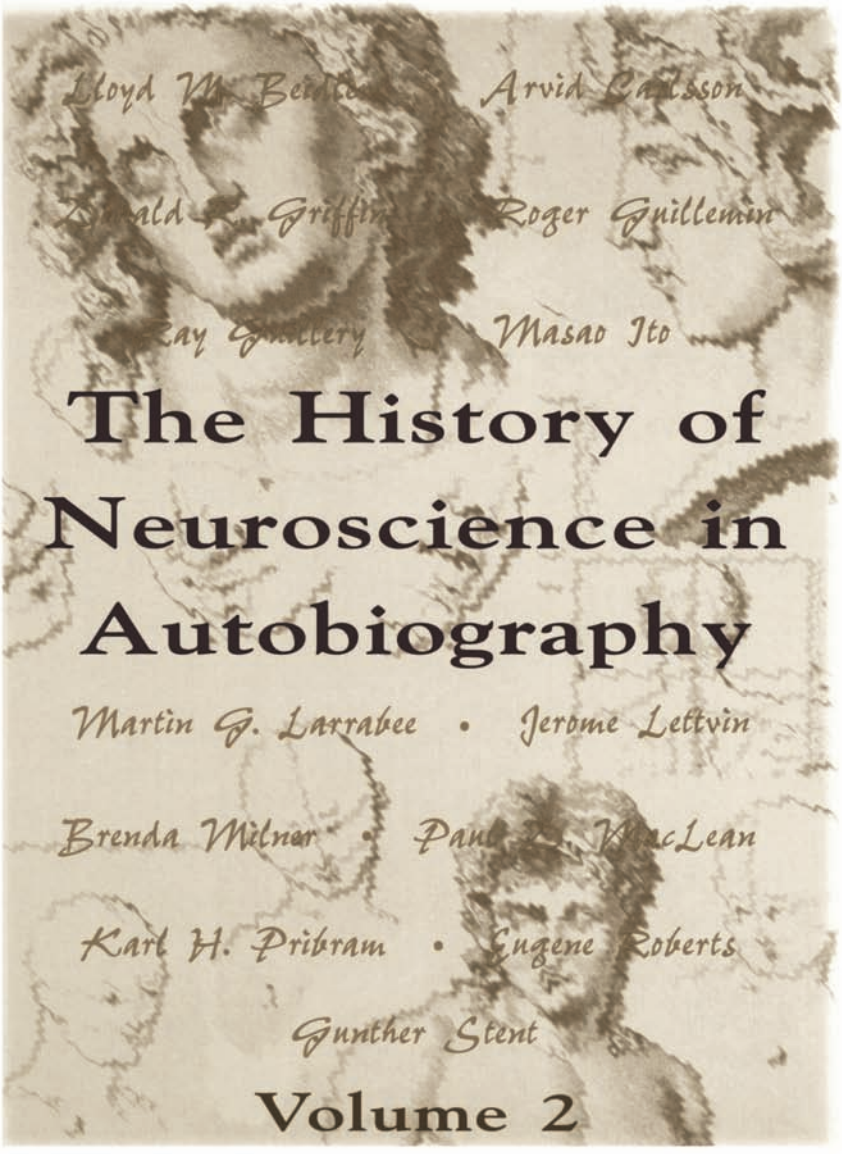


Edited by Larry R. Squire

The History of Neuroscience in Autobiography

VOLUME 2

Edited by Larry R. Squire

ACADEMIC PRESS

San Diego London Boston New York Sydney Tokyo Toronto

This book is printed on acid-free paper. ♾

Academic Press
a division of Harcourt Brace & Company
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.apnet.com

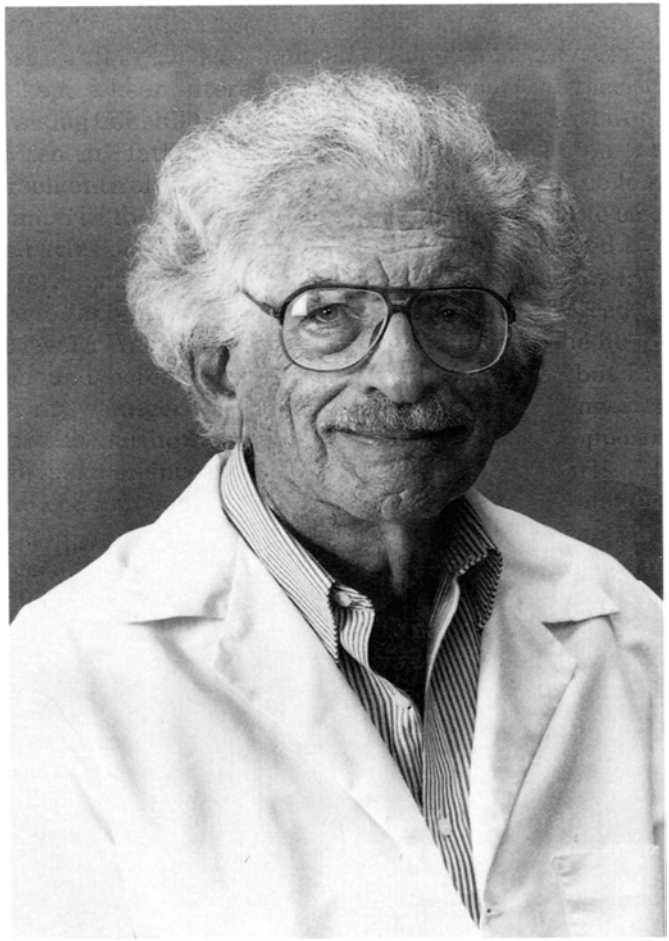
Academic Press
24-28 Oval Road, London NW1 7DX, UK
http://www.hbuk.co.uk/ap/

Library of Congress Catalog Card Number: 98-87915

International Standard Book Number: 0-12-660302-2

PRINTED IN THE UNITED STATES OF AMERICA
98 99 00 01 02 03 EB 9 8 7 6 5 4 3 2 1

Contents



Eugene Roberts

BORN:

Krasnodar, Russia
January 19, 1920

EDUCATION:

Wayne University, B.S. (1940)
University of Michigan, M.S. (1941)
University of Michigan, Ph.D. (1943)

APPOINTMENTS:

Washington University School of Medicine (1946)
City of Hope Research Institute (1954)

HONORS AND AWARDS:

National Academy of Sciences, U.S.A. (1988)
American Academy of Arts and Sciences (1995)

Eugene Roberts reported the discovery of γ-aminobutyric acid (GABA) in the brain in 1950, and then pioneered the immunohistochemical localization of neurotransmitter-specific neural systems at the light and electronmicroscopic levels using antisera to the synthetic enzyme glutamic acid decarboxylase. His work was instrumental in establishing GABA as the major inhibitory neurotransmitter in the vertebrate central nervous system.

The History of Neuroscience in Autobiography, Volume 2

Eugene Roberts

Almost immediately after sitting down to write this piece, I felt myself drowning in a Proustian whirlpool of a lifetime of multisensory recollections running back in time in zig–zag fashion. This was not amenable to encapsulation for transmission to a reader. I believe as Picasso did: "There are so many realities that in trying to encompass them all one ends in darkness. That is why, when one paints a portrait, one must stop somewhere, in a sort of caricature. Otherwise there would be nothing left at the end," (Picasso, cited by Ashton, 1988).

Therefore, at the outset, I undertake a broad brush-stroke, impressionistic approach, presenting a few vignettes from my life that relate to important formative influences. Perhaps one or another of them, in holographic fashion, may reveal me almost *in toto.*

Escape from Lenin's Tomb

At the age of 2 years and 9 months, in November 1922, I was brought by my parents from Russia to the United States. My parents were natives of Kerch, a Black Sea port in the Crimea. They were on the move after the Russian revolution and were living in Krasnodar in the Caucasus, where I was born in 1920. In 1921 they moved to Leningrad (now, once again, St. Petersburg), whence they came to the United States, leaving by boat from Lebau, Latvia. The intricate and expensive travel arrangements had been made by my father's brother, a wealthy real estate developer in Detroit, Michigan. We were met upon docking in New York City by his agent and taken immediately to a train departing for Detroit. In this manner, I was deprived of the classical immigrant experience of passing through Ellis Island, which I saw only recently at a distance from the deck of a ship that my wife and I were taking to England.

In Russia, my father had been a successful merchant with international connections. In the period between the Russian revolution in 1917 and the time we left Russia in 1922, merchants like my father were encouraged by the communist government to import from abroad and to sell on the flourishing black market items such as food, clothing, and soap to help keep the Russian population from starving or freezing to death or dying from inordinate spread of infections. Deciding in February of 1923 that the emergency was over, Lenin's government arrested and summarily executed all of the merchants like my father and either killed members of their families

or sent them into exile in Siberia. By the slim margin of 3 months, our little family had survived and made it to America!

I was an only child and received much love, attention, and encouragement from my parents. I spoke only Russian with them at home and in public as long as they lived, although eventually they spoke English well. This contrasted with friends who were older than I was when they came from Russia, many of whom repressed their knowledge of Russian, hoping to escape the "greenhorn" label so scornfully applied to immigrants in those days. Knowledge of Russian proved to be very useful during three separate month-long visits to the Soviet Union between 1962 and 1975, twice as a member of cultural exchange groups sent by the U.S. Department of State and once as a guest of the Soviet Academy of Sciences.

Dr. Lakoff's Magic

Shaken by chills and only half conscious, lying on a narrow bed in a dimly lit room in Detroit Receiving Hospital after having suffered a ruptured appendix, on a pre-sulfa drug, pre-antibiotic day in April 1936, I heard my death sentence pronounced to my father, just outside the door: "Mr. Rabinowitch, your son has less than 1 chance in 100 of living. Perhaps we should do a laparotomy." A blanket of sadness descended upon me and a tableau of things never to be experienced flowed through my mind as though on a receding tide—high school graduation, college, Microbe Hunterian and Arrowsmithian ecstatic commitment to science, a true love of my own. . . . To this day I marvel that I felt no panic or fear. Now in my 78th year, having experienced the thrills and rewards of the scientific chase and deep, reciprocated love, I wonder how it will be when death and I make our final rendezvous.

I remember a kind, roundish face looking down at me while saying to my parents standing alongside, "We must get this boy out of here and into my hospital. He'll die here. He may die in the ambulance, but we must take that chance." Twelve weeks later, 1 week after leaving the hospital, having regained 40 pounds and having lost the horrible mirror-repelling jaundiced yellow color I had acquired, I strode jauntily into the office of Dr. Charles Lakoff, arguably the best abdominal surgeon in Detroit, for my only post-treatment visit. Upon seeing me, he turned pale and sat down. I became alarmed and asked him whether or not he was ill. He said, "Eugene, I never thought I would see you walking again." I replied, "It's a good thing you didn't let me know." As I was leaving the office, the secretary handed me a bill for $50 to be paid "at my convenience." She told me that Dr. Lakoff thought I should be charged this amount so that my self-respect and dignity would be preserved. They were.

Among the things I learned from this experience was never again to scoff at artistic representations of halos. I saw a halo three times daily for the last 9 of the 11 weeks I lay in the hospital, where Dr. Lakoff was chief

of staff. My heart would begin to pound when I heard the sounds signaling the approach of Dr. Lakoff and his entourage. A golden glow enveloped him as he crossed the threshold to my room. Amazed and completely unbelieving, I checked this out daily. It always was there.

In 1960, I made a special trip to Detroit to present to Dr. Lakoff an inscribed volume of *Inhibition in the Nervous System and γ-Aminobutyric Acid* (Roberts et al., 1960) containing the proceedings of a neuroscience conference which I had organized together with colleagues from UCLA and Cal Tech at the City of Hope the previous year. I asked Dr. Lakoff, then already retired from surgery, how he had known what to do to save my life. He had touched me only to check the drain in my abdomen and to pat me on the head. He had never allowed even a single aspirin to be given me. His only reply was to quote his wife as having said, "Charlie, why do you run around so much? They'll die anyway." Then, smiling broadly he said, "You know, more than half of them lived."

Raphael's Mantle

My uncle, Raphael Dorfman, husband of my father's sister, Hannah, lived in the flat next door, the back door of which opened onto the same breezeway as did ours. In Russia he had been a teacher in a *gymnasium,* a secondary school that differed from our high schools in that the curriculum included material at the level usually covered in the first 2 years of our colleges and universities. Because of restrictions of immigration quotas, he resided in Latvia for several years after his wife and son had come to the United States. He arrived in the depth of the depression and eventually found work as a house painter. He had poor rapport with a son who was absolutely refractory to any kind of intellectual stimulation. However, Raphael found fertile soil in me. He had taught Latin and mathematics in Russia. When I began Latin and algebra in the eighth grade, he took over supervision of my homework and other aspects of my intellectual development.

A trim, athletic man with a full head of wavy luxurious brown hair and a heavy, attractively kept mustache, a cigarette hanging from the right side of his mouth, wearing his beautiful silk shirt with brightly embroidered collar, he would say in Russian, "Well, Eugene, what have we tonight?" By that time I would have done my math problems and Latin assignment. He would give me additional problems to do and various puzzles to solve. He helped me achieve elegance in Latin translation while informing me about life in ancient Rome and life, in general. He also taught me some Yiddish, which was not spoken in my home because my mother came from a Russified environment in which Yiddish was disdained. I learned to read the *Jewish Daily Forward,* to which my father subscribed, but I never learned to speak Yiddish.

Raphael's liberal views made sense to me during the dark days of the Depression. I followed with great interest and approval the inhibitory con-

trols that the New Deal was applying to the unfettered practices of free enterprise capitalism that had led to the depression. To this day, my social and economic views are colored by Raphael's tutelage. It probably is one of the reasons that I felt comfortable when I came to the City of Hope Medical Center in 1954, which at that time was in transition from being a tuberculosis sanatorium to becoming a cancer hospital and basic research center. At its inception in 1912, the institution had been called the Jewish Consumptive Relief Association and was largely supported by Yiddish-speaking Jews with a socialistic orientation and labor union loyalties. However, only kosher food was served in order to accommodate orthodox Jewish patients who might require it. At that time, many in the San Gabriel Valley called it the "Jew hospital." By the terms of the original charter of the City of Hope, free care of the highest possible quality would be given to patients without consideration of race, creed, or color. Upon arrival to the hospital, patients were given a brochure addressed "to a VIP" so that they would not feel themselves to be recipients of charity. Money to cover the costs was raised through a system of auxilaries throughout the country and from labor unions. Compulsory free care now has been discontinued because of fiscal exigencies and changes in health care delivery practices. It was beautiful while it lasted and was as empowering to staff as it was to patients.

Raphael must have noticed how much I admired his beautiful shirt with the embroidered collar. Upon my graduation from junior high school he made me a gift of it. I had put on Raphael's mantle, so to speak. I wore it nightly until it wore out completely. Psychologically, I still am wearing it. When I went to see him in 1943, after having received my doctorate in biochemistry from the University of Michigan, the look on his face said it all. I had achieved his great ambition. My gratitude to him knows no bounds. He had made it possible for me to make a life for myself. He was my archangel Raphael.

Miss André's Biology Class

Throughout my school years, from grade school through graduate school, I was fortunate in having well-educated, perceptive, and sometimes inspiring teachers. One stood out above all the rest. Miss Elonia André was a biology teacher to whose class I was assigned during my first semester in high school. She was a little, gray-haired lady with metal-rimmed glasses, a kind face that could turn stern, and two little bags, one underneath each side of her chin, that jiggled asynchronously when she said the words, "beetles and bugs."

Noticing my apparently insatiable curiosity, she invited me to use the well-stocked and well-equipped laboratory to do whatever I wished when classes were over, during which time she sat in her office reading, correcting papers, and occasionally coming into the laboratory to observe my activities or just to chat. I became fascinated with the unicellular creatures found in

her hay infusions, particularly with the antics of slipper-like paramecia as they went through their endless, apparently random swims. Wondering how I might perturb their activities, I decided to put a salt crystal at the edge of a drop in which a paramecium was moving about. Not only did it avoid touching the crystal, but the region it avoided increased progressively with time. Although I correctly deduced that the spreading invisible barrier was attributable to the diffusion of dissolved salt away from the crystal, I had no idea as to why the increased salt concentration affected the behavior of the paramecium. I observed dozens of paramecia in this manner and summarized my results in a short paper which I submitted to Miss André. She approved it enthusiastically. I loved Miss André, I loved biology, and I was "hooked" on science. She had stayed late after school solely on my account. She could have gone home promptly at four o'clock like the other teachers.

Miss André retired at the same time that my class graduated from high school in 1936 and, as one of the editors of the high school annual, the *Centralite,* I arranged to dedicate it to her. I am writing this, 61 years later, while looking at the wonderful, full-page picture of Miss André on the frontispiece and the dedication to her that I had written: "This *Centralite* is dedicated to Miss Elonia André, teacher and friend, who since 1896 has communicated the language and love of Nature to students of Central and won a permanent place in their hearts."

One cold winter day, after completing courses in zoology and comparative anatomy at Wayne State University, I visited Miss André and her sister, both living in retirement in their home in Detroit, Michigan. I remember with great pleasure the cup of hot mulled grape juice and the attractive plate of cookies that they served while they queried me about my life at the university.

I never saw Miss André again. Something of her always will remain with me.

The Most Difficult Letter I Ever Have Had to Write

Dr. Seymour S. Kety
National Institute of Mental Health
National Institutes of health
Bethesda 14, Maryland

Dear Seymour:

I hope this letter isn't as hard for you to read as it is for me to write. Some time after I had spoken to you and had sent the letter to Bierman refusing his job, he called me and asked me why I had not taken the position which he had offered me. I

> frankly told him a number of the objections that I had. He gave me assurance that these objections would be overcome and agreed to make his promises concrete by placing them in contract form. In the interim my wife and I had become increasingly apprehensive about working for the government at the present time, and it became apparent that it would be psychologically difficult for us to operate successfully and happily in the present environment, as we imagine it to be. I have, therefore, decided to take my chances in California, fully realizing what a superior scientific opportunity and environment I will be giving up. I am mortified to think of the concern and work that my vacillation will have caused you and only hope that you find it in your heart to forgive me. . . .

I wrote the letter in April 1954. I had been offered and had accepted the job I had wanted above all others. After my discovery of brain γ-aminobutyric acid (GABA) in 1949 and the subsequent realization of its potential importance in brain function, I dearly wanted to spend full time working on it. At that time I was a research associate in the Division of Cancer Research at Washington University in St. Louis and my major obligation was to pursue cancer research. Although the director, the eminent E.V. Cowdry, had most generously permitted me to work on GABA, I felt guilty at pulling less than my full weight on work of interest to him. As soon as I had learned from friends at the National Institutes of Health (NIH) in 1951 that a Mental Health Institute was being formed in Bethesda, with Seymour Kety as research director, I knew that was where I most wanted to be. With enthusiastic recommendations by scientists on the staff of the NIH and in academe, I was given an excellent civil service appointment by Kety with more than adequate support in terms of personnel, space, and equipment. In 1953 and early 1954 I was making relatively frequent trips to Bethesda to oversee construction of laboratories and installation of equipment.

A dark cloud had descended over Washington and was spreading over the rest of the country, as well. The witch hunts of the Cold War were gaining momentum. On successive visits I could sense progressive tightening of the vise. People were removing books from their shelves at home or turning them upside down. There was a palpable hesitation of individuals to join small groups talking in the halls of the Clinical Center at the NIH, where previously, friendly gregariousness had been the norm. My friends in other branches of government spoke on the phone with me only reluctantly, often suggesting that we talk only when we met face to face. Because I had been cleared by the FBI a number of times during my years on the Manhattan Project and because I could not recall having said or done anything that could be interpreted as subversive, I felt invulnerable to personal

attack. Little did I realize that the situation had become a serious test of personal courage and conscience even for the "untainted" ones, somewhat akin to that faced by those who had to decide whether or not to hide or defend Jews during the Nazi years in Germany.

On my next visit to Bethesda, I dropped in to visit the biochemical group at the National Cancer Institute. Among others, I usually visited with an old friend, Vernon Riley. This time he was nowhere to be found. No one, including the head of the biochemistry group, Jesse Greenstein, whom I admired greatly for his scientific ability and personal courage, would tell me the whereabouts of Riley. On inquiry, Greenstein shrugged his powerful wrestler's shoulders and said, "I don't know what these guys do. I work 18 hours a day on my research and don't keep track of them." Finally, after persistent questioning around the laboratories, I learned that Riley had been summarily ordered off the grounds by a letter signed by Oveta Culp Hobby, Secretary of the Department of Health, Education and Welfare, with no reason being given. In a state of utter disbelief, I went directly to G. Borroughs (Bo) Mider, director of the National Cancer Institute, whom I had known well at the University of Rochester during Manhattan Project days. His reply to my query as to whether he would demand an explanation for Riley's dismissal was, "Can we risk the jobs of 3300 scientists for the sake of one?" I said forlornly as I left his office, "You're all in danger if you don't." Things went no better at the Mental Health Institute.

I could not quietly allow human beings to be destroyed in this fashion. In spite of this dilemma, with trepidation I still planned to go to Bethesda and turned down an offer at the City of Hope Medical Center in Duarte, California, in a note I sent the Friday of the week during which the preceding incidents had taken place. The following Monday morning I joined others in the medical students' lounge at Washington University Medical School to watch on television the first day of the Army–McCarthy hearings. There appeared on the screen the hateful, sneering countenance of Senator Joseph McCarthy, going through his act of shuffling papers with supposed lists of subversives while muttering irresponsible accusations of communism and disloyalty. My spirits had fallen to ground zero when the departmental secretary came in and told me that she was holding a long distance call for me from California. Returning to the departmental office, I lifted the receiver to hear from Howard Bierman, "Eugene, why did you turn down my job?" My instantaneous reply was, "I didn't. I have just accepted it."

I now have spent more than 43 productive years at the City of Hope and have never regretted the decision I so hastily arrived at. Seymour Kety and I have been good friends ever since. McCarthy was destroyed by the hearings and subsequently drank himself to death. Riley got a job at Memorial-Sloan Kettering Institute and thereafter continued doing good work until his death. Eventually, I was told that the unsubstantiated accusation in Riley's file leading to his dismissal was that as a student at the University

of Chicago, years before, someone had said that he had been seen contributing a few coins for the benefit of Americans who had fought as members of the Abraham Lincoln Brigade of the Spanish Republican Army against Francisco Franco, subsequently the long-time fascist dictator of Spain and Hitler's ally. Come to think of it, so had I!

Just as I was completing this article, the following item appeared in the Los Angeles Times on April 28, 1997:

> *Spain*
>
> Germany Admits Guilt over Guernica
>
> Germany's president expressed remorse for the 1937 bombing of Guernica, making his country's first atonement for what he called "the most terrible atrocities." Roman Herzog sent a message to survivors of the German bombing raid—immortalized in a painting by Pablo Picasso—one day after the 60th anniversary of the first air attack on an undefended town in history. German warplanes dropped 100,000 pounds of bombs on the civilians of the Spanish town, killing between 1000 and 1650 people.
>
> (Times Wire Reports)

Imagine the letter Pablo Picasso might have gotten from Oveta Culp Hobby had he been working at the NIH in 1954!

A Brief Career Sketch

I received my early education in excellent public schools near my home. I won a scholarship to Wayne State University in Detroit, where I majored in chemistry and minored in biology, and from which I received a B.S. degree, magna cum laude, in 1940. I had become fascinated by organic chemistry and originally had planned to work toward a doctorate in this field at the University of Michigan.

When I first became acquainted with the subject of biochemistry in 1939, the course was not popular with those who wished to make a career in science. It appeared to be a static discipline, concerned in the laboratory largely with medically related analyses of constituents of blood and urine and based on theories that viewed living organisms as combustion engines and on Folin's hypothesis of the existence of independent exogenous and endogenous types of metabolism. But by 1940, the time at which I had to make a graduate career choice, the situation had been changed completely. Using compounds labeled with the newly discovered stable isotopes deuterium and ^{15}N, Rudolf Schoenheimer and his associates, through brilliantly

designed experiments, showed a dynamic state of body constituents to exist: "The simile of the combustion engine pictured the steady flow of fuel into a fixed system, and the conversion of this fuel into waste products. The new results imply that not only the fuel but the structural materials are in a steady state of flux. The classical picture must thus be replaced by one which takes account of the dynamic state of body structure." This swept me and a number of others with both chemical and biological interests into the suddenly overwhelmingly exciting activities of metabolic biochemistry. This important watershed and the events surrounding it seem largely to have been forgotten. Not one of my last five postdoctoral associates, trained in leading centers in the United States, Japan, and Switzerland and all well versed in techniques of modern biochemistry and molecular biology, recognized Schoenheimer's name when I brought it up in our first interviews. It is maladaptive and even tragic that such historical disconnectedness should exist. May I recommend for reading, or rereading, a small and beautifully written, posthumously published book by Schoenheimer, *The Dynamic State of Body Constituents* (Schoenheimer, 1942), and an excellent summary of earlier as well as of some more recent relevant studies by one of his original associates, Sarah Ratner (Ratner, 1979).

During an interview prior to my admission to graduate school, Professor H. B. Lewis, Chairman of Biochemistry at Michigan, told me discouragingly that jobs for biochemists generally were very scarce, but virtually impossible to obtain for "Jewish boys," unless it was in some "choice" location such as southern Alabama. Noting the look of disappointment on my face, he said, "Well, young man, if you feel that way, come along, anyway." I did.

During my first year at Ann Arbor, I learned from a variety of unsolicited sources with supporting data that the name Rabinowitch on an application would virtually assure that I would not even be granted an interview for a job, either in industry or in academe. I changed my name to Roberts with that in view, while maintaining my Jewish identity in all other respects. My parents did not take it lightly, but agreed with my decision.

The practicality of this move was borne out in 1946 when I applied for a position at Washington University. At the interview with Prof. Cowdry, there was instant rapport between us and he offered me the job, forthwith, even though early in our conversation I had informed him of my Jewishness. Shortly after I came to work in St. Louis, the senior scientist of our group told me that it was a good thing that it was not evident from my application that I was Jewish. Learning of the opening, the dean of the medical school was heard to have said to Dr. Cowdry, "Vincent, please don't start a synagogue." Although times have changed, it is important to remember both the scientific and social past so that we may transcend previous limitations and errors.

At the University of Michigan, a scholarship from the McGregor Foundation and a University Fellowship aided me in earning my M.S. degree in

1941 and Ph.D. in 1943. I was fortunate in that my doctoral thesis director, the eminent lipid biochemist, Prof. H.C. Eckstein, left the thesis work entirely to my own devisal and execution, all of which was accomplished in a 9-month period. Titled *Factors Influencing the Deposition of "Fat" in the Liver,* it focused on problems of biological methylation. My master's and doctoral theses resulted in three publications in the *Journal of Biological Chemistry,* a matter of great pride in those days.

Immediately upon completion of my thesis, just as I was about to be drafted into the army, I was commandeered as a civilian to the Manhattan Project at the University of Rochester in Rochester, New York, to work on the toxicology of uranium dusts. Starting from scratch, with little precedent to draw upon, our group quickly developed the technologies necessary for the preparation, dispersal, sampling, and analysis of uranium dusts and studied in eight species of animals the effects of chronic inhalation exposure to different concentrations of dusts of various uranium salts used in industrial processes related to subsequent production of nuclear weapons. Sensitive techniques were devised for monitoring and evaluating the toxicities of these substances. Safe limits for human exposure were established and assiduously enforced so that health records in the plants involved in this work were superior to those in many diverse industries at that time. After multiple experimental repetitions over the years, our original values were declared to be the best available and were voted into law by Congress. In the course of this demanding 80-hour-a-week effort, I found the time to conduct and complete a study on mechanisms of drug-stimulated vitamin C synthesis in the rat. This "extra" work was carried out late at night, usually between 10 PM and 3 AM. It also was published in the *Journal of Biological Chemistry.*

I left the Manhattan Project in 1946 and joined the Division of Cancer Research at Washington University in St. Louis, spending three months each summer between 1947 and 1954 at the Jackson Memorial Laboratory in Bar Harbor, Maine. I developed a comprehensive program of study of nitrogen metabolism in carcinogenesis in an attempt to localize and characterize the differences between normal and neoplastic tissues that result in the greater growth potential of the latter. Even though it may seem naive by today's standards, in those prehistoric days when the structure and function of DNA were unknown and its genetic role unsuspected, this work was highly regarded and well funded.

In 1954, I left Washington University to join the staff of the City of Hope Medical Center at the inception of its research endeavor. There I became Chairman of Biochemistry and Associate Director of Research. I assembled a staff of eight independent scientists in the biochemistry department and assisted in recruiting members for other departments. In addition, I organized and staffed electronic and machine shops and a chemical stock room and solicited journals, books, and funds to create and staff a library. Originally housed within the confines of my department, these ac-

tivities eventually were taken over and enlarged by the central administration to serve the needs of the institution as a whole. After helping set the pattern for the research effort, which consisted of hiring talented and committed young scientists and leaving them alone to seek their own unique destinies while furnishing them with moral and material support, and after having convinced administrators and the board of directors of the validity of this approach to management of basic science research activities, I resigned as Associate Director of Research and the position was not reactivated.

When I came to the City of Hope, I was committed to do research on cancer and the nervous system. In 1949 I had discovered the unique presence of large quantities of GABA in the brain, now known to be the major inhibitory neurotransmitter in the nervous system. Its potential importance in brain function became apparent by 1957, at which time my attention began to turn more toward nervous system function and away from cancer research. In 1968 I was asked separately by two major universities to organize an interdisciplinary division of neurosciences, and planned to leave to accept one of these offers. However, I was persuaded by the Board of Directors of the City of Hope to stay on and organize such a division there. At its peak, the Division of Neurosciences had 13 senior independent scientists, all in different areas of endeavor, and a total staff of more than 70 employees.

In 1983 I requested to be relieved of all administrative duties so that I could devote myself entirely to my own research. I was made Distinguished Scientist and Director of Neurobiochemistry, essentially a one-man department. At the same time, I resigned from all committees and editorial boards, and as advisor to foundations.

My attention currently is focused largely on identifying major inhibitory command-control mechanisms and their nesting at levels of membrane, metabolism, genome, brain, and society.

I also am continuing some work on GABA, particularly on GABA transport, and have expanded my interests to include the effects of steroids and of amyloid on nervous system function. In the latter areas, the work on memory, attenuation of progression of degeneration after spinal cord injury, aging, and Alzheimer disease has aroused considerable interest. When time allows, I work on a book to be entitled *The Inhibited Brain.*

I have trained a number of American postdoctoral students as well as those from many other countries. Of my awards, I prize most highly election to membership in the National Academy of Sciences and the American Academy of Arts and Sciences and an honorary degree from the University of Florence.

Beyond Measurement: The Pattern Is the Thing

As in everyday life, so in science, ultimately we search for patterns. We are pattern-recognizing creatures. What usually begins as a single-minded de-

votion to the in-depth analysis of one or a small number of variables leads to questions of how the results might relate to the whole living unit, whether it be cell, tissue, organism, or society. Under primitive circumstances, the survival of individuals and species may have depended on the recognition of similarities of patterns and on the discernment of small differences among similar patterns. We know intuitively what pattern recognition is and recognize it by the relief of anxiety or joy when we experience its occurrence. We also can recognize superior pattern-recognizing abilities in others, whatever the nature of their activities. In the latter context, I am reminded of an incident many years ago when a newly trained pediatrician whom I knew became utterly distressed on failing to diagnose his own child's illness, which was characterized by a sore throat, a high fever, and general malaise. Upon seeing the child, his mother, whose medical education consisted of raising six children, laughed and said immediately, "Well, of course, the child has measles." The next morning her diagnosis was confirmed when the tell-tale rash appeared. We have internalized models, often difficult or even impossible to express verbally, to which we constantly are matching environmental patterns that arise from our sensory perceptions.

As I look back over my work of the last 50 years, it becomes clear to me that it can be epitomized as a search for patterns. Among my several biochemical endeavors, the discovery of GABA in brain and the consequent studies of its role in inhibitory processes afforded the best opportunity to begin to seek fundamental patterns in nature. To date, the major conclusion of my pattern search is the following: Progressive disinhibition of tonically inhibited living systems is coupled to increased variability generation in such a manner that the probability of making an optimally adaptive choice of behavior from among those available remains approximately constant over a wide range of increasing force parameters. Healthy living systems operate under conditions of freedom without license (Roberts, 1991).

All living systems are pattern-recognizing or generalizing entities, from single cells to complex human organizations. When a living system, unicellular or international, is presented with a new information pattern in its environment (external and internal), it is activated in a unique fashion. The types of impinging influences and their sequences, intensities, and rates of change result in an activation pattern that is likely to be different from any experienced previously. Even in well-controlled experiments in which single variables are manipulated, it is the change in the pattern of the environment which is the stimulus (stress, pressure, forcing function) for the system. Progress has been made toward establishing a universal law of generalization or pattern recognition that may be helpful in estimating the probability of whether or not an organism will react to a novel stimulus pattern in accordance with consequences associated with previously experienced stimulus patterns (Shepard, 1987; Ennis, 1988).

At all levels of observation, from genomic expression to freeway driving, progressive disinhibition is coupled to increased variability generation in

healthy organisms (Roberts and Matthysse, 1970; Roberts, 1976a, 1986c, 1991; Hikosaka and Wurtz, 1983). A released system, like a wound spring, has the tactical advantage over a driven one in that it does not have to overcome the inertia of start-up when it switches from an inactive or minimally active to a fully active state. Metaphorically, metabolically generated energy is used to wind the biological springs. Ever-present, tonically active inhibitory influences, together with phasically active ones, maintain barriers to physicochemical perturbations, so that the interactions within the system in yin-yang fashion produce asymmetric, graded local changes. Transient signals are transduced by a variety of devices at hand to release processes that govern amounts and turnovers of substances, their locations, and their relations to each other.

Coupling exists between the driving force (pressure) and the generation of variability (information-processing capacity) among the subunits that participate in the nest of relations comprising the particular system being considered, i.e., healthy living systems have an expansible capacity for processing information in relation to demand. Paradoxical as it may seem initially, facile traverse of the adaptive functional range largely is made possible by diverse activities of inhibitory (attenuating and/or time-delaying) influences. In the central nervous system (CNS) inhibitory projection and local circuit neurons play crucial roles in information processing (Roberts, 1986a–c, 1991).

I surmise that increases in activities with increases in forcing function occur according to principles of nonlinear dynamics. It would be expected according to the latter concept that with progressively increasing force parameter, each living system considered would show three characteristic behaviors: smooth, periodic (oscillatory), and turbulent (chaotic). With full participation of inhibition and with tight coupling between degrees of disinhibition and variability generation, the region of smooth flow (efficiently adaptive behavior) would extend over a much greater range of force parameters than in their absence (Lorenz, 1963; Rössler, 1976; Mandell, 1983).

When healthy living systems are effectively stimulated, processes are released to operate at rates and for durations that enable them to react adaptively in a manner compatible with their individual behavioral repertoires. Cascades of processes are generated by actions of environmental factors that reduce transmembrane potential and/or interact with specific membrane receptors. Cascades of other processes are released by expression of genetic potential (Ames et al., 1986; Morgan et al., 1987; Saffen et al., 1988). When environmental pressures are increased, the number of such countercurrent cascades and their extents are increased in such a way that the probability of their meshing to give system-typical adaptive patterns tends to remain approximately constant. The relative constancies of structural, compositional, and functional features of cells, tissues, and organs in mature animals under various environmental conditions are in-

dicative of the existence of remarkable biochemical and biophysical servomechanisms which coordinate a variety of complex biosynthetic and degradative pathways and which continuously adjust the rates of flow of substances between the organism and its environment, between extracellular and intracellular compartments of tissues, between cytosol and organelles, and among cytosolic aggregates (molecular ensembles) of individual cells (Schoenheimer, 1942; Roberts and Simonsen, 1962; Ratner, 1979; Goldberg et al., 1983; Goldberg and Walseth, 1985; Wheatley and Inglis, 1986).

Because several response options may be available even to the simplest cell in a particular instance, because the particular choices made among the options often are unpredictable, and because the exercise of options results in functional and structural changes of varying extents and durations, it may be said that creativity and memory exist in every living unit (Tam et al., 1986). Although increasing in levels of complexity, the operations of multicellular organisms in their environments are not different in basic principle from those of single cells.

The Saga of GABA

Patterns of Free Amino Acids in Normal and Neoplastic Tissue

For a number of years, beginning with my joining the staff at Washington University in St. Louis in 1946, I was interested in the study of amino acid metabolism in both normal and neoplastic tissues in experimental animals. Early on, it appeared desirable to determine the composition of pools of nonprotein amino acids and related substances. I anticipated that the patterns of steady-state concentrations of these constituents would reflect characteristics of the tissues in a way that might reveal key metabolic differences among them. However, as in almost every biochemical field at that time, progress was slow and curiosity limited until methods became available that enabled a large number of determinations to be made in a reasonably short period of time. The development of simple and rapidly performed two-dimensional paper chromatographic procedures allowed the detection of microgram quantities of substances for which other adequate microanalytical procedures were not available and made it feasible to survey rapidly the distribution of free or loosely bound amino acids and other ninhydrin-reactive substances in biological fluids. I hastened to apply these techniques for the first time to animal tissues (Roberts and Tishkoff, 1949).

The paper chromatographic procedures furnished tools that were ideally suited for giving simultaneous information rapidly about the maximal number of constituents and, although often employed in a semiquantitative fashion, could give valuable hints about the presence of new materials and

indicate which substances should be studied further in particular biological situations. Column chromatographic methods already were being applied extensively, but the procedures, although quantitative, were more time-consuming and allowed far fewer samples to be examined. In addition, unknown substances were much easier to detect and identify by the paper chromatographic procedures.

As the work developed, it became apparent that the perceptions of the patterns of the spots of the different constituents on the chromatograms as well as their relative sizes and color values were more meaningful than the same data given in printouts of names of the constituents followed by numbers designating the amounts, in bar graphs, or in other forms of data presentation. Most human computers seem to be able to store and retrieve the patterned pictorial information more effectively and to relate it to metabolic events more rapidly than they can deal with the numerical representation of the same information. In general, it appears that the transmittal of some types of numerical information about multivariant situations from nonhuman computers to human recipients would be more effective if the information were transformed into quantitative, pictorial patterns resembling those seen on two-dimensional paper chromatograms! Indeed, recently the visual display of quantitative information has become an object of concerted scientific inquiry.

One of the great advantages of the paper chromatographic procedures, long since discarded in most laboratories, was the easy detection of new substances. For example, employing these methods we detected and identified aminoethylphosphonic acid, the first compound with a carbon–phosphorus bond found in animal tissues (Kittredge and Roberts, 1969). In this instance, the finding of a new spot on a chromatogram led to a new field of phosphorus biochemistry. Surveys of ninhydrin-reactive substances found in members of the major invertebrate phyla have revealed the presence of many unknown substances (Kittredge et al., 1962). Young scientists who may not wish to take part in the frenetic competition that exists in some areas of modern biochemistry and molecular biology may find many years of satisfying and exciting work in studies of such substances.

The earliest observations with the paper chromatographic method showed that, in a given species at a particular state of development, each normal tissue has a distribution of easily extractable amino acids and related substances that is characteristic for that tissue and is remarkably stable to external perturbations (Roberts and Simonsen, 1962). On the other hand, quite similar patterns are found in many different types of transplanted and spontaneous tumors (Roberts and Frankel, 1949; Fig. 1). The latter findings agreed with Greenstein's generalization (1954) based on enzyme assays: "No matter how or from which tissues tumors arise, they more nearly resemble each other chemically than they do normal tissues or than normal tissues resemble each other."

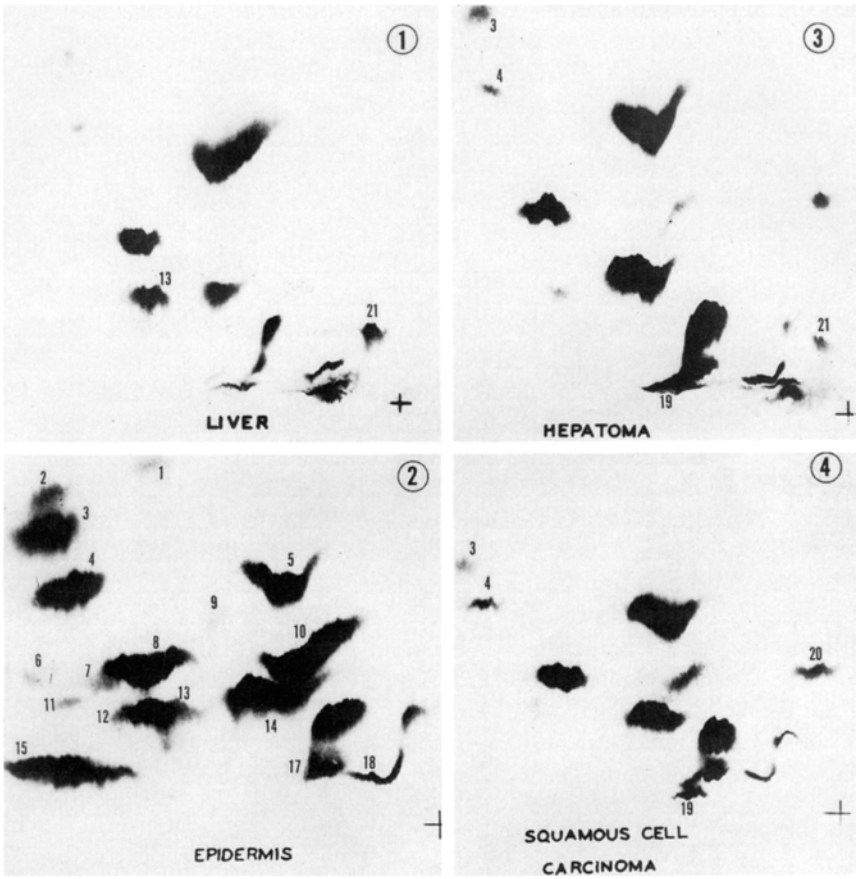


Figure 1. Comparison of the free amino acid patterns of mouse liver (1) and epidermis (2) with those found in a transplantable hepatoma (3) and squamous-cell carcinoma (4). Extracts obtained from 75 mg of fresh weight of tissue were employed for descending two-dimensional paper chromatography (phenol, right to left; lutidine, bottom to top). Constituents on chromatograms: tyrosine, 1; phenylalanine, 2; leucine and isoleucine, 3; valine, 4; taurine, 5; proline, 6; hydroxyproline, 7; alanine, 8; threonine, 9; serine, 10; histidine, 11; glycerylphosphorylethanolamine and/or β-alanine, 12; glutamine, 13; glycine, 14; arginine, 15; lysine, 16; glutamic acid, 17; aspartic acid, 18; ethanolamine phosphate, 19; cystine (cysteic acid), 20; glutathione, 21. Reprinted from *Cancer Res* 1949;9: 645–648, with permission of American Association for Cancer Research.

Employing new techniques of molecular biology, explanations for these findings now are being sought in interplay of expression of genetic potential with transmembrane potential and with second-messenger-initiated cascades.

Discovery of GABA in Brain

Working during the summer of 1949 at the Roscoe B. Jackson Memorial Laboratory in Bar Harbor, Maine, where an unusually good selection of transplantable mouse tumors carried in a number of inbred mouse strains were available for study, I analyzed the free amino acid content of the C1300 transplantable neuroblastoma. I chromatographed several mouse brain extracts for comparison with the neuroblastoma. Much to my surprise, relatively large quantities of an unidentified and previously unobserved ninhydrin-reactive material appeared on the chromatograms. At most, only traces of this material had appeared in a large number of extracts of many other normal and neoplastic tissues previously examined, or in samples of urine and blood. This immediately excited my curiosity.

Upon returning to my laboratories at Washington University in St. Louis, I isolated the unknown material from suitably prepared paper chromatograms. A study of the properties of the substance revealed it to be GABA. I submitted an abstract (Roberts and Frankel, 1950a) reporting the presence of GABA in brain. An abstract reporting the presence of an "unidentified amino acid in brain only" appeared from the Houston laboratory of Jorge Awapara (Awapara, 1950) in the proceedings of the same meeting. Because of a room shortage at the meetings where these findings were reported, I was assigned to share a room with Awapara who knew by that time that the unknown material he had found in brain was GABA. In addition to GABA, work in both of our laboratories had revealed the presence of large amounts of taurine in extracts of many rodent tissues. Awapara and I agreed that my laboratory would continue to work on GABA since I already was deeply engaged in relevant enzymatic studies, while he would put future emphasis on the study of metabolism of taurine. The first complete papers from our laboratories dealing with the occurrence of GABA in brain appeared in the same issue of the *Journal of Biological Chemistry.*

My identification of GABA in brain extracts was expedited by a report in 1949 that GABA was prominent among the soluble nitrogenous components detectable by two-dimensional paper chromatography in the potato tuber (Steward et al., 1949). This caused me to write semifacetiously in my notebook, "This proves that the brain is like a potato!" Unfortunately, little has happened to the state of the world since that time to change my mind. GABA had been found in nature long before. In 1910, D. Ackermann (Ackermann, 1910; Ackermann and Kutschler, 1910) found it to be produced in putrefying mixtures by the action of bacteria. Subsequently, many reports had been made about the occurrence of GABA and/or its formation in bacteria, fungi, and plants. I was thrilled to receive a letter from Ackermann with congratulations on my first report of the presence of GABA in brain.

The Emergence of a Physiological Role for GABA

For several years, the unique presence of relatively large amounts of GABA in the tissue of the central nervous system (CNS) of various species remained a puzzle. The great neurochemist Heinrich Waelsch once discouragingly remarked that GABA probably was a metabolic wastebasket. My continuing efforts to convince some of the eminent neurophysiologists working at Washington University at that time to apply GABA to various nerve preparations at the end of their planned experiments met with complete failure, even though I brought solutions of GABA personally to their laboratories in the hopes of persuading them to test it.

In the first review on the subject in 1956, written after I had moved to my present position, I concluded in desperation, "Perhaps the most difficult question to answer would be whether the presence in the gray matter of the CNS of uniquely high concentrations of γ-aminobutyric acid and the enzyme which forms it from glutamic acid has a direct or indirect connection to conduction of the nerve impulse in this tissue" (Roberts, 1956). However, later that year, the first suggestion that GABA might have an inhibitory function in the vertebrate nervous system came from studies by T. Hayashi's group in Tokyo. They found that topically applied solutions of GABA exerted inhibitory effects on electrical activity in the brain. In 1957, from pharmacological studies with convulsant hydrazides, the suggestion was made by K. Killam and associates that GABA might have an inhibitory function in the CNS. Also in 1957, evidence was adduced for an inhibitory function for GABA in studies by E. Florey and co-workers that showed GABA to be the major factor responsible for the inhibitory action of brain extracts on the crayfish stretch receptor neuron. Within a brief period, interest in GABA increased greatly. Research then being carried out ranged from the study of the effects of GABA on ionic movements in single neurons to clinical evaluation of the role of the GABA system in, for example, epilepsy, schizophrenia, and various types of mental retardation. This warranted the convocation of a memorable interdisciplinary conference in 1959 at the City of Hope Research Institute (now the Beckman Research Institute of the City of Hope). It was attended by most of the individuals who had a role in opening up this exciting field and who presented summaries of their work at the meeting.

This first GABA conference was the greatest learning experience of my life. Having spent most of my scientific career in the narrow confines of organic chemistry, classical biochemistry, and the bare beginnings of molecular biology, I was thrust into the world of membranes, electrodes, voltage clamps, neuroanatomy, EEG and seizures, neuroembryology, and animal behavior. I had the privilege of meeting a number of the world's leading neuroscientists among the participants, a few of whom still are active scientifically and have remained close personal friends. What a mind-boggling

intellectual feast! The meeting itself was overwhelming to me. The excitement was pervasive because all of the participants sensed that a new era was beginning. The subject of neural inhibition finally had returned to front stage and center. It was obvious that much of the future progress in the field would depend on interdisciplinary efforts and that we all would have to begin to learn each other's languages and ways of thinking. At times the proceedings resembled what one imagines might have taken place at the Tower of Babel. However, we all shared the optimistic feeling that we could help each other learn enough so that effective communication soon would take place. For some of us this turned out to be true, and many students in the laboratories of the participants reaped the benefit of the "new enlightenment." It was a particularly heartening social occasion because approximately 80 individuals from Australia, Canada, England, France, Hungary, Japan, the Soviet Union, and the United States met in enthusiastic amity and forged long-lasting scientific and personal links. As a result of this meeting, I was given the opportunity to organize the first interdisciplinary division of neurosciences anywhere, with a generous allotment of 13 senior positions!

GABA as Inhibitory Neurotransmitter—A Rocky Road

Perhaps the subject of inhibition had languished in the wings for so many years because there was no material basis for it. Inhibitory neurons had not been identified, an inhibitory neurotransmitter had not been isolated and characterized, and postsynaptic sites for neural inhibition had not been shown. It is well to remember that it was not until 1952, 2 years after the discovery of GABA in brain, that the controversy as to whether synaptic transmission in the CNS is largely electrical or chemical in nature was settled in favor of the latter (Eccles, 1982).

It made no difference to me initially whether or not GABA were a neurotransmitter. My goal was the elucidation of its function in the nervous system, whatever it might be. However, I did sense that the "transmitter question" seemed to agitate a number of physiologists. Much skepticism arose with regard to the possible transmitter function of GABA because of the large quantities of GABA present in brain, three to four orders of magnitude higher than those of acetylcholine, the only proven neurotransmitter at that time. It would have been much more acceptable to some if the first identified inhibitory neurotransmitter would have had a more exotic chemical structure than that of GABA. At a later date, the situation was even worse for glycine, the simplest amino acid, which was identified as a putative inhibitory neurotransmitter in the spinal cord. Some time after the legitimization of GABA as an inhibitory neurotransmitter, I received an SOS signal from the discoverers of the glycine phenomenon, M. Aprison and R. Werman, whose findings were being met with much skepticism and

even abuse. After visiting their laboratories and becoming thoroughly acquainted with their data, for several years I made it a point in my lectures on GABA to mention the glycine work and to indicate my support for the convincing findings of these excellent scientists. I vividly remember sitting at a symposium in Stockholm near a leading neurophysiologist who kept shaking his head and muttering angrily to himself during Werman's presentation of the data documenting the inhibitory role of glycine in spinal cord.

The resistance to acceptance of GABA as a naturally occurring neurotransmitter was supported by negative experiments and interpretations, many of which, in retrospect, were technically flawed. At the 1959 GABA symposium (Roberts et al., 1960), statements were made that seemed to rule out a neurotransmitter role for GABA. It finally was remarked by someone that GABA entered the conference as a proud transmitter candidate and left it as a poor metabolite. For example, E. Florey, whose brilliant work had pioneered in the identification of GABA as a factor in brain extracts that exerted inhibitory effects on invertebrate preparations, stated, "Although one likes to ascribe to neurogenic compounds an action on postsynaptic membranes, there is every possibility that they affect intraneuronal processes which in turn control the state of the membrane. At present, it is not possible to decide whether GABA normally occurs outside of nerve cells as a product of secretion (modulator substance). All available evidence speaks, however, against it playing a role as inhibitory transmitter in vertebrates." Florey made this statement, at least in part, because at that time GABA had not yet been shown to possess most of the "classical" requirements for neurotransmitter, chief among which are proof of identity of postsynaptic action with that of the natural transmitter, presence in inhibitory nerves, releasibility from terminals of identified nerves, and the presence of a rapid inactivating mechanism at synapses. The most critical among these is the first. As aptly stated by P. Fatt, "The characterization of the postjunctional response must logically precede any attempt to identify the transmitter, since the criterion for the identification is its ability to duplicate the effect of prejunctional nerve stimulation."

Also damaging to GABA's transmitter candidacy was Florey's failure to detect GABA in the crustacean nervous system and his report of the discovery of an incredibly more potent inhibitory substance, Factor I. The latter findings unquestionably resulted from a concatenation of technical errors, because enormous concentrations of GABA later were found in inhibitory axons of crustacea and the existence of a Factor I independent of GABA has never been established. Faulty chemical work by H. McLennan led to conclusions similar to Florey's. Also in the 1959 conference, W. Van der Kloot, discoverer of the blocking effect of picrotoxin on peripheral inhibition in invertebrates, noted, "Yet there is a compelling reason to deny that GABA itself is the transmitter. The inhibitory effects always persisted without diminution after GABA was perfused into claws. If there is an enzymatic

breakdown of GABA, the disappearance of the molecule is inconspicuous." The latter statement was based on the mistaken notion that the removal of all neurotransmitters would have to take place by a mechanism strictly analogous to the extremely rapid enzymatic destruction of acetylcholine by acetylcholinesterase at the vertebrate neuromuscular junction. Actually, the case of acetylcholine appears to be an exception. GABA and a variety of other neurotransmitters now are known to be removed rapidly from synaptically active sites by carrier-mediated transport into pre- and postsynaptic neuronal sites as well as into glial cells. Although usually operating effectively as a GABA "vacuum cleaner" in a physiologically coordinated manner with the natural release mechanisms, the GABA transport systems can be more easily overwhelmed with an exogenous flood of GABA than can the cholinergic system with acetylcholine, because acetylcholinesterase is an enzyme with a very high turnover number. Van der Kloot's notion was derived from an incorrect assumption followed by an inappropriate experiment. D.R. Curtis and colleagues (Curtis et al., 1959) concluded that GABA was "not a specialized inhibitory substance." This was based on their failure to observe in neurons onto which GABA was applied iontophoretically the hyperpolarization that is known to occur during natural inhibition in cat spinal motoneurons. They stated, "The possible specific inhibitory character of these acids (β-alanine and GABA) is excluded by their failure to produce a hyperpolarization of motoneuronal membranes." It seems probable that the failure to observe hyperpolarization by the latter workers was attributable to the fact that, in the ingeniously devised coaxial microelectrodes used in their studies, there was sufficient electrical coupling between the barrels so as to preclude detection of the small potentials required to show hyperpolarization. Although glycine probably is the major inhibitory neurotransmitter in ventral regions of the spinal cord, numerous GABAergic synapses have been found immunocytochemically on the somata of spinal motoneurons and throughout the spinal cord, in general.

C.A.G. Wiersma, who had laid the groundwork for the use of crustacean sensory and neuromuscular synapses in quantitative physiological studies, also stated in the 1959 symposium, "At one time it looked as if GABA would meet the requirements of an inhibitory transmitter substance for many places, but in the last year or so it has become more doubtful that this is the case." This was based on a paper published by G. Hoyle and Wiersma in 1958 in which is found the following statement: "We have applied this substance (GABA) in concentrations of 10^{-9}–10^{-3} M to several preparations of crayfish and crab opener muscles. It did not produce mechanical inhibition in any of them, nor did it raise the resting potential in muscle fibers in which inhibitory action had this effect." Although never acknowledged publicly, this result later was found to be attributable to a mislabeling of the bottle from which Hoyle took the substance to be tested. They probably had applied α-aminobutyric acid and not GABA. I personally made this discov-

ery when looking over the chemicals on a shelf in Wiersma's laboratory in his presence after Hoyle had left Cal Tech to go to the University of Oregon.

In the summary to the whole conference, although conceding the probable existence of true inhibition on ventral horn neurons in the spinal cord and on the crustacean sensory cell, George Bishop, the great collaborator of J. Erlanger and H. Gasser, remarked, "In spite of considerable experimentation and even more extensive interpretation, the position is still tenable that little or no such inhibition, that accompanying a positively oriented impulse and a decrease of resistance of the synaptic membrane, occurs in the cortex of the mammal. Postimpulsive depression resembling refractoriness is on the contrary of very prevalent occurrence there." The curtain appeared to have been brought down on the candidacy of GABA as inhibitory neurotransmitter!

At a meeting of the Federated Societies held in the spring of 1959, I was approached by Stephen Kuffler and asked by him whether or not I thought there was still a chance that GABA might be a neurotransmitter and whether or not it would be worthwhile to hire a biochemist to work with him and other physiologists on the action of GABA in the crustacean nervous system as part of a team he was going to constitute upon his move from Johns Hopkins to Harvard. My affirmative answer encouraged him to hire E. Kravitz as biochemist for his group. The most convincing evidence for the role of GABA as an inhibitory neurotransmitter eventually was obtained by the Harvard group and by A. and N. Takeuchi in Japan from studies at crustacean neuromuscular junctions. The postsynaptic action of applied GABA mimics exactly that found on stimulating inhibitory nerves. Inhibitory axons contain enormous concentrations of GABA, while less than 1% of these levels can be detected in excitatory nerves. GABA is released from lobster inhibitory nerves in amounts proportional to the number and frequency of stimuli applied to the nerve and is not released by stimulation of excitatory nerves. The enzyme that forms GABA from L-glutamate, glutamic acid decarboxylase (GAD), is preferentially distributed in inhibitory nerves. Synaptically released GABA probably is inactivated by an uptake mechanism that is capable of acting against large concentration gradients. Picrotoxin, a convulsant agent, blocks the inhibitory action of GABA and the natural inhibitory transmitter similarly. Thus, in the crustacean peripheral nervous system GABA possesses impeccable credentials as an inhibitory transmitter, perhaps more convincingly documented than in the case of any other known transmitter at any site of action. Over the years, strong evidence had been adduced from many physiological and pharmacological studies for an inhibitory neurotransmitter role for GABA in the vertebrate CNS. However, the necessary definitive chemical correlative work and release experiments are much more difficult to achieve in the tightly packed vertebrate CNS than in the crustacean peripheral nervous system. Even using the latter, the definitive experiments

on release of GABA were formidable, M. Otsuka losing approximately 14 kg in body weight while performing the arduous work required to achieve valid results.

GABA is liberated from presynaptic terminals of inhibitory nerves on dendrites, usually close to the cell body, on initial axon segments, on cell bodies, or on terminals of neurons employing other transmitters. It increases the permeability of membranes to specific ions in such a way as to cause the membranes to resist depolarization. For example, by acting on a particular class of receptors ($GABA_A$), GABA produces an increase in permeability to Cl^- ions that is measured as an increase in membrane conductance. GABA also produces increases in K^+ conductance by action on another distinct class of receptors ($GABA_B$) that are not colocalized with $GABA_A$ receptors (Bowery et al., 1983). In general, GABA accelerates the rate of return of the resting potential of all depolarized membrane segments which it contacts and stabilizes undepolarized membrane segments by decreasing their sensitivity to stimulation. Thus, at many sites in the nervous system, GABA exercises inhibitory command-control of membrane potential.

Remarkably, GABA alone of the amino acids commonly found in proteins and/or in tissues and body fluids in the free or easily extractable form is electroneutral, bearing no net charge at the physiological pH of 7.3 (Roberts and Sherman, 1993, and Appendix I). The obvious advantage of this property for a neural informational molecule is that it confers the highest probability of successfully traversing the densely packed extracellular synaptic domain between presynaptic release sites and postsynaptic receptor sites (100–200 nM) without becoming coulombically attracted or repelled by charged entities en route. The electroneutrality of the GABA molecule also is resistant to fluctuations of pH in the physiological pH ranges, since the pK_1 (COOH) and pK_2 (NH^+_3) values are 4.031 and 10.556, respectively. Thus, the number of transmitter molecules released from a nerve terminal as a result of nerve activity in a variety of circumstances would reliably and quantitatively transmit messages from presynaptic nerves to the postsynaptic sites upon which they impinge. With the exception of GABA, all known major neurotransmitters are charged at physiological pH. Glutamic and aspartic acids and glycine are anionic, and acetylcholine, serotonin, norepinephrine, and dopamine are cationic. Numerous structure–activity studies in GABA-responsive systems show that no α-, β-, or ω-amino acid known to occur in any abundance in animal tissues approaches GABA in molar efficacy at the $GABA_A$ receptor. Therefore, the noise level created by nonspecific effects at the $GABA_A$ receptor would be minimal, ensuring fidelity and specificity of the neural messages delivered by GABA.

Coordinate enhancement occurs in GABAergic inhibitory function with progressive acidification because GABA formation and its anion channel-opening efficacy are increased while its metabolic destruction by transamination and removal by transport are decreased (Roberts and Sherman,

1993). Diminution occurs upon alkalinization. Contrawise, acidification decreases postsynaptic efficacy of glutamate, the major excitatory neurotransmitter. In the above manner the delicate balance between excitation and inhibition in the brain is maintained within the adaptive range in response to local or global activity that acidifies the environment in which it occurs. Accelerated metabolism following nerve activity results in accelerated formation of CO_2 and lactic acid, the accompanying acidification applying physiological "brakes," so to speak, slowing down neural activity while recovery takes place. This keeps the system from "overheating," thereby helping prevent structural and functional damage from taking place. When GABAergic–glutamatergic relations are unbalanced by glutamatergic overactivity, seizures may occur. For example, the excitement experienced at an athletic event with the attendant hyperventilation not infrequently causes seizures to occur in susceptible individuals. Overbalancing in favor of the GABA system can lead to maladaptive decrement in neural activity and even to coma.

The properties of the simple GABA molecule and of the machinery built to support its function make it eminently suitable to guide the brain to function in a "civilized" manner. The yin–yang between the glutamatergic excitatory and GABAergic inhibitory systems is played out on the tight rope of a delicate balance, prolonged imbalances in relations between them leading to serious disorders.

The "charm" of GABA lies in nature's choice of this simple molecule, made from the common metabolic soil of glutamic acid, for the all-important role as major controller of the infinitely complex machinery of the brain, allowing it to operate in the manner best described as freedom without license. Try as one might, one cannot come up with a better choice for the job.

Immunocytochemistry of GABA Neurons

For a number of years I felt that my laboratory, largely biochemical, was wandering in the wilderness of the complexities of the metabolism of the vertebrate CNS (Appendix II) without definitively coming to terms with problems related to GABAergic transmitter functions and the roles of GABA neurons in information processing. The history of chemical work on GABA goes back more than 47 years and it recapitulates most of the modern history of neurochemical endeavor. At the time that GABA was discovered in brain and the first experiments were being performed on its biochemistry and pharmacology, an accepted approach to neurochemistry was to study the whole brain or some grossly defined regions. Acetone powders, homogenates, slices, and other types of preparations were made from whole rodent brain and the projected studies were performed on such preparations. In due course it became possible to analyze for the components of

the GABA system in different brain regions, and laminar analyses were performed on such suitably layered structures as the cerebellum, hippocampus, retina, and superior colliculus. Although the functions of most brain regions in terms of physiology, morphology, and behavior still were not well understood, a certain degree of definition was attained relevant to quantitative aspects of the amounts of GABA and the enzymes most importantly involved in its formation and degradation, GAD and GABA transaminase. The localization of GABA neurons was inferred by correlating microchemical, electrophysiological, pharmacological, and iontophoretic studies with what was known of the cytoarchitecture of specific regions of brain and spinal cord. Analyses of GABA contents and GAD activities were performed in almost all identifiable brain structures and spinal cord. Some studies combined biochemical analyses with various types of lesioning procedures in an attempt to correlate specific neural degenerations with losses of GAD and GABA.

The distributions of the components of the GABA system also were studied extensively by subcellular fractionation techniques in preparations from whole brain or selected regions. Interpretation of results from the above types of analyses always suffered from lack of definition, attributable to the presence of millions of cells of different types in any dissected region, and definitive conclusions were not possible about specific synaptic connections. Even when individual cell bodies of large neurons (e.g., cerebellar Purkinje cells) were dissected out and subjected to microanalytical examination, presynaptic endings from the axons of other neurons adhered to the neuronal somata, and it was impossible to estimate the proportions of a particular measured variable contributed by somata or presynaptic endings. Exquisite dissection techniques made possible the determination of GABA contents in membrane-containing and membrane-free portions of individual Deiters' neurons. However, none of these approaches clarified the manner in which GABA neurons might participate in information processing in different parts of the vertebrate CNS.

A critical examination of our own work and that of others led me to the inevitable conclusion that direct visualization of components of the GABA system, particularly GABA neurons and their terminals, was necessary to obtain unequivocal proof of the existence of components of the GABA system at specific synaptic sites in neural tissues. The most likely approaches to achieve this goal appeared to be those that might lead to visualization of the pertinent proteins (GAD, GABA transaminase, and the GABA transport and receptor proteins) at the light and electron microscopic levels. This had never been done before for any neurotransmitter, and the technical difficulties to be overcome were formidable, to say the least.

Early in 1968 I decided with great trepidation to "go for broke," so to speak, and to begin with GAD, the rate-limiting enzyme in GABA formation, a portion of which I knew to be present in an easily solubilized form

and in high concentration in synaptosomes prepared from mouse brain. Many attempts had been made by us to develop chemical procedures for the visualization of GAD, but all failed because of the difficulties in demonstrating histochemically the products of the enzymatic reaction, GABA and CO_2. The difficult alternative approach was to locate GABA neurons by immunocytochemical procedures. This required the preparation of pure GAD from brain, development of antibodies to the enzyme, and then visualization of the antibodies by a suitable labeling technique specifically at those cellular and subcellular sites where GAD, the antigen, is located. It is to this task that I and a group of talented young colleagues dedicated ourselves for several years, the work finally resulting in the first immunocytochemical visualization ever of a neurotransmitter synthesizing enzyme at various sites in the central nervous system at both light and electron microscopic levels (see Figs. 2–4 for examples and Appendix III for steps along the way). Much subsequent work from our own laboratories and that of others on GAD as well as on many other proteins has appeared over the years using variations of the techniques we developed. A useful atlas has been made of the distribution of GABAergic neurons and terminals in the rat central nervous system (Mugnaini and Oertel, 1985).

The development of the immunocytochemical techniques ended what I consider to be the "romantic" era of the GABA saga. We now are in the period of molecular biology in which aspects of all components of the GABA system are being explored at structural and genetic levels. My long association with the simple GABA molecule has been like peeling an onion, one problem inevitably leading to another in endless succession. The "peeling" continues at a greatly accelerated rate in laboratories throughout the world.

Inhibitory Command-Control by the Extended Brain: Instinctual Needs, Social Inhibition, and Release of Human Behavior

The brain, its function enhanced by invention and uses of language, tools, and social devices, has assumed a dominant position among the nested command-control systems that have evolved on the way from primordial single cell to conscious human being. The neuroendocrine system, the control of which is centered in the brain, regulates to a greater or lesser extent all aspects of structure and function throughout the body. The extended brain with its emergent properties increasingly is learning how to manipulate various aspects of the environment, terrestrial and even extraterrestrial.

Neural inhibition is not sufficient by itself to enable individuals to govern their activities in an adaptive manner in settled communities. For modern civilization to exist at all, it is necessary for there to be attenuation and time-delay of the gratification of instinctual needs or of their symbolic

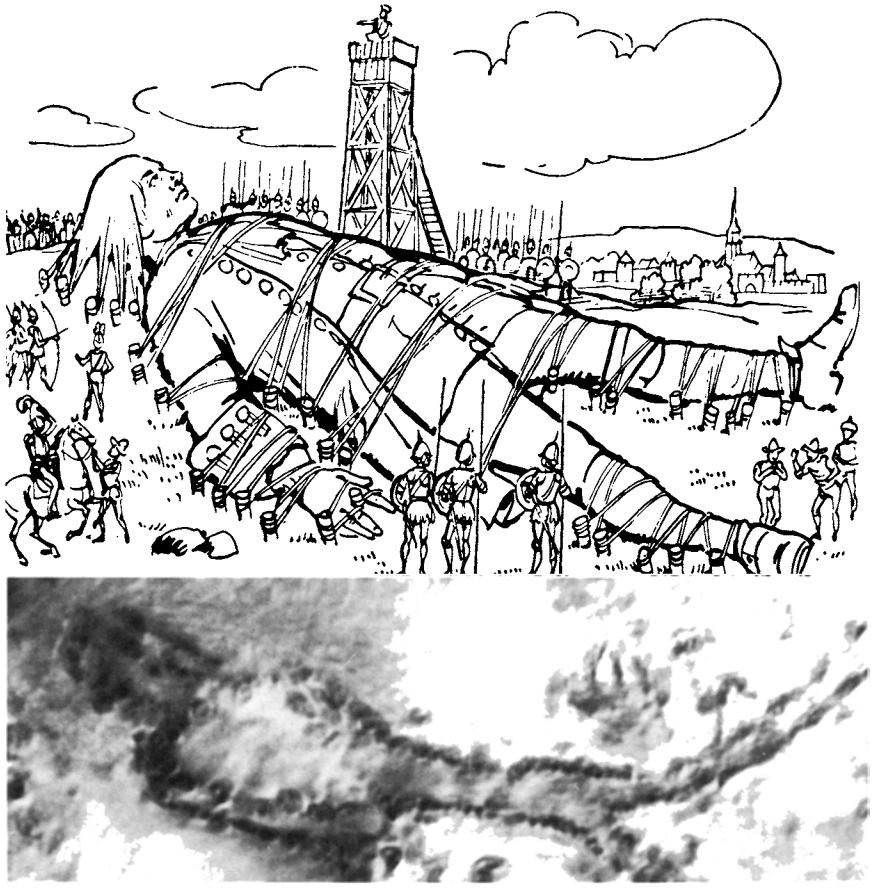


Figure 2. The inhibited nervous system. An unretouched photograph taken with Nomarski optics of a neuron in the rat nucleus interpositus studded with GAD-positive terminals, presumably Purkinje cell axonal terminals, is placed below a picture of Gulliver, showing him when he awoke to find himself pinioned to the ground. The latter picture is taken from the 1956 edition of Vol. 7 of *The Book of Knowledge,* The Grolier Society Inc., New York. (Figure prepared by Robert Barber).

equivalents. For this purpose have been devised religions, taboos, and laws which, assiduously taught by word and example from early childhood on and reinforced by rewards and punishments, help to prepare individuals in their adulthood to walk the tightropes of the tensions arising from the interplay between instinctual demands and social inhibitions. These social inhibitions consist of the learned restrictions that have become part of the extended brain (conscience) and externally existing policing systems.

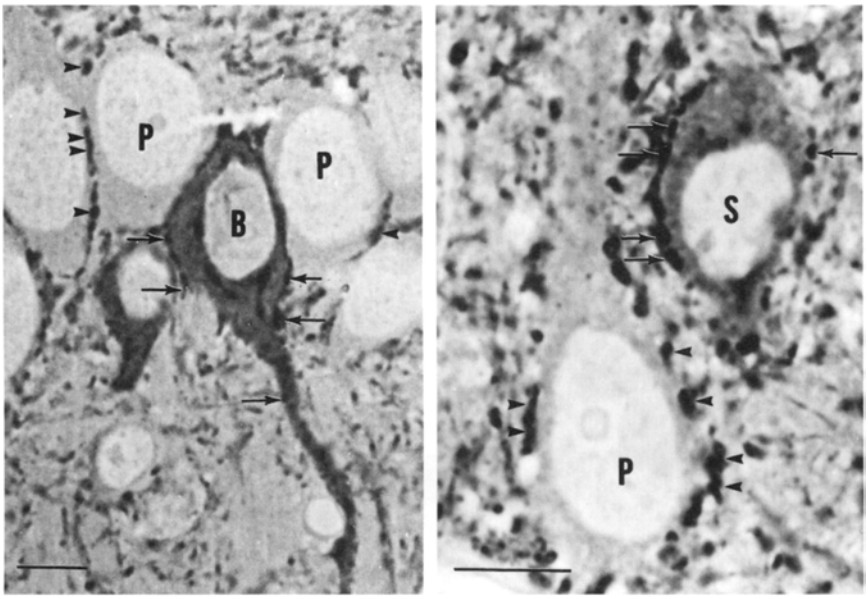


Figure 3. GABA neurons and terminals in rat hippocampus and cortex. Left: a presumptive basket cell (B) in rat hippocampus that stains positively for L-glutamic acid decarboxylase (GAD) and is, therefore, a GABAergic neuron. It is studded with numerous GAD-positive terminals (arrows). Right: soma of a GAD-positive stellate cell (S) in layer V of the rat visual cortex that also is studded with GAD-positive terminals. In both micrographs, it is seen that somata of pyramidal neurons (P), which are not GAD-positive, are contacted by numerous GAD-positive terminals. The bar represents 10 μm. (Figure kindly furnished by C. E. Ribak and J. E. Vaughn.)

The essence of my argument is that the extents and variabilities of the cascades of multichannel informational flows, inward and outward, are coupled to demand at all times at all levels of observation in healthy organisms. Changes in activities with changes in forcing function occur everywhere in accord with principles of nonlinear dynamics. I posit that this would be as true for overt behavior of an individual in a complex social setting as for the activities within a membrane of an individual neuron. It now is of greatest importance to build bridges between neurobiology and physics so that we come to understand the physical principles that underlie our lives and how we live them.

The necessarily intimate coupling between brain and sensorium is easily seen from behavioral abnormalities that result from brain lesions or during sensory isolation. However, it is the dominant extended brain that ultimately abstracts and interprets environmental patterns and supervises

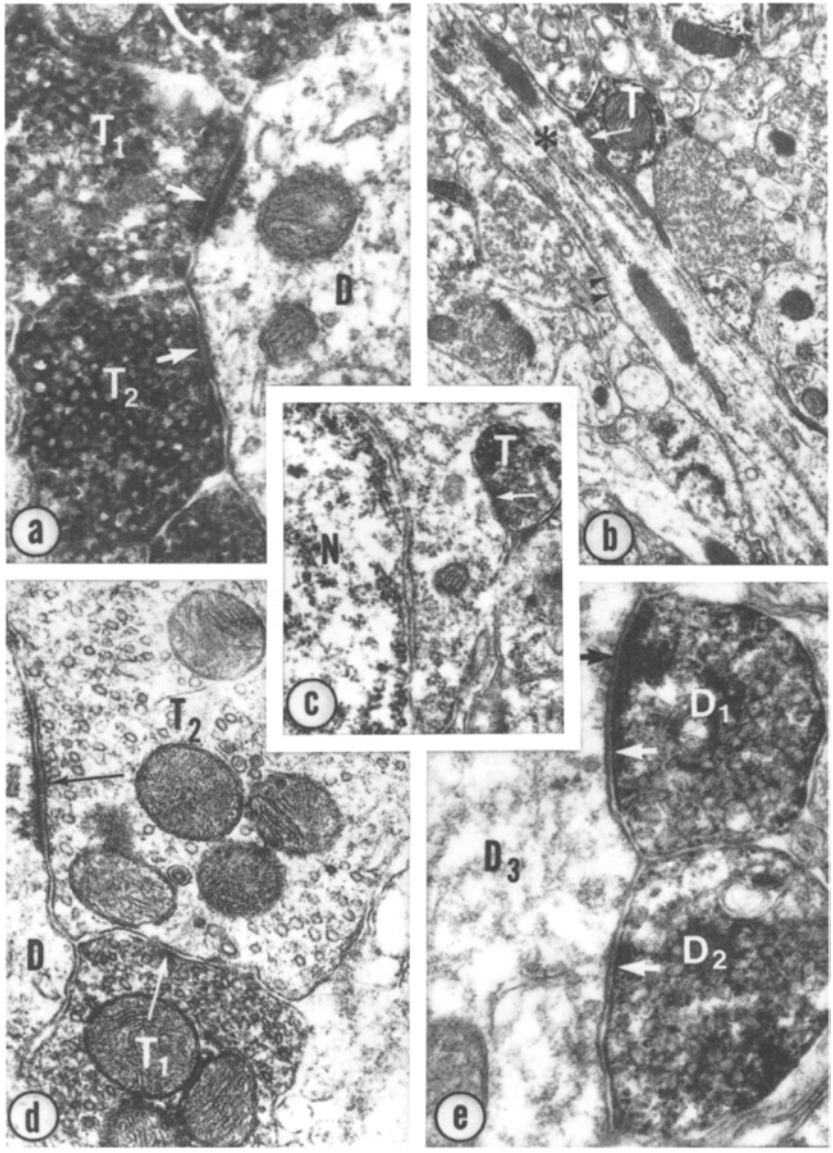


Figure 4. Electron micrographs of various types of synaptic junctions formed by presynaptic terminals that contain glutamate decarboxylase (GAD), the enzyme that synthesizes the neurotransmitter γ-aminobutyric acid (GABA). All specimens were obtained from rat CNS, and all were prepared according to immunoperoxidase procedures developed for the ultrastructural localization of GAD. (a) Axodendritic synapses in the substantia nigra. Two axon terminals (T_1 and T_2) filled with electron-opaque, GAD-positive reaction product are shown to synapse with a dendritic shaft (D) in the pars reticulata. One of the terminals (T_1)

the organization and release of adaptive behavior by enabling appropriate choices to be made from among the available options.

To cite from an article written with Matthyssee in 1970: "We feel that social progress, and, indeed, the probability of survival, depend in large part on the range of behavioral options available to individuals and to nations. It may be that an integrated approach to the nervous system will reveal options yet undiscovered, for example by increasing the capacity for learning and for control of aggressive drives."

We scientists must not despair at the relative lack of social progress in the intervening 28 years. "No, our science is no illusion. But an illusion it would be to suppose that what science cannot give us we can get elsewhere" (Freud, 1961).

A Final Note

My parents brought me from Russia to the United States when I was two years old. Unquestionably, the day of arrival was the luckiest one in my life. Nowhere else in the world would I have been given the opportunity to follow my inclinations so freely and with such good support and encouragement for so many years.

I was blessed with excellent teachers and humane and sensitive mentors. The young scientists who joined me at various times throughout my career have enlightened me and have contributed critically to whatever success the work may have attained.

I have had the freedom to travel and to meet colleagues from many countries. Whatever the state of the world, sitting in my study in the quiet

forms an asymmetric synaptic junction (arrow); the other terminal (T_2) forms a symmetric synapse (arrow) (original magnification × 44,000). (b) An axoaxonic synapse in the cerebral cortex. A GAD-positive axon terminals (T) is shown forming a symmetric synapse (arrow) with an axon initial segment identified by a dense undercoating of the axolemma (arrowheads) and a fasciculation of microtubules (e.g., asterisk) (original magnification × 20,000). (c) An axosomatic synapse in the dorsal horn of the spinal cord. A probable synaptic junction (arrow) is shown between a GAD-positive terminal (T) and neuron (N) in the substantia gelatinosa (original magnification × 26,000). (d) An axoaxonic synapse in the dorsal horn of the spinal cord. A synaptic junction (white arrow) is shown between the GAD-positive presynaptic terminal (T_1) and another synaptic terminal (T) that is not GAD-positive. In addition, T_2 is the presynaptic component of another synaptic junction (black arrow) with a dendrite (D) (original magnification × 38,000). (e) Dendrodendritic synapse in the glomerular layer of the olfactory bulb. Two GAD-positive gemmules (D_1, D_2) from dendrites of periglomerular neurons form synapses with a mitral/tufted dendritic shaft (D_3). One gemmule (D_1) appears to form a reciprocal synapse, and the other gemmule (D_2) appears to be presynaptic only (original magnification × 54,000). Directions of synaptic transmission are indicated by arrows in a to e. Electron micrographs provided by RP Barber, BJ McLaughlin, CE Ribak, and JE Vaughn. Reprinted from *Nerve Cells, Transmitters, and Behaviour,* Levi-Montalcini R, ed. Vatican City, Italy: Pontifical Academy of Sciences, 163–213, with permission of the author.

of evening I have warm thoughts about those whose work I admire and whom I have been fortunate enough to know personally. What great scientists and fine human beings there are among them!

Selected Bibliography

Ackermann D. Über ein neues auf bakteriellem Wege gewinnbares Aporrhegma. *Z Physiol Chem* 1910;69:273–281.

Ackermann D, Kutscher F. Über die Aporrhegmen. *Z Physiol Chem* 1910;69: 265–272.

Ames A III, Walseth TF, Heyman RA, Barad M, Graeff RM, Goldberg ND. Light-induced increases in cGMP metabolic flux correspond with electrical responses of photoreceptors. *J Biol Chem* 1986;261:13034–13042.

Ashton D. *Picasso on art* (unabridged republication of edition published in New York in 1972), New York: Da Capo Press, 1988.

Awapara J. Detection and identification of metabolites in tissues by means of paper chromatography. *Fed Proc* 1950;9:148.

Awapara J, Landua AJ, Fuerst R, Seale B. Free γ-aminobutyric acid in brain. *J Biol Chem* 1950;187:35–39.

Barber RP, Saito K. Light microscopic visualization of GAD and GABA-T in immunocytochemical preparations of rodent CNS. In: Roberts E, Chase TN, Tower, DB, eds. *GABA in nervous system function.* New York: Raven Press, 1976; 113–132.

Barber RP, Vaughn JE, Saito K, McLaughlin BJ, Roberts E. GABAergic terminals are presynaptic to primary afferent terminals in the substantia gelatinosa of the rat spinal cord. *Brain Res* 1978;141:35–55.

Bazemore AW, Elliott KAC, Florey E. Isolation of Factor I. *J Neurochem* 1957;1: 334–339.

Bowery NG, Hill DR, Hudson AL. Characteristics of $GABA_B$ receptor binding sites on rat whole brain synaptic membranes. *Br J Pharmacol* 1983;78:191–206.

Curtis DR, Duggan AW, Felix D. GABA and inhibition of Deiter's neurons. *Brain Res* 1970;23:117–120.

Curtis DR, Phillis JW, Watkins JC. The depression of spinal neurones by γ-amino-*n*-butyric acid and β alanine. *J Physiol London* 1959;146:185–203.

Diamond S, Balvin RS, Diamond FR. *Inhibition and choice.* New York: Harper and Row, 1963.

Dudel J, Finger W, Stettmeier H. GABA induced membrane current noise and the time course of the inhibitory synaptic current in crayfish muscle. *Neurosci Lett* 1977;6:203–208.

Eccles JC. *The inhibitory pathways of the central nervous system.* Springfield, IL: Charles C. Thomas, 1969.

Eccles JC. The synapse: From electrical to chemical transmission. *Annv Rev Neurosci* 1982;5:325–339.

Edwards C. Physiology and pharmacology of the crayfish stretch receptor. In: Roberts E, Baxter, CF, van Harreveld A, Wiersma CAG, Adey WR, Killam KF, eds. *Inhibition in the nervous system and gamma-aminobutyric acid.* Oxford: Pergamon Press, 1960;386–408.

Ennis DM. Toward a universal law of generalization. *Science* 1988;242:944.

Florey E. Über einen nervösen Hemmungsfaktor in Gehirn und Ruckenmark. *Naturwiss* 1953;40:295–296.

Florey E, McLennan H. The release of an inhibitory substance from mammalian brain, and its effect on peripheral synaptic transmission. *J Physiol* 1955;129: 384–392.

Freud S. *Standard edition of complete psychological works,* Vol. 21, Strachey J, ed. London: Hogarth, 1961;1–145.

Gale EF. The bacterial amino acid decarboxylases. *Adv Enzymol* 1946; 6:1–32.

Goldberg ND, Ames A III, Gander JE, Walseth TF. Magnitude of increase in retinal cGMP metabolic flux determined by ^{18}O incorporation into nucleotide α-phosphoryls corresponds with intensity of photic stimulation. *J Biol Chem* 1983;258:9213–9219.

Goldberg ND, Walseth TF. A second role for second messengers: Uncovering the utility of cyclic nucleotide hydrolysis. *Biotechnology* 1985;3:235–238.

Graham LT, Jr., Shank RP, Werman R, Aprison MH. Distribution of some synaptic transmitter suspects in cat spinal cord: glutamic acid, aspartic acid, γ-aminobutyric acid, glycine and glutamine. *J Neurochem* 1967;14:465–472.

Greenstein JP. *Biochemistry of cancer.* New York: Academic Press, 1954:362.

Greenstein JP, Winitz M. *Chemistry of the amino acids,* Vol. 1. New York: Wiley, 1961;486–489.

Hall ZW, Bownds MD, Kravitz EA. The metabolism of gamma aminobutyric acid in the lobster nervous system. Enzymes in single excitatory and inhibitory axons. *J Cell Biol* 1970;46:290–299.

Hayashi T, Nagai K. Action of ω-amino acids on the motor cortex of higher animals, especially γ-amino-β-oxybutyric acid as the real inhibitory principle in brain. In: *20th International Physiology Congress, Brussels, 1956. Abstracts of Reviews: Abstracts of Communications,* 1956;410.

Hayashi T, Suhara R. Substances which produce epileptic seizures when applied on the motor cortex of dogs, and substances which inhibit the seizure directly. In: *20th International Physiology Congress, Brussels. 1956. Abstracts of Reviews: Abstracts of Communications,* 1956;411.

Hikosaka O, Wurtz, RH. Visual and oculomotor functions of monkey substantia nigra pars reticulata. IV. Relation of substantia nigra to superior colliculus. *J Neurophysiol* 1983;49:1285–1301.

Hori N, Ikeda K, Roberts E. Muscimol, GABA and picrotoxin: effects on membrane conductance of a crustacean neuron. *Brain Res* 1978;141:364–370.

Hoyle G, Wiersma CAG. Inhibition at neuromuscular junctions in Crustacea. *J Physiol London* 1958;143:426–440.

Ito M. Roles of GABA neurons in integrated functions of the vertebrate CNS. In: Roberts E, Chase TN, Tower, DB, eds. *GABA in nervous system function.* New York: Raven Press, 1976;427–448.

Killam KF, Bain JA. Convulsant hydrazides I: *In vitro* and *in vivo* inhibition of vitamin B_6 enzymes by convulsant hydrazides. *J Pharmacol Exp Ther* 1957; 119:255–262.

Killam KF. Convulsant hydrazides II: comparison of electrical changes and enzyme inhibition induced by the administration of thiosemicarbazide. *J Pharmacol Exp Ther* 1957;119:263–271.

Killam KF, Dasgupta SR, Killam EK. Studies of the action of convulsant hydrazides as vitamin B_6 antagonists in the central nervous system. In: Roberts E, Baxter CF, van Harreveld A, Wiersma CAG, Adey WR, Killam KF, eds. *Inhibition in the nervous system and gamma-aminobutyric acid.* Oxford: Pergamon Press, 1960;302–316.

Kittredge JS, Roberts E. A carbon-phosphorus bond in nature. *Science* 1969;164: 37–42.

Kittredge JS, Simonsen DG, Roberts E, Jelinek B. Free amino acids of marine invertebrates. In: Holden JT, ed. *Amino acid pools.* New York: Elsevier, 1962;176–186.

Kravitz EA, Iversen LL, Otsuka M, Hall ZW. Gamma-aminobutyric acid in the lobster nervous system; release from inhibitory nerves and uptake into nerve-muscle preparations. In: von Euler C, Skoglund S, Soderberg U, eds. *Structure and function of inhibitory neuronal mechanisms.* Oxford: Pergamon Press, 1968;371–376.

Krnjevic K. Inhibitory action of GABA and GABA-mimetics on vertebrate neurons. In: Roberts E, Chase TN, Tower DB, eds. *GABA in nervous system function.* New York: Raven Press, 1976;269–281.

Kuffler SW. Synaptic inhibitory mechanisms. Properties of dendrites and problems of excitation in isolated sensory nerve cells. *Exp Cell Res Suppl* 1958;5:493–519.

Kuffler SW, Edwards C. Mechanism of gamma aminobutyric acid (GABA) action and its relation to synaptic inhibition. *J Neurophysiol* 1958;21:589–610.

Lorenz EN. Deterministic non-periodic flow. *J Atmos Sci* 1963;20:130–141.

Mandell AJ. From intermittency to transitivity in neuropsychobiological flows. *Am J Physiol* 1983:245:R484–R494.

Matsuda T, Wu J-Y, Roberts E. Immunocytochemical studies on glutamic acid decarboxylase (EC 4.1.1.15) from mouse brain. *J Neurochem* 1973;21:159–166.

Matsuda T, Wu J-Y, Roberts E. Electrophoresis of glutamic acid decarboxylase (EC 4.1.1.15) from mouse brain in sodium dodecyl sulfate polyacrylamide gels. *J Neurochem* 1973;21:167–172.

McLaughlin BJ, Wood JG, Saito K, Barber R, Vaughn JE, Roberts E, Wu J-Y. The fine structural localization of glutamate decarboxylase in synaptic terminals of rodent cerebellum. *Brain Res* 1974;76:377–391.

McLaughlin BJ, Wood JG, Saito K, Roberts E, Wu J-Y. The fine structural localization of glutamate decarboxylase in developing axonal processes and presynaptic terminals of rodent cerebellum. *Brain Res* 1975;85:355–371.

McLaughlin BJ, Barber R, Saito K, Roberts E, Wu J-Y. Immunocytochemical localization of glutamate decarboxylase in rat spinal cord. *J Comp Neurol* 1975;164: 305–321.

McLennan H. The fractionation and purification of Factor I. *J Physiol* 1960;151: 31–39.

Morgan JI, Cohen DR, Hempstead JL, Curran T. Mapping patterns of c-*fos* expression in the central nervous system after seizure. *Science* 1987;237:192–197.

Mugnaini E, Oertel WH. An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. In: Bjorklund A, Hokfelt T, eds. *Handbook of chemical neuroanatomy,* Vol. 4, *GABA and neuropeptides in the CNS,* Part I. New York: Elsevier, 1985.

Nicoll RA. Pharmacological evidence for GABA as the transmitter in granule cell inhibition in the olfactory bulb. *Brain Res* 1971;35:137–149.

Obata K, Ito M, Ochi R, Sato N. Pharmacological properties of the postsynaptic inhibition by Purkinje cell axons and the action of γ-aminobutyric acid on Deiters neurones. *Exp Brain Res* 1967;4:43–57.

Obata K, Takeda K. Release of γ-aminobutyric acid into the fourth ventricle induced by stimulation of the cat's cerebellum. *J Neurochem* 1969;16:1043–1047.

Onodera K, Takeuchi A. An analysis of the inhibitory post-synaptic current in the voltage-clamped crayfish muscle. *J Physiol London* 1979;286:265–282.

Otsuka M. GABA in the crustacean nervous system: A historical review. In: Roberts E, Chase TN, Tower DB, eds. *GABA in nervous system function.* New York: Raven Press, 1976;245–249.

Otsuka M, Iversen LL, Hall ZW, Kravitz EA. Release of gamma-aminobutyric acid from inhibitory nerves of lobster. *Proc Natl Acad Sci USA* 1966;56:1110–1115.

Otsuka M, Konishi S. GABA in the spinal cord. In: Roberts E, Chase TN, Tower DB, eds. *GABA in nervous system function.* New York: Raven Press, 1976;197–202.

Potter DD. The chemistry of inhibition in crustaceans with special reference to gamma-aminobutyric acid. In: von Euler C, Skoglund S, Soderberg U, eds. *Structure and function of inhibitory neuronal mechanisms.* Oxford: Pergamon Press, 1968;359–370.

Ratner S. The dynamic state of body proteins. *Ann NY Acad Sci* 1979;325:189–209.

Ribak CE. Aspinous and sparsely-spinous stellate neurons in the visual cortex of rats contain glutamic acid decarboxylase. *J Neurocytol* 1978a;7:461–478.

Ribak CE. Immunocytochemical localization of glutamic acid decarboxylase (GAD) in the rat corpus striatum. *Anat Rec* 1978b;190:521.

Ribak CE, Harris AB, Vaughn JE, Roberts E. Inhibitory GABAergic nerve terminals decrease at sites of focal epilepsy. *Science* 1979;205:211–214.

Ribak CE, Vaughn JE, Saito K. Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport. *Brain Res* 1978;140:315–332.

Ribak CE, Vaughn JE, Saito K, Barber R, Roberts E. Immunocytochemical localization of glutamate decarboxylase in rat substantia nigra. *Brain Res* 1976;116: 287–298.

Ribak CE, Vaughn JE, Saito K, Barber R, Roberts E. Glutamate decarboxylase localization in neurons of the olfactory bulb. *Brain Res* 1977;126:1–18.

Robbins J, Van der Kloot WG. The effect of picrotoxin on peripheral inhibition in the crayfish. *J Physiol London* 1958;143:541–552.

Roberts E. Formation and utilization of γ-aminobutyric acid in brain. In: Korey SR, Nurnberger JI, eds. *Progress in neurobiology. 1. Neurochemistry.* New York: Hoeber-Harper, 1956;11–25.

Roberts E. Disinhibition as an organizing principle in the nervous system—The role of the GABA system. Application to neurologic and psychiatric disorders. In: Roberts E, Chase TN, Tower DB eds. *GABA in nervous system function.* New York: Raven Press, 1976a;515–539.

Roberts E. Immunocytochemistry of the GABA system—A novel approach to an old transmitter. In: Ferrendelli JA, McEwen BS, Snyder SH, eds. *Neurotransmitters, hormone and receptors: Novel approaches.* Bethesda, MD: Society for Neuroscience, 1976b;123–138.

Roberts E. Roles of GABA neurons in information processing in the vertebrate CNS. In: Karlin A, Tennyson VM, Vogel HJ, eds. *Neuronal information transfer.* New York: Academic Press, 1978;213–239.

Roberts E. γ-Aminobutyric acid (GABA): From discovery to visualization of GABAergic neurons in the vertebrate nervous system. In: Bowery NG, ed. *Actions and interactions of GABA and benzodiazepines.* New York: Raven Press, 1984; 1–25.

Roberts E. Failure of GABAergic inhibition: A key to local and global seizures. *Adv Neurol* 1986a;44:319–341.

Roberts E. GABA: The road to neurotransmitter status: In: Olsen RW, Venter JC, eds. *Benzodiazepine/GABA receptors and chloride channels: Structural and functional properties.* New York: Liss, 1986b;1–39.

Roberts E. What do GABA neurons really do? They make possible variability generation in relation to demand. *Exp Neurol* 1986c;93:279–290.

Roberts E. Living systems are tonically inhibited, autonomous optimizers, and disinhibition coupled to variability generation is their major organizing principle: inhibitory command-control at levels of membrane, genome, metabolism, brain, and society. *Neurochem Res* 1991;16:409–421.

Roberts E, Baxter CF, Van Harreveld A, Wiersma CAG, Adey WR, Killam KF, eds. *Inhibition in the nervous system and gamma-aminobutyric acid.* Oxford: Pergamon Press, 1960.

Roberts E, Chase TN, Tower DB, eds. *GABA in nervous system function.* New York: Raven Press, 1976.

Roberts E, Frankel S. Free amino acids in normal and neoplastic tissues of mice as studied by paper chromatography. *Cancer Res* 1949;9:645–648.

Roberts E, Frankel S. γ-Aminobutyric acid in brain. *Fed Proc* 1950a;9:219.

Roberts E, Frankel S. γ-Aminobutyric acid in brain: its formation from glutamic acid. *J Biol Chem* 1950b;187:55–63.

Roberts E, Frankel S. Glutamic acid decarboxylase in brain. *J Biol Chem* 1951;188: 789–795.

Roberts E, Matthysse S. Neurobiochemistry: At the Crossroads of Neurobiology. *Annv Rev Biochem* 1970;39:777–820.

Roberts E, Sherman MA. GABA—The quintessential neurotransmitter: Electroneutrality, fidelity, specificity, and a model for the ligand binding site of $GABA_A$ receptors. *Neurochem Res* 1993;18:365–376.

Roberts E, Simonsen DG. Free amino acids in animal tissue. In: Holden JT, ed. *Amino acid pools.* Amsterdam: Elsevier, 1962;284–349.

Roberts E, Tishkoff GH. Distribution of free amino acids in mouse epidermis in various phases of growth as determined by paper partition chromatography. *Science* 1949;109:14–16.

Rössler OE. Chaotic behavior in simple reaction systems. *Z Naturforsch A* 1976;31: 259–264.

Saffen DW, Cole AJ, Worley PF, Christy BA, Ryder K, Baraban JM. Convulsant-induced increase in transcription factor messenger RNAs in rat brain. *Proc Natl Acad Sci USA* 1988;85:7795–7799.

Saito K. Immunochemical studies of GAD and GABA-T. In: Roberts E, Chase TN, Tower DB, eds. *GABA in nervous system function.* New York: Raven Press, 1976; 103–111.

Saito K, Barber R, Wu J-Y, Matsuda T, Roberts E, Vaughn, JE. Immunohistochemical localization of glutamate decarboxylase in rat cerebellum. *Proc Natl Acad Sci USA* 1974;71:269–273.

Saito K, Roberts E, Barber R. Immunocytochemical identification of GABAergic neurons. In: Kuriyama K, ed. *Structure and function of synapses.* Tokyo: Ishiyaku Shuppan, 1977;143–157.

Saito K. Schousboe A, Wu J-Y, Roberts E. Some immunochemical properties and species specificity of GABA-α-ketoglutarate transaminase from mouse brain. *Brain Res* 1974;65:287–296.

Saito K, Wu J-Y, Matsuda T, Roberts E. Immunochemical comparisons of vertebrate glutamic acid decarboxylase. *Brain Res* 1974;65:277–285.

Sano K, Roberts E. Binding of γ-aminobutyric acid by brain preparations. *Biochem Biophys Res Commun* 1961;4:358–361.

Sano K, Roberts E. Binding of γ-aminobutyric acid by mouse brain preparations. *Biochem Pharmacol* 1963;12:489–502.

Schoenheimer R. *The dynamic state of body constituents.* Cambridge: Harvard University Press, 1942.

Schousboe A, Wu J-Y, Roberts E. Purification and characterization of the 4-aminobutyrate-2-ketoglutarate transaminase from mouse brain. *Biochemistry* 1973; 12:2868–2873.

Schousboe A, Wu J-Y, Roberts E. Subunit structure and kinetic properties of 4-aminobutyrate-2-ketoglutarate transaminase purified from mouse brain. *J Neurochem* 1974;23:1189–1195.

Shepard RN. Toward a universal law of generalization for psychological science. *Science* 1987;237:1317–1323.

Steward FC, Thompson JF, Dent CE. γ-Aminobutyric acid. A constituent of the potato tuber? *Science* 1949;110:439–440.

Storm-Mathisen J, Leknes AK, Bore AT, Vaaland JL, Edminson P, Haug F-M S, Ottersen OP. First visualization of glutamate and GABA in neurones by immunocytochemistry. *Nature* 1983;301:517–520.

Takeuchi A. Studies of inhibitory effects of GABA in invertebrate nervous systems. In: Roberts E, Chase TN, Tower DB, eds. *GABA in nervous system function.* New York: Raven Press, 1976;255–267.

Takeuchi A, Takeuchi N. The effect on crayfish muscle of iontophoretically applied glutamate. *J Physiol* 1964;170:296–317.

Takeuchi A, Takeuchi N. Electrophysiological studies of the action of GABA on the synaptic membrane. *Fed Proc* 1967;26:1633–1638.

Takeuchi A, Takeuchi N. The structure-activity relationship for GABA and related compounds in the crayfish muscle. *Neuropharmacology* 1975;14:627–634.

Tam S-P, Hache RJG, Deeley RG. Estrogen memory effect in human hepatocytes during repeated cell division without hormone. *Science* 1986;234:1234–1237.

Thom R. *Structural stability and morphogenesis.* Reading, MA: Benjamin, 1975. [English edition].

Udenfriend S. Identification of γ-aminobutyric acid in brain by the isotope derivative method. *J Biol Chem* 1950;187:65–69.

Varon S, Weinstein H, Kakefuda T, Roberts E. Sodium-dependent binding of γ-aminobutyric acid by morphologically characterized subcellular brain particles. *Biochem Pharmacol* 1965;14:1213–1224.

Vaughn JE, Barber RP, Saito K, Roberts E, Famiglietti EV, Jr. Immunocytochemical identification of GABAergic neurons in rat retina. *Anat Rec* 1978;190:571–572.

von Bekesy G. *Sensory inhibition.* Princeton: Princeton University Press, 1967.

von Euler C, Skoglund S, Söderberg U. *Structure and function of inhibitory neuronal mechanisms.* Oxford: Pergamon Press, 1968.

Wang Y-J, Salvaterra P, Roberts E. Characterization of [^{3}H]muscimol binding to mouse brain membranes. *Biochem Pharmacol* 1979;28:1123–1128.

Weinstein H, Varon S, Muhleman DR, Roberts E. A carrier-mediated transfer model for the accumulation of ^{14}C-γ-aminobutyric acid by subcellular brain particles. *Biochem Pharmacol* 1965;14:273–288.

Wheatley DN, Inglis MS. Protein turnover during cell growth: A re-examination of the problem of linear incorporation kinetics of radioactively-labeled amino acids into protein and its relationship to growth characteristics. *Cytobios* 1986;47: 187–210.

Wiersma CAG, Furshpan E, Florey E. Physiological and pharmacological observations on muscle receptor organs of the crayfish, *Cambarus clarkii* girard. *J Exp Biol* 1953;30:136–150.

Wong E, Schousboe A, Saito K, Wu J-Y, Roberts E. Immunochemical studies of brain glutamate decarboxylase and GABA-transaminase of six inbred strains of mice. *Brain Res* 1974;68:133–142.

Wood JG, McLaughlin BJ, Vaughn JE. Immunocytochrmical localization of GAD in electron microscopic preparations of rodent CNS. In: Roberts E, Chase TN, Tower DB, eds. *GABA in nervous system function.* New York: Raven Press, 1976; 133–148.

Wu J-Y. Purification, characterization, and kinetic studies of GAD and GABA-T from mouse brain. In: Roberts E, Chase TN, Tower DB, eds. *GABA in nervous system function.* New York: Raven Press, 1976;7–55.

Wu J-Y, Matsuda T, Roberts E. Purification and characterization of glutamate decarboxylase from mouse brain. *J Biol Chem* 1973;248:3029–3034.

Wu J-Y, Roberts E. Properties of brain L-glutamate decarboxylase: Inhibition studies. *J Neurochem* 1974;23:759–767.

Yoneda Y, Roberts E, Dietz GW, Jr. A new synaptosomal biosynthetic pathway of glutamate and GABA from ornithine and its negative feedback inhibition by GABA. *J Neurochem* 1982;38:1686–1694.

Additional Publications

Steroid-Related

Roberts E, Bologa L, Flood JF, Smith GE. Effects of dehydroepiandrosterone and its sulfate on brain tissue in culture and on memory in mice. *Brain Res* 1987;406: 357–362.

Bologa L, Sharma J, Roberts E. Dehydroepiandrosterone and its sulfated derivative reduce neuronal death and enhance astrocytic differentiation in brain cell cultures. *J Neurosci Res* 1987;17:225–234.

Flood JF, Smith GE, Roberts E. Dehydroepiandrosterone and its sulfate enhance memory retention in mice. *Brain Res* 1988;447:269–278.

Flood JF, Roberts E. Dehydroepiandrosterone sulfate improves memory in aging mice. Brain Res 1988;448:178–181.

Roberts E. Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) as neural facilitators: effects on brain tissue in culture and on memory in young and old mice. A cyclic GMP hypothesis of action of DHEA and DHEAS in nervous system and other tissues. In: Kalimi M, Regelson W, eds. *The biological role of dehydroepiandrosterone (DHEA).* New York: Walter de Gruyter & Co, 1990; 13–42.

Flood JF, Morley JE, Roberts E. Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. *Proc Natl Acad Sci USA* 1992; 89:1567–1571.

Guth L, Zhang Z, Roberts E. Key role for pregnenolone in combination therapy that promotes recovery after spinal cord injury. *Proc Natl Acad Sci USA* 1994;91: 12308–12312.

Roberts E. Pregnenolone—From Selye to Alzheimer and a model of the pregnenolone sulfate binding site on the $GABA_A$ receptor. *Biochem Pharmacol* 1995;49: 1–16.

Flood JF, Morley JE, Roberts E. Pregnenolone sulfate enhances post-training memory processes when injected in very low doses into limbic system structures: The amygdala is by far the most sensitive. *Proc Natl Acad Sci USA* 1995;92: 10806–10810.

Alzheimer-Related

Flood JF, Morley JE, Roberts E. Amnestic effects in mice of four synthetic peptides homologous to amyloid β protein from patients with Alzheimer disease. *Proc Natl Acad Sci USA* 1991;88:3363–3366.

Flood JF, Roberts E, Sherman MA, Kaplan BE, Morley JE. Topography of a binding site for small amnestic peptides deduced from structure-activity studies: Relation to amnestic effect of amyloid β protein. *Proc Natl Acad Sci USA* 1994;91: 380–384.

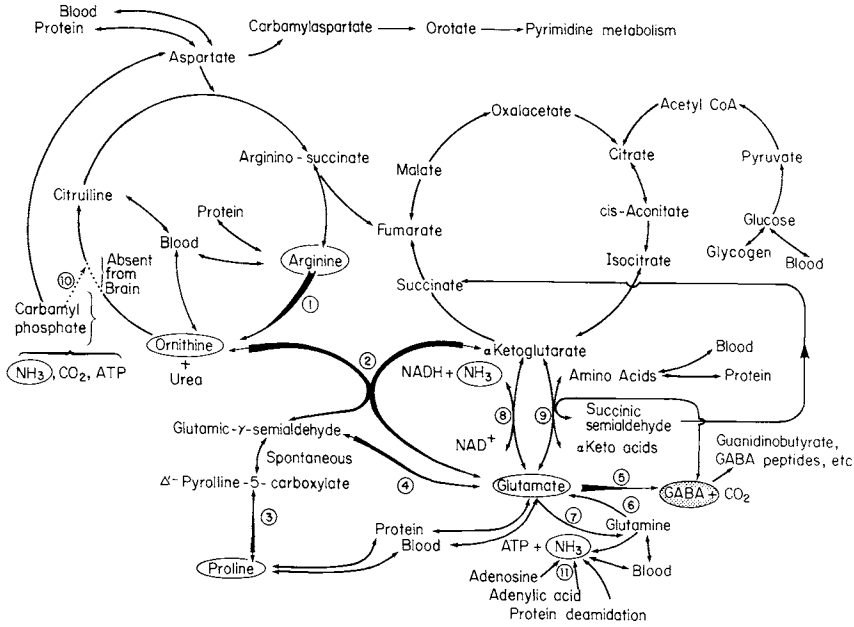
Flood JF, Morley JE, Roberts E. An amyloid β-protein fragment, Aβ[12-28], equipotently impairs post-training memory processing when injected into different limbic system structures. *Brain Res* 1994;663:271–276.

Appendix I. Isoelectric Points of Major Naturally Occurring Amino Acids and Peptides in Animal Tissues[a]

Amino acid	pI
Aspartic acid	1.77
Glutamic acid	3.22
Cystine	5.03
Taurine	5.12
Asparagine	5.41
Phenyalanine	5.48
Homocystine	5.53
Threonine	5.64
Glutamine	5.65
Tyrosine	5.66
Serine	5.68
Methionine	5.74
Hydroxyproline	5.74
Tryptophan	5.89
Citrulline	5.92
Isoleucine	5.94
Valine	5.96
Glycine	5.97
Leucine	5.98
Alanine	6.00
Sarcosine	6.12
Proline	6.30
β-Alanine	6.90
Cysteine	6.94
Homocysteine	7.05
γ-Aminobutyric acid	7.30[b]
Histidine	7.47
δ-Amino-*n*-valeric acid	7.52
ε-Amino-*n*-caproic acid	7.60
l-Methylhistidine	7.67
Carnosine	8.17
Anserine	8.27
Lysine	9.59
Ornithine	9.70
Arginine	11.15

[a]Data taken from Greenstein and Winitz, 1961

[b]Physiological pH is 7.30



Appendix II. Metabolic relationships potentially relevant to control of GABA formation and utilization. Many of these have been explored in studies in my laboratories and in those of others. Reprinted from *Benzodiazepine / GABA receptors and chloride channels: Structural and functional properties.* Olsen RW, Venter JC, eds. New York, NY: Liss, 1986; 1–39, with permission of the author.

Appendix III. Immunocytochemistry of GABA-Related Enzymes

Steps along the way	Date	Authors
1. Purification and properties		
GAD		
Purification and characterization of glutamate decarboxylase from mouse brain	1973	Wu, Matsuda, and Roberts
Electrophoresis of glutamic acid decarboxylase from mouse brain in sodium dodecyl sulfate polyacrylamide gels	1973	Matsuda, Wu, and Roberts
Properties of brain L-glutamate decarboxylase: inhibition studies	1974	Wu and Roberts
GABA-T		
Purification and characterization of the 4-aminobutyrate-2-ketoglutarate transaminase from mouse brain	1973	Schousboe, Wu, and Roberts
Subunit structure and kinetic properties of 4-aminobutyrate-2-ketoglutarate transaminase purified from mouse brain	1974	Schousboe, Wu, and Roberts
Summary		
Purification, characterization, and kinetic studies of GAD and GABA-T from mouse brain	1976	Wu
2. Immunological studies		
GAD		
Immunochemical studies on glutamic decarboxylase from mouse brain	1973	Matsuda, Wu, and Roberts
Immunochemical comparisons of vertebrate glutamic acid decarboxylase	1974	Saito, Wu, Matsuda, and Roberts
Immunochemical studies of brain glutamate decarboxylase and GABA-transaminase of six inbred strains of mice	1974	Wong, Schousboe, Saito, Wu, and Roberts
GABA-T		
Some immunochemical properties and species specificity of GABA-α-ketoglutarate transaminase	1974	Saito, Schousboe, Wu, and Roberts
Summary		
Immunochemical studies of glutamate decarboxylase and GABA-α-ketoglutarate transaminase	1976	Saito

Appendix III—*Continued*

Steps along the way	Date	Authors
3. Immunocytochemical approaches		
GAD		
Immunohistochemical localization of glutamate decarboxylase in rat cerebellum	1974	Saito, Barber, Wu, Matsuda, Roberts, and Vaughn
The fine structural localization of glutamate decarboxylase in synaptic terminals of rodent cerebellum	1974	McLaughlin, Wood, Saito, Barber, Vaughn, Roberts, and Wu
The fine structural localization of glutamate decarboxylase in developing axonal processes and presynaptic terminals of rodent cerebellum	1975	McLaughlin, Wood, Saito, Roberts, and Wu
Immunocytochemical localization of glutamate decarboxylase in rat spinal cord	1973	McLaughlin, Barber, Saito, Roberts, and Wu
Immunocytochemical localization of glutamate decarboxylase in the substantia nigra of the rat	1976	Ribak, Vaughn, Saito, and Barber
Immunocytochemical localization of glutamate decarboxylase in rat substantia nigra	1976	Ribak, Vaughn, Saito, Barber, and Roberts
Immunocytochemical localization of glutamate decarboxylase (GAD) in the olfactory bulb	1976	Ribak, Vaughn, and Saito
Glutamate decarboxylase (GAD) localization in neurons of the olfactory bulb	1977	Ribak, Vaughn, Saito, Barber, and Roberts
Immunocytochemical localization of GAD in somata and dendrites of GABAergic neurons following colchicine treatment	1976	Ribak and Vaughn
The immunocytochemical localization of GAD within stellate neurons of rat visual cortex	1977	Ribak
Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport	1978	Ribak, Vaughn, and Saito
GABAergic terminals are presynaptic to primary afferent terminals in the substantia gelatinosa of the rat spinal cord	1978	Barber, Vaughn, Saito, McLaughlin, and Roberts

Appendix III—*Continued*

Steps along the way	Date	Authors
Immunocytochemical localization of GAD in electron microscopic preparations of rodent CNS	1976	Wood, McLaughlin, and Vaughn
Aspinous and sparsely spinous stellate neurons contain glutamic acid decarboxylase in the visual cortex of rats	1978[a]	Ribak
Immunocytochemical identification of GABAergic neurons in rat retina	1978	Vaughn, Barber, Saito, Roberts, and Famiglietti
Immunocytochemical localization of glutamic acid decarboxylase (GAD) in the rat corpus striatum	1978[b]	Ribak
GABAergic axon terminals decrease at experimental seizure foci in monkey cerebral cortex	1978	Ribak, Harris, Anderson, Vaughn, and Roberts
GABA-T		
See Wood et al.		
Summaries		
Light microscopic visualization of GAD and GABA-T in immunocytochemical preparations of rodent CNS	1976	Barber and Saito
ImmunochemistryoftheGABAsystem—a novel approach to an old transmitter	1976	Roberts
Immunocytochemical identification of GABAergic neurons	1977	Saito, Roberts, and Barber
Roles of GABA neurons in information processing in the vertebrate CNS	1978	Roberts