and Woodward, 1983; Waterhouse et al., 1982; Llano and Gerschenfeld, 1993; Yeh et al., 1981; Moises et al., 1979; Parfitt et al., 1990; Mori-Okamoto and Tatsuno, 1988; Mori-Okamoto et al., 1991; Sessler et al., 1989). However, our data differs from these studies in that norepinephrine was never observed to reduce spontaneous activity in our experiments and in only one cell was it shown to enhance glutamate effects. In addition, our investigation has also revealed a suppression of GABA-mediated inhibition by norepinephrine as well as a biphasic effect on GABA, neither of which have been previously reported. Earlier studies have suggested that biphasic responses, in which a neurochemical has two opposing effects on the same neuron, are due to the differences in affinities of multiple noradrenergic receptor subtypes that are inversely linked to a common second messenger system, for example the adenylate cyclase/cyclic adenosine monophosphate (cAMP) pathway (Crepel et al., 1987; Mori-Okamoto and Tatsuno, 1988; Mori-Okamoto et al., 1991; Basile and Dunwiddie, 1984; Parfitt et al., 1988; Granholm and Palmer, 1988). In these and other studies augmentative effects of norepinephrine on both glutamatergic excitation and GABAergic inhibition were shown to be mediated by a β1 adrenergic receptor-coupled increase in cAMP (Llano and Gerschenfeld, 1993; Waterhouse et al., 1982; Yeh and Woodward, 1983; Siggins et al., 1971a; Mori-Okamoto and Tatsuno, 1989; Siggins et al., 1971b; Siggins et al., 1973; Sessler et al., 1989) whereas suppression of glutamate-induced activity has been suggested to be mediated via an α2 adrenergic receptor-coupled inhibition of the adenylate cyclase system (Granholm and Palmer, 1988; Basile and Dunwiddie, 1984; Parfitt et al., 1988; Mori-Okamoto et al., 1991). At present it is not clear whether the varied
responses observed in our study are due to the activation of different receptor subtypes, nor is it clear whether the differences between our study and previous investigations reflect species variations in the expression of particular receptors or methodological differences.

**Dopamine effects**

In the present study we have provided the first report that dopamine can modulate cerebellar activity. Dopamine suppresses glutamate-induced activity while having no effect on spontaneous activity of Purkinje cells, and has varied or biphasic effects on GABA-mediated inhibition of Purkinje cells. Previous studies on the physiological effects of dopamine in other regions of the central nervous system have also shown it to have biphasic effects that are mediated by multiple receptor subtypes (Martin and Waszczak, 1994; Cepeda et al., 1992; Shen et al., 1992). Multiple subtypes of dopamine receptors have been reported in the cerebellum of several species (Panagopoulos et al., 1991; Mansour et al., 1992; Gehlert, 1993; Levant et al., 1993; Camps et al., 1990; Wamsley et al., 1989; Dawson et al., 1988; Martres et al., 1985; Mengod et al., 1989; Bouthenet et al., 1991; Bouthenet et al., 1987; Panagopoulos et al., 1988; Panagopoulos et al., 1993; Panagopoulos and Matsokis, 1994). Therefore, like norepinephrine, it appears that dopamine might also be acting upon multiple receptor subtypes within the cerebellum, or even on single Purkinje cells, to produce multiple responses. Because our experiments did not take measures to block synaptic transmission in the slices we cannot rule out potential presynaptic effects of dopamine that could alter other inputs to Purkinje cells as an explanation for the varied responses to dopamine.
In our study it is apparent that dopamine and norepinephrine have very similar effects on Purkinje cell activity, particularly with respect to their effects on glutamate and their lack of effects on spontaneous activity. Previously, the effects of norepinephrine have been suggested to be mediated via alterations in the concentration of intracellular cAMP (Mori-Okamoto et al., 1991; Siggins et al., 1971b; Siggins et al., 1971a; Mori-Okamoto and Tatsuno, 1989; Siggins et al., 1973; Sessler et al., 1989). Although we did not attempt to determine the cellular mechanisms by which dopamine effects Purkinje cell activity, dopamine receptors are known to act principally upon the cAMP system as well (Kebabian and Calne, 1979). Thus, it is possible that dopamine and norepinephrine work through a similar intracellular messenger pathway to produce their effects on Purkinje cells. Both catecholamines exerted varied effects on GABAergic inhibition of Purkinje cells. However, in less than half of the cases (6 of 15) in which each was applied separately to the same neuron in conjunction with GABA did they have similar or identical effects. Therefore, it is more difficult to speculate whether norepinephrine and dopamine might be acting through similar biochemical mechanisms to modulate GABA effects on Purkinje cells.

One discrepancy that must be addressed concerns the apparent mismatch between cerebellar dopaminergic afferents, dopamine receptors, and the location of the physiological recordings done in the present study. Because dopaminergic receptors have been localized by other researchers to vermal lobules IX and X of the rat cerebellum (Mengod et al., 1989; Bouthenet et al., 1991; Bouthenet et al., 1987; Levant et al., 1993; Gehlert, 1993; Camps et al., 1990) we restricted our recordings to vermal lobule IX in our mouse model, although,
to date, no localization studies of dopamine receptors have been done in the mouse cerebellum. However, after completing a set of double-label experiments in the mouse looking for potential dopaminergic origins of cerebellar TH-immunopositive fibers we found that lobule X appears to be the primary site of termination of a dopaminergic projection from the hypothalamus (see Ch. 2). Similarly, a mismatch between the location of cerebellar dopamine receptors (in vermal lobules IX and X) and the proposed termination site of VTA TH-immunoreactive projections to the cerebellum (in the lateral hemispheres) (Ikai et al., 1992) also occurs in the rat. A common explanation for receptor mismatch is the "volume transmission" theory that allows for the diffusion of chemical messengers some distance between their site of release and the receptor site at which they act (Fuxe and Agnati, 1991; Agnati et al., 1992). However, it is not known whether such a phenomenon could account for the relatively large disparity in the release sites and binding sites observed for dopamine in the rodent cerebellum.

Despite these discrepancies, it remains evident that a functional dopaminergic input to the cerebellum exists. Further studies involving the localization of dopaminergic receptors, fibers and synapses within the mouse cerebellum will be required in order to sort out these inconsistencies.

**Functional considerations**

The principal finding of this study is that both norepinephrine and dopamine suppress glutamate-induced excitatory activity and either augment or reduce the inhibition due to GABA whereas neither have any effect on spontaneous activity of Purkinje cells.
Glutamate is the putative neurotransmitter of parallel fibers and climbing fibers, the two classically defined excitatory inputs to Purkinje cells, and GABA is the neurotransmitter of basket cells, which make inhibitory synaptic contacts on the somata of Purkinje cells (for a review see: Ito, 1984). Thus, it is apparent that one function of catecholamines in the cerebellum is to alter the responsiveness of Purkinje cells to their afferent inputs rather than to affect the intrinsic membrane properties that regulate spontaneous activity. Such modulation of the excitatory and inhibitory inputs to Purkinje cells would alter the flow of neural information from the cerebellar cortex to the cerebellar nuclei and would therefore result in a modification of cerebellar output to motor systems. For example, decreasing Purkinje cell activity, either by suppressing the effects of glutamate or by enhancing the inhibition due to GABA, would result in disinhibition of nuclear cells, and thus would increase cerebellar output. In the case of vermal lobules IX and X, where dopaminergic receptors and the terminals of dopamine afferents (of hypothalamic origin) are located, elevating cerebellar output would have stimulatory effects on the descending vestibulospinal tracts involved in maintaining equilibrium. In addition, these lobules are involved in the oculomotor aspects of vestibular function.

It is expected that norepinephrine would have more widespread effects on cerebellar function based on its more divergent distribution within the cerebellum. It has been hypothesized that norepinephrine acts to "gate in" (Woodward et al., 1991) or increase the "signal-to-noise ratio" (Woodward et al., 1979) of the responses of Purkinje cells to their inputs, thus modulating their output to the cerebellar nuclei and, in turn, output of the
cerebellum. Such adjustments in responsiveness might be important during periods when heightened arousal or attentiveness are required. The locus coeruleus, which is primarily noradrenergic and gives rise to a majority of the catecholaminergic afferents to the cerebellum, has been shown to be involved in the circuitry responsible for altering the vigilance states of the organism in parallel with increased activation of the sympathetic nervous system (Aston-Jones et al., 1991). The posterior hypothalamus, which contains the A11 dopaminergic cell group, is also involved in sympathetic nervous system function (Ranson, 1934). Thus, based on the similarities in the effects of dopamine and norepinephrine in the present study, and on the hypothalamic origins of dopaminergic inputs to the cerebellum, a similar function might soon be assigned to dopamine in the modulation of cerebellar circuitry relative to the physiological state of the organism.
Fig. 14. (Top) Chart recording demonstrating that norepinephrine (NE) and dopamine (DA) have no effects, whereas GABA has an inhibitory effect, on the spontaneous activity of Purkinje cells. (Bottom) Chart recording showing biphasic effects of DA on GABAergic inhibition and a suppression of GABA effects by NE.
Fig. 14
Fig. 15. (A) Chart recording showing the suppression of glutamate (GLUT)-induced activity by both DA and NE. When applied together NE and DA have a partial additive effect in suppressing GLUT. (B) Oscilloscope traces during the application of 1) GLUT alone; 2) GLUT + DA; 3) GLUT + NE; and 4) GLUT + NE + DA. Time = 200 msec; spike amplitude = 60-75 mV.
Fig. 15
Fig. 16. (A) Chart recording illustrating the dose-dependent suppression of GABAergic inhibition by both DA and NE. (B) Oscilloscope traces during spontaneous activity (1 and 5) and during the application of GABA alone (2 and 6); DA alone (3); DA + GABA (4); NE alone (7); and NE + GABA (8). Time = 200 msec; spike amplitude = 75-100 mV.
Fig. 16
Fig. 16 (continued)
Fig. 17. (A) Chart recording showing the suppression of GABA by both DA and NE including a partial additivity of NE and DA effects on GABA. (B) Oscilloscope traces during the application of GABA alone (1 and 3); 2) DA + GABA; 4) NE + GABA; 5) NE + DA + GABA; and 6) spontaneous activity. Time = 200 msec; spike amplitude = 100-175 mV.
Fig. 17
Fig. 18. Chart recording depicting the dose-dependent suppression of GABA by NE and biphasic effects of DA on GABA, particularly at higher application currents (eg. +70 nA).
Fig. 18
Fig. 19. Chart recording showing an augmentation of GABA by NE while having no effect on spontaneous activity.
Fig. 20. (A) Chart recording illustrating an enhancement of GABA effects by NE and biphasic effects of DA, particularly at higher application currents (eg. +30 nA). (B) Oscilloscope traces during 1) GABA alone before NE application; 2) NE + GABA; 3) GABA alone after NE application; 4) GABA alone before DA application; 5) DA + GABA, showing an initial augmentation of GABA inhibition; and 6) DA + GABA, showing a secondary suppression of GABA. Time = 200 msec; spike amplitude = 35-50 mV.
Fig. 20
Fig. 21. Chart recording illustrating a suppression of GABAergic inhibition by DA and an enhanced effect of GABA by NE. The effects negate each other when DA and NE are applied simultaneously.
Fig. 21
Fig. 22. Chart recording illustrating no effect of either DA or NE on spontaneous activity, a reduction of GABA inhibition by DA, and variable effects of NE on GABA. Lower application currents (e.g., +20 nA) of NE result in enhanced GABA effects whereas higher currents (e.g., +80) result in a suppression of GABA.
Fig. 22
Fig. 23. (A) Chart recording showing suppression of GABA by DA and NE at higher current levels (eg. +70 nA and +80 nA, respectively) and biphasic effects at lower levels. (B) Oscilloscope traces during the application of 1) GABA alone; 2) GABA + NE (at +35 nA), showing an initial enhancement of GABA effects; 3) GABA + NE (at +35 nA), showing a secondary suppression of GABA inhibition; 4) GABA alone; 5) GABA + NE (at +80 nA). Time = 200 msec; spike amplitude = 60 mV.
Fig. 24. Chart recording illustrating a dose-dependent enhancement of GABA at lower current levels of NE (< +35 nA) and biphasic effects of NE at higher application currents (> +35 nA). Increasing the application current of GABA accentuates the biphasic response elicited at higher currents of NE (eg. +50 and +70 nA). DA also has biphasic effects on GABA inhibition, particularly at higher currents (eg. +70 nA).
Fig. 24
Fig. 25. Chart recording showing no effect of DA on spontaneous activity and variable effects on GABA inhibition. At lower current levels (eg. +10 nA) of DA GABAergic inhibition is enhanced whereas at higher current levels (eg. +80 nA) GABA is suppressed.
LIST OF REFERENCES


Ikai, Y., M. Takada, Y. Shinonaga, and N. Mizuno (1992) Dopaminergic and non-dopaminergic neurons in the ventral tegmental area of the rat project, respectively, to the cerebellar cortex and deep cerebellar nuclei. Neurosci. 51:719-728.


Kerr, C.W. and G.A. Bishop (1992) The physiological effects of serotonin are mediated by the 5HT\textsubscript{1A} receptor in the cat's cerebellar cortex. Brain Res. 591:253-260.


