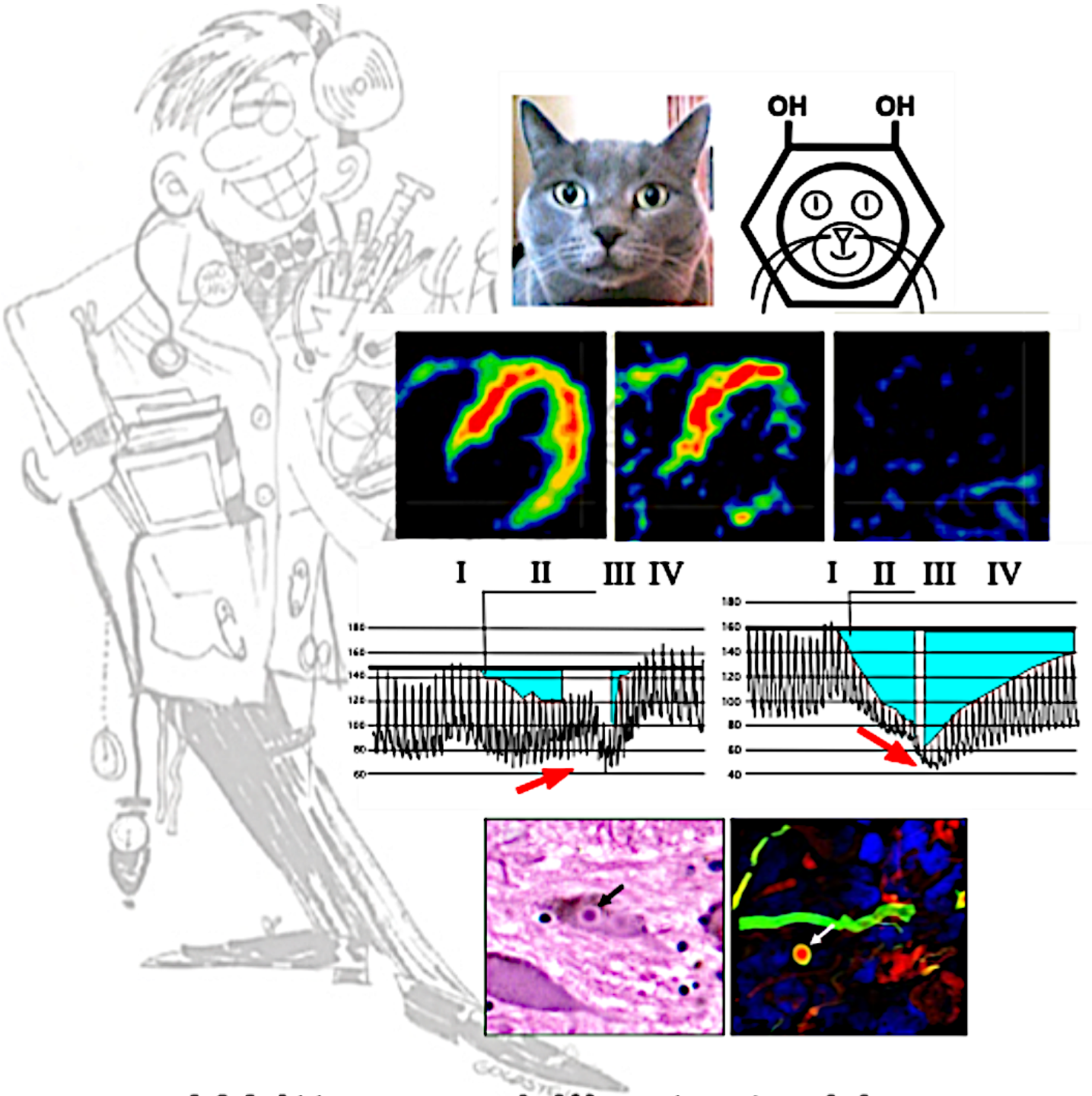
An Autonomic Life



Written and Illustrated by

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DISCLAIMERS

The views and opinions expressed here are the author's and do not necessarily represent the views of the National Institute of Neurological Disorders and Stroke, the National Institutes of Health, the Department of Health and Human Services, or the United States Government.

DEDICATION

I dedicate this book to my wife Minka, to our family, to the memory of my mentor Irv Kopin, to my team at the NIH who have enriched my life by their dedication and support, and to the patients who by participating in our research have enabled me to experience the joy of insight.

INTRODUCTION

Here I look back at five decades studying, observing, discovering, theorizing, writing, and teaching about the autonomic nervous system (ANS). The ANS is the part of the nervous system that is responsible for the automatic, unconscious, involuntary processes that regulate the body's "inner world" throughout life, in emergencies, in activities of daily living, and in a variety of common and rare diseases. The ANS functions at the ineffable border of the body and mind and so is at the core of brain-body medicine.

The ANS works via chemical messengers. Prominent among these are the catecholamines dopamine, norepinephrine, and epinephrine (adrenaline). I'm a chronic catecholaholic, having studied these "biogenic amines" and their effects since college days. I use the term, biogenic, here for two reasons. One is that living things make and use these biochemicals. The second is that catecholamines are absolutely required for surviving the vicissitudes of life.

Two teachings from my Jewish heritage induced me to consolidate and relate my professional story here. The first is, "It is not incumbent on you to finish the task; but you are not free to desist from it." As a founder of the fields of clinical catecholamine neurochemistry and sympathetic neuroimaging, I believe I've helped establish autonomic medicine as a clinical and scientific discipline. I will not complete this task. In a sense it will never be completed, just as for any academic field that depends on continuous accretion of new knowledge. By this writing I'm demonstrating that I haven't desisted from the task. The second teaching is "If I am not for myself, who will be for me. And if I am only for myself, what am I? And if not now, when?" I'm writing this in the hope that your reading this retrospective will inspire you to learn and apply some of the insights and lessons conveyed here in your own autonomic lives. If I didn't write this, no one else would. With me near retirement, the timing seems right.

What is an autonomic life?

The meaning of the word, “autonomic,” is ambiguous. It has at least two definitions, and both are relevant to this book.

One definition refers to autonomic as automatic, involuntary, and unconscious. The English physiologist John Newport Langley had this in mind when he coined the phrase, “autonomic nervous system,” (ANS) about a century ago. Autonomic can also mean “autonomous”—i.e., independent or self-governing. The French physiologist Claude Bernard and the American physiologist Walter B. Cannon were referring to autonomic in this sense when they taught that numerous processes in the body’s inner world enable organisms to keep levels of key variables within bounds (homeostasis, a word Cannon invented), despite the fluctuations of the outer world we occupy during life. By both meanings of the term, I feel I’ve lived an autonomic life.

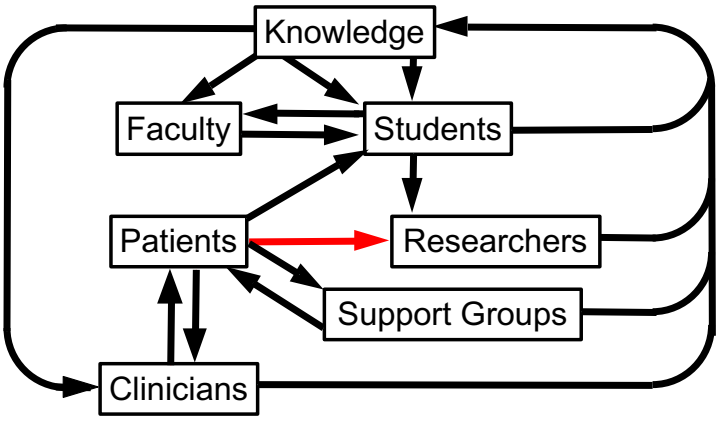
Within intramural research programs at the National Institutes of Health (NIH) I’ve long directed Independent Sections, distinctly different from the much more common career paths in extramural programs that receive NIH grants—another autonomic aspect of my life.

The physiology and pathophysiology of the ANS have occupied me for most of my adult life. At scientific meetings, when I ask questions I always first identify myself with, “Goldstein, NIH.” The institution has been my identity. Here I tell the stories behind many research findings but more importantly convey some of the discoveries, thought processes, and insights that have been on my mind and continue to preoccupy me. I’ll leave to the reader whether these reflect providence or serendipity (fortune favoring the prepared mind, to paraphrase Louis Pasteur).

Nature’s “secret mysteries”

I hope to relate in this book that patient-oriented research (POR) based on developing and applying new technologies, especially in individuals with rare diseases, has led to many discoveries, and the discoveries have induced new concepts about functions and disorders of the ANS.

Accretion of new knowledge in this area, as in all areas of biomedical research, never ends. T.S. Eliot wrote, “We shall not cease from exploration/And the end of all our exploring/Will be to arrive where we started/And know the place for the first time.” Rather than circles, I think of upward spirals.



The accretion of new medical knowledge occurs in cycles. Information provided by patients to researchers (red arrow) is a key part of this process.

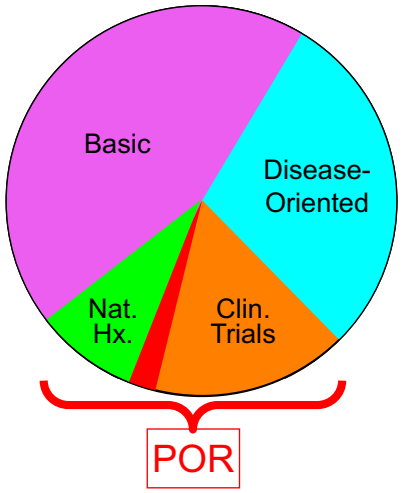
Most of medical research is basic or disease-oriented. Disease-oriented research consists mainly of animal, tissue, or cellular models of diseases. You’re doing patient-oriented research (POR) when you shake hands with the subject matter. POR is only a small slice of the biomedical research pie, and most of POR is clinical trials of new experimental therapeutic agents or natural history studies. Only a tiny sliver is about elucidating pathophysiological mechanisms—yet another feature of my autonomic life.

Patients are a unique scientific resource. They tell us what is wrong; from their test results they show us what is wrong. Our job is to try to learn what they teach. In this book I summarize and pass on some of what I’ve learned so far.

In this regard I feel I’ve been following a tradition that goes back to the time of William Harvey, the father of modern medical research. Harvey was

the first to describe the circulation of the blood. In 1657, the year he died, in a letter to a colleague he wrote the following.

> Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature, by the careful investigation of cases of rarer forms of disease.



In the pie of biomedical research, patient-oriented research (POR) is a slice. Clinical trials and natural history studies constitute most of POR. The red sliver corresponds to pathophysiological POR, in which mechanisms of diseases are studied based on information from the patients who are suffering from those conditions.

William Harvey (1578-1657), father of modern medical research.

Discovering nature's secret mysteries has been my quest and passion. The business of biomedical research is testing hypotheses; the thrill is making discoveries. I remember stating this once when I was defending a clinical protocol at a meeting of the Institutional Review Board of the National Institute of Neurological Disorders and Stroke (NINDS). They thought I was joking, but I wasn't.

FOUNDATIONS

A foundation of my autonomic life was growing up in an attic.

An attic childhood

When I was born my parents were living in a shack that had been built hastily in Newark, New Jersey for veterans coming home from World War II. My father had been stationed on the South Pacific island of Guam. Guam was the staging area for B-29s such as the *Enola Gay* that dropped the atomic bomb on Hiroshima. My father never told my siblings or me what exactly he did on Guam. For many years I used a transparent plastic clipboard he gave me that he claimed was from the material used for the windows of B-29s.

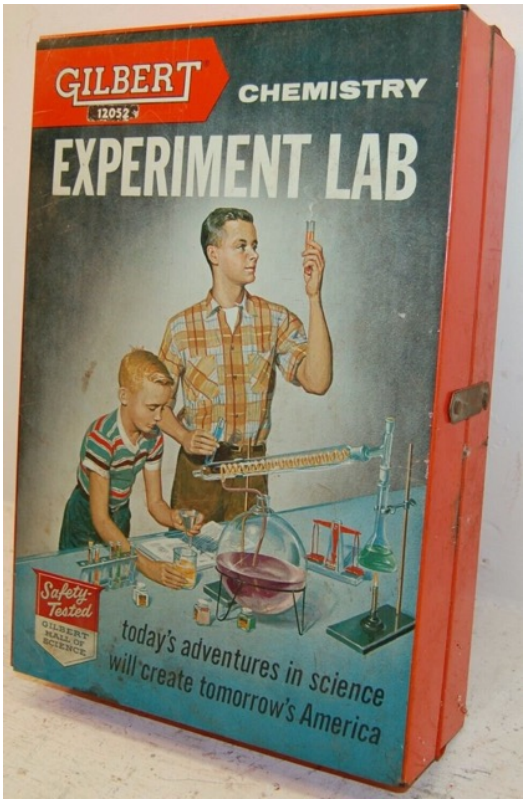
We lived for a while in an apartment house in North Bergen before moving to a single-family house at 169 Renner Avenue, just up the block from Bergen Street in Newark's South Ward. Both my parents worked for the US Government, which is to say they had stable, middle class, Civil Service jobs.

My father had aspired to become a lawyer. Instead, my mother had me. Afterward came Annie, Janet, Harris, and Cliffy.

The house became small, and I was moved to an attic room in the gabled third story. In the attic of the house on Renner I came and went as I wished.

In the attic I puttered with my Gilbert chemistry set and learned how to measure with phenolphthalein the acidity of a solution.

When we moved several blocks away to a larger house, at 49 Vassar Avenue, also near Bergen Street, I lived in the attic there too. I had no problem being alone.



A Gilbert chemistry set from the 1950s. Note the caption, "today's adventures in science will create tomorrow's America."

The arrow points to the window of my room in the attic at 49 Vassar Avenue.

I drew my first cartoons in the attic at 49 Vassar. I learned caricature from John Guderian at Newark's Arts High School Saturday mornings. My father would drop me off and pick me up. At 15 I painted a still life that featured my father's old combat boots, along with one of my US Keds canvas sneakers. I'm still proud of that painting. I thought I had the potential to become another Norman Rockwell.

My first and only oil painting, done at about age 15 when I lived in the attic of our house on Vassar Avenue. Depicted are my father's combat boots from World War II, as well as one of my US Keds sneakers.

In both houses I shared the attic with my widowed grandmother on my mother's side, Bessie Rich. She grew guppies in a large aquarium tank, the motor of which droned day and night. She would spend hours propped in bed staring blankly in the glow of her TV. Her bedroom always smelled of Ben-Gay.

She would become animated, however, when watching professional wrestling. She would warn the good guys about lurking enemies such as Skull Murphy, whose signature move was to butt opponents senseless with his vaselined bald head. It was through viewing wrestling on TV with Grandma Bessie that I learned about the "sleeper hold."

In professional wrestling you can win in three ways—by the referee

slapping the mat three times, disqualification, and submission. The sleeper hold was a submission hold. The wrestler would suddenly circle around the opponent and grab his neck from behind. Instead of choking the opponent (that would be a disqualification), he would rub the upper neck on both sides. After several seconds, the opponent would seem to fall asleep and slump to the mat unconscious.

Over the years I grew to doubt the genuineness of professional wrestling, but I do believe there is a kernel of truth to the sleeper hold. As you'll read later, for years I've used it to teach about the carotid sinus baroreflex.

Several blocks away in Newark lived David and Daniel Fink and their family. I was the top student at Maple Avenue School, and David and Daniel, fraternal twins, were the top students at Chancellor Avenue School. These elementary schools fed Weequahic High School (made famous by Philip Roth). We became academic rivals but close friends. David called me "Goldstein." I called him "Fink."

David J. Fink. I think this photo was taken when he was Chairman of the Department of Neurology at the University of Michigan.

David Fink will be returning a few times in this narrative. He and I often would think the same thing at the same time. I called this "brain waves."

Brain waves led to our first academic publication, which was based on research we did as college students—on the autonomic nervous system.

David, Daniel, and I attended Hebrew school after school at Temple B'nai Abraham, which at the time was on Clinton Avenue in Newark. It's probably fair to say that most Jewish American kids who attended Hebrew school in the 1950s-1960s hated it. It was boring, and it interfered with extracurricular activities. I'm convinced this is why I never developed as a baseball player at Weequahic. I was a whiz at ping-pong, however. I remember Sy Mullman, a starting pitcher on Weequahic's baseball team, commenting that he wished I could play baseball like I played ping-pong. I'll return to ping-pong and its significance shortly.

B'nai Abraham was different. For the Fink boys and me there were flashes of inspiration, mainly because of two personalities, Edwin Soslow and Joachim Prinz.

I don't remember Soslow for his religious teaching. I don't remember the curriculum much at all; however, I do remember his cosmopolitan political philosophy.

For instance, he taught, with a knowing grin and squinted eyes, that successful revolutions are not instigated and led by the oppressed minority. They are led by the seconds in command, who know the inner workings of the government, have the connections, and see the opportunities for overthrowing the ruling elite. He expounded on Hegel's dialectic, which would lead eventually to synthesis, and about the pendulum of history. These ideas were exciting for us young teenagers. A decade later, when Minka and I married, Rabbi Soslow officiated at our wedding. David and Daniel Fink were attendees.

Rabbi Joachim Prinz was inspirational for different reasons but also not for his religious teachings. Instead, I remember him for his own personal history, as a courageous young rabbi in Berlin at the beginning of the Nazi era, his leadership in the civil rights movement, his incisive writing, and especially his stirring rhetoric.

At the March on Washington in 1963, Prinz spoke just before Dr. Martin Luther King, Jr. To appreciate Prinz's skills as a speaker you have to hear him. You can, by viewing the trailer for the movie about him, *I Shall Not Be Silent* (https://www.youtube.com/watch?v=8Hw8N2XF6Hk). When I view the segment of his speech at the March on Washington beginning at about 1:22, it brings tears to my eyes.

Rabbi Edwin Soslow. Soslow was teaching at B'nai Abraham when David and Daniel Fink and I attended Hebrew school there. When Minka and I married, Soslow officiated. Daniel (no beard) and David (with beard) attended the ceremony.

Rabbi Joachim Prinz, a close associate of the Rev. Martin Luther King, Jr. co-organized the 1963 March on Washington for Jobs and Freedom.

Brian Buffini and the analogy of the 3 bowls

A much more recent source of inspiration has been the real estate coach Brian Buffini. To teach about achieving life goals he has used an analogy to 3 bowls. Picture 3 bowls, one on top of another. There is a small bowl at the top, a larger middle bowl under it, and an even larger bowl at the bottom. When you pour into the top, small bowl, eventually it overflows into the middle, larger bowl, and then that overflows into the bottom, largest bowl.

Brian Buffini and his 3 bowl analogy.

The 3 bowls signify stability, success, and significance. Stability comes from recognizing one's talents and honing them into skills by practice and learning. The upper bowl overflows to the middle bowl, success. Success comes from consistently and continuously pouring into the top bowl of learning and practice. The bottom bowl, the bowl of significance, means success beyond oneself. The middle bowl of success overflows to the bottom bowl—the bowl of legacy and transcendence.

For me, as you'll read throughout this monograph, pouring into the top bowl has meant devising, validating, and applying technologies and learning from assessing functions of the autonomic nervous system (ANS) in people. These technologies include clinical neurochemistry, for measuring levels of catecholamines (pronounced cat-a-COAL-a-means) and related compounds, clinical imaging of the sympathetic nervous system, which is a key component of the ANS, clinical autonomic physiology, and, relatively recently, clinical sympathetic microimaging. I review here these technologies, the discoveries that came from applying them, and the concepts that the discoveries induced. The middle bowl of success has filled from these discoveries, insights, and ideas. Some achievements are the discovery of cardiac sympathetic denervation in Parkinson's disease (PD), objective indices (biomarkers) that predict PD in at-risk individuals, and the notion of catecholamine autotoxicity, where diseases result not only from genetics and life experiences (nature and nurture, genes and environment) but also from cumulative harmful effects of chemicals that are produced normally in the body all the time. I'll be referring to particular breakdown products of the catecholamines dopamine, norepinephrine, and epinephrine (synonymous with adrenaline). I've been conducting patient-oriented research on catecholaminergic systems for most of my life.

By continuously pouring into the top bowl, I've achieved success, as measured by publications that are widely cited, support of the research, recognition by peers, and opportunities to lead. Now I'm beginning to fill the bottom bowl, by inspiring the larger community to use my concepts with the overall goal to delay the onset or slow the progression of a class of diseases that importantly result from degeneration of catecholaminergic neurons, by teaching as broadly as possible about autonomic medicine, and by mentoring rising researchers in the field.

An oddball introduction to separation and detection technology

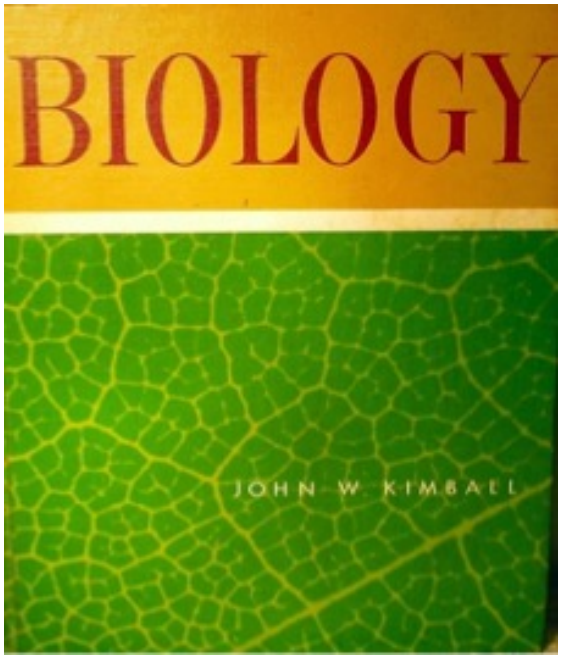
I liked playing ping-pong because I was good at it, and vice versa. I'd play on Sundays for hours at the South Ward Boys' Club. Independently I

came up with a way to hold the racket favoring my backhand. I served with a quick backhand stroke. Later I played Friday nights at the local YMHA on Chancellor Avenue. Others were dancing to the Stroll, the Mashed Potatoes, or the Monster Mash. I played ping-pong. I represented our Boys' Club in ping-pong tournaments. I also sold raffle tickets, printed to look like bricks, to raise funds for the new Club building. In 1964 I was named the Boys' Club Regional Boy of the Year. The prize was a scholarship for me to attend the Summer Session at Phillips Academy, in Andover, Massachusetts. The flight from Newark to Boston was my first time in an airplane. I landed in what was for me a new world.

Andover was the top prep school in the country. Numerous young men who subsequently gained national fame attended Andover, including Presidents George Bush (both father and son). The campus was idyllic, with large green lawns, towering old trees, and a variety of architecturally impressive buildings such as the iconic Samuel Phillips Hall.

Samuel Phillips Hall, at Phillips Academy, Andover, Massachusetts.

The courses I took that summer were biology and English composition. I relished both. At the time, Dr. John Ward Kimball was editing the first edition of his textbook, *Biology.* Imagine coming from a public high school in Newark to be taught by a biologist who'd just completed a single-authored textbook! The book's 6th edition was published in 1994.



John W. Kimball, PhD, was editing the first edition of his biology textbook when I attended the Summer Session at Andover.

For my project I needed to learn to use paper electrophoresis to separate the proteins in different plants. The program supplied me with a new electrophoresis machine. The project was partly successful, in that the chromatographic pattern for lettuce was different from that for cabbage. To visualize the electrophoretic bands required spraying the paper strips with

ninhydrin dye. Ninhydrin is used to detect amines (a part of a molecule that is present in all amino acids, the building blocks of proteins, as well as in the catecholamines) by converting the amines to dark blue-purple derivatives. Ninhydrin is used in fingerprinting to this day because of the proteins that are sloughed off the skin and deposited on surfaces that can be sprayed. I learned the hard way *not* to get ninhydrin spray on my fingers. They instantly were stained blue-purple and stayed that way for days.

The electrophoresis project during the Summer Session at Andover foretold the far more sophisticated separation and detection technology, liquid chromatography with electrochemical detection, which I pioneered at the NIH about 15 years later and have used in my lab for more than 40 years. The principle of both assays is the same: first separate, then detect.

From my eye-opening experiences during the Summer Session I applied to Andover for my senior of high school year and got in. Phillips Academy was on a trimester program. The first trimester ended at Thanksgiving. This meant that I'd have to get excellent grades and cultivate relationships with faculty who'd be willing to write letters of recommendation for me for college, all within a few months. I've never worked so hard mentally as during that trimester. One of the courses was Advanced Placement (AP) Chemistry. "AP" meant I could get college credit if I passed the AP exam at the end of the academic year. It was clear from the beginning that I was completely lost. I couldn't figure out what a mole was. On my first test I received a score of 26 out of 100. Not only did I flunk but it was also obvious I had no idea what I was doing. The instructor, thankfully, was Dr. Ronn Minne. Calmly, reassuringly, he went over the material and looked over my homework many times. I wrote about what I was thinking at each step. Eventually I aced the AP exam. Much later I became an expert on the chemistry of catecholamines, and for several years I was the Chief of the Clinical Neurochemistry Section at the NIH.

My left hand

Phillips Academy was so renowned that college recruiter teams came

to the campus for on-site interviews. My heart was set on Yale, but I doubted I could compete. When the time came for my interview there were no rooms available in the administration building. I offered to walk with the interviewer to my dorm.

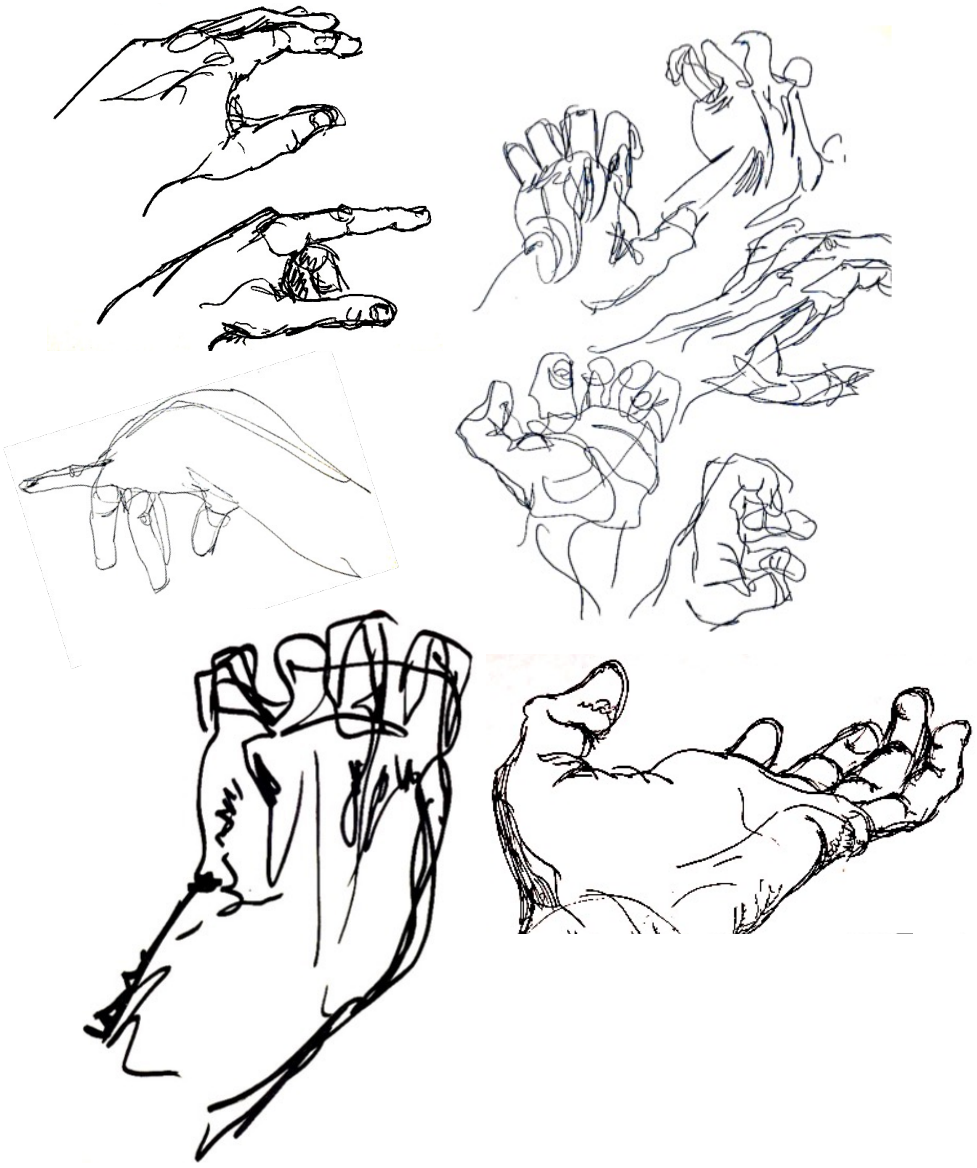
Fuess House, at Phillips Academy. The arrow indicates where my room was. I don't remember the tree.

My single room on the second floor of the recently constructed Fuess House had a picture window the full length of the room that looked out onto the winding road to the rest of the campus. There was a bookshelf near the ceiling above the window; below the window was my bed. The door to the room was opposite the window, shelf, and bed.

As soon as I opened the door, I realized I hadn't straightened the room at all. I'd thrown my laundered undershirts, underpants, and socks up on the shelf rather than place them neatly in my dresser. There were more white briefs than books on that shelf. At least they were clean.

The interviewer didn't seem to notice. What drew his attention were numerous sketches I'd done of my left hand. I'd taped scores of them all over the walls. I'd become acquainted with books by George Bridgman, a famous art teacher at the Art Students League in New York, who had a system of "constructive anatomy" that I adopted.

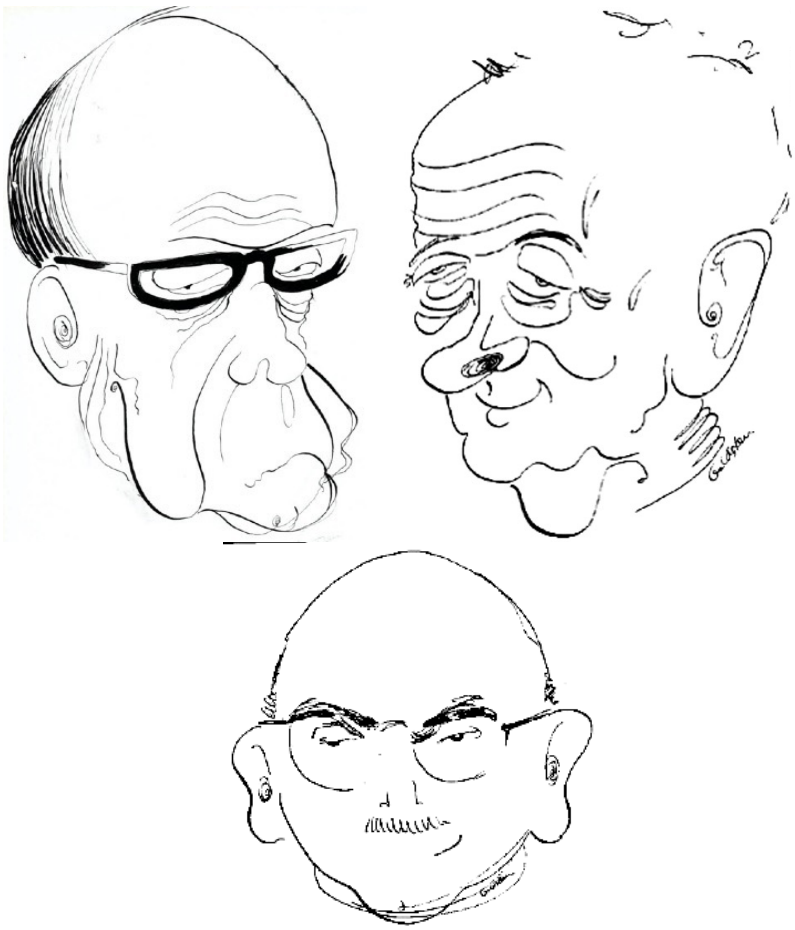
The drawings were very quick, with an emphasis on gesture and strokes and minimal detail. They were caricatures more than detailed renderings. I became a caricaturist. A few I did of faculty were published in the graduation yearbook, the *Pot Pourri*.



Some sketches of my left hand from my days at Phillips Academy, Andover.

Cartooning, caricature, and facility with drawing turned out to be key determinants of my success. I have a knack for conveying ideas visually. I

teach by what I call the “3 As of sticky teaching”—art, analogy, and anecdote. I believe the drawings of my left hand helped me get into Yale.



Caricatures of Phillips Academy faculty published in the class yearbook. Depicted are G. Grenville Benedict (Dean of Students), Fred Allis (Instructor in History), and Richard Pieters (Head of the Mathematics Department).

The last all-male class at Yale

I was in the last all-male class at Yale College, the Class of 1970.

Even a glance at the photo of classmates in Calhoun College would teach a lesson in political incorrectness. In addition to being men, we were,

with rare exception, white and of Anglo-Saxon ethnicity (WASPs).

The residential college itself, named after the white supremacist and anti-abolitionist John C. Calhoun, was re-named in 2017 after Grace Murray Hopper, Yale ’30 M.A., ’34 Ph.D., a rear admiral in the US Navy

Freshman photos of students in Calhoun College, in the Yale College Class of 1970, the last all male class at Yale. I’m in the 2d row, 6th from the left.

noted for her contributions to the early development of computer technology.

At Yale I aimed to become a professional cartoonist. I drew editorial cartoon panels and caricatures for the *Yale Daily News.* Eventually I joined the Editorial Board. I had a column, “Land of Makebelieve,” which I illustrated.

I put together a portfolio and visited a professional cartoonist named Will Elder and asked him to look it over. Elder was a founder of *Mad* magazine. I came to appreciate his work in his “Little Annie Fannie” series in *Playboy.*

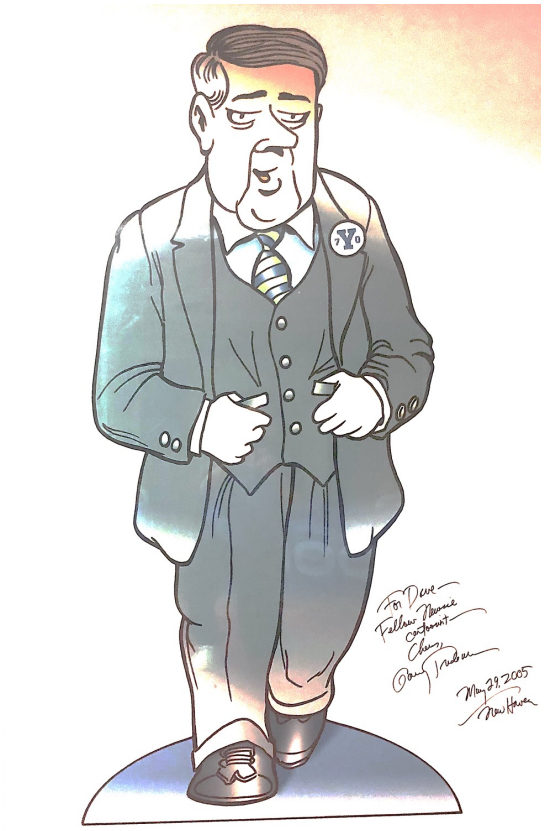
After perusing my portfolio, he said he thought I could make it as a cartoonist, but he wouldn't recommend it. I asked, "Why not?" He replied that from the artwork in the portfolio he could see that for me cartooning was just so much fun. But if I had to depend on it for my bread and butter it'd lose its appeal, and that'd be a shame. I'd have deadlines I'd have to meet and compromises I'd have to make. He then commented, "You're a bright kid. You should become a doctor. Then you'll be rich, and you can pursue cartooning as a hobby. That way it'll always be fresh."

A cartoon self-portrait by Will Elder.

This proved to be the biggest irony of my life. While I did the editorial panels and caricatures, there was another cartoonist for the *Yalie Daily* who had a strip. The strip was "Bull Tales," and the cartoonist was (and is) Garry Trudeau. Garry's "Doonesbury" went on to become one of the most popular and successful comic strips in history. He grew rich, while for most of my life I've been working for the Government on a middle-class salary, with deadlines I have to meet and compromises I have to make.

Playboy and my first research publication

At Yale my friend from childhood days in Newark, David Fink, and I were Intensive Majors in Psychology. “Intensive” meant we had to carry out a research project. We came up with a project idea when we both realized that in our class notes from Richard Nisbett’s course in social psychology we’d both written the same question.



A poster of a Doonesbury character (based on Yale’s President at the time, Kingman Brewster) autographed for me by Garry Trudeau.

The general topic was the experience of emotion. Psychology researchers at Columbia, Stanley Schachter and Jerome Singer, had recently published evidence that the experience of emotion requires two things, cognitions and physiological arousal. Think of an analogy to Einstein’s $E=mC^2$; this would be E=PA * C.

Stewart Valins, a graduate student in Schachter's group, had proposed that to experience an emotion an individual would not need physiological arousal; just the cognition of physiological arousal would suffice. This was in line with the James-Lange theory.

Valins devised a "false heart rate biofeedback" experiment. From projected photos male subjects rated *Playboy* nudes on attractiveness, and the subjects were given false information about their heart rates (in those days there were no ethical qualms about deceiving subjects). They were told that each heartbeat would produce an audible click. Subjects rated the nudes as sexier when the clicks were more rapid. This finding supported Valins's hypothesis that PA is relevant to the experience of emotion when the individual uses the PA as a source of information.

David Fink and I shared "brain waves." In the margins of our lecture notes we asked the same question: "What happened to the real heart rate?"

At that time, Yalies taking introductory psychology were required to be subjects in a psychology experiment. The sign-up sheets were tacked to a bulletin board in the hallway outside the lecture room in Linsly-Chittenden Hall. To publicize our experiment, we displayed a *Playboy* nude, with the sign-up sheet below the photo. It didn't take long before the sign-up sheet was completely filled. Additional sheets that were taped on eventually went all the way down to the floor.

The key to the experiment was the clicks. Fink made a tape recording of clicks that were timed so that for nudes #4 and #7 of a total of 8 the "heart rate" would increase. As a pilot, we tested my Calhoun classmate Jim Stryker. Stryker was a cynical, suspicious sort, but he volunteered to be the guinea pig for the trial run. We hooked him up to an electrocardiographic apparatus and told him that each time his heart beat, he would hear a click, but he should just ignore this artifact. Using a borrowed overhead projector, we displayed nude #1 on the wall. He heard click…click…click. For nude #2 the same thing, click…click…click. For

nude #3 he heard a rapid CLICKCLICKCLICK. Fink and I looked at each other. The fast heart rate was supposed to be for nude #4, not nude #3; but whatever, we continued. For nude #5 there was click…click…click. For nude #6 there was CLICKCLICKCLICK, so we knew we were off by 1 nude. For nude #7 there was click…click…click, and then there was silence. It was the end of the tape recording. Stryker turned around immediately with a shocked, panicked look. He thought his heart had stopped! Fink and I had a great laugh. I don't think Stryker ever forgave us.

The study was a great success. We found that when subjects felt more emotional their actual heart rates changed, regardless of the false heart rate biofeedback. When we graduated, David Fink and I shared Yale's Angier Prize for Research in Psychology.

David Fink went on to Harvard Medical School, post-graduate training in neurology, a distinguished academic career at the University of Pittsburgh, and finally Chair of the Department of Neurology at the University of Michigan.

Our article was published in the *Journal of Personality and Social Psychology* in 1972. Over the years it became a "citation classic," meaning that it has been cited more than 100 times—the first of 147 citation classics as of this writing.

Journal of Personality and Social Psychology
1972, Vol. 21, No. 1, 41–51

COGNITION OF AROUSAL AND ACTUAL AROUSAL AS DETERMINANTS OF EMOTION[1]

DAVID GOLDSTEIN, DAVID FINK, AND DAVID R. METTEE[2]

Yale University

Valins' false heart rate procedure was replicated in the first part of the present study. Results showed that increases in false heart rate feedback (cognition of physiological arousal) led to increases in reported emotionality. (Nudes for which "heart rate" increased were rated higher than nudes for which heart rate remained constant.) In addition, while subjects were being exposed to the nudes, their actual heart rates were determined. It was found that cognition of physiological arousal led to changes in actual physiological arousal (change in heart rate), but reported emotionality was not consistently related to those changes. In the second part of the study, an attempt was made to experimentally vary the level of emotion, in particular, the feeling of being offended, by exposing some subjects to male nudes (offensive condition). These subjects reported that they were more offended by the experiment than did subjects exposed only to female nudes. However, in the offensive condition, speedups in false heart rate feedback did *not* produce any systematic effects either on actual physiological arousal or on ratings of emotionality (in this case, offensiveness), but, there *was* a significant relationship between actual physiological arousal (heart rate change) and reported emotionality. The implication of these results is that physiological arousal apparently functions as more than a mere cognitive cue to be interpreted by the individual along with many other cognitive cues. The present data suggest that actual physiological arousal was a crucial mediator of emotion, since, regardless of a subject's artificially provided cognition of physiological arousal, he seemingly did not experience much emotion unless there was actual physiological arousal.

Abstract of my first published report of original research related to the autonomic nervous system, more than a half century ago. The topic was whether physiological arousal is a crucial mediator of emotion, so that cognition of physiological arousal alone is insufficient. "Physiological arousal" here referred to heart rate.

"You're all on the track, and the race goes to the swiftest."

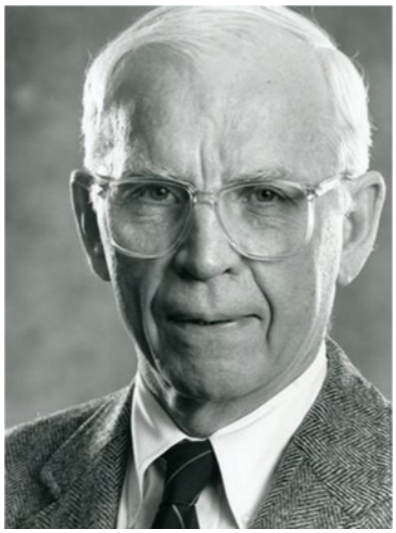
When David E. Rogers, Dean of the Johns Hopkins University School of Medicine, delivered his welcoming address to the incoming medical students in the Class of 1974, he mentioned that as the premier medical school in the country Hopkins could be extremely selective in their admissions decisions. Everyone in the class had something extra, something special, to offer that made that person stand out. For instance, among the class was a cartoonist. That was me.

Cartoons from "A Guide to Baltimore and the Johns Hopkins Medical Institutions." The textbook title is, "The Basic Basic Fundamental Essentials of Introductory Medicine."

Our textbook was Harvey's *Principles and Practice of Medicine.* All the chapters were written by Hopkins faculty. It was a massive tome, just like the competing Harrison's *Principles of Internal Medicine,* used at the other premier medical school, Harvard, where David Fink was a medical student. In the pamphlet, *A Guide to Baltimore and the Johns Hopkins Medical Institutions,* I drew a cartoon of a medical student asleep in a lounge chair, spread-eagled with a huge textbook opened on his lap. The title of the book was *Basic Basic Fundamental Essentials of Introductory Medicine.*

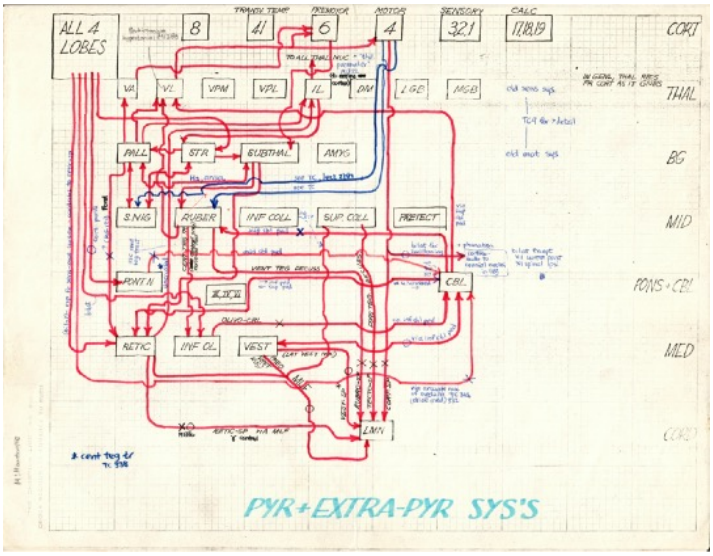
The coursework was grueling, but the faculty were superstars—A. McGehee Harvey in medicine, Albert Lehninger in biochemistry, Barry Wood in infectious diseases, Victor McKusick in human genetics, Daniel Nathans in basic genetics, Owsei Temkin in the history of medicine, Grover Hutchins in pathology, Sol Snyder in psychopharmacology, and Vernon Mountcastle in physiology. I had the idea of asking faculty to distribute their

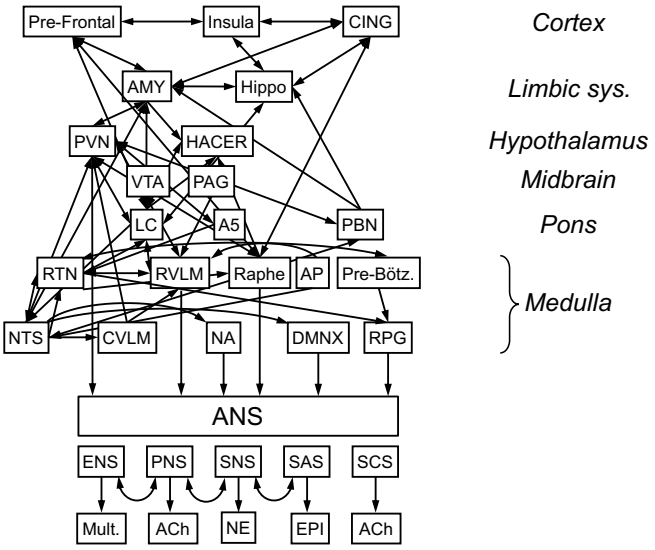
lecture notes, with additions such as concept diagrams and edits to take into account omissions or errors or student queries. This would be beneficial for learning and review. I approached Vernon Mountcastle, Chair of the Physiology Department, with my suggestion. I remember Mountcastle less for his research accomplishments, which were substantial (he shared a Lasker Prize in 1983 for discovering sensory columns in the cerebral cortex) than for his tight-lipped cold-bloodedness. Mountcastle dismissed the proposal on the grounds that it is irrational to help competitors. He stated, “You’re all on the track, and the race goes to the swiftest.”



Professor Vernon Mountcastle, Chair of the Department of Physiology at Johns Hopkins when I was a medical student. Mountcastle succeeded Philip Bard, of the Cannon-Bard theory of emotion, discussed later. By the time I was a medical student, Mountcastle’s Medical physiology textbook was in its twelfth edition.

Neuroanatomy was especially difficult because of its extreme complexity. With my drawing skills I diagrammed the centers and pathways in networks. This helped organize the subject matter not only for me but also for my classmates. I still have a knack for drawing concept diagrams.





Diagrams of (left) neuroanatomic pathways of the pyramidal and extra-pyramidal systems, created in 1971, and (right) neuroanatomic pathways of the central autonomic network, created a half century later, in 2021.

The Oxford Technique

William Milnor was a grandfatherly Professor who introduced the class to cardiovascular physiology (he wrote a single-authored textbook with that title). In early 1971 he announced an opportunity to spend the summer at Oxford in England as a Fellow in cardiovascular physiology. I applied and was accepted. That summer I took up residence in St. Catherine's College under the tutelage of Dr. Derek Bergel. Bergel was an amiable, pipe-smoking, basic physiologist who was an authority on mechano-transduction of baroreceptors. Somehow these specialized cells could turn being distorted into an electrical neural signal transmitted to the brain, arousing a reflex, the baroreflex, that would potentially decrease blood pressure and blood flow to the brain.

I don't think I visited the Bergel lab once during that summer. Instead, I spent hours alone in the stacks of the venerable Bodleian Library. I meticulously entered color coded notes about one article after another. I also tried to understand McDonald's *Blood Flow in Arteries.*

During that summer I learned about the “Oxford technique.” In 1969, Harley Smyth, Peter Sleight, and George Pickering of Oxford’s Radcliffe Infirmary had published a method for assessing baroreflex sensitivity. With an arterial catheter they monitored blood pressure continuously, and they injected drugs that constrict or relax blood vessels throughout the body without directly affecting the heart rate. Vasoconstriction would increase blood pressure, and via the baroreflex heart rate would fall. The extent of decrease in heart rate (they actually used the cardiac interbeat interval) for a given increase in blood pressure would be a measure of baroreflex sensitivity.

When I returned to Hopkins, I was prepared to test baroreflex sensitivity in the baboons on the 6th floor of the Traylor Building, the domain of Colonel Joseph V. Brady. Brady was a founding father of behavioral biology.

Recall the sleeper hold from when I watched professional wrestling with Grandma Bessie in the attic. The carotid sinus is a specialized structure at the crotch where the common carotid artery splits into the external carotid artery (delivering blood to the face and scalp) and the internal carotid artery (delivering blood to the brain.) The carotid sinus contains distortion receptors called baroreceptors (meaning pressure receptors). One of the peculiarities of the human body is that it has no way to track blood flow to the brain. Instead, when the carotid sinus is stretched, information from the distortion receptors travels to the brainstem and elicits a reflex that changes autonomic nervous outflows in ways that relax blood vessels and decrease the heart rate. Massaging the carotid sinus region stimulates the baroreceptors. The combination of vascular relaxation and slowed heart rate decreases the blood pressure. If the blood pressure falls to a low enough level consciousness cannot be maintained.

An offer I couldn’t refuse

The Johns Hopkins School of Medicine and University inaugurated a 6-year MD-PhD Program in Behavioral Science in 1970. In order to generate product as quickly as possible, our class of medical students were

recruited to join. Hopkins made me an offer I couldn't refuse. My medical school tuition would be waived, and I would receive a stipend. I joined the MD-PhD Program. The stipend enabled my wife Minka and me to buy our first house, a row house on North Calvert Street a short walk from the Johns Hopkins Homewood Campus.

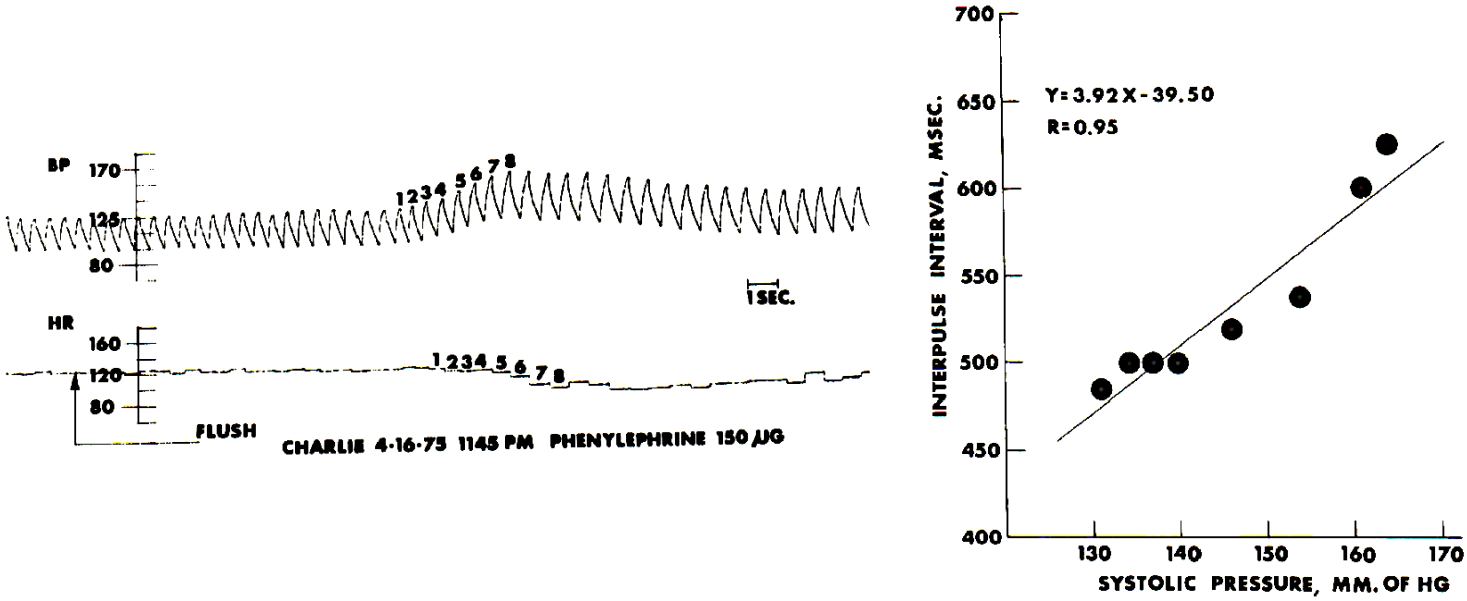
Joseph V. Brady and Richard S. Ross. These respective giants of behavioral biology and cardiology supervised my PhD research. Ross volunteered to be a subject in my project on biofeedback heart rate training.

A baboon introduction to allostasis

Among many of Joe Brady's projects one headed by Alan Harris was about using cardiovascular biofeedback to create a baboon model of neurogenic hypertension. I rarely saw the baboons, as they were kept inside large wooden boxes in the lab. At 12 noon each day a light would go on in the box. A white light meant that all was well, and a pellet treat would be delivered if the animal stayed "in white" for long enough. Red meant that an electric shock would be coming. The trials last 12 hours, each day,

every day. Using a psychological technique called shaping Harris trained the animals to raise their blood pressure just enough to keep the white light on.

It was amazing to me that an experimenter could literally dial a particular blood pressure, and the animals would reach and maintain exactly that pressure, for days, weeks, even years.



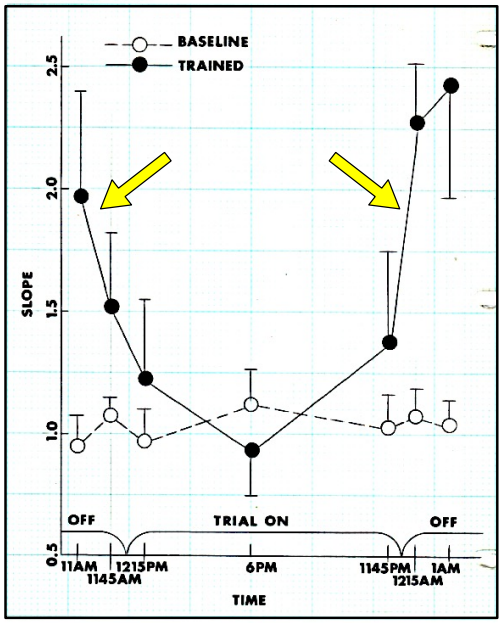
Baroreflex slope in a baboon, calculated using the Oxford technique, which I had just learned at Oxford in 1971.

Harris and Brady thought they had a model of neurogenic hypertension. They didn't. Each night at midnight the lights in the box would be turned off, and the blood pressure would rapidly return to the baseline level. There was little evidence of a chronic hypertensive state developing, despite what would seem to be prolonged, repeated episodes of distress. On the other hand, this was a tremendous model to study mechanisms of blood pressure regulation. How did the baboons raise their blood pressure? My job was to find out. As a lowly medical student, I wasn't allowed to interfere with the animals' performance in any way. There was an arterial catheter coming out of a hole in the box, connected to a polygraph for

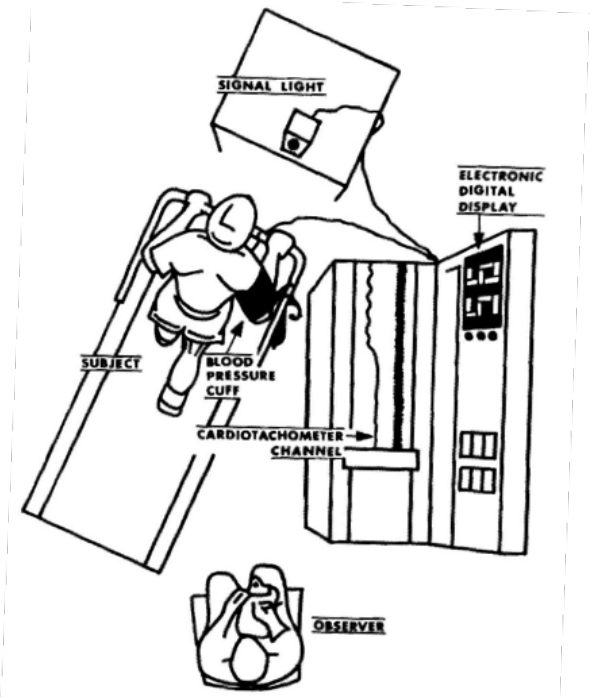
recording the blood pressure and heart rate. There was a venous catheter entering another hole in the box, by which drugs could be injected. That was sufficient for me to check the sensitivity of the baroreflex using the Oxford technique. I could bolus inject phenylephrine, which rapidly produces vasoconstriction and raises blood pressure, and measure the slope of the relationship between the interbeat interval and blood pressure for each heartbeat; and bolus inject nitroglycerine, which evokes the opposite cardiovascular effects.

The baboons all showed the same pattern of baroreflex slope before, during, and after the 12-hour trials. Concurrently with—actually preceding—the increase in blood pressure, baroreflex sensitivity would fall. The minimum sensitivity was at the 6 PM observation point. By 11:45 PM the baroreflex sensitivity was increasing, and at 12:15 AM and 1 AM the sensitivity was back to baseline, just as was the blood pressure. This meant that the gain of the baroreflex could change rapidly and even in advance of the anticipated need. The animals could react to a stimulus (in this case the light in the box) before they were exposed to it. This might seem to throw on its head the expected temporal sequence of stimulus followed by response. Actually, it's easy to understand by classical (or Pavlovian) conditioning. The baboons had learned associations between daily events, such as the caretaker Willie inspecting them in the morning each day, with the lights coming on, and their conditioned responses included dropping baroreflex gain. I appreciated much later that this anticipatory response, temporarily altering the stimulus-response relationship, is an example of "allostasis."

My dissertation title was "Instrumental Cardiovascular Conditioning." In addition to studying Joe Brady's baboons I studied human subjects, including Richard S. Ross, then the head of the cardiology division and later Dean of the Medical School. I found that with biofeedback humans can be trained to decrease their heart rates during repeated bouts of exercise. Drs. Brady and Ross were my dissertation committee.



Graph showing baroreflex slope as a function of time in baboons before and after operant blood pressure conditioning with trials from 12 noon to 12 midnight. Note the changes in slope in anticipation of the beginning and ending of the trial (yellow arrows)—an example of allostasis.



Sketch of the experimental setup for my study, "Biofeedback heart rate training during exercise." The publication is a citation classic.

The WAMI alternative

For internal medicine residency I matched with the University of Washington Affiliated Hospitals. The UW system included several different types of hospitals (university, private, VA, county shock-trauma) all located near Interstate 5, which bisects the city from north to south. House staff could live anywhere along I-5, as opposed to the situation in the northeastern USA, where house staff had to live in slums or in barricaded enclaves near the teaching hospitals.

Robert G. Petersdorf, Chair of the Department of Medicine, instituted a program of clinic-based as opposed to hospital-based experiences called the “WAMI” program (Washington, Alaska, Montana, Idaho), to meet regional needs for general internists especially in rural areas via a decentralized medical educational system. Junior residents had to spend 2 months in a clinic. I picked a small private cardiology clinic that was across the street from a private hospital in downtown Seattle.

Early in the externship I was rounding with one of the cardiologists in the intensive care unit of the hospital. A patient with a stroke had been admitted who had a bizarre EKG and elevated cardiac-specific enzymes. In those days it was a diagnostic challenge distinguishing a heart attack causing a stroke from a stroke causing a heart attack. It was well known that stroke patients could have abnormal EKGs. I asked the attending about which EKG changes actually are new at the time of a stroke, as opposed to being there before the stroke. He didn’t know, and he suggested I find out. Soon afterward I carried out a case control study, reviewing medical records from 150 acute stroke patients who had an EKG on file before their stroke and 150 patients admitted for a different acute problem who had an EKG on file before their admission. While I was supposed to be learning outpatient medicine in a cardiology clinic, I actually spent most of my time in medical records and in the library. The result was a single-authored publication in *Stroke* in 1979.

Just like the report based on my study at Yale, this article went on to become a citation classic. It must be highly unusual for an individual to

have first- or single-authored publications based on original research carried out as a college student and then as a medical resident.

The Electrocardiogram in Stroke: Relationship to Pathophysiological Type and Comparison with Prior Tracings

DAVID S. GOLDSTEIN, M.D., PH.D.

SUMMARY The author reviewed electrocardiographic records of 150 patients with acute stroke and 150 age- and sex-matched controls, to assess the relative frequencies of ECG abnormalities among the pathophysiologic categories of stroke, and to distinguish new abnormalities at the time of the stroke from those noted on prior tracings. Of the 150 patients with stroke, 138 (92%) showed ECG abnormalities. The most common abnormalities were also changes from prior tracings: QT prolongation (68 patients, 45%), ischemic changes (59, 35%), U waves (42, 28%), tachycardia (42, 28%), and arrhythmias (41, 27%). Patients with cerebral embolus had a significantly increased frequency of atrial fibrillation (9 patients, 47%); and with subarachnoid hemorrhage an increased frequency of QT prolongation (20, 71%) and sinus arrhythmia (5, 18%). The frequencies of QT prolongation and ischemic changes related strongly to admission systolic pressure but not to mortality. Stroke patients had an increased frequency of pathologic Q waves (30 patients, 20%) and left ventricular hypertrophy (39, 26%), but these were not new findings at the time of the stroke.

The results are consistent with an interaction of underlying hypertensive or atherosclerotic cardiovascular disease, sympathetic hyperactivity, and possibly myocardial necrosis, in producing ECG changes.

Stroke Vol 10, No 3, 1979

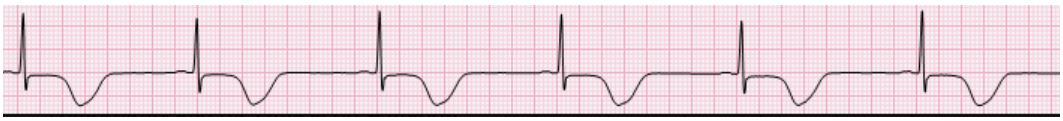


TABLE 1 *ECG Findings in 150 Acute Stroke Patients and 150 Age- and Sex-Matched Controls*

Finding	Cerebral thrombosis	Stroke, indeterminate etiology	Subarachnoid hemorrhage	Cerebral embolus	Intracerebral hemorrhage	Stroke, overall	Controls
Normal	7 (14%)	4 (11%)	0 (0%)	0 (0%)	1 (6%)	12 (8%)	53 (35%)
Abnormal	42 (86%)	34 (89%)	28 (100%)	19 (100%)	15 (94%)	138 (92%)	97 (65%)†††
Prolonged QT	18 (37%)	15 (39%)	20 (71%)**	7 (37%)	8 (50%)	68 (45%)	18 (12%)†††
T wave inversion	12 (24%)	14 (37%)	7 (25%)	4 (21%)	6 (38%)	43 (29%)	32 (21%)
U waves	13 (27%)	9 (24%)	9 (32%)	4 (21%)	7 (44%)	42 (28%)	14 (9%)†††
Increased HR	7 (14%)	10 (26%)	10 (36%)	8 (42%)	7 (44%)	42 (28%)	12 (8%)†††
ST depression	12 (24%)	12 (32%)	8 (11%)	5 (26%)	4 (25%)	41 (27%)	15 (10%)†††
LVH	12 (24%)	10 (26%)	7 (25%)	3 (16%)	7 (44%)	39 (26%)	18 (12%)††
Q waves	11 (22%)	6 (16%)	6 (21%)	4 (21%)	3 (19%)	30 (20%)	14 (9%)††
Atrial fib.	0 (0%)	8 (21%)	3 (11%)	9 (47%)***	1 (6%)	21 (14%)	6 (4%)††

My case-control study on EKG changes associated with stroke was conducted when I was a Junior Resident in Internal Medicine at the Univ. of Washington. I was supposed to be learning outpatient cardiology in a private clinic.

Building 10 and Nature's secret mysteries

David Fink was at the National Institutes of Health (NIH) in Bethesda, Maryland when he suggested I apply for a Clinical Associate position in the National Heart, Lung, and Blood Institute (NHLBI). Most of NIH is grants administration, but a small part of NIH-sponsored research is done intramurally at the NIH, especially at the NIH Clinical Center, Building 10.

Fink thought intramural NIH would be a good place for me. He was right. I applied after the deadline had passed, but there was an unexpected opening, and I was accepted. I began at the NIH in July, 1978.

Building 10, with the Hatfield Clinical Research Center added in front of it, is the largest research hospital on earth. Titans of academic medicine have passed through Building 10 during their training. I came here fresh from internal medicine residency. I've been working in Building 10 ever since. The yellow arrow points to where I sit.

Building 10, the NIH Clinical Center. I first worked in Building 10 as a Clinical Associate in the National Heart, Lung, and Blood Institute (NHLBI). The arrow shows where my office/lab is currently.

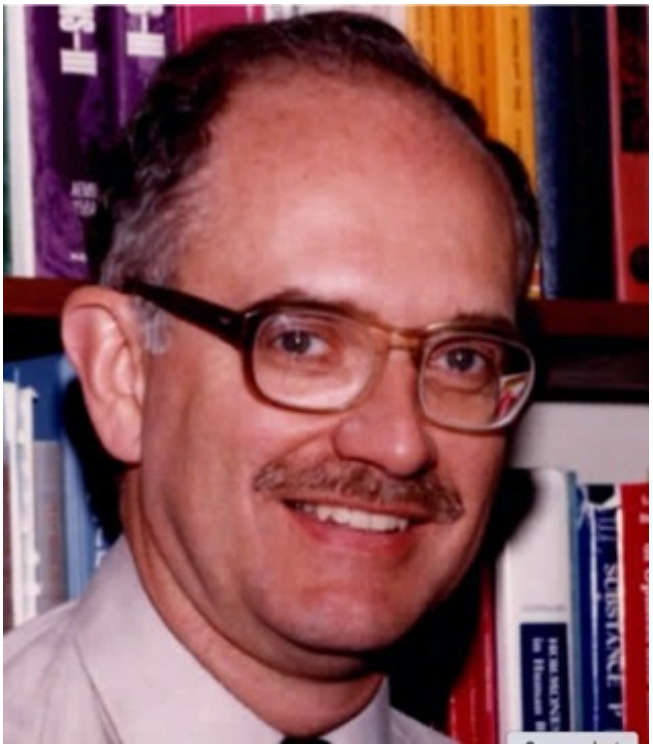
In Building 10 I've been privileged to develop several clinical autonomic tests and apply them for the first time—especially in patients with uncommon or unique conditions. The combination of new technology and patients with rare diseases sets the stage for discoveries and based on those discoveries insights that induce new concepts—exactly in line with William Harvey's statement about nature's secret mysteries.

As Clinical Associate I was a "resi-tern," rotating in 2-month periods among the inpatient programs of Branches of the NHLBI. This experience counted toward my third year of medical residency, and I took the Board exam in internal medicine in Bethesda, at what was then the Bethesda Naval Hospital, across the street from the NIH campus. During the year I was expected to choose a Branch in which I'd work as a Fellow. I interviewed with Stephen Epstein, Chief of the Cardiology Branch, Art Nienhuis, Chief of the Hematology Branch, Ron Crystal, Chief of the Pulmonary Branch, Bryan Brewer, Chief of the Molecular Disease Branch, and Harry Keiser, Chief of the Hypertension-Endocrine Branch. I chose Harry Keiser.

CLINICAL CATECHOLAMINE NEUROCHEMISTRY

“You have to measure something.”

Harry Keiser was a paradigm of reasonableness and fairness. He gave me free rein to conduct my research independently. This stood out from the other Branches, where I’d have to apprentice by helping more senior investigators in their own projects. Mainly I feel thankful to Harry for his giving me a piece of advice that put me on a sure path to success at the NIH.



Harry R. Keiser was my supervisor when I came to the NIH as a Clinical Associate in 1978. At that time, he was the Chief of the Hypertension-Endocrine Branch and Clinical Director of the National Heart, Lung, and Blood Institute.

After listening attentively as I described my interest in whether hypertensives are “hyper-tense,” with an overactive stress response or repressed aggression or a hypertensive personality, he interrupted my monologue and asked, “What are you going to measure?” Science isn’t

only about ideas. It's about measurements that test ideas or inspire ideas more than the ideas themselves. Actually, people care little about your ideas, but if you can measure something that nobody else can measure, or you are skilled in carrying out a procedure that no one else can carry out, then the world will beat a path to your door, and the ideas that others have in applying your technology may be even better than yours. Harry opined, correctly, that the NIH is an assay- and procedure-intoxicated place.

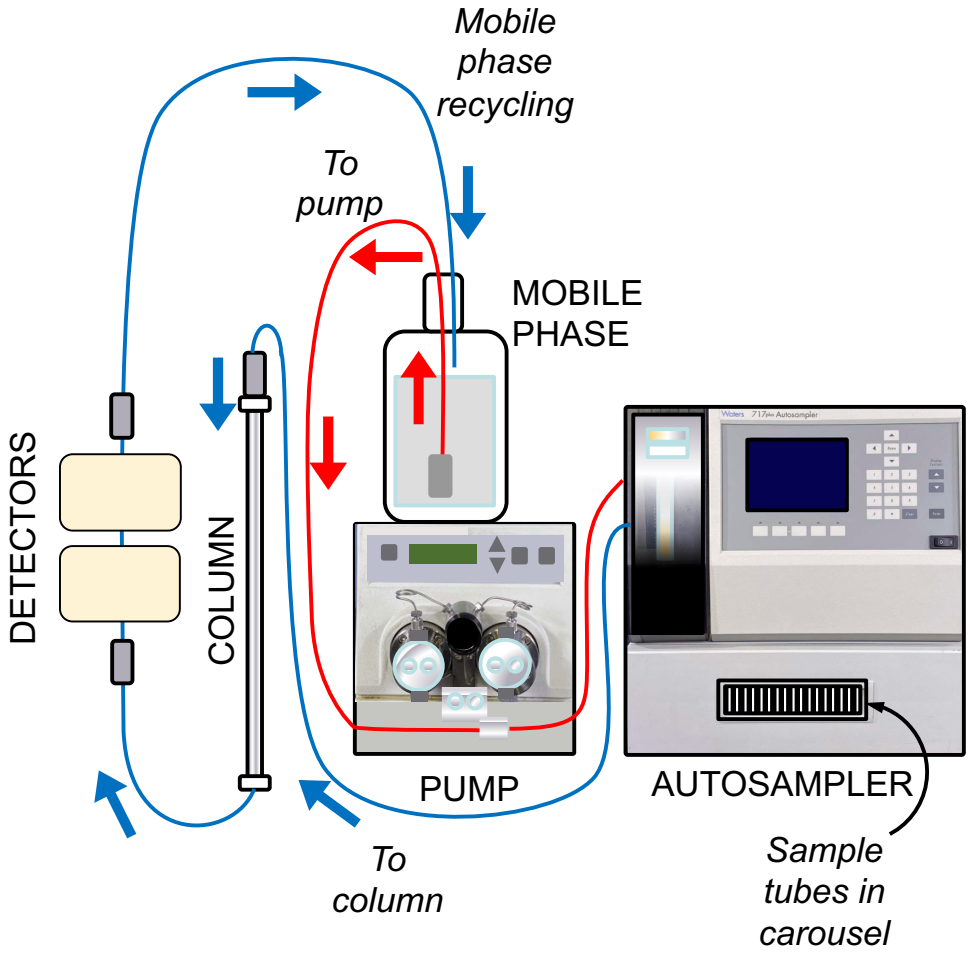
Keiser was an expert on a class of chemicals called kinins that produce effects on blood vessels (vasoactive compounds). I could measure bradykinin, or activity of the renin-angiotensin-aldosterone system, or indices of activation of the hypothalamic-pituitary-adrenocortical system, or vasopressin, or vasoactive factors produced inside blood vessel walls, or the catecholamines norepinephrine, epinephrine, and dopamine; but I'd have to measure something. I asked about what was already being measured in the lab. One was levels of catecholamines, by a Fellow, Joe Izzo, using a technology called a radioenzymatic assay. Joe was going to be departing for a position at the University of Buffalo, and he'd be leaving behind his technician, Marian Warner. I would just have to keep track of quality control, results reporting, data analysis, that sort of thing. I told Harry I'd take charge of measuring catecholamines by the radioenzymatic assay.

The assay didn't work, at least in the lab of the Hypertension-Endocrine Branch. After several frustrating months, Marian Warner left. So, there I was. I consulted my old friend, David Fink. David asked if I'd tried HPLC as an alternative to the radioenzymatic assay. I didn't know what the abbreviation stood for.

HPLC stands for high pressure liquid chromatography. A stainless steel tube (a column) is filled with a powder of tiny silica beads. The beads have attached to them molecules with particular properties. A solution (mobile phase) is pumped through the column at high pressure. High pressure is needed, because the silica beads are so compacted that it takes high pressure to get the mobile phase through the column. When injected onto the column, different biochemicals such as catecholamines

stick for characteristic times (retention times) before coming out the other end of the column.

In retrospect, this was analogous to the paper electrophoresis I did during the Summer Session at Andover in 1964.

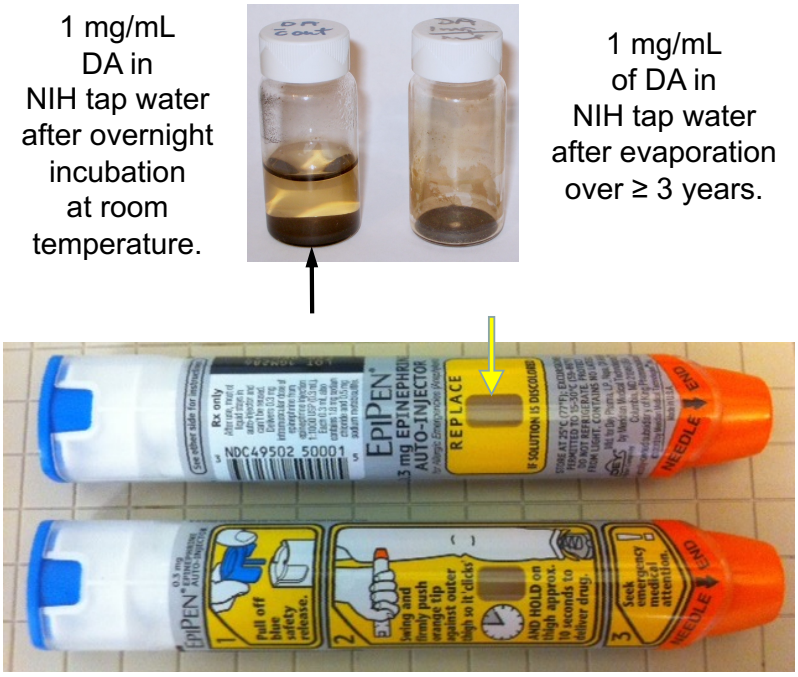


In a typical HPLC setup, mobile phase is pumped through a refrigerated automated injector (autosampler), which injects samples into the flow stream pumped through the chromatographic column. The column separates the analytes, which have particular retention times. The detector electrodes quantify the amounts of analytes. The mobile phase is recycled.

The effluent from the column was passed through an electrode set at a low oxidation potential. Any catecholamine would be oxidized, producing a current, and the greater the current, the larger the amount of

catecholamine. This would be analogous to the ninhydrin spray I had used at Andover to quantify proteins in the paper electrophoresis experiment.

The combination of HPLC (later called simply liquid chromatography, or LC) with electrochemical detection (ED) was a powerful way to separate and quantify levels of catecholamines.

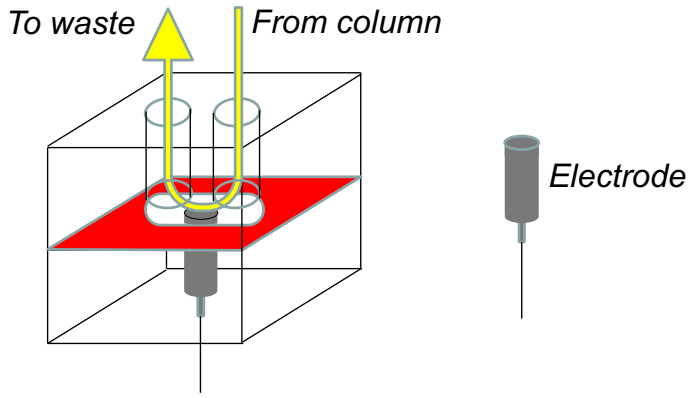


Catecholamines are highly susceptible to oxidation. Dopamine dissolved in tap water auto-oxidizes to a black powder (melanin). Oxidation of epinephrine to a tan solution in an EpiPen™ is the basis for the indicator window (yellow arrow) showing that the device should be replaced.

Setting up the LC-ED method took 6 long, frustrating months. The key problem was background noise. When I called up Bioanalytical Systems (BAS), the manufacturer of the electrochemical detector, about this, they claimed the problem was radiofrequency interference. What city was I in?

I replied that I was in Bethesda, Maryland, a suburb of Washington, DC. They said there couldn't be a worse city for radiofrequency interference. But I'm pretty resourceful. On the 13th floor of the NIH Clinical Center the National Eye Institute had a walk-in Faraday cage for

electroretinography, and it was completely isolated electrically. I recall that for a ground it had a braided copper cable that literally went from the 13th floor to the ground. I brought the Waters HPLC system with BAS electrochemical detector and strip chart recorder on a cart up to the walk-in Faraday cage and got in. I could see the same background noise on the strip chart recorder. So, it wasn't the city I was in. Pat Pastore, the Waters rep, rummaged through her contacts book and found that there was a lab at Johns Hopkins in Baltimore that had the same HPLC-electrochemical setup and that the system was working. Maybe that lab would be willing to help. I don't recall the person who kindly let us test our system in his lab. I arranged a US Government station wagon from the motor pool.

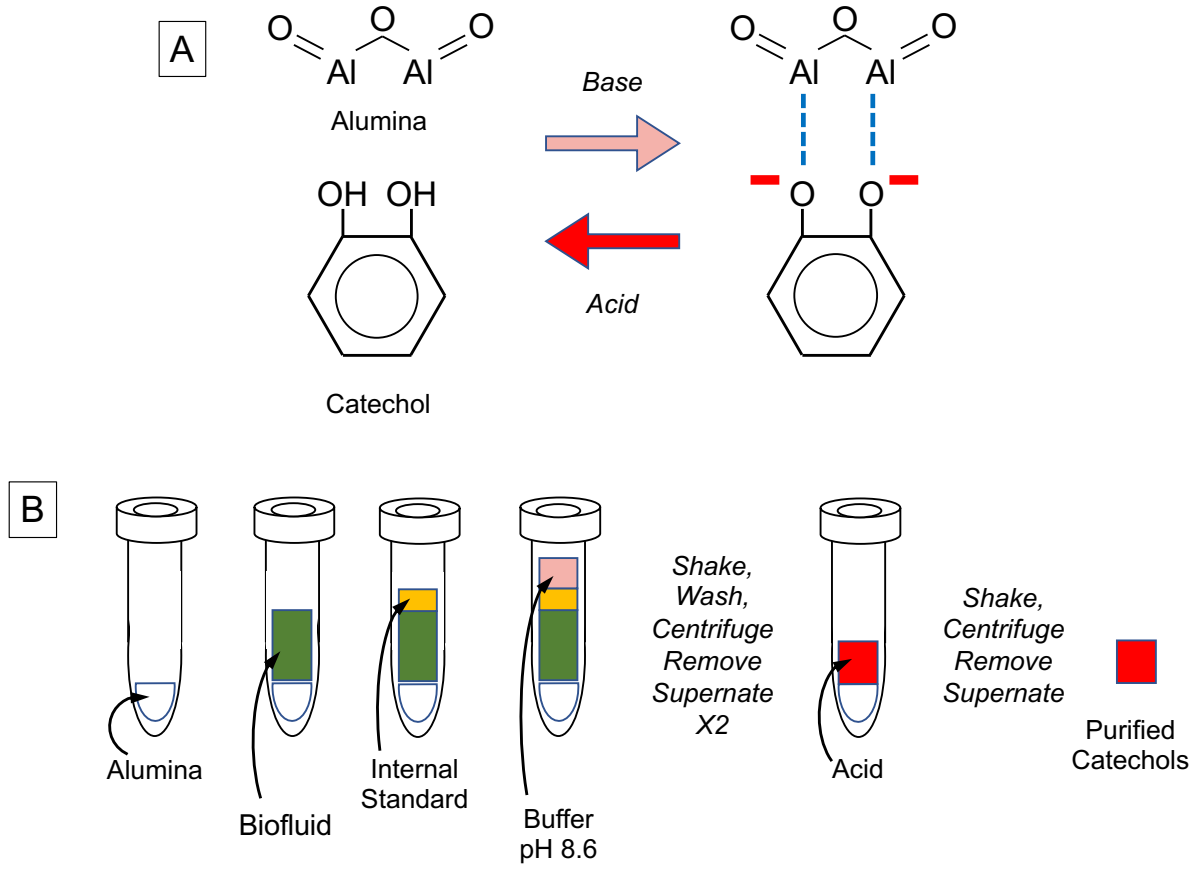


Electrochemical detection is based on exposure of the fluid exiting the HPLC column to an oxidizing potential.

Our technician, Rod Turner, and I loaded the HPLC and detector system in the back and drove to Baltimore. We double parked outside the Traylor Building (the same building that had housed Brady's baboons). Rod stayed in the car while I brought up individual pieces of equipment and plugged each into the functioning system, which did have good quality chromatographic recordings with low background noise. When I switched our thin-layer flow cell electrode for theirs, instantly there was the background noise we'd seen in Bethesda. So, the problem was the thin layer flow cell. I called the company to ask if they'd do a QC check on their electrodes before sending them to us, but they refused. I ended up buying a bunch of electrodes, testing each one and using the electrodes that gave the best signal-to-noise ratio.

Magic powder

To assay catecholamines in biofluids like plasma requires a sample cleanup step to partially purify the catecholamines. The plasma epinephrine level in a healthy person at rest can be as low as a few picograms per milliliter (pg/mL), which is to say a millionth of a millionth of a gram per mL. The human mind cannot comprehend what a picogram is. Plasma contains many thousands of chemicals at higher concentrations than epinephrine. Directly injecting plasma into an LC-ED system wouldn't work. The sample would have to be cleaned up first to get rid of the much more abundant non-catecholamine compounds.

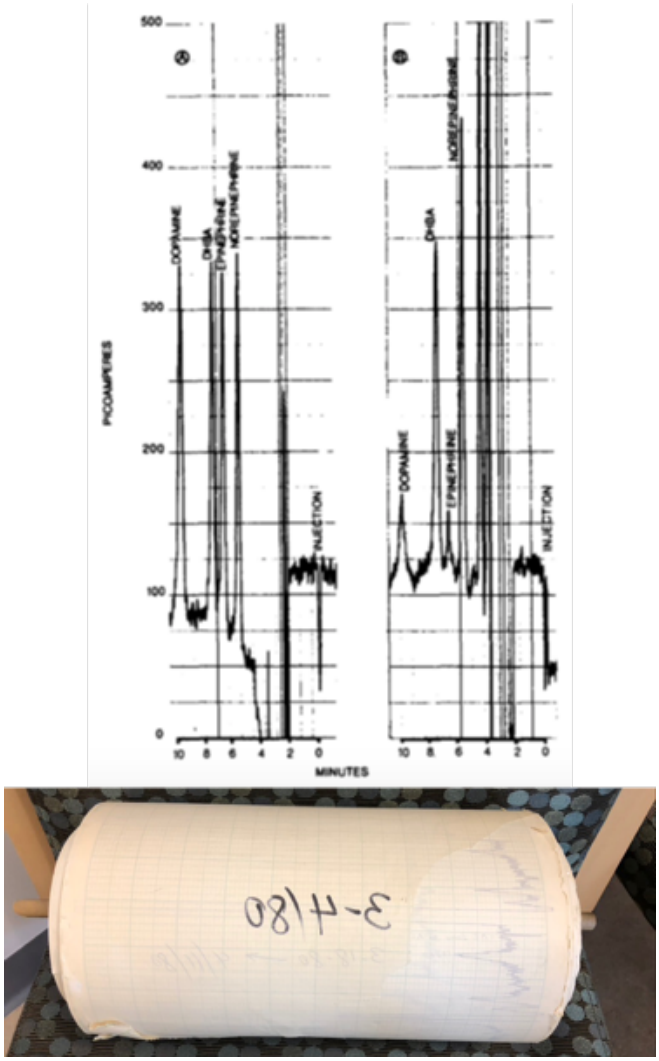


Principle of batch alumina extraction for partially purifying catechols. (A) Catechols have adjacent hydroxyl groups on a benzene ring. (B) The catechols are attracted to the aluminum atoms in the alumina under basic conditions and desorb from the alumina under acidic conditions.

To remove non-catecholamine chemicals from the plasma I used batch alumina extraction. Alumina, or aluminum oxide, is one of the most abundant chemicals in the earth's crust. Alumina powder is basically a type of crushed rock. For partially purifying catecholamines, though, it's a magic powder. The chemical formula is Al_2O_3, meaning that the molecule has 2 aluminum atoms, which tend to have a positive charge. Catecholamine molecules contain 2 adjacent hydroxyl groups on a benzene ring (a catechol nucleus). It happens that the 2 aluminum atoms in the alumina molecule line up with the 2 hydroxyl groups on the benzene ring of the catecholamine molecule. Under basic conditions catecholamines stick (adsorb) to the alumina, and under acidic conditions the catecholamines come off (desorb) from the alumina.

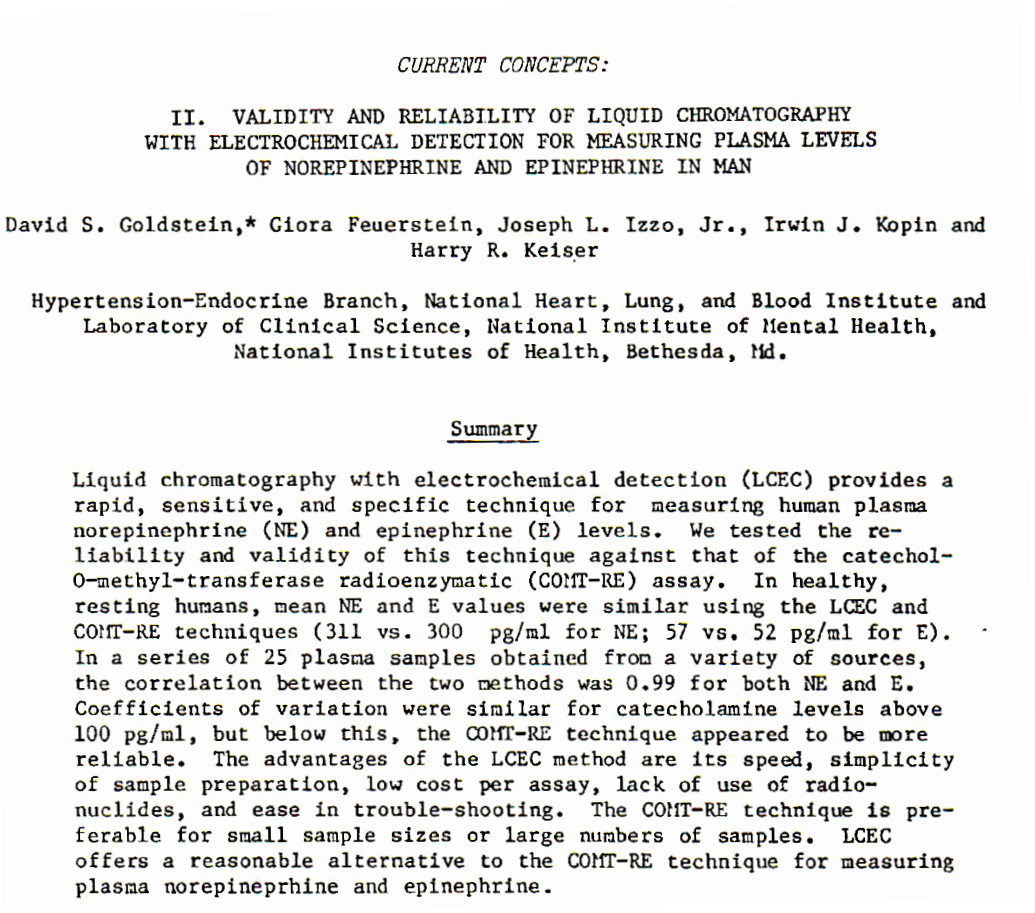
To assay the catecholamines in human plasma, I would add alumina powder to the sample, raise the pH with a TRIS/EDTA buffer, shake the sample vigorously so that the catecholamines had a chance to adsorb to the alumina, spin down the sample in a centrifuge (since alumina powder is crushed rock this is quick), remove the supernatant, and replace it with a wash solution, centrifuge again, and do this again until the alumina, with the catecholamines adsorbed, was washed. Then, after removing the supernatant a last time, I'd add acid to the alumina and shake the sample to get the catecholamines to desorb from the alumina. The supernatant, now containing the partially purified catecholamines, could be transferred to microvials and injected into the HPLC system.

For a few years I recorded the signals from the electrochemical detector on a strip chart recorder, so there was a paper record. I rolled the paper onto a wooden spindle, and the paper eventually accumulated into scrolls.



(Top) LC-ED chromatographs of (left) catecholamine standards and the internal standard (DHBA) and (right) catecholamines in a human plasma sample. (Bottom) A scroll of chromatographs recorded on a strip chart recorder (time period March 18-April 11, 1980).

To validate the LC-ED method I collaborated with Giora Feuerstein, who was measuring catecholamines successfully using the radioenzymatic assay in the Laboratory of Clinical Science, directed by Irwin J. (“Irv”) Kopin in the National Institute of Mental Health (NIMH). Ours was the first validated LC-ED method for measuring plasma levels of catecholamines in humans. The article, published in *Life Sciences* in 1981, is a citation classic.



CURRENT CONCEPTS:

II. VALIDITY AND RELIABILITY OF LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION FOR MEASURING PLASMA LEVELS OF NOREPINEPHRINE AND EPINEPHRINE IN MAN

David S. Goldstein,* Giora Feuerstein, Joseph L. Izzo, Jr., Irwin J. Kopin and Harry R. Keiser

Hypertension-Endocrine Branch, National Heart, Lung, and Blood Institute and Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, Md.

Summary

Liquid chromatography with electrochemical detection (LCEC) provides a rapid, sensitive, and specific technique for measuring human plasma norepinephrine (NE) and epinephrine (E) levels. We tested the reliability and validity of this technique against that of the catechol-O-methyl-transferase radioenzymatic (COMT-RE) assay. In healthy, resting humans, mean NE and E values were similar using the LCEC and COMT-RE techniques (311 vs. 300 pg/ml for NE; 57 vs. 52 pg/ml for E). In a series of 25 plasma samples obtained from a variety of sources, the correlation between the two methods was 0.99 for both NE and E. Coefficients of variation were similar for catecholamine levels above 100 pg/ml, but below this, the COMT-RE technique appeared to be more reliable. The advantages of the LCEC method are its speed, simplicity of sample preparation, low cost per assay, lack of use of radionuclides, and ease in trouble-shooting. The COMT-RE technique is preferable for small sample sizes or large numbers of samples. LCEC offers a reasonable alternative to the COMT-RE technique for measuring plasma norepineprhine and epinephrine.

Cover page from the first report validating liquid chromatography with electrochemical detection for measuring plasma levels of norepinephrine and epinephrine in humans.

Validation of the LC-ED method for plasma catecholamines was a foundation of clinical catecholamine neurochemistry. I consider pioneering clinical catecholamine neurochemistry to be one of my main contributions to science.

Are hypertensives hypertense?

One of my first applications of the LC-ED method was in testing whether plasma norepinephrine (NE) levels indicate activity of the sympathetic nervous system and are increased in patients with essential hypertension. “Essential” refers to high blood pressure without an identified cause. NE is the main chemical messenger of the sympathetic nervous system in cardiovascular regulation.

While developing the LC-ED method I reviewed the available literature about plasma NE levels in essential hypertension. The literature was already substantial but remarkably inconsistent. Only about 40% of the studies were positive (reporting statistically significant hypertensive-normotensive differences). Typical studies compared age-matched groups of patients with control subjects. Although there was dramatic variability in catecholamine values within and across studies, virtually all studies of plasma NE in young hypertensive patients (mean age less than 40 years old) were positive.

From the results of these reviews I proposed that increased sympathetic nervous activity might play a pathophysiologic role early in the development of the disease. The articles went on to become citation classics. By now the concept of a role of sympathetic hyperactivity in early essential hypertension has been amply supported.

Eventually, in collaboration with several researchers at the NIH and across the street at the new Uniformed Services University of the Health Sciences we accumulated enough original data to test the hypothesis that the difference between hypertensive and normotensive groups depend on age. The report of our observational data also became a citation classic.

In this period we also reported results from our collaboration on plasma NE and epinephrine (EPI) levels in essential hypertension and plasma catecholamines in patients with secondary forms of hypertension.

In all these studies there was a large amount of scatter in the data in the hypertensive and normotensive groups, probably meaning there are many pathophysiological mechanisms underlying essential hypertension. This led us to ask whether there is a way to identify the subgroup of hypertensives in whom there is an augmented sympathetic nervous contribution to the high blood pressure.

Plasma Norepinephrine in Essential Hypertension

A Study of the Studies

DAVID S. GOLDSTEIN, M.D., PH.D.

SUMMARY Of 32 studies comparing plasma norepinephrine concentrations in hypertensive and normotensive groups, 28 (88%) reported higher levels in the hypertensive group. However, only 13 (41%) of the studies reported *statistically significant* hypertensive-normotensive differences in norepinephrine, leading the present attempt to identify factors differentiating "positive" studies (those reporting significant hypertensive-normotensive differences) from "negative" studies (those reporting nonsignificant differences). Hypertensive norepinephrine levels were similar in positive and negative studies (281 vs 288 pg/ml), but normotensive levels were lower in the positive studies (177 vs 269 pg/ml). When compared with the fluorimetric technique, the radioenzymatic type of assay was associated both with a lower frequency of positive results (25% vs 100%) and greater intrastudy standard deviations (152 vs 72 pg/ml). Hypertensive-normotensive differences varied inversely with age ($r = -0.37$). Resolution of the persisting controversy about norepinephrine levels in essential hypertension will require more attention to the causes of variability associated with the assay technique, to the sources, characteristics, and treatment of the normotensive controls, and to the age of the patient population. (Hypertension 3: 48–52, 1981)

Plasma Catecholamines and Essential Hypertension

An Analytical Review

DAVID S. GOLDSTEIN, M.D., PH.D.

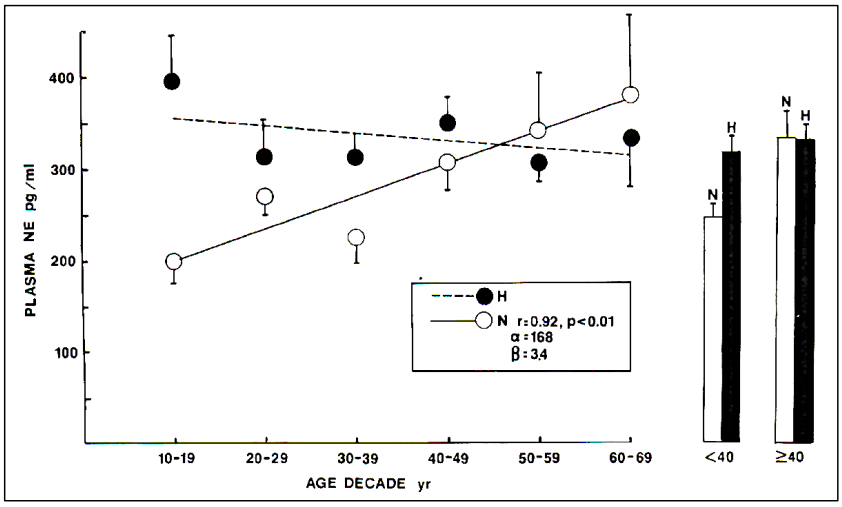
SUMMARY Of 78 comparative studies of plasma catecholamines in patients with essential hypertension and in normotensive controls, most reported higher catecholamine levels in the hypertensives, although only about 40% of the studies were positive (reporting statistically significant hypertensive-normotensive differences). Although there was dramatic variability in catecholamine values within and across studies, virtually all studies of norepinephrine in *young, consistently hypertensive* patients were positive. The likelihood that a study was positive with respect to norepinephrine was independent of the likelihood with respect to epinephrine, so that total catecholamine values, or else the sum of norepinephrine plus epinephrine, differentiated hypertensives from normotensives to a greater extent than levels of either substance alone. The preponderance of literature on the subject supports the hypothesis that increased plasma catecholamine concentrations occur in some patients with essential hypertension. Elevated plasma norepinephrine in relatively young, established hypertensive patients is consistent with a pathophysiologic role for increased sympathetic neural activity in this subgroup. (Hypertension 5: 86–99, 1983)

Based on reviews of the literature about plasma catecholamines in essential hypertension, I proposed that increased sympathetic nervous activity may occur during development of the disease

Age-Dependence of Hypertensive-Normotensive Differences in Plasma Norepinephrine

DAVID S. GOLDSTEIN, M.D., PH.D., C. R. LAKE, M.D., PH.D., BART CHERNOW, M.D.,
MICHAEL G. ZIEGLER, M.D., MICHAEL D. COLEMAN, M.D.,
ADDISON A. TAYLOR, M.D., PH.D., JERRY R. MITCHELL, M.D., PH.D.,
IRWIN J. KOPIN, M.D., AND HARRY R. KEISER, M.D.

SUMMARY We compared venous plasma norepinephrine (NE) concentrations in 191 resting, supine patients with essential hypertension and 129 normortensive controls. Among normotensives, plasma NE increased significantly with age, but among hypertensives, no age-related increase occurred, due to relatively high NE values among young hypertensives. When patients and controls less



Our original observational data confirmed that hypertensive-normotensive differences in plasma norepinephrine (NE) levels depend on age, consistent with increased sympathetic noradrenergic activity playing a role in the development of essential hypertension.

The finding of increased sympathetic outflow in hypertensive patients does not necessarily mean that the increase is pathophysiologically significant. To identify “hypernoradrenergic hypertension” we devised a combined neurochemical/pharmacological approach in which the alpha-2 adrenoceptor antagonist yohimbine or the alpha-2 adrenoceptor agonist clonidine was administered. At the time I had no idea this sort of combined analysis would reverberate many times in subsequent years and lead eventually to clinical laboratory diagnosis by convergence (consilience).

Alpha-2 adrenoceptors located on sympathetic nerves provide a “brake” on NE release. If a patient had an increased sympathetic contribution to blood pressure, then there would be an augmented increase in blood pressure in response to yohimbine, associated with a high baseline NE level. Among hypertensive patients, those with large responses to yohimbine typically reported a history of anxiety, depression, or other psychopathology and of marked pressor or tachycardic episodes during emotional stress. Across the subjects, baseline NE concentrations predicted the magnitude of pressor responses to yohimbine. Therefore, yohimbine challenge testing could distinguish patients with pressor hyperresponsiveness due to excessive sympathetic reactivity. Analogously, hypertensives with high plasma NE levels at baseline had large decreases in blood pressure in response to clonidine, which acts in the brain to suppress sympathetic outflow and acts on alpha-2 adrenoceptors to suppress NE release for a given amount of sympathetic outflow.

The report about clonidine suppression testing in essential hypertension, published in the *Annals of Internal Medicine* in 1985, became a citation classic.

Clonidine Suppression Testing in Essential Hypertension

DAVID S. GOLDSTEIN, M.D., Ph.D; PAUL D. LEVINSON, M.D.; REUVEN ZIMLICHMAN, M.D.; ARTHUR PITTERMAN, M.D.; ROBIN STULL; and HARRY R. KEISER, M.D.; Bethesda, Maryland

To assess the contribution of sympathetic outflow to blood pressure in patients with essential hypertension, we measured blood pressure and plasma norepinephrine responses to clonidine, an antihypertensive agent that decreases central sympathetic outflow, in 44 patients and in 41 normotensive control subjects of similar age. Among the hypertensive patients, the resting level of plasma norepinephrine was significantly related to the decrease in mean arterial pressure 3 hours after a single oral dose of clonidine, 300 μg ($r=0.62, p<0.001$). The magnitude of the depressor response in the patients also was correlated significantly with the decrease in plasma norepinephrine after clonidine ($r=0.60, p<0.001$). These results suggest that increased sympathetic outflow plays a pathophysiologic role in some patients with essential hypertension.

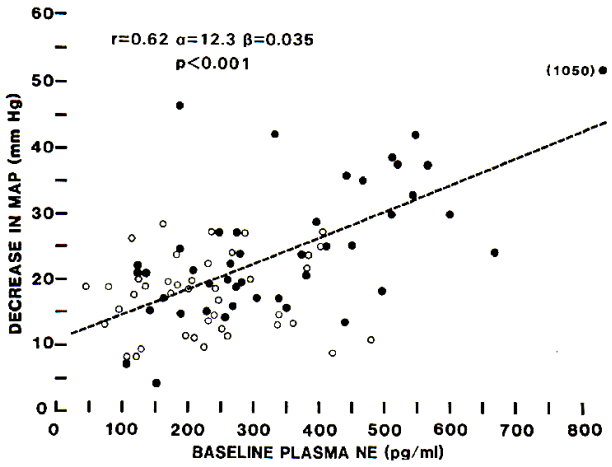


Figure 1. Decrease in mean arterial pressure (*MAP*) as a function of baseline plasma norepinephrine (*NE*) in patients with essential hypertension (*black dots*) and in normotensive control subjects (*open circles*). Each point represents an individual subject. The dashed line is the linear regression line of best fit for the hypertensive group. Baseline plasma norepinephrine predicted the magnitude of the depressor response to clonidine in the hypertensive but not the normotensive subjects.

Our report showing that clonidine suppression testing can identify hypernoradrenergic hypertension.

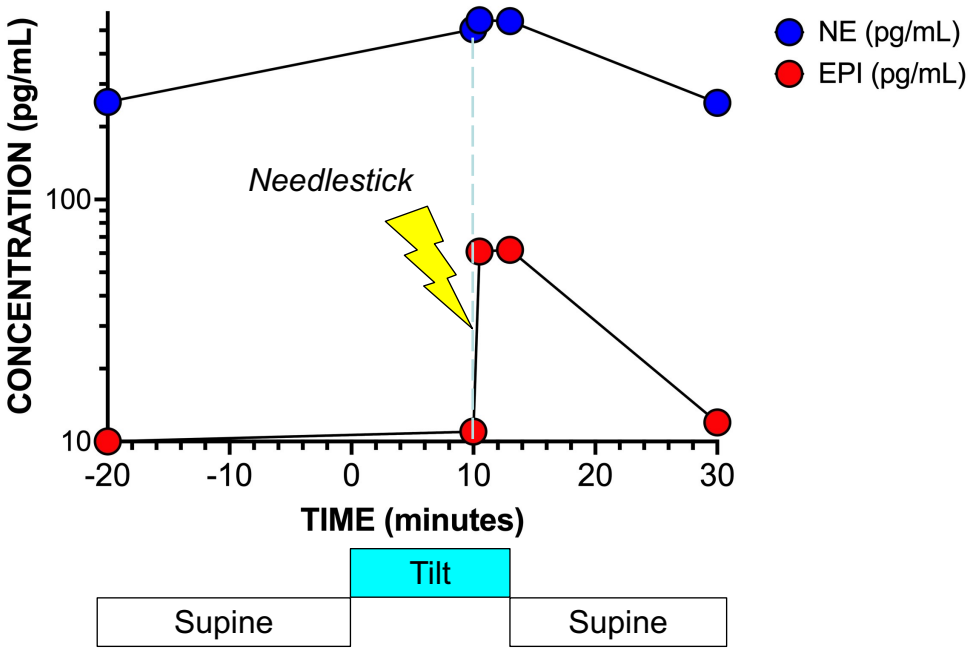
Fight, flight, faint

In the early 20th century Walter B. Cannon introduced the phrase, “fight or flight,” referring to an emergency situation in which activation of the “sympathico-adrenal” system would maintain “homeostasis” (a word he invented). Researchers as well as the lay public and media still refer to this monolithic pattern. It was presumed that fight or flight situations would arouse the sympathetic nervous and adrenal hormonal systems as a functional unit.

Soon after publishing the LC-ED assay I was asked by the Cardiology Branch to consult on a patient with frequent fainting, which could be evoked by a needlestick if she looked at the needle. She was able to mount a plasma NE response when she was tilted head-up as an orthostatic stimulus, when she received an infusion of nitroprusside, which relaxes

blood vessels and releases the sympathetic noradrenergic system from baroreflex restraint, and when she exercised; however, when she had needlestick-induced syncope there was no increase in plasma NE, despite a large fall in blood pressure. At the same time there was a large increase in plasma EPI. From the results I proposed that “vasodepressor syncope” in this patient resulted from a coordinated pattern of central nervous origin, in response to a threatening situation where she could neither fight nor flee.

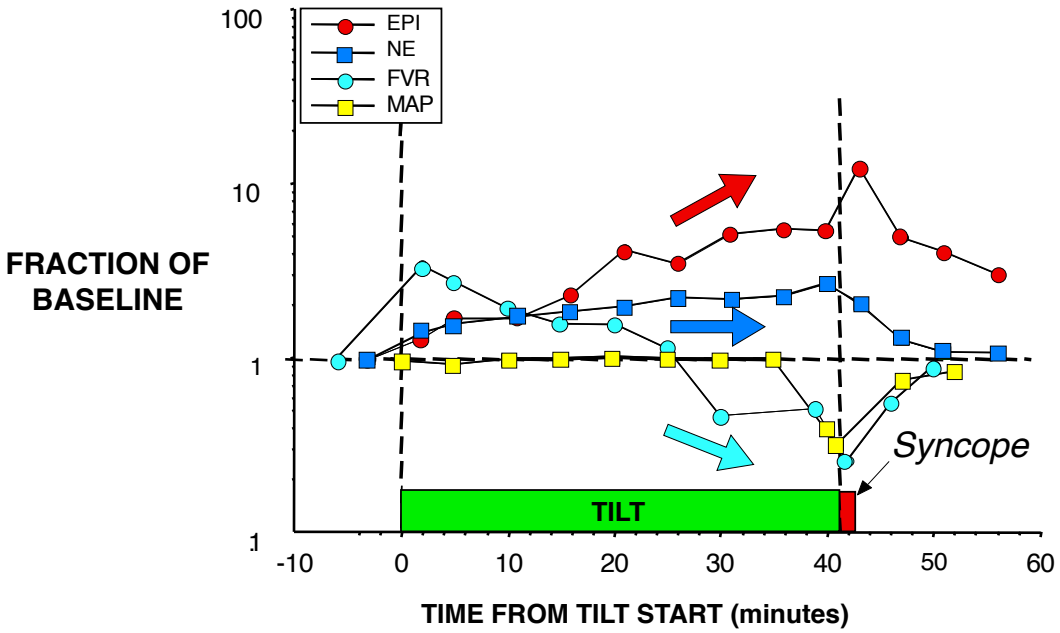
This case report, published in the *American Journal of Cardiology* in 1982, became a citation classic.



First demonstration of “sympathoadrenal imbalance” in a patient with frequent vasodepressor syncope. The Figure is based on data in the case report. Head-up tilting increased plasma NE, but needlestick-induced hypotension and syncope did not increase plasma NE further. Meanwhile, plasma EPI increased substantially.

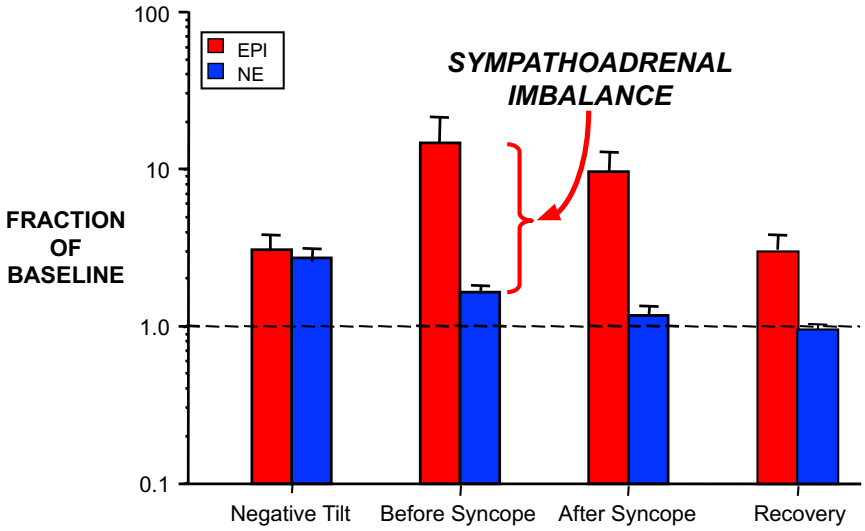
Sympathoadrenal imbalance may be pathophysiologically significant. EPI potently stimulates beta-2 adrenoceptors on vascular smooth muscle cells in skeletal muscle. This relaxes the arterioles and decreases skeletal muscle vascular resistance. In the skin EPI stimulates alpha-adrenoceptors

on vascular smooth muscle cells and sweat gland cells, evoking pallor and sweating—classic signs in fainting reactions.

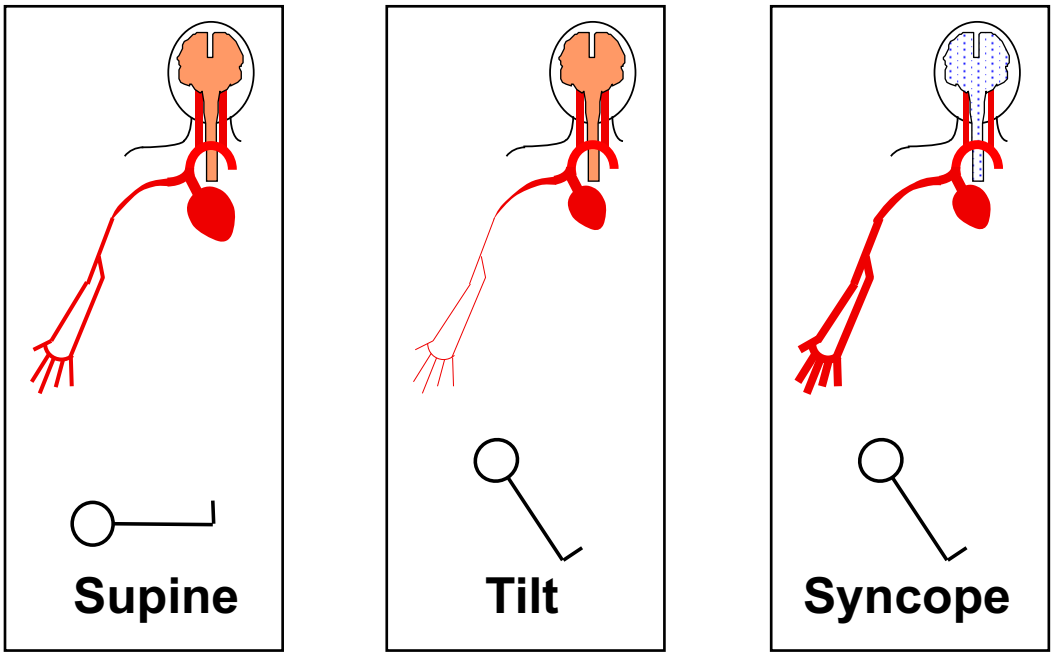


Sympathoadrenal imbalance preceding tilt-evoked sudden hypotension and syncope. Plasma epinephrine (EPI) increases progressively (red arrow) while norepinephrine (NE) changes by relatively little (blue arrow) before the acute drop in mean arterial pressure (MAP, yellow). Note that forearm vascular resistance (FVR, aqua) at first increases during tilting but then decreases as EPI increases (aqua arrow).

Normally, when a person is tilted head-up, venous return to the heart decreases, but reflexive sympathetic noradrenergic stimulation results in generalized vasoconstriction, and the increase in total peripheral resistance maintains mean arterial pressure. In sympathoadrenal imbalance there is a “break” on sympathetic noradrenergic outflows, and the skeletal muscle vasodilator effect of EPI is relatively unopposed by reflexive sympathetically-mediated vasoconstriction. This means that the distribution of the cardiac output is shunted toward the limbs—and away from the brain. Eventually the patient notices something is amiss, and a distress response leads to more EPI secretion—a positive feedback loop that is inherently unstable.



Sympathoadrenal imbalance before tilt-evoked syncope. In sympathoadrenal imbalance there is a greater proportionate increase in plasma epinephrine (EPI) than norepinephrine (NE).



Concept diagram illustrating how sympathoadrenal imbalance during head-up tilt may evoke a fainting reaction.

It seems reasonable to propose that in a distressing situation where a person can neither fight nor flee, an alternative is to faint. Evocation of the

sympathoadrenal imbalance pattern by the brain might be a primitive protective behavior analogous to an opossum's "playing dead."

Tracer catecholamine kinetics

The concentration of NE in the plasma is determined not only by the rate of entry into the circulation but also by the rate of removal (clearance) from the circulation. How could we separately assess the two very different processes?

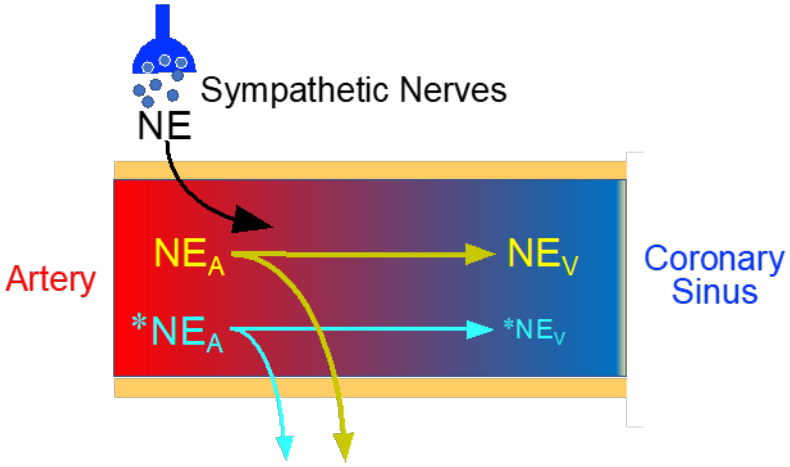
In the early 1980s Murray D. Esler introduced a method to separate "spillover" from clearance of NE using tracer kinetics. A trace amount of NE tagged with radioactivity (^{3}H or ^{14}C) was infused intravenously to attain a steady-state, plateau level. From the infusion rate (in dpm/min) divided by the concentration of ^{3}H (in dpm/mL) one could calculate the clearance of plasma NE—i.e., the volume of plasma emptied of NE per minute (units mL/min). Then, from the steady-state concentration of NE and the clearance, the spillover rate (in pmoles/min) could be calculated. The spillover rate was the NE concentration (in pmoles/mL) times the clearance (in mL/min). In other words, the NE concentration was the ratio of spillover to clearance. Another way to think about it is that endogenous NE entering the bloodstream diluted the radioactive tracer, and the extent of dilution was the measure of NE spillover.

We adopted the tracer kinetic method, which we used in many studies over about two decades. The first of these was to measure the spillover rate of NE in essential hypertension. The report was published in the *Journal of Clinical Investigation* in 1983. We found that the elevated plasma NE in some hypertensive patients reflected increased NE spillover, not decreased NE clearance.

Murray analogously calculated the NE spillover rates in the main organs of interest, the heart and kidneys, based on the percent removal of ^{3}H, the arteriovenous increment in plasma NE. Although Murray Esler's tracer kinetic approach for measuring NE spillover was an important advance, it had a key limitation. Most of the NE released from sympathetic

nerves is removed by a recycling process, Uptake-1. This recycling is now known to be mediated by a specific transporter called the cell membrane NE transporter, or NET. Murray's method could not distinguish increased sympathetically mediated NE release from decreased NE reuptake as determinants of increased NE spillover.

Extension of the LC-ED assay beyond the catecholamines to include the NE metabolite 3,4-dihydroxyphenylglycol (DHPG) was a fundamental development that had implications for clinical assessments of sympathetic function in a variety of disorders. Before telling the story and presenting some of the data, one must understand what the "catechols" of the body are.

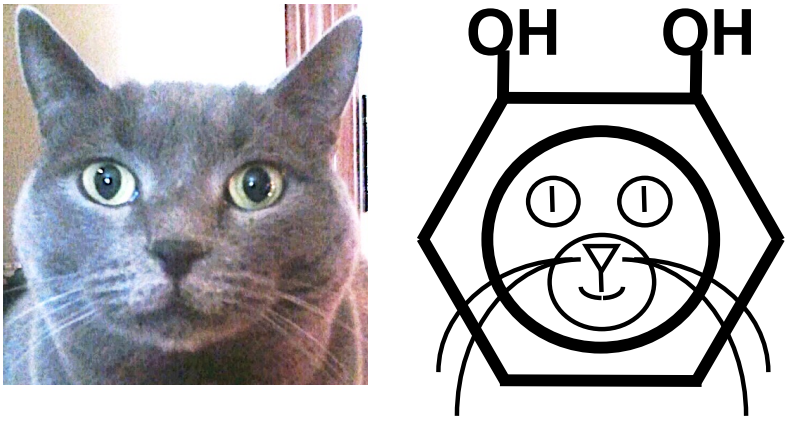


The tracer kinetics principle, in this case applied to calculate the rate of entry of norepinephrine (NE) into the bloodstream from sympathetic nerves in the heart. Some of the tracer-labelled NE in the arterial blood is removed in passage through the heart, so the coronary sinus concentration of radioactive NE is less than the arterial concentration. One may presume the same percent removal of endogenous NE in the arterial blood. The specific activity of NE (radioactivity divided by total NE content) in the venous drainage is less than that in the arterial blood, because endogenous NE from sympathetic nerves dilutes the tracer.

across the organ, and the rate of local plasma flow. He inferred from his results that patients with essential hypertension have increased renal and cardiac spillovers of NE.

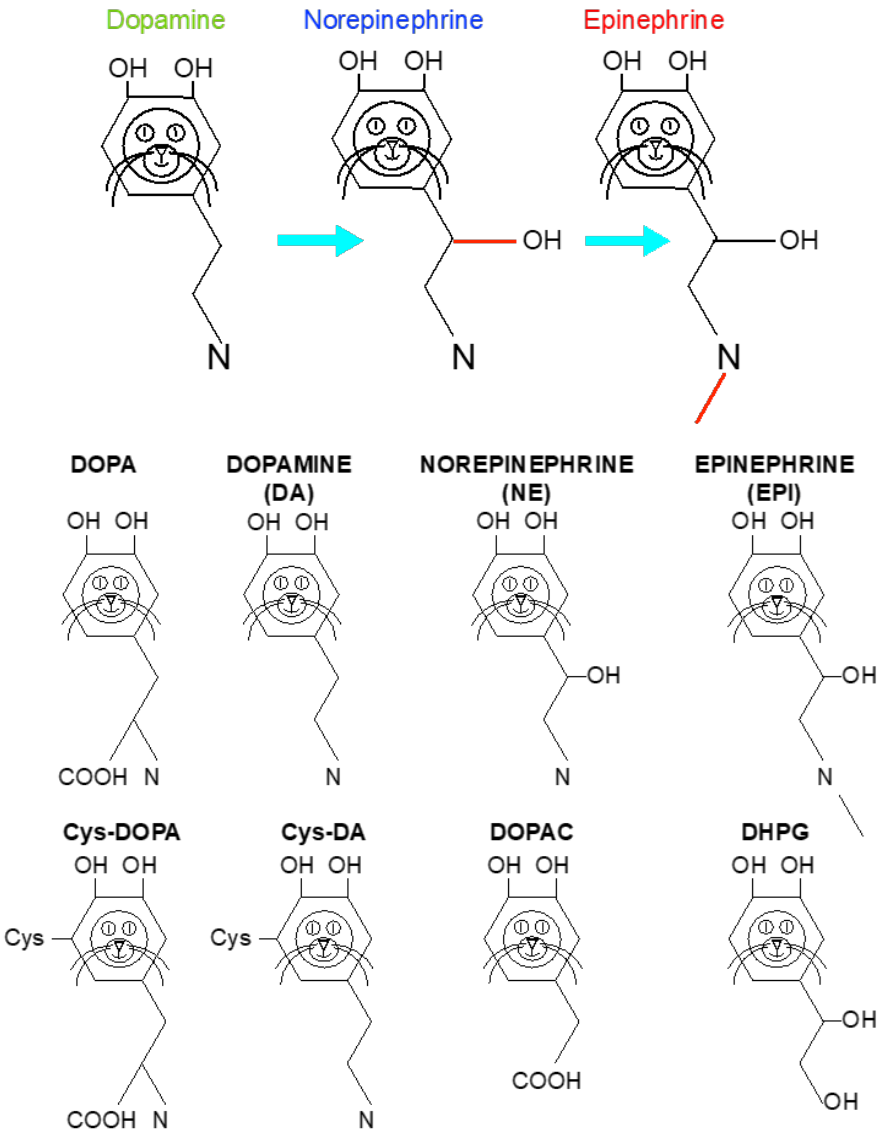
Catecholamines look like cats.

Catechols are analogous to the head of a cat. The two pointy ears are the adjacent hydroxyl (-OH) groups on the hexagonal benzene ring. A catecholamine has a hydrocarbon tail ending in an ammonia (amine) group. Think of the ammonia smell of the entire cat, from head to tail, in its litterbox.



Catechols look like the head of a cat. The two pointy ears are the adjacent hydroxyl groups on the benzene ring.

In thin layer flow cells such as those marketed by Bioanalytical Systems, only a small percentage of the oxidizable compounds passing over the glassy carbon electrode would be oxidized. A major advance was the introduction of a porous electrode by Environmental Sciences Associates (ESA) in the early 1980s. Because the surface area for the electrochemical reactions was essentially infinite, if there were a series of post-column electrodes, one could perform a complete oxidation at the first electrode and apply a reducing potential at a second (or third) electrode and record from the reducing electrode.



The catecholamines (dopamine, NE, EPI) have a hydrocarbon tail with an amine group. The human body has several catechols, one of which is DHPG, the main neuronal metabolite of NE.

This meant there would be signal produced only species that were reversibly oxidized. Most of the compounds oxidized at the first electrode would be oxidized irreversibly. An exception is catechols. The adjacent hydroxyl groups on the benzene ring are oxidized (to quinones), but upon exposure to a reducing potential the quinones would be converted back to the parent catechols.

There were two key consequences of using the series electrode

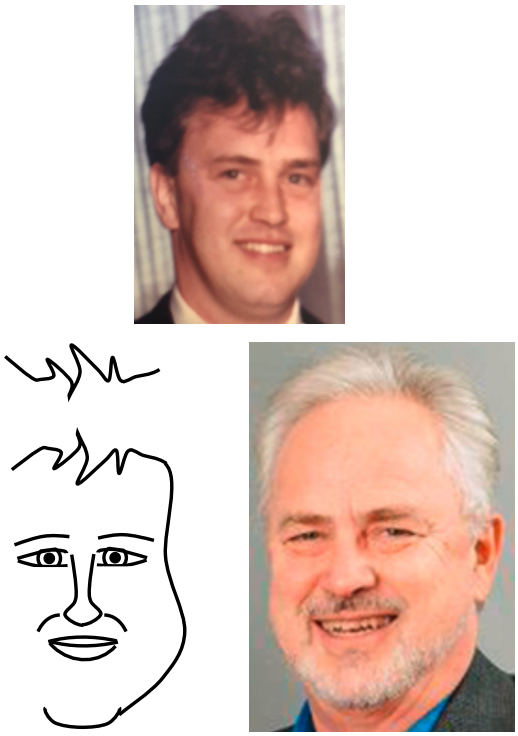
arrangement. First, the baseline “noise” in the chromatographs was reduced, meaning greater sensitivity than with the thin layer flow cell arrangement. Second, the solvent front was much smaller and narrower, meaning that earlier-retaining species could be separated from the solvent front. One of the compounds with a short retention time was DHPG.

Graeme Eisenhofer came to the NIH from New Zealand as a post-doc after obtaining his PhD at the University of Otago in 1983. His thesis was on the acute and chronic effects of ethanol on water metabolism and the sympathetic nervous system in humans. Graeme had (and has) an extraordinary talent for developing biochemical assay methodology. In 1986 we published the first report about plasma levels of catechols, including DHPG and 3,4-dihydroxyphenylacetic acid (DOPAC, the main neuronal metabolite of dopamine), in humans. That report is a citation classic.

In the report we noted the effects of monoamine oxidase (MAO) inhibitors on plasma DHPG and DOPAC levels. One of the tested MAO inhibitors was deprenyl (also called selegiline). There are two isoforms of MAO, MAO-A and MAO-B. In test tubes, deprenyl selectively inhibits MAO-B; however, in vivo in humans the drug clearly decreased plasma DHPG and DOPAC levels, suggesting inhibition of MAO-A. This apparent paradox, which we will return to later in the story, has never been resolved, but other investigators using independent methods have reported the same phenomenon.

The ability to assay DHPG and NE simultaneously was crucial for our investigation of the sources and meaning of plasma DHPG in humans. Two years later, in 1988, we published in the *Journal of Clinical Investigation* about the relationship between DHPG and the intra-neuronal metabolism of NE in sympathetic nerves. This report also was a citation classic. Our interpretation of the results has stood the test of time: (1) plasma DHPG in humans is derived mainly from sympathetic nerves; (2) increments in plasma DHPG during stimulation of NE release result from uptake of NE into sympathetic nerve endings and subsequent intraneuronal conversion to DHPG; (3) plasma DHPG under basal conditions probably is determined

mainly by net leakage of NE into the axonal cytoplasm from storage vesicles; and (4) increments in NE concentrations at neuronal uptake sites can be estimated by simultaneous measurements of DHPG and NE during NE infusion and NE release. Measurement of NE and DHPG provides unique clinical information about sympathetic function.



Graeme Eisenhofer, then and now.

We also found that there is far more DHPG production in the heart than in other organs. As explained later, this may have implications for recent discoveries related to cardioselective NE deficiency in Parkinson's disease.

CLIN. CHEM. 32/11, 2030–2033 (1986)

Simultaneous Liquid-Chromatographic Determination of 3,4-Dihydroxyphenylglycol, Catecholamines, and 3,4-Dihydroxyphenylalanine in Plasma, and Their Responses to Inhibition of Monoamine Oxidase

Graeme Eisenhofer,[1] David S. Goldstein,[2] Robin Stull,[2] Harry R. Keiser,[2] Trey Sunderland,[3] Dennis L. Murphy,[3] and Irwin J. Kopin[1]

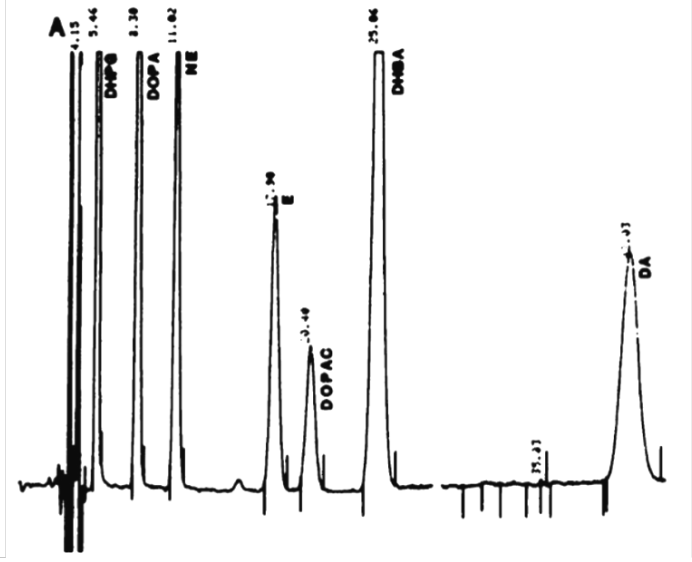
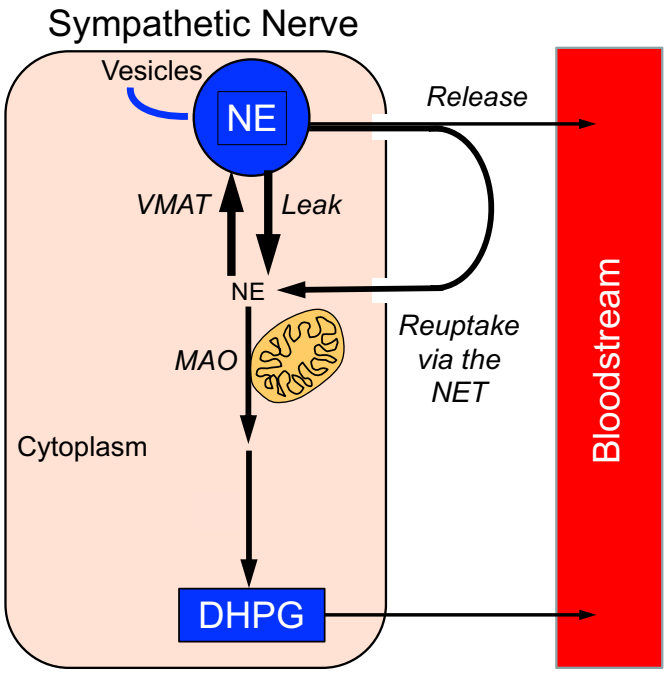


Table 1. Concentrations (ng/L) of DOPA, Norepinephrine, Epinephrine, DHPG, and DOPAC in Plasma before and after Administration of Deprenyl and Tranylcypromine

	Baseline	Deprenyl	Baseline	Tranylcypromine
	Mean ± SEM (n = 12)		Mean ± SEM (n = 6)	
DOPA				
Supine	3386 ± 321	2920 ± 341	3551 ± 354	2971 ± 331
Upright	3173 ± 262	2717 ± 262	3469 ± 261	2869 ± 407
Norepinephrine				
Supine	318 ± 61	343 ± 45	346 ± 66	260 ± 37
Upright	626 ± 102	696 ± 119	584 ± 102	453 ± 98
Epinephrine				
Supine	34 ± 8	30 ± 4	40 ± 8	45 ± 18
Upright	57 ± 27	43 ± 8	105 ± 31	79 ± 45
DHPG				
Supine	821 ± 96	242 ± 37[b]	849 ± 101	213 ± 120[a]
Upright	840 ± 76	236 ± 37[b]	861 ± 77	230 ± 115[a]
DOPAC				
Supine	731 ± 118	368 ± 99[b]	713 ± 126	63 ± 19[c]
Upright	665 ± 147	385 ± 121[a]	427 ± 193	83 ± 19[c]

[a,b] Significantly different from baseline value. [a] $p < 0.05$, [b] $p < 0.01$. [c] n = 3.

The first report of simultaneously assaying plasma levels of 3,4-dihydroxyphenylglycol (DHPG) and 3,4-dihydroxyphenylacetic acid (DOPAC), the catecholamine precursor DOPA, and the catecholamines in human plasma. The aqua rectangle highlights decreases in plasma DHPG and DOPAC in people treated with the MAO-B inhibitor deprenyl.



Concept diagram comparing the sources of circulating NE and DHPG. Delivery of NE to the bloodstream depends on release and reuptake. Delivery of DHPG to the bloodstream depends on NE release and reuptake but mainly on the balance of vesicular uptake via the vesicular monoamine transporter (VMAT) and passive leakage of NE from vesicular stores.

Our concept about plasma levels of NE and DHPG has been supported by numerous experiments and observations over the years. Here are the key elements.

The rate of NE delivery to the bloodstream depends on two processes—release from the sympathetic nerves by exocytosis of vesicles containing NE and neuronal reuptake of NE.

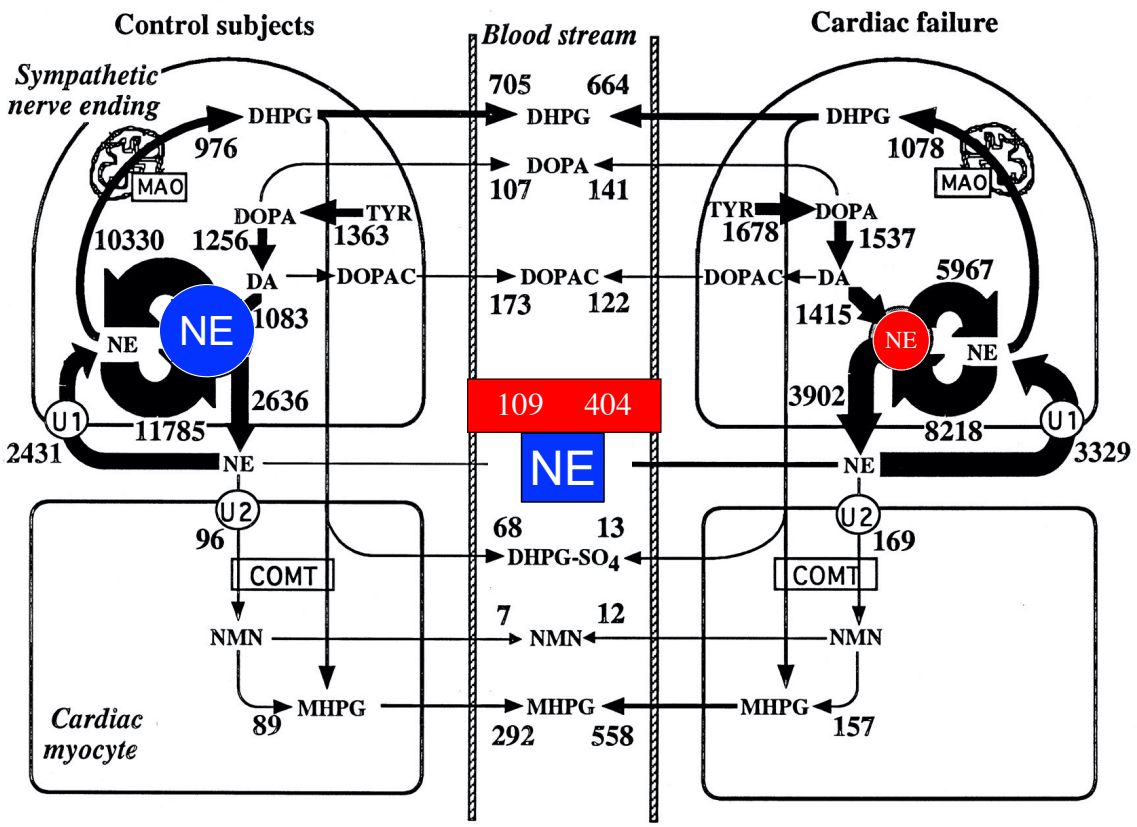
The rate of delivery of DHPG to the bloodstream depends on four processes—vesicular uptake of cytoplasmic NE vis the vesicular monoamine transporter (VMAT), passive leakage of NE from the vesicles to the cytoplasm, vesicular uptake of cytoplasmic NE, monoamine oxidase (MAO) in the outer mitochondrial membrane metabolizing NE to DHPG (via an intermediate that we'll get to later), and neuronal reuptake of NE.

We soon realized that if we applied the tracer kinetics approach and measured both endogenous and radiolabeled NE and DHPG in arterial and coronary sinus plasma, we'd be able to calculate Uptake-1 activity for the first time in vivo in the human heart. We reported this novel methodology in *Circulation* in 1988, another citation classic.

At about that time, Graeme's post-doc position had to end. He worked with Murray Esler in Australia for the next 3 years and introduced in Murray's lab the improved tracer kinetics approach. Graeme returned to the NIH in 1991.

In 1996 I was privileged to be the senior author for what I consider to be Graeme's *tour de force.* Based on arterial and coronary sinus concentrations of endogenous and tracer-labelled catechols he was able to estimate the rates and amounts of all the reactions and reactants determining NE synthesis, storage, release, reuptake, and metabolism in sympathetic nerves, in control subjects and in patients with congestive heart failure. The article, also published in *Circulation,* is one of the most widely cited articles in the field. Among other things, the report resolved the apparent paradox of high circulating NE levels despite depletion of NE. stores.

In 2002, applying the same tracer kinetic technique during right heart catheterization, we obtained evidence for different abnormalities of cardiac sympathetic function in two chronic orthostatic intolerance syndromes, frequent neurocardiogenic syncope (NCS) and postural tachycardia syndrome (POTS). The data indicated that POTS and NCS both involve substantial, different, tonic abnormalities of cardiac sympathetic function, qualifying both conditions as cardiac sympathetic dysautonomias. POTS seems to involve increased and NCS decreased NE release from intact cardiac sympathetic nerves, without evidence for fixed abnormalities in activity of the NET, NE synthesis, or sympathetic innervation density in either disorder. The article, also published in *Circulation,* is a citation classic.



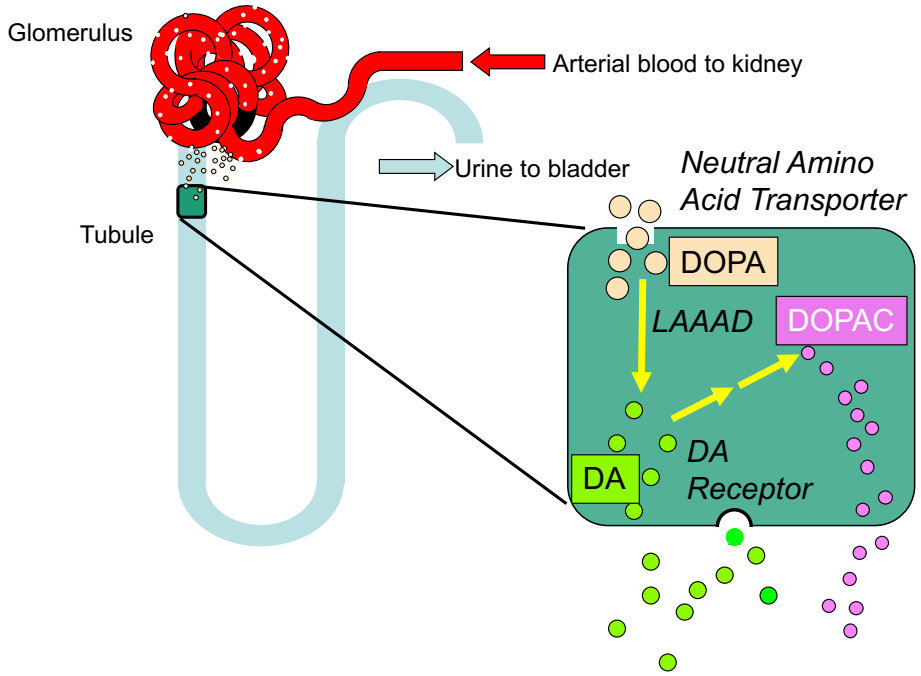
Graeme Eisenhofer's 1996 tour de force calculation of the rates of all the major processes in NE synthesis, storage, release, reuptake, and metabolism in the human heart, in control subjects and in patients with congestive heart failure.

In 2019 we published with Mark Pekker, a Professor of Mathematics at the University of Alabama, Huntsville, the results of a similar project to identify the determinants of myocardial NE depletion in Lewy body diseases such as Parkinson's disease. The tracer kinetic technique, based on sophisticated applications of clinical catecholamine neurochemistry, therefore has led to major discoveries and concepts over more than 35 years.

Three catecholamine systems

In human urine there is a far higher dopamine (DA) concentration than concentrations of either NE or EPI. The pattern in urine differs markedly from that in plasma, where the catecholamine with the highest

concentration is NE. By applying the LC-ED assay for catechols to plasma and urine of healthy humans we discovered that most of the DA in urine comes not from release as a neurotransmitter from sympathetic nerves, nor from glomerular filtration of DA in the plasma, but from renal uptake and decarboxylation of circulating DOPA in kidney parenchymal cells.

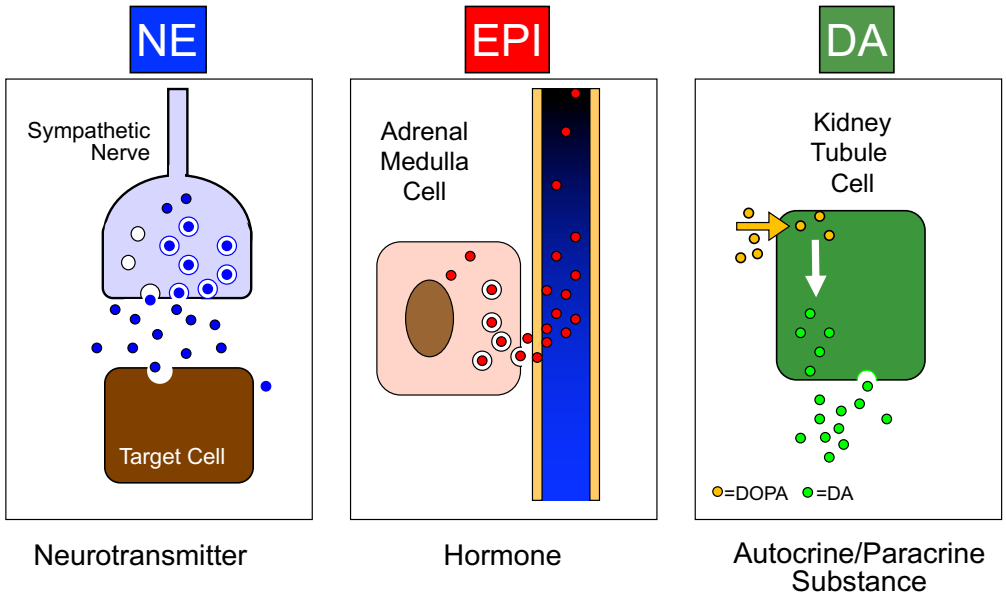


Our group discovered that all of the endogenous dopamine (DA) in human urine is derived from uptake and decarboxylation of circulating DOPA.

This discovery led to the concept of a third catecholamine system in which DA is an autocrine-paracrine substance, produced in, released from, and acting on the same or nearby cells. The body's three catecholamines therefore have quite different actions. NE is a neurotransmitter, EPI is a hormone, and DA is an autocrine-paracrine substance.

In a series of experiments Graeme Eisenhofer, several other colleagues, and I found that most of the catecholamine made and metabolized in the body is in the gut. Considering that primitive animals that lack a brain or circulation contain DOPA and DA, it seems that the DA autocrine-paracrine systems of the body are ancient in evolutionary terms and probably were superseded by hormonal and neurotransmitter systems.

The functions of these systems and their interactions remain poorly understood.



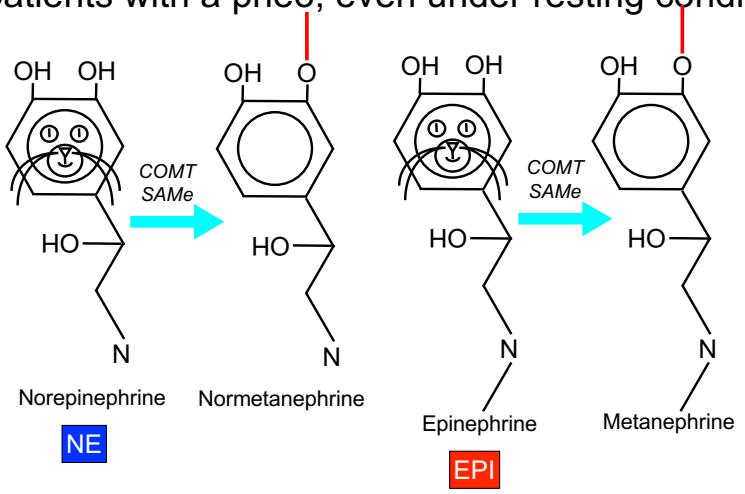
Three types of catecholamine system—neurotransmitter, hormone, and autocrine/paracrine

With John Gill we discovered that patients with salt-sensitive hypertension have increased urinary DOPA excretion and a low urinary DA/DOPA ratio, consistent with decreased renal DA production from DOPA.

Pheo

Harry Keiser was an authority on pheochromocytomas, or “pheos.” Pheos are tumors that make catecholamines and therefore often manifest with high blood pressure. Although rare, they are clinically important. First, most pheos are benign, meaning that pheo represents a situation where hypertension is potentially surgically curable. Second, if left undiagnosed, the catecholamines secreted by pheos can be toxic. In a report published in the *New England Journal of Medicine* in 1989 we reported the case of a child who had reversible heart failure from a pheo (a citation classic). Third, a patient harboring a pheo can have a hypertensive paroxysm in response to a minor manipulation such as induction of general anesthesia.

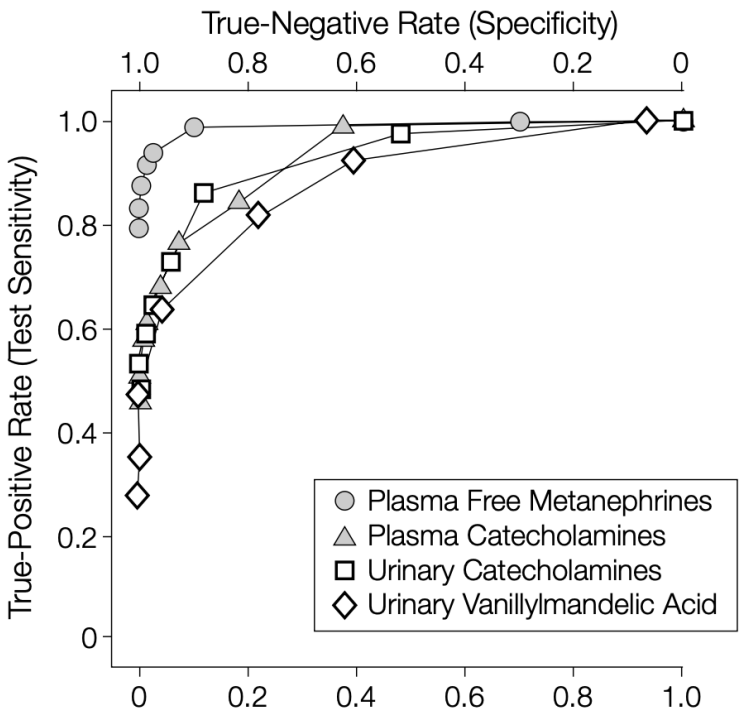
Graeme Eisenhofer and Jacques Lenders developed an LC-ED method for measuring metanephrines. Metanephrines are not catechols. NE in the circulation is taken up extensively by non-neuronal cells that express catechol-O-methyltransferase (COMT) and convert the NE to normetanephrine (NMN). EPI in the circulation is converted extra-neuronally to metanephrine (MN). Graeme and Jacques found that pheos, as well as normal adrenomedullary chromaffin cells, express COMT. In this way the metabolism of catecholamines in the sympathetic adrenergic system differs substantially from metabolism in the sympathetic noradrenergic system or brain catecholamine neurons. Graeme and Jacques reasoned that since catecholamines stored in vesicles leak passively continuously into the cytoplasm, plasma metanephrines could be increased in patients with a pheo, even under resting conditions.



Catecholamines are converted to metanephrines via COMT. S-Adenosyl-methionine (SAMe) is the methyl group donor for the reaction.

They were spectacularly correct. Their reports on plasma metanephrines as a sensitive test for pheo have become citation classics.

After Harry Keiser retired, Karel Pacak, in the National Institute of Child Health and Development (NICHD), became the expert on pheo in intramural NIH. He, Graeme, and Jacques (now in retirement) continue to collaborate on pheo, especially in familial forms of the disease.

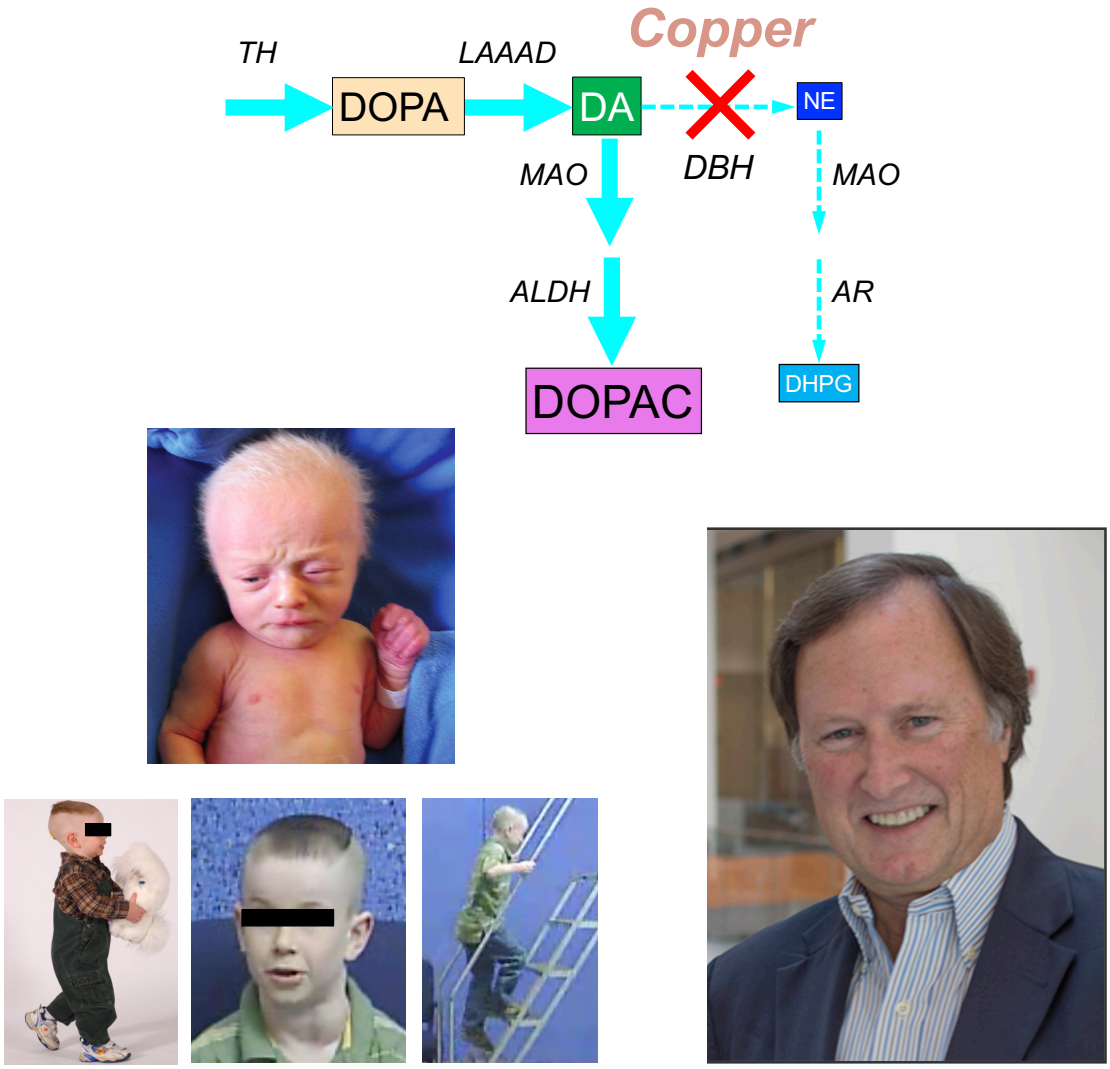


Extraordinary sensitivity of plasma free metanephrines for diagnosing pheo.

Kinky hair disease

Menkes disease is a rare, X-linked recessive disorder of copper metabolism that is due to mutation of the gene that encodes the copper ATP-ase, *ATP7A*, which is used to transport copper across membranes. Dopamine-beta-hydroxylase (DBH), the enzyme that catalyzes the conversion of DA to NE, contains, and its activity absolutely requires, copper.

In the late 1980s a pediatric researcher, Stephen G. Kaler, asked me whether a tracer-kinetic study might detect Menkes disease from decreased levels of tracer-labelled NE after administration of tracer-labelled dopamine (DA). I suggested that before this complex testing involving radioactivity, why not assay levels of NE, DHPG, DA, and other catechols in the plasma?



(Top) Since DBH is a copper enzyme, Menkes disease has a catechol pattern involving buildup of DA and its metabolites and decreased NE and its metabolites. (Lower left top) Typical appearance of an infant with Menkes disease. (Lower left bottom) This patient with Menkes disease began receiving copper injections soon after being diagnosed. The treatment virtually normalized his nervous system development. (Lower right) Stephen G. Kaler developed the first successful treatment of Menkes disease.

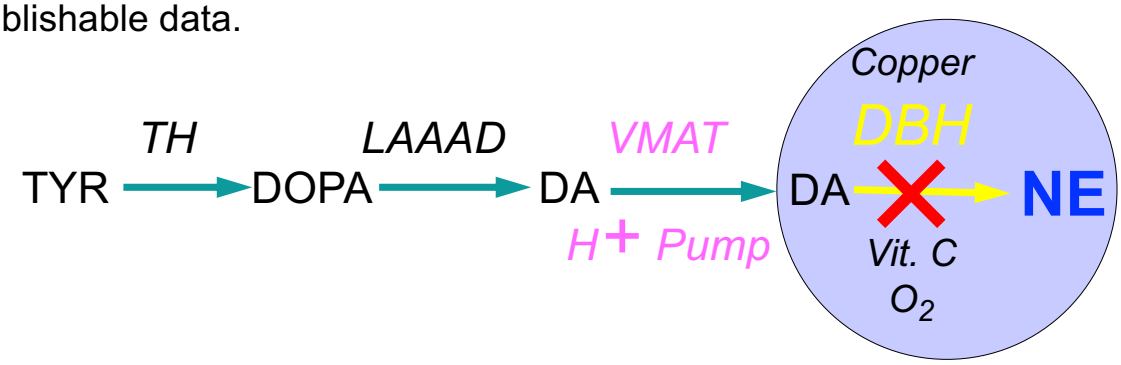
We found that all patients with Menkes disease had the same abnormal pattern of plasma levels of catechols. Because of the decreased DBH activity, plasma levels of DOPA, DA, and DOPAC were high with

respect to NE and DHPG. We went on to show that in at-risk newborns, measuring plasma catechols provided a perfectly sensitive and specific test for diagnosing Menkes disease. Steve Kaler found that treatment with copper injections could result in dramatic improvement in outcome. Our reports published in 1994 in *Nature Genetics* in 2008 in the *New England Journal of Medicine* are citation classics.

Our lab has been certified for many years under the Clinical Laboratory Improvement Amendments (CLIA) to carry out plasma catechol assays for diagnostic purposes for free as a public service. For diagnosing Menkes disease in at-risk newborns the assay has proven to be perfectly sensitive and specific for more than a quarter century.

Dopamine-beta-hydroxylase (DBH) deficiency

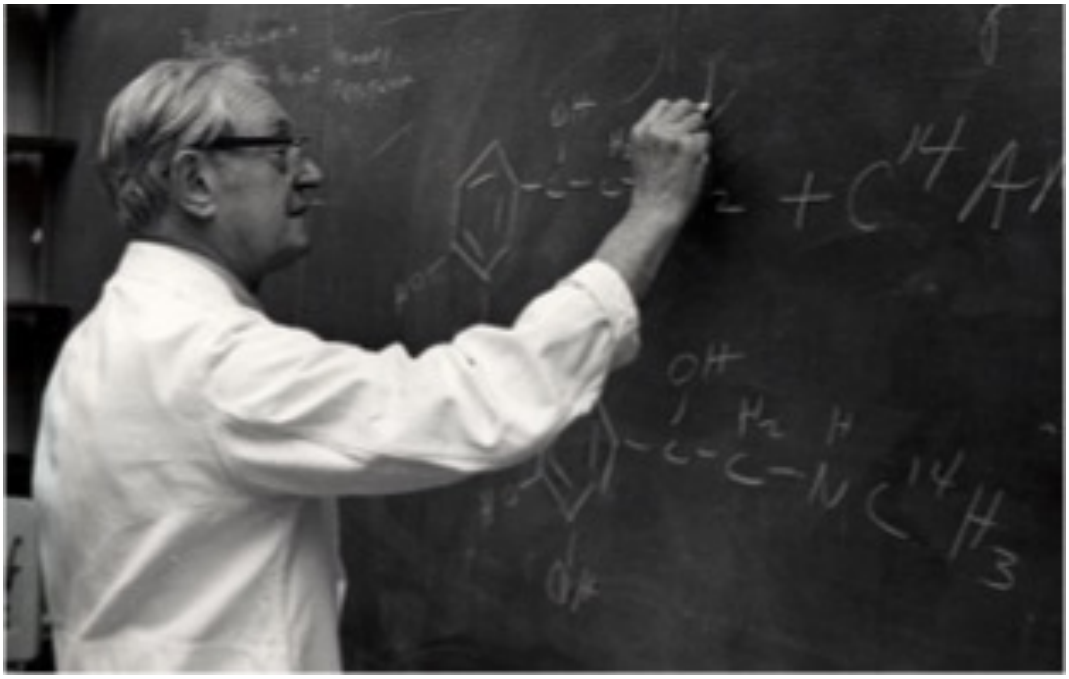
Any condition producing DBH deficiency would be expected to manifest clinically by symptoms and signs of decreased ability to synthesize norepinephrine, such as orthostatic hypotension. DBH deficiency should also be characterized by the same abnormal plasma catechol pattern as in Menkes disease. This is what we found in a study done in conjunction with David Robertson and Italo Biaggioni of the Vanderbilt Autonomic Dysfunction Center. As I recall, David visited our lab and brought with him plasma samples from 2 DBH-deficient patients. Courtney assayed the samples straightaway, and within one day we had publishable data.



Dopamine-beta-hydroxylase (DBH) catalyzes the conversion of dopamine (DA) to norepinephrine (NE). DBH is localized to vesicles.

On the shoulders of giants

In developing clinical catecholamine neurochemistry at the NIH, I stood on the shoulders of giants—Julius (“Julie”) Axelrod and Irwin J. (“Irv”) Kopin.



Julie Axelrod at the blackboard. The chemical drawn below his right elbow is C^{14}-labelled EPI.

Julie Axelrod shared a Nobel Prize in Physiology of Medicine 1970 for his discovery of neuronal reuptake (Uptake-1) as the main means for inactivation of NE. This discovery, and several others he made, depended on the kinetics of radiolabeled catecholamines. The tracer kinetic approaches used by Murray Esler, Graeme Eisenhofer, and me were rather direct descendants. Julie also discovered catechol-O-methyltransferase (COMT), the enzyme that catalyzes the metabolism of the catechols in non-neuronal and adrenomedullary chromaffin cells.

Administratively, Irv was Julie’s boss when Axelrod received the Nobel Prize. I first met Irv when he was high up on the totem pole in the NIMH, while I was a lowly Fellow in the NHLBI. We shared an interest in plasma

levels of NE as an index of activity of the sympathetic nervous system in hypertension. In our discussions our age and hierarchical differences disappeared. Irv was a whiz at calculus and exploited his mathematical skills repeatedly to model biochemical processes involved in the synthesis and fate of catecholamines in the body. He thought mathematically. I thought visually. It was a perfect match.

Irv Kopin, Fred Goodwin, and Julie Axelrod celebrating Julie's being awarded a Nobel Prize in Physiology or Medicine in 1970.

In 1960 Irv and Julie discovered DHPG. The clinical scientific significance of this discovery was unknown until Graeme Eisenhofer and I developed the assay method using LC-ED to measure it.

Chevrusah

After Irv Kopin stepped down as Scientific Director of the NINDS and Chief of the Clinical Neuroscience Branch, he served with distinction as a valued Scientist Emeritus for many years. He always had a desk in our lab. I benefited greatly from his mastery of mathematical modeling and pharmacokinetics. He had an encyclopedic memory of the relevant literature and controversies. He knew catecholamine metabolism inside out, because he had been there personally from the founding of the field.

You don't know what you have until it's gone. At Irv's funeral in August of 2017, I recalled our special relationship. Our style of interaction was to debate about the science. We would argue about interpretations of the data, about which experiments to do and how to do them, about conceptual models, about getting the historical facts straight, and about how to link genetics and molecular medicine with integrative physiology. I think we were following the Jewish tradition of the *chevrusah*, the talmudic learning partner. Two people try to understand the material by discussing it together. I will always feel thankful and honored that Irv was my medical scientific *chevrusah*.

(Left) Photo of Irv Kopin at about the time of his retirement as Scientific Director of the NINDS. (Right) My caricature of Irv.

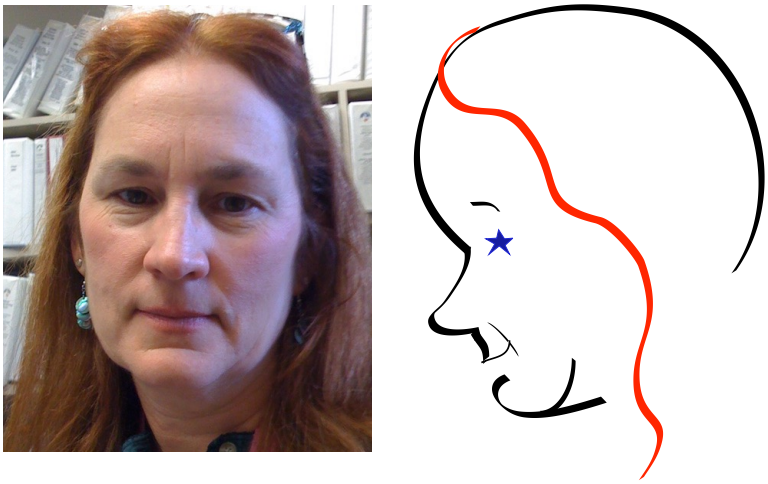
Irv Kopin, Karel Pacak, Julie Axelrod, Gal Yadid, and me at the Eighth International Catecholamine Symposium in 1996.

The Cal Ripken of clinical catecholamine neurochemistry

Cal Ripken played baseball for the Baltimore Orioles for his entire 21-year career and was inducted into the Hall of Fame in 2007, not for home runs or batting average or hits but for consistency. He was the “iron man” who played an unprecedented 2,632 consecutive games. This monumental accomplishment reflected practice, attention to detail, and work ethic. He made it look easy.

Courtney Holmes, who ran our lab for 28 years, was the Cal Ripken of catecholamine neurochemistry. Courtney personally carried out thousands of clinical assays of levels of catechols in samples of plasma, microdialysate, cerebrospinal fluid, and tissues with reliability and sensitivity unmatched by any other lab in the world. Over the years she expanded the repertoire of catechols to the NE pro-drug L-DOPS, fluorodopamine and its metabolites, fluorodopa, sulfoconjugated catecholamines, and synthetic catecholamines. She pointed me unerringly to the truth. If there were a catecholamine Hall of Fame, Courtney Holmes surely would be in it.

Thanks to Courtney’s technical mastery and deep understanding of chromatographic recordings, we made many discoveries together. I called them “sparkles of insight.”



(Left) Courtney Holmes, the Cal Ripken of clinical catecholamine neurochemistry. (Right) My caricature of Courtney.

For instance, we did a study in which we infused the drug tyramine into healthy subjects. Tyramine is known to be taken up into sympathetic nerves and to release NE. Courtney noticed that the increase in plasma DA was far greater than the increase in NE. Eventually we showed that this was from contamination of the tyramine infusate by DA.

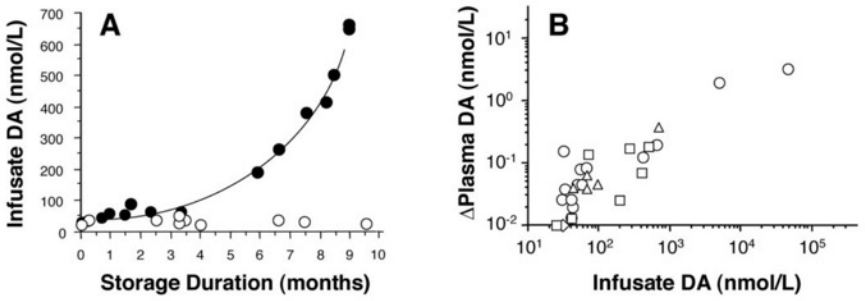
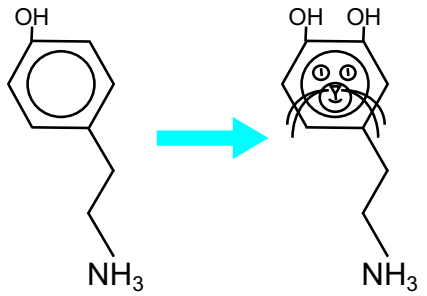


Fig. 1. Concentrations of DA in TYR solutions as a function of duration of storage at 4 °C and −70 °C (*A*), and increment in arterial plasma DA concentration as a function of infusate DA concentration in humans undergoing intravenous infusion of TYR (*B*).
(*A*), ●, 4 °C; ○, −70 °C. Equation for the line: $y = 38^{(0.31x)}$; $r = 0.99$. (*B*), ○, patients with chronic orthostatic intolerance; □, patients with chronic autonomic failure; △, patients after bilateral thoracic sympathectomies for hyperhidrosis; ◇, healthy volunteer.



Tyramine Dopamine

Courtney Holmes discovered that tyramine in solution stored in an ordinary freezer can oxidize spontaneously to form DA. This resolved the "tyramine paradox."

Her discovery resolved the tyramine "paradox." Tyramine was expected to produce vasoconstriction because it would release NE. Instead, tyramine produced vasodilation, due to DA in the infusate.

Courtney's daughter's wedding

If genius is seeing what everyone else has seen and thinking what no one else has thought, then Courtney was a genius at troubleshooting the alumina-HPLC-electrochemical method for catechols.

Hypertension
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ORIGINAL ARTICLE

Tyramine-Induced Vasodilation Mediated by Dopamine Contamination

A Paradox Resolved

Giris Jacob, Alfredo Gamboa, André Diedrich, Cyndya Shibao, David Robertson, and Italo Biaggioni

The discovery of contamination of tyramine solution by dopamine resolved the tyramine paradox.

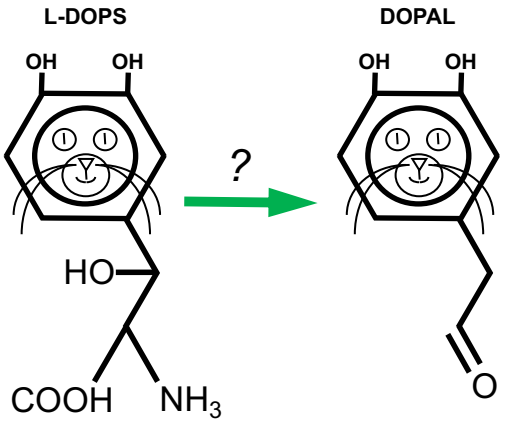
There was a period of time when the chromatographs showed a small, variable amount of contamination by dopamine, from 0 to about 100 picograms. Her troubleshooting strategy was first to check the HPLC system and then to check the reagents injected into the system. By methodically substituting system components—the injector, HPLC pump, guard column, main column, filters, sample tubes, connection tubing, electrodes—even using another system entirely—the hardware itself was exonerated. She replaced all the reagents, made new mobile phase, used another batch of alumina, and used a new bottle of HPLC grade water, but the contamination was still there. If it wasn't the system, and it wasn't what was injected into the system, then what could be the source of the contamination?

In a stroke of genius, Courtney asked whether the problem could be herself. She always wore new disposable gloves when setting up samples, the counter was kept clean, and she never ate in the lab, so these factors were excluded. Her desk was in a cubbyhole in the lab with a barn door closure, so she could eat at her desk with the door closed. This had been her practice for years. Her daughter was going to be married, and Courtney wanted to lose weight to fit in the dress she planned to wear for the wedding. She had gone on a diet. Each morning she would eat a banana at her desk before donning gloves for the assay. Bananas contain a huge amount of DA. Aha! It didn't take long for her to show that her eating banana was the source of the contamination. It could have been banana on her

fingers, even though her fingers were gloved; it could even have been banana on her breath. The human mind cannot comprehend what a picogram is.

Courtney also found that after people had taken the NE pro-drug L-DOPS they had a small amount of the potentially toxic compound DOPAL in their plasma. L-DOPS and DOPAL both are catechols. L-DOPS is L-3,4-threo-dihydroxyphenylserine, and DOPAL is 3,4-dihydroxyphenylacetaldehyde. Courtney observed a virtually perfect correlation between plasma DOPAL and plasma L-DOPS. Either the L-DOPS was contaminated with a trace amount of DOPAL, or else L-DOPS was converted to DOPAL in the body.

It has been known for many years that EPI in solution can be converted to DOPAL by acidifying the solution and heating it briefly. Recall that the last step in the alumina extraction is acidifying the solution. Perhaps DOPAL was made during the acidification step. Meanwhile, ingested L-DOPS would be expected to be exposed to stomach acid. The HPLC-electrochemical assay method is so sensitive, it is possible that even the tiniest amount of conversion of L-DOPS to DOPAL by either route could explain the obtained results. The exact explanation for Courtney's finding remains elusive. The point is that the data were the truth.



Courtney Holmes found that L-DOPS ingestion increases plasma DOPAL levels.

THE REARRANGEMENT OF EPINEPHRINE

By Dr. J. H. FELLMAN

Division of Neurology, University of Oregon Medical School, Portland 1, Oregon

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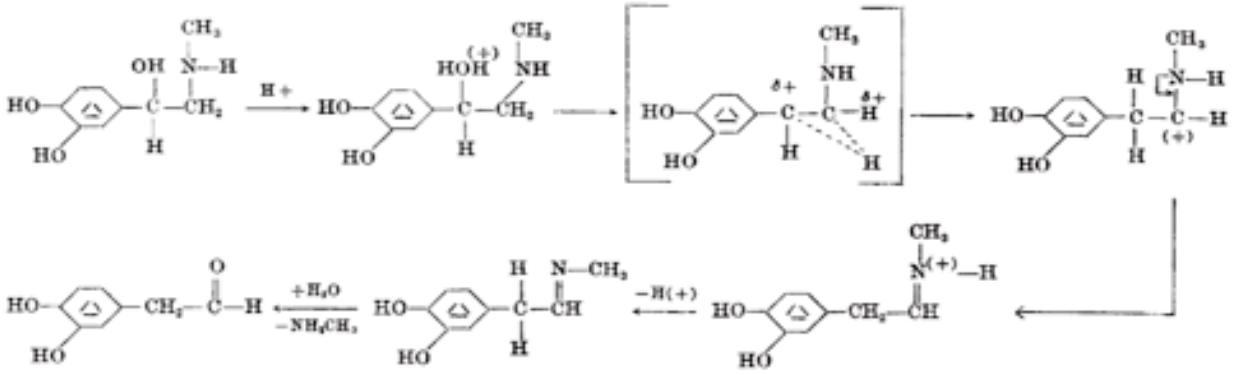


Model for conversion of the catechol epinephrine to the catecholaldehyde DOPAL.

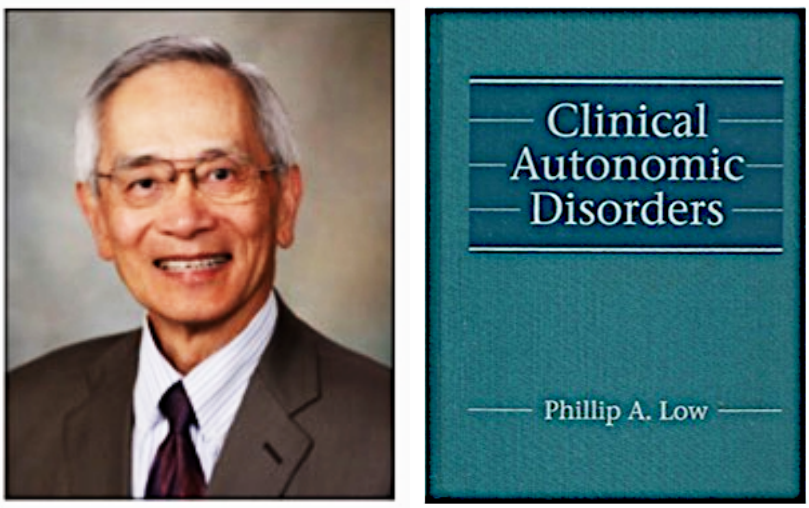
Crossroads

In 1984 Irv Kopin became the Scientific Director of the National Institute of Neurological and Communicative Disorders and Stroke. In 1990 he recruited me to leave the NHLBI to head the Clinical Neurochemistry Section in the Clinical Neuroscience Branch.

After 12 years in the NHLBI studying the sympathetic noradrenergic system (SNS) in hypertension, I had come to a crossroads. It was by then obvious to me that to understand mechanisms of hypertension I'd have to assess simultaneously many effectors or systems that regulate blood pressure. One of these, but only one, would be the SNS.

In retrospect, I was at this crossroads when I attended the first meeting of the American Autonomic Society (AAS) in Nashville in 1992. My presentation was on sympathetic mechanisms of hypertension. It seemed everyone else was presenting on autonomic failure syndromes.

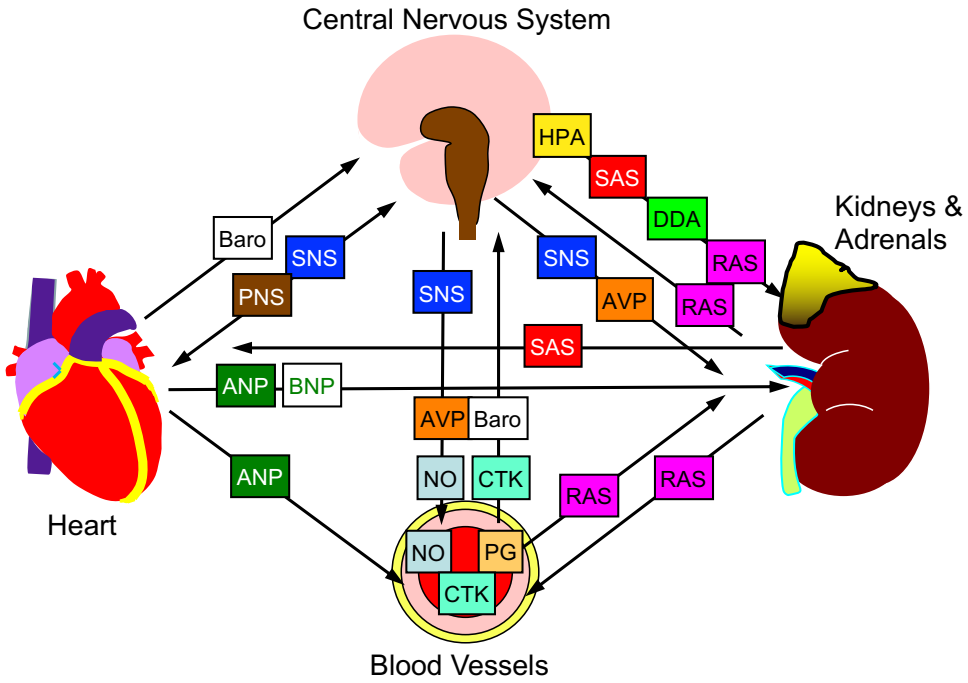
At that meeting Phillip Low, of the Mayo Clinic, introduced his multi-authored textbook, *Clinical Autonomic Disorders.* Among the many chapters there was none on the SNS in hypertension.



The first edition of Phillip A. Low's multi-authored textbook, Clinical Autonomic Disorders, was published in 1993. There was no chapter on the sympathetic nervous system in hypertension. Primary chronic autonomic failure consisted of pure autonomic failure (PAF) and the Shy-Drager syndrome (later referred to as multiple system atrophy, MSA).

Should I continue to study hypertension, along with other diseases of interest in the NHLBI at the time, such as hypertrophic cardiomyopathy and "Syndrome X" (referring to chest pain with normal coronary arteries), or should I shift to diseases of the SNS itself?

To understand the role of the SNS in cardiovascular regulation, would it be better to study hypertensive patients or patients who lack a functioning SNS?



Some systems regulating blood pressure. There are numerous bi-directional interactions among the central nervous system, heart, kidneys, adrenal glands, and blood vessels. Abbreviated, in alphabetical order: ANP=atrial natriuretic peptide (atriopeptin); AVP=arginine vasopressin; Baro=baroreflex afferents; CTK=cytokines; DDA=DOPA-dopamine autocrine-paracrine system; HPA=hypothalamic-pituitary-adrenocortical axis; NO=nitric oxide; PG=prostaglandins PNS=parasympathetic nervous system; RAS=renin-angiotensin-aldosterone system; SAS=sympathetic adrenergic system

In the same letter in which William Harvey wrote about nature's secret mysteries, he continued with the following.

> For it has been found in almost all things, that what they contain of useful or of applicable, is hardly perceived unless we are deprived of them, or they become deranged in some way.

I decided to study patients with rare forms of orthostatic hypotension (OH), a cardinal manifestation of SNS failure. Such patients had been followed and studied by Dr. Ronald J. Polinsky.

Ron left the NIH for another institution and then industry. I inherited his patients, as well as a freezer in the basement of Building 13 containing thousands of their plasma and cerebrospinal fluid specimens. A sad day in my scientific life came when Courtney and I emptied that freezer. The samples had to be trashed, because the tubes were labeled with Ron Polinsky's coding and the code was long since gone or because the tubes were labeled with personal identifying information and had to be discarded for confidentiality reasons.

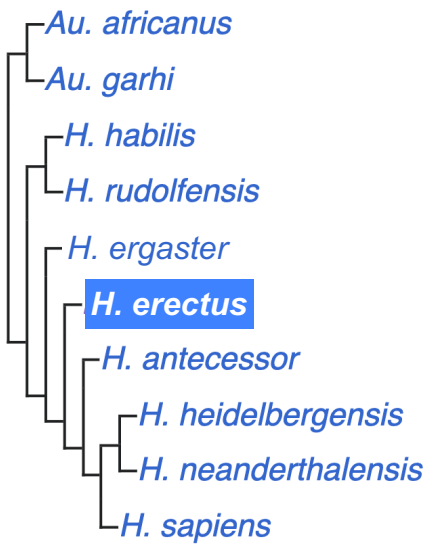
Homo erectus

A characteristic feature of us humans is that we stand. It is thought that in Africa about 5-6 million years ago a drier climate resulted in a humanoid shift from jungle to savannah life. Bipedalism enabled seeing further distances while migrating across savannahs, reaching via the freed-up arms, tool-making, carrying objects and infants, communication via hand gestures or arm waving, and more powerful striking and manipulating in agonistic encounters. Bipedalism is also more energy efficient and makes it easier to control body temperature. Even a small reduction in the energy used for movement would offer a huge selective advantage. According to cladographic data our ancestor *Homo erectus* came on the scene about 2.3 million years ago.

Standing up (orthostasis) necessarily imposes a gravitational stress. In order to tolerate standing the individual must be able to tighten blood vessels below the level of the heart and increase the force and rate of cardiac contraction, to maintain blood flow to the brain. Standing up is a relatively recent event in evolutionary terms. I think this is why, in contrast with the multiplicity of systems regulating blood pressure, only one system, the sympathetic noradrenergic system (SNS), regulates the total peripheral resistance to blood flow via arteriolar constrictor tone and the pattern of distribution of the cardiac output so as to maintain blood flow to the brain during orthostasis.

I think this is why orthostatic hypotension (OH), a fall in blood pressure during standing, is a cardinal sign of SNS failure.

Norepinephrine (NE) is the main chemical messenger of the SNS in cardiovascular regulation. Plasma NE levels normally approximately double within 5 minutes of standing up from lying down.



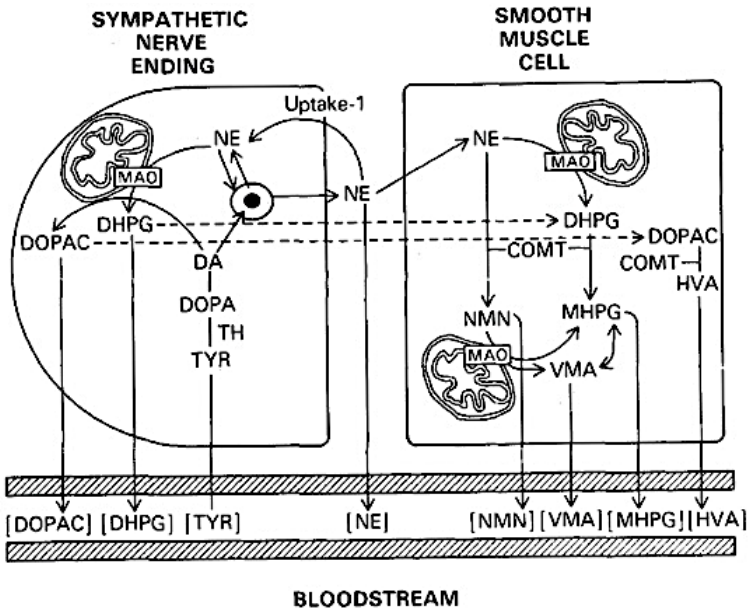
Cladogram of evolution of Homo erectus about 2.3 million years ago. A characteristic feature of H. erectus was bipedalism. The upright posture afforded survival advantages but also introduced the problem of maintaining blood flow to the brain during prolonged standing. The sympathetic noradrenergic system (SNS) enabled regional changes in vascular caliber and shifts in the distribution of cardiac output.

Ron Polinsky, Irv Kopin, and I collaborated on the first application of the plasma catechols assay in autonomic failure syndromes involving OH. The article, published in *Annals of Neurology* in 1989, is a citation classic. We found that pure autonomic failure (PAF) and multiple system atrophy (MSA) differed in that plasma NE and DHPG were low in PAF and not in MSA.

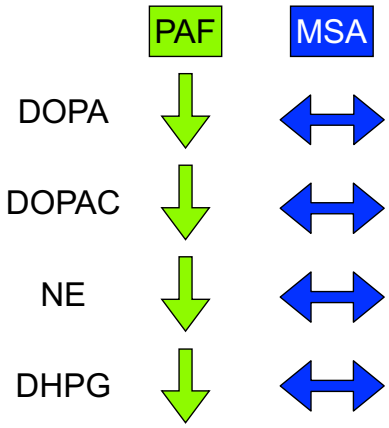
In retrospect, the *Annals of Neurology* report provided an example of how concepts used to interpret data can be incomplete or erroneous and can be superseded by newer concepts, whereas the data themselves are forever. The finding I have in mind was the ratio of DHPG/NE in plasma. The patient group with pure autonomic failure (PAF) had an elevated mean

DHPG/NE ratio. As indicated by inspection of the concept diagram in the article (reproduced below), there were several potential explanations for this finding. The one we advocated for was that newly synthesized NE is preferentially released during sympathetic stimulation but also leaks preferentially from the vesicles into the cytoplasm. Even if NE release were markedly reduced, DHPG could still be produced because of this leakage process. In the group with PAF, the mean ratio of the DHPG/NE ratio level was almost 4 times that of the control subjects.

At the time we did not consider an alternative possibility, decreased ability to recycle NE back into the vesicles, which would shift the fate of cytoplasmic NE toward metabolism by monoamine oxidase (MAO) to form DHPG. Our subsequent work indicated that a vesicular storage defect seems to be a major problem in PAF and other Lewy body diseases such as Parkinson's disease with OH. The data were correct, but the interpretation was incomplete.



Concept diagram illustrating the neuronal and extraneuronal fates of norepinephrine (NE). High plasma DHPG/NE ratios could have a variety of explanations. One that wasn't mentioned in the report was an inability to recycle NE efficiently back into vesicles, which would deliver more NE to monoamine oxidase (MAO), forming DHPG.



The pattern of plasma catechols in PAF indicated decreased catecholamine biosynthesis, such as from sympathetic denervation.

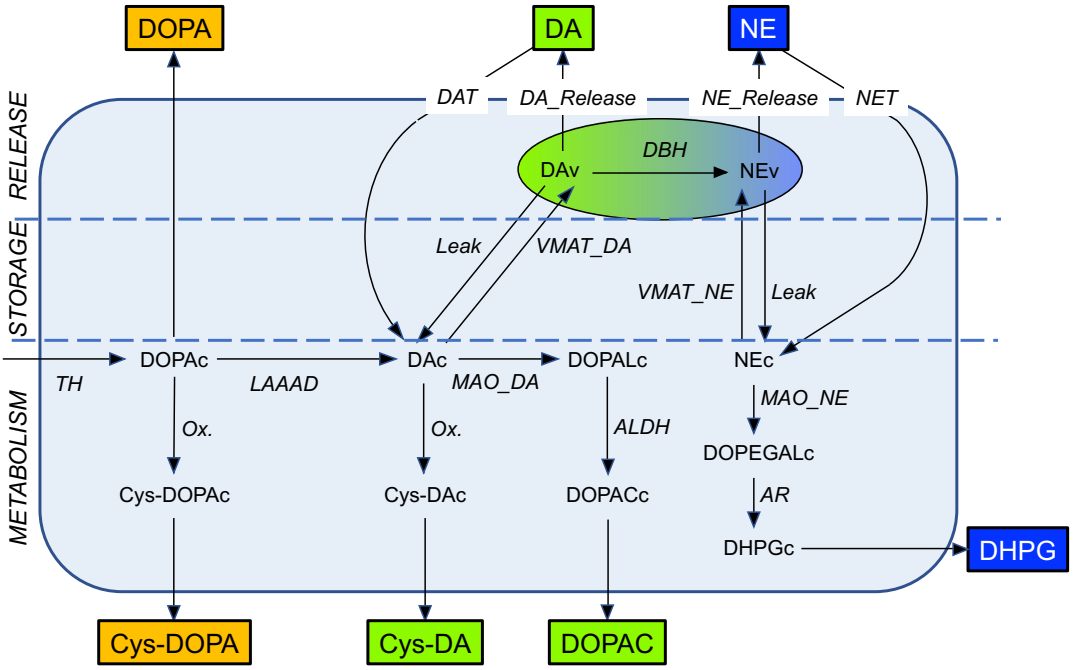
A neurochemical window on the brain

In the 1960s Julie Axelrod and colleagues showed that there is an efficient blood-brain barrier for catecholamines. This meant that assaying cerebrospinal fluid (CSF) levels of catechols might provide a neurochemical window on the status of catecholamine systems in the brain.

For instance, as indicated in the concept diagrams, if there were central DA deficiency, then CSF DOPAC would be decreased. If there were central NE deficiency, then CSF DHPG would be decreased. If there were a shift in the fate of cytoplasmic DA away from enzymatic metabolism by monoamine oxidase (MAO) towards spontaneous oxidation (Ox.) to form 5-S-cysteinyldopamine (Cys-DA), then the ratio of Cys-DA/DOPAC would be increased. if there were a vesicular storage defect, then CSF DOPAC would be increased relative to CSF DA, and CSF DHPG would be increased relative to CSF NE. If there were inhibition of the cell membrane catecholamine transporters (the DAT and the NET), then the CSF DOPAC/DA and DHPG/NE ratios would be decreased. If there a decrease in the activity of L-aromatic-amino-acid decarboxylase (LAAAD), then there would be a buildup of DOPA and 5-S-cysteinylDOPA(Cys-DOPA) with respect to DA, NE, and its metabolites. And so forth.

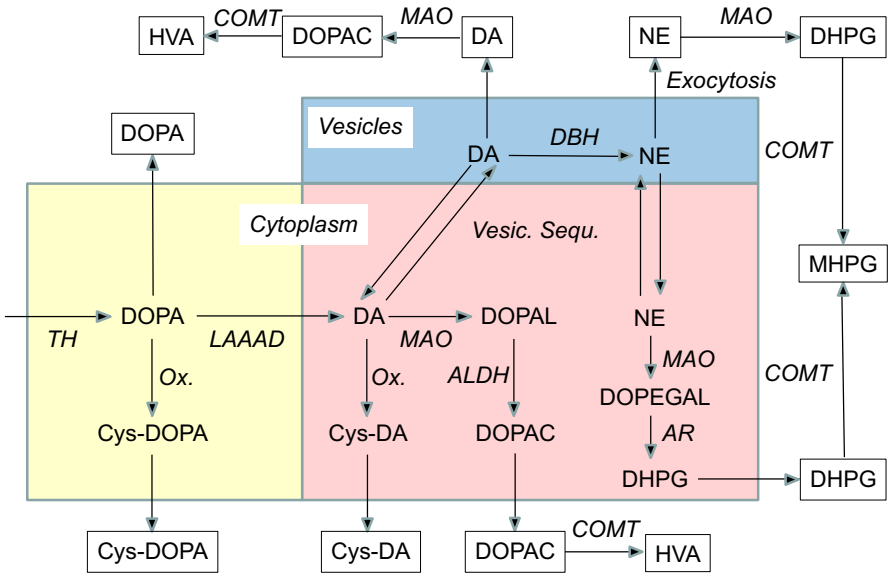
Applying our LC-ED methodology for catechols we reported decreased CSF levels of NE and DHPG in patients with neurogenic OH associated with multiple system atrophy (MSA) and pure autonomic failure (PAF).

The combination of low CSF and DHPG pointed to central NE deficiency in nOH from MSA or PAF. We elaborated on this theme over the next two decades, by extending the analyses to Parkinson's disease (PD). PAF and PD are Lewy body forms of alpha-synucleinopathy, and MSA is a non-Lewy body form. CSF levels of DHPG and DOPAC were low in all three forms of synucleinopathy, indicating the central catecholamine deficiency occurs in this class of diseases regardless of the occurrence of Lewy bodies. Since DOPAC was lower and DHPG higher in PD than in PAF, the two Lewy body diseases seemed to differ in the extents of dopaminergic vs. noradrenergic deficiency. Finally, in a subgroup of PD patients who had CSF sampled before or within 2 years after the onset of parkinsonism, CSF DOPAC was low, suggesting that this neurochemical index of central DA deficiency may identify early PD.

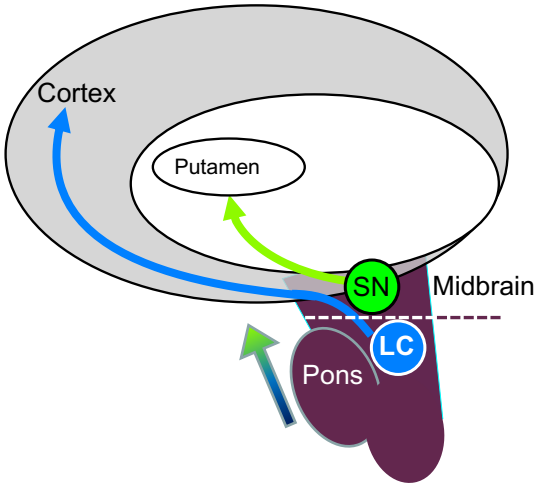


Just as measuring levels of catechols in plasma enables examining specific processes in sympathetic nerves, measuring the same biochemicals in cerebrospinal fluid provides a means to assess intra-neuronal functions in catecholaminergic terminals in the brain.

In 2021 we extended the neurochemical analyses to CSF levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), which is the main central neural metabolite of NE, and homovanillic acid (HVA), the main metabolite of DA.



By directly injecting small amounts of CSF directly into the LC-ED system we extended the neurochemical methodology to MHPG, the main end-product of central NE metabolism, and to HVA, the main end-product of DA metabolism.



The CSF neurochemical data fit with an ascending pathogenetic sequence for evolution of PAF to PD+OH, with early involvement of the pontine noradrenergic locus ceruleus (LC) followed by involvement of the midbrain dopaminergic substantia nigra (SN).

We accomplished this by simply injecting small amounts of CSF directly into the LC-ED system. We could get by with small volumes of injectate because of the relatively high concentrations of MHPG and HVA compared to their catechol precursors DHPG and DOPAC. The difference between PAF and PD was now clearer, since in PAF CSF HVA levels were normal, whereas in PD CSF HVA levels were decreased. Both diseases entailed decreased CSF MHPG levels.

DOPAL and catecholamine autotoxicity

It is generally accepted that neurodegenerative diseases involving catecholamine systems result from an interplay of genetic predispositions and environmental exposures—"nature" and "nurture," or the "seed" and the "soil." This view does not address well the aging-relatedness of major neurodegenerative diseases such as Parkinson's disease. A third causal factor is aging, but how aging adds to the nature/nurture concept is unclear.

I've written about "autotoxicity" exerted by catecholamines or their metabolites. This section is about extending the repertoire of neurochemical analytes to include the catechol 3,4-dihydroxyphenylacetaldehyde (DOPAL). Although all the neuronal enzymatic metabolism of dopamine passes through DOPAL, the world's literature on DOPAL consists of only about 160 articles.

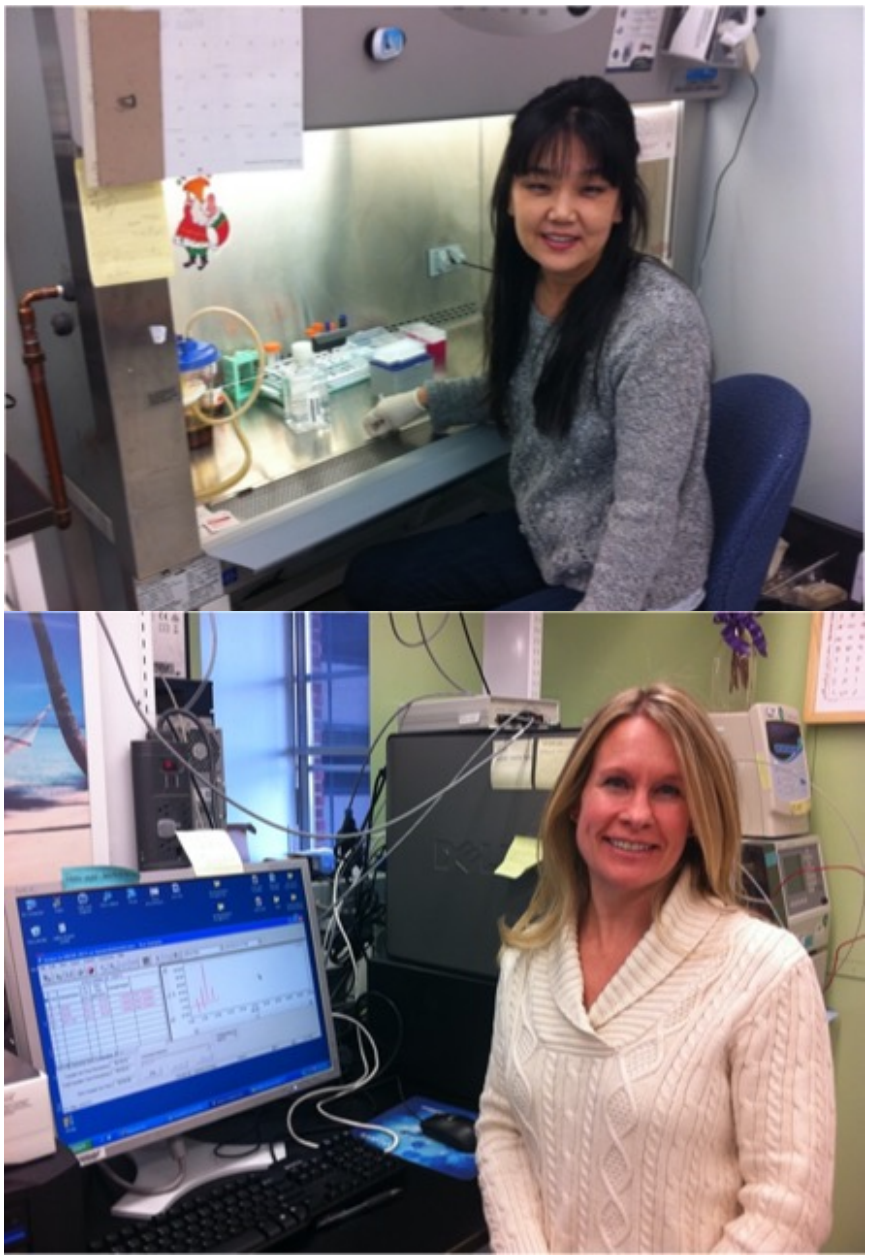
The DOPAL story in our lab began with Itzchak Lamensdorf, Graeme Eisenhofer, and Judy Harvey-White, who supervised by Irv Kopin discovered in 2000 that incubation with the metabolic inhibitor rotenone increases the formation of endogenous DOPAL in rat pheochromocytoma PC12 cells. Moreover, DOPAL was toxic to the cells. Treatment to decrease rotenone-induced DOPAL production attenuated rotenone's toxic effects.

I had these observations in mind when Yunden Jinsmaa joined our lab. Jinsmaa came from the lab of Jonathan Doorn at the University of Iowa and already has some experience with DOPAL, since the Doorn group had found that lipid peroxidation products increase DOPAL formation.

Jinsmaa was highly skilled at culturing rat pheochromocytoma PC12 cells. We found these cells were special in that they not only produced dopamine but also produced DOPAL and DOPAC endogenously. Jinsmaa, Patti, and I conducted a series of experiments about the sources and significance of DOPAL in PC12 cells.

One of the first was to block vesicular uptake via the vesicular monoamine transporter (VMAT) using reserpine. As predicted from the concept diagram, reserpine depleted the cellular content of dopamine (DA) while increasing the content of DOPAL in the cells and medium. The notion of a shift in the fate of cytoplasmic DA from vesicular uptake to formation of DOPAL via monoamine oxidase (MAO) became a key part of the “catecholaldehyde hypothesis” for the pathogenesis of Lewy body diseases such as Parkinson’s disease (PD).

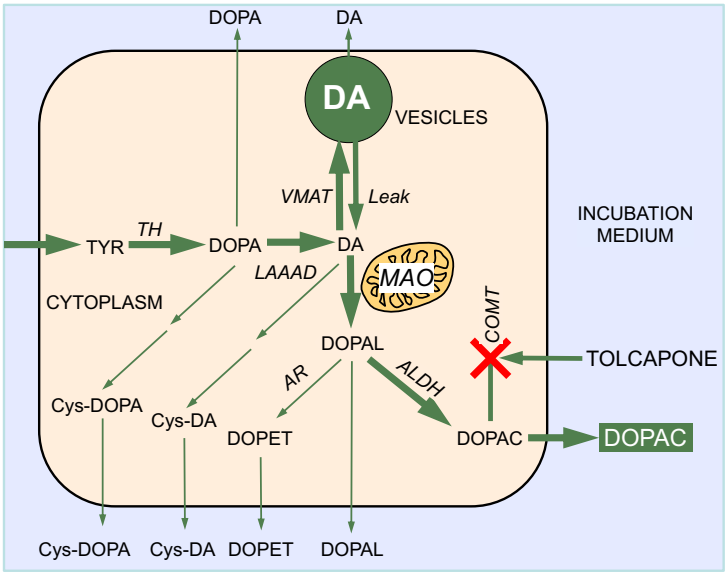
Cytoplasmic DA has a third fate besides uptake into vesicles and oxidative deamination catalyzed by MAO. This is spontaneous oxidation to form DA-quinone and then a variety of downstream oxidation products, one of which is 5-S-cysteinyldopamine (Cys-DA). The ability to assay Cys-DA simultaneously with DOPAL and DOPAC enabled the discovery of the ”MAOI tradeoff.” As indicated in the diagram, treatment with a MAO inhibitor (MAOI) would be expected to increase vesicular uptake of cytoplasmic DA and also increase Cys-DA production, and this is what Jinsmaa and Patti found. Since both DOPAL and spontaneous oxidation products such as Cys-DA are toxic, the beneficial effects of MAOI in terms of decreasing levels of DOPAL could be offset by the harmful effects in terms of increasing levels of the spontaneous oxidation products. As explained later, the MAOI tradeoff may help explain why experimental therapeutic trials using MAO inhibitors failed to slow the neurodegenerative process in patients with symptomatic PD.



Yunden Jinsmaa (“Jinsmaa”) (top) and Patti Sullivan (bottom) carried out key experiments about factors determining endogenous production of DOPAL, DOPAL interactions with numerous proteins including alpha-synuclein, and mitigation of DOPAL-induced protein modifications by N-acetylcysteine (NAC).

Probably Jinsmaa’s most important contribution was demonstrating that DOPAL potently modifies the protein alpha-synuclein (αS). αS is a key component of Lewy bodies, a histopathologic hallmark of PD, and αS

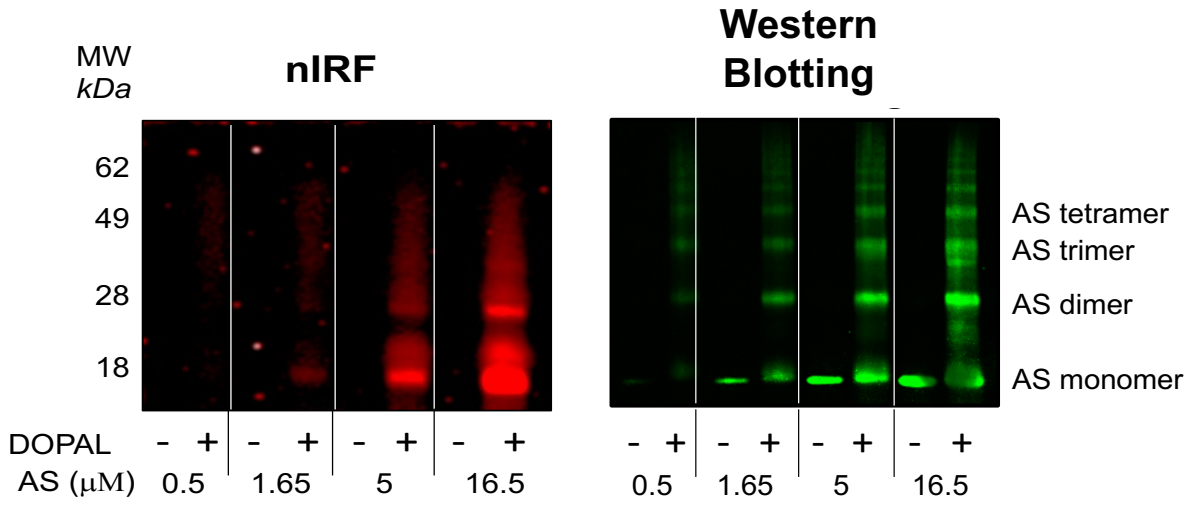
misfolding is thought to be a major pathogenetic step in the loss of DA neurons in PD. Jinsmaa found that DOPAL potently induces αS to form oligomers, which are thought to be the toxic form of the protein. Incubation of cells with DOPAL also results in quinoprotein adducts with αS and a variety of other proteins ("quinonization"). Finally, co-incubation of cells with DOPAL and αS results in intracellular aggregates of αS. Importantly, Jinsmaa discovered that the antioxidant, N-acetylcysteine (NAC), mitigates all these effects of DOPAL on αS and other intracellular proteins.



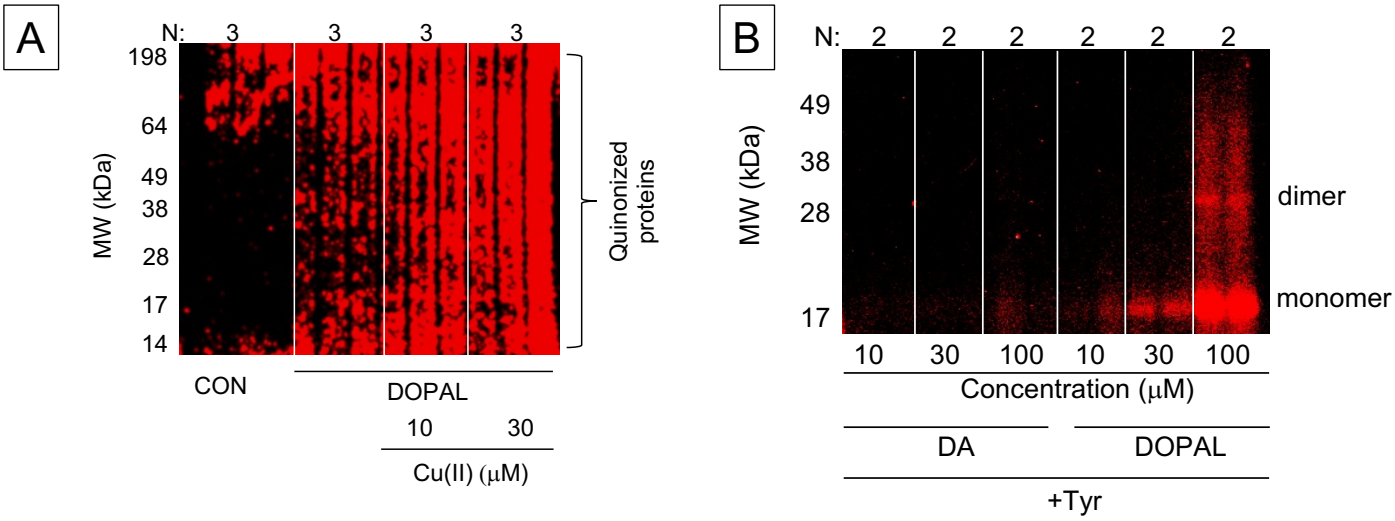
Experimental setup for measuring endogenous catechols in rat pheochromocytoma (PC12) cells and incubation medium. The cells were pre-incubated with tolcapone to block metabolism of DOPAC by catechol-O-methyltransferase (COMT). The main catechol in the cells is dopamine (DA), due to efficient storage of DA in vesicles. The main catechol in the incubation medium is DOPAC, due to the incubation medium acting as a "sink" for accumulation of all the compounds released from the cells.

Jinsmaa discovered that DOPAL spontaneously oxidizes to DOPAL-quinone, and DOPAL-quinone might mediate the toxic interactions between DOPAL and αS. Jinsmaa's findings inspired us to propose combining a MAOI with NAC, to avert the MAOI tradeoff and decrease spontaneous oxidation of DOPAL to DOPAL-quinone, a novel potential treatment for PD. Unfortunately, this entire line of research had to be ended based on the

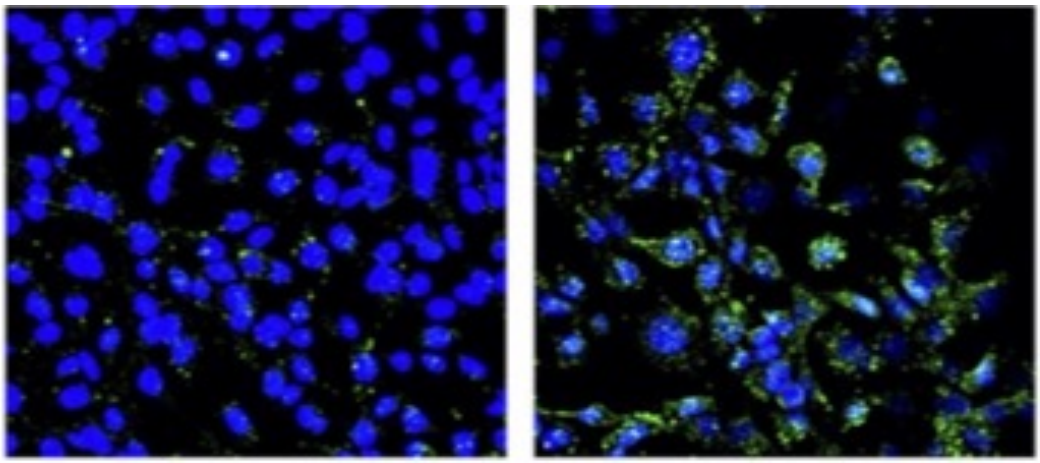
NINDS Board of Scientific Counselors' (BSC's) review of our program. The BSC thought our basic cellular work wasn't competitive, and our budget was cut. Jinsmaa went on to head a laboratory in industry. We continued to conduct cellular catechol neurochemistry experiments in collaborations with other groups.



Jinsmaa discovered that DOPAL (left) forms quinoprotein adducts with (quinonizes) and (right) oligomerizes alpha-synuclein (AS).



Jinsmaa also discovered that DOPAL quinonizes myriad intracellular proteins (A) and in this respect is far more potent than dopamine (B).



Jinsmaa discovered that compared to incubation of human glial cells with alpha-synuclein (AS) alone (left), incubation with AS+DOPAL resulted in aggregates of AS (right), demonstrated by the green specks.

Tissue catechols

Epileptic foci

HPLC with electrochemical detection after batch alumina extraction can be used to measure levels of catechols in essentially any biological matrix—plasma, urine, cerebrospinal fluid, skeletal muscle microdialysate, ovarian cystic fluid, umbilical cord blood, mosquito hemolymph, fruit fly heads, and human tissues biopsied during life or harvested after death.

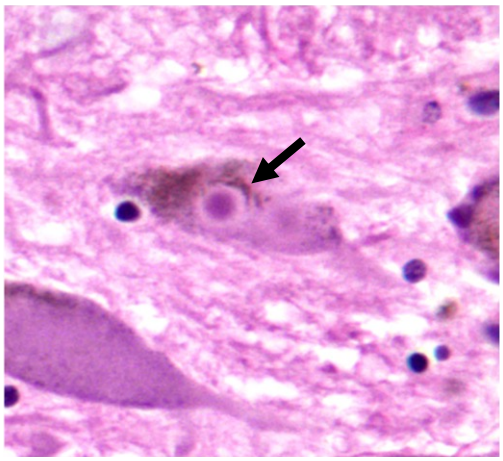
The first time we assayed catechols in human brain tissue was in a study in collaboration with N. Suzan (“Sue”) Nadi, an epilepsy researcher. In those days (the late 1980s) epileptic foci were being removed neurosurgically at the NIH under electrocorticographic guidance, and a rim of normal surrounding tissue would also be excised. We asked whether tissue concentrations of catechols differed between the affected and unaffected regions of cerebral cortex. There were differences, but actually what made the study special was that we obtained the first values for tissue levels of catechols in normal human cortex.

The ultimate act of philanthropy

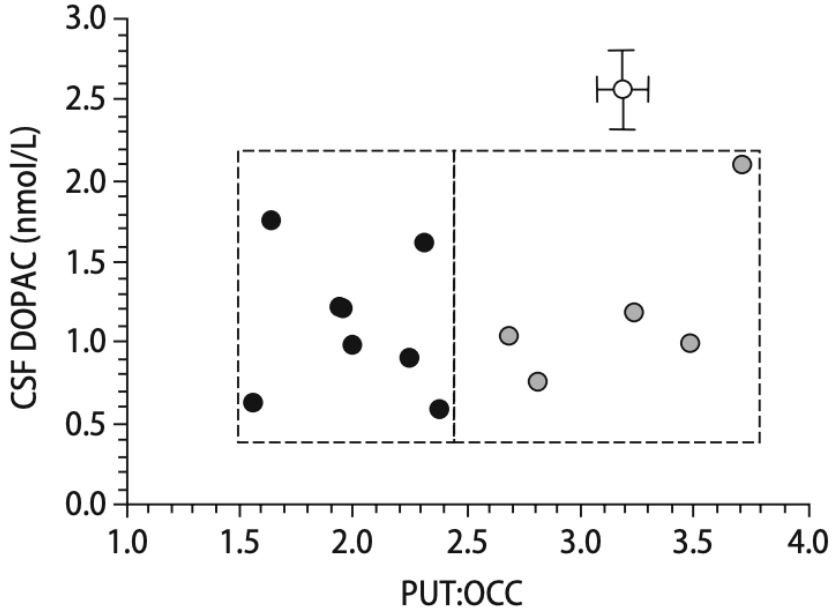
Many patients I've evaluated and followed over the years at the NIH Clinical Center have expressed the wish to have their tissues harvested at autopsy, to increase understanding of their diseases and benefit our research. I consider this to be the ultimate act of philanthropy. In this section I highlight a discovery that came from acceding to their wishes.

An adult woman had severe orthostatic hypotension (OH, a fall in blood pressure every time she stood up). The OH was neurogenic, meaning that it was the result of a failure of the sympathetic noradrenergic system. Since she had no identified secondary cause (such as from medications or diabetes) and had no signs of central neurodegeneration like parkinsonism or cognitive dysfunction, she was diagnosed with pure autonomic failure (PAF).

At autopsy many years later, she had low tissue concentrations of dopamine and of tyrosine hydroxylase activity (a marker of catecholaminergic neurons) in the substantia nigra, which is a major source of dopamine in the brain. She also had typical Lewy bodies in her brainstem, demonstrating that PAF can be a Lewy body disease, as in Parkinson's disease (PD). Meanwhile, there was no evidence of loss of dopaminergic terminals in the basal ganglia. This was the first evidence for central dopamine deficiency in PAF.



A Lewy body (arrow) in the brainstem of a patient with PAF.

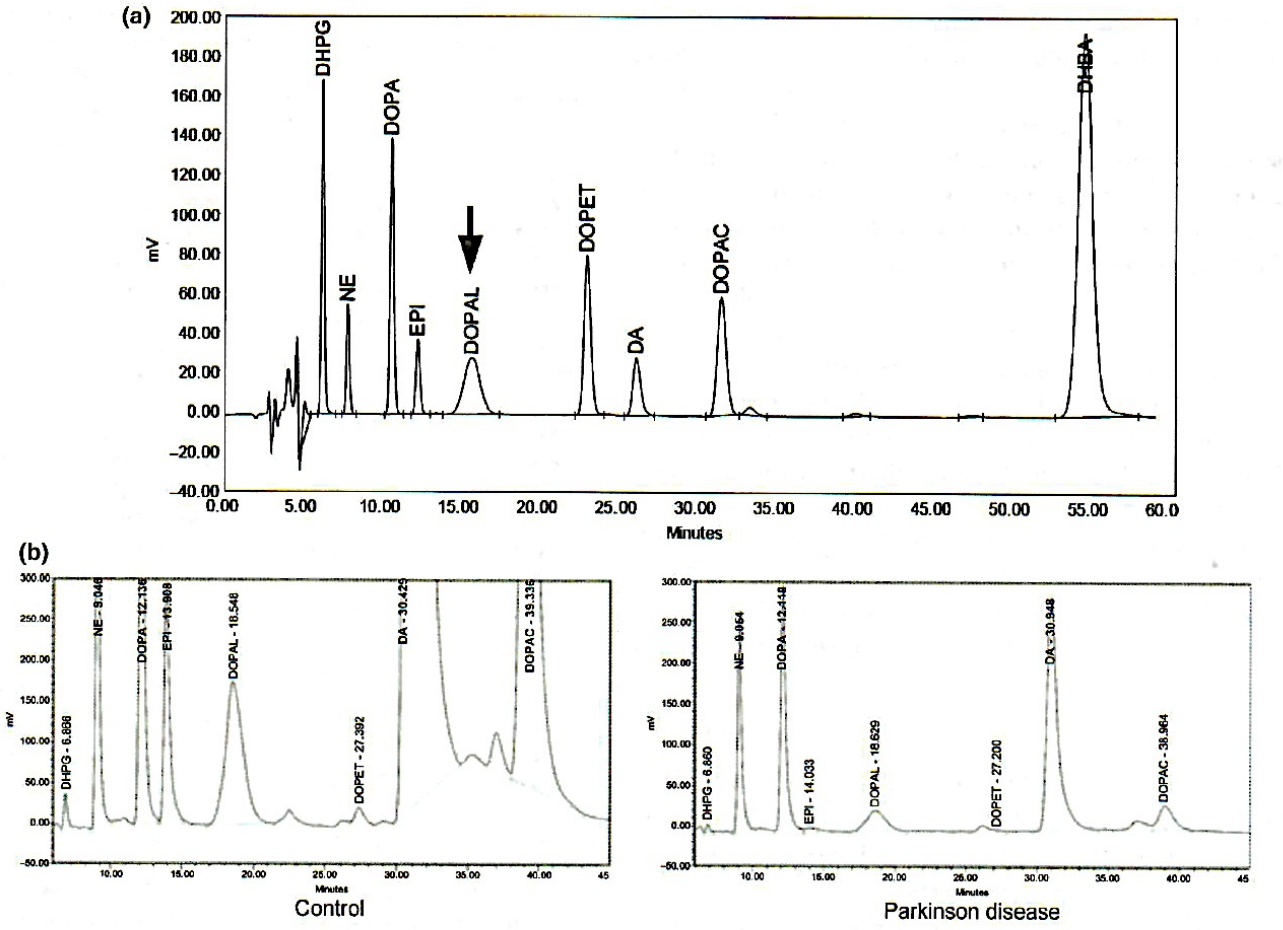


PAF patients (gray) and Parkinson's disease patients (black) have in common low CSF DOPAC, indicating central dopamine deficiency in both diseases. The groups differ in that in PAF putamen dopaminergic innervation is intact.

DOPAL in post-mortem brain tissue

Patti Sullivan was already a veteran in assaying catechols and metanephrines by LC-ED when she converted from a Technologist in the Clinical Pathology Department of the NIH Clinical Center to a Biologist in our Section in 2008. After Courtney retired, Patti took charge of our catecholamine assay lab. She continued a tradition of technical excellence, reliability, extraordinary productivity, and proactive problem-solving for both the clinical and collaborative preclinical research of our group.

Jinsmaa's and Patti's data about sources and significance of endogenous DOPAL in PC12 cells led us to explore whether DOPAL is present in post-mortem brain tissue. It was.



Chromatographs of (top) catechol standards, (bottom left) catechols in post-mortem putamen from a control subject, and (bottom right) catechols in putamen from a Parkinson's disease (PD) patient. Note that the DOPAL peak is short and fat. In the PD patient DOPAL is built up with respect to dopamine (DA) and DOPAC.

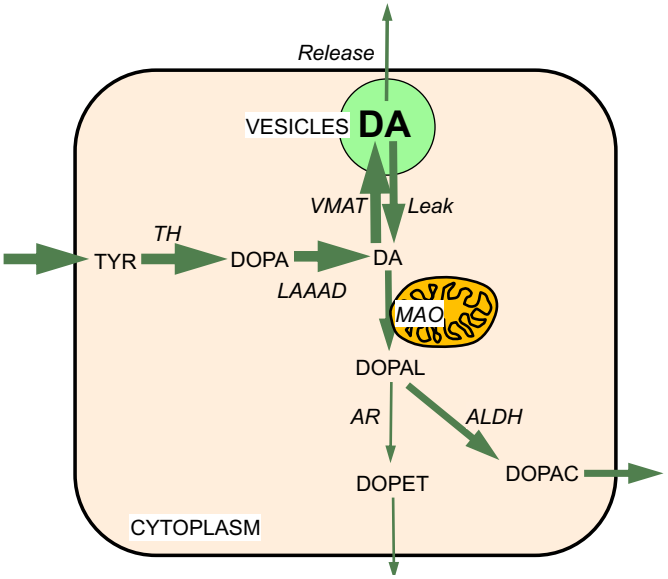
We discovered that DOPAL has a peculiar appearance in LC-ED chromatographs. Ordinarily, as the retention time increases the chromatographic peaks get shorter and broader. In the case of DOPAL, the peak was too short and too broad. Subsequent peaks were spikier and taller. Sometimes the DOPAL peak also wasn't a smooth bell-shaped curve. Instead, there could be a "horn" superimposed on the DOPAL peak. An explanation is that DOPAL, as an aldehyde, can exist in multiple forms, called tautomers. Tautomers are defined as isomers of chemical

compounds that readily interconvert. The interconversion commonly reflects relocation of a hydrogen atom within the compound. Since the molecular weights and structures of the tautomers are essentially the same, the DOPAL chromatographic peak actually consists of multiple peaks with about the same retention time. The result is that the DOPAL peak is too short and too fat.

From the chromatographs one can appreciate several abnormalities. First, the dopamine (DA) and DOPAC peaks are on scale rather than being way above scale. This demonstrates substantial putamen DA depletion. Second, compared to the DA peak, the DOPAL peak is big. Third, the DOPAL peak is also big with respect to DOPAC. Fourth, the norepinephrine (NE) and DHPG peaks are small. Fifth, the epinephrine (EPI) is larger in the control subject than the PD patient.

The concept diagrams help understand the abnormal neurochemical pattern in PD. The depletion of DA and DOPAC reflects loss of dopaminergic terminals (denervation) or decreased ability to take up DA from the cytoplasm into the vesicles (an example of the sick-but-not-dead phenomenon). The decreased DOPAC/DOPAL ratio indicates decreased activity of aldehyde dehydrogenase (ALDH), the enzyme that converts DOPAL to DOPAC.

We found that putamen tissue harvested at autopsy from patients with PD had a buildup of DOPAL after adjusting for DA deficiency. We identified 2 determinants of the DOPAL buildup. The first is a vesicular storage defect. That is, DA produced in the cytoplasm is not taken up efficiently into the vesicles or leaks from the vesicles back into the cytoplasm, where monoamine oxidase (MAO) in the outer mitochondrial membrane can catalyze the conversion of DA to DOPAL. Second, there is decreased activity of aldehyde dehydrogenase (ALDH), the enzyme that converts DOPAL to the acid catechol 3,4-dihydroxyphenylacetic acid (DOPAC).



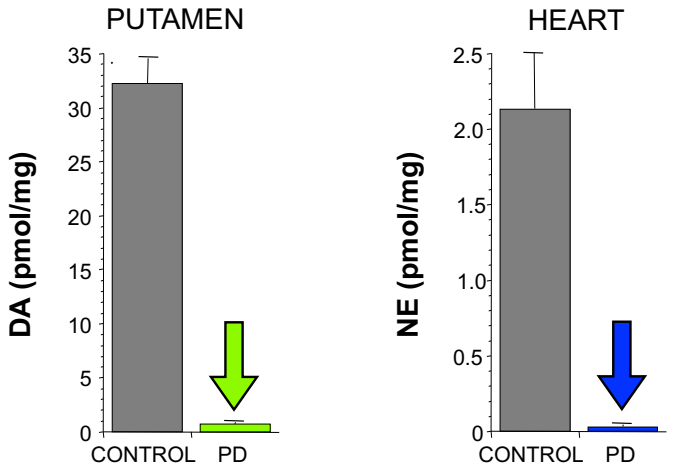
Concept diagram to explain the abnormal pattern of tissue catechols in PD.

Cardiac noradrenergic deficiency

As discussed later in the section on neuroimaging, our 1997 report of evidence for loss of sympathetic noradrenergic nerves in the heart in PD provided an early indication that in PD the disease process in not confined to the brain. The gold standard for identifying cardiac noradrenergic deficiency is a decrease in the tissue content of norepinephrine (NE), which is the main neurotransmitter of the sympathetic nervous system in cardiovascular regulation. By applying our catechols assay to post-mortem tissue harvested from patients with PD and PAF we confirmed cardiac NE deficiency in both these Lewy body diseases.

We found that the extent of NE deficiency in the left ventricular myocardium in PD was about equally severe as the extent of dopamine (DA) deficiency in the striatum that causes the movement disorder.

I've been asked many times what the functional significance is of cardiac NE deficiency in PD. I don't know for sure. You don't need sympathetic innervation for your heart to beat—if you had a heart transplant there'd be no nervous connections to the donor heart at all, but it still would beat.



Kish et al., NEJM 1988;318:876-880

Goldstein et al., JCI Insight 2019; doi: 10.1172/jci.insight.130441

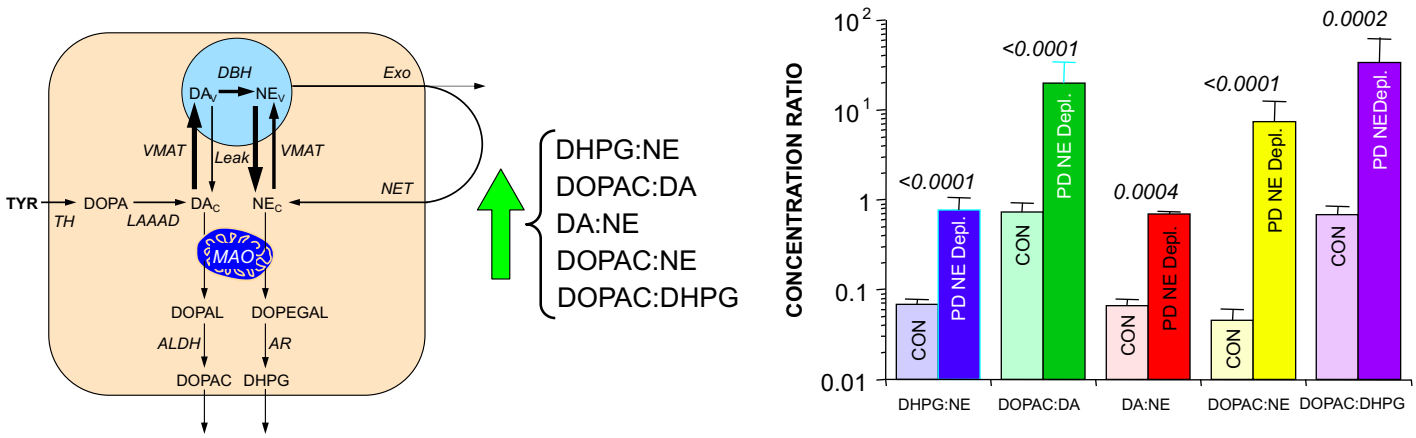
In PD the extent of decrease in myocardial norepinephrine (NE) content is about the same as the decrease in putamen dopamine (DA) content.

I do think you need cardiac sympathetic noradrenergic nerves for appropriate increases in the rate and force of heart contraction during stress. If so, cardiac noradrenergic deficiency would produce non-specific symptoms such as shortness of breath with exercise and fatigue. Sympathetic nerves may also be needed for most efficiently regulating the conduction of electrical impulses in the heart.

The sick-but-not-dead phenomenon

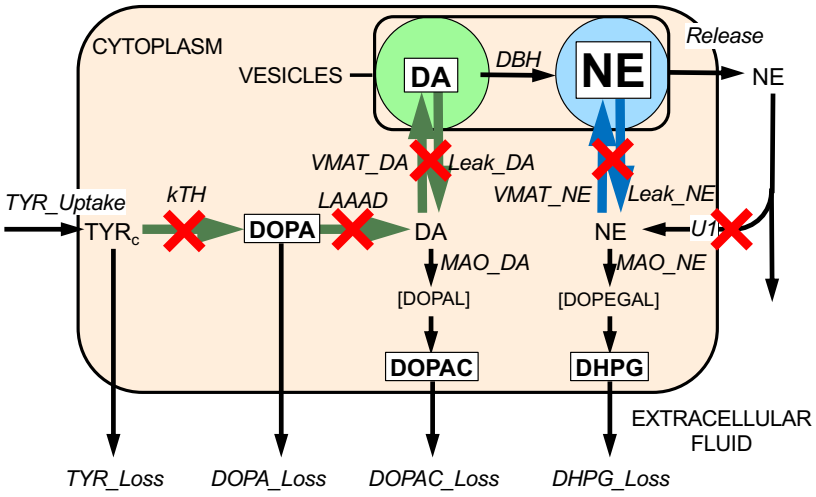
The occurrence of cardiac noradrenergic deficiency in PD does necessarily mean that the deficiency directly and solely reflects loss of sympathetic nerves. If there were a decreased ability to take up catecholamines from the cytoplasm into the vesicles or decreased NE synthesis and recycling there would be depletion of NE stores, even without a loss of nerves. I've called this the "sick-but-not-dead" phenomenon.

We devised 5 post-mortem biochemical indices that would indicate a vesicular storage defect. PD patients with myocardial NE depletion had all 5 indices.



(Left) Concept diagram according to which 5 different catechol ratios would indicate decreased vesicular sequestration of cytoplasmic catecholamines. (Right) By all 5 indices patients with PD and cardiac NE depletion had a vesicular storage defect.

From empirical data about post-mortem tissue levels of catechols and in vivo cardiac NE kinetics we developed a mathematical approach to evaluate all the known processes determining cardiac NE stores in Lewy body diseases (LBDs). The modeling revealed multiple functional abnormalities in cardiac sympathetic nerves in LBDs, giving meaning to the sick-but-not-dead phenomenon.

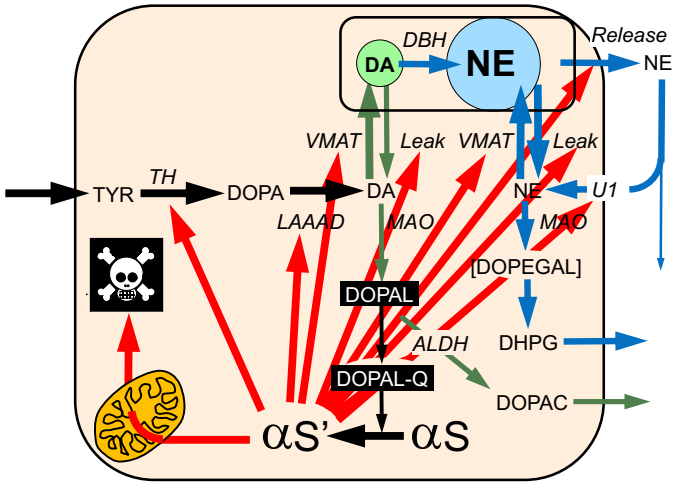


Computational modeling revealed multiple functional abnormalities (red X marks) in residual sympathetic nerves in patients with Lewy body diseases.

The sick-but-not-dead phenomenon has profound implications for treatment and prevention. You can't treat dead neurons. Neurons that are dysfunctional but alive might be salvaged.

The catecholaldehyde hypothesis

The world's literature about DOPAL consists of fewer than 200 articles. Nevertheless, all of the neuronal metabolism of dopamine passes through DOPAL.



Concept diagram depicting harmful effects of DOPAL-alpha-synuclein (αS) interactions.

Intra-neuronal DOPAL buildup is toxic, by a variety of mechanisms. One of these seems to be via its spontaneous oxidation to DOPAL-quinone (DOPAL-Q) and then the DOPAL-Q binding to and modifying numerous intracellular proteins—especially alpha-synuclein (αS). According to the "catecholaldehyde hypothesis," DOPAL-induced αS modifications interfere with a variety of intraneuronal processes, decreasing the ability to maintain neuronal homeostasis.

The getaway car analogy

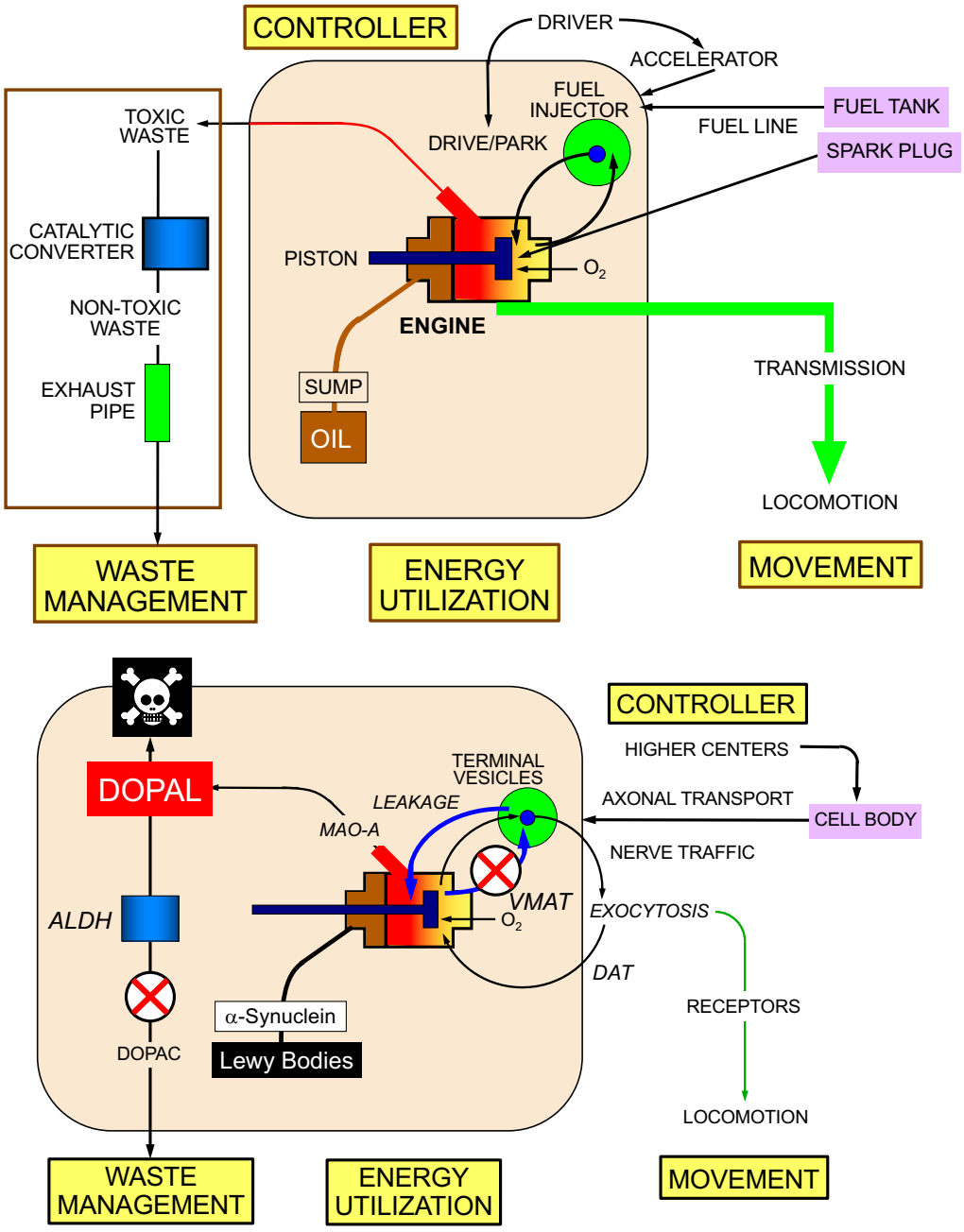
One way to grasp the catecholaldehyde hypothesis is by analogy to a bank robber's getaway car. Automobiles use energy under the direction of

a controller for locomotion. A getaway car is in idle all the time. This means toxic byproducts of combustion are being produced all the time. The toxic waste is converted to non-toxic waste by a catalytic converter. Oil lubricating the pistons cycles from and to a sump. For the sake of analogy let's assume that uncombusted fuel can be returned to the fuel injector and recycled.

Catecholamine neurons are like a getaway car engine. They're in idle all the time. This enables rapid shifting to fight-or-flight behaviors. The vesicles are leaky, so that even without exocytotic release there is ongoing enzymatic oxidation of cytoplasmic catecholamine. Because of the leaky vesicles there is a relatively rapid ongoing rate of catecholamine synthesis. This enables a longer period of increased exocytosis during stress before the vesicular stores are exhausted. The immediate product of the oxidation is DOPAL, which is toxic, but DOPAL normally is detoxified by the enzyme ALDH (enzymes literally are catalytic converters). PD involves decreased ALDH activity and decreased efficiency of catecholamine recycling back into the vesicles, promoting DOPAL accumulation. DOPAL aggregates αS, deposits of which characterize Lewy bodies. αS deposits are like gunk building up in the engine. Eventually there is enough gunk that the engine is less efficient; more fuel is injected to keep the idling rate. This is a positive feedback loop that eventually destroys the engine. A "post-mortem" would reveal frozen pistons and gunk and likely not reveal the original causes that led to engine failure.

The MAOI tradeoff

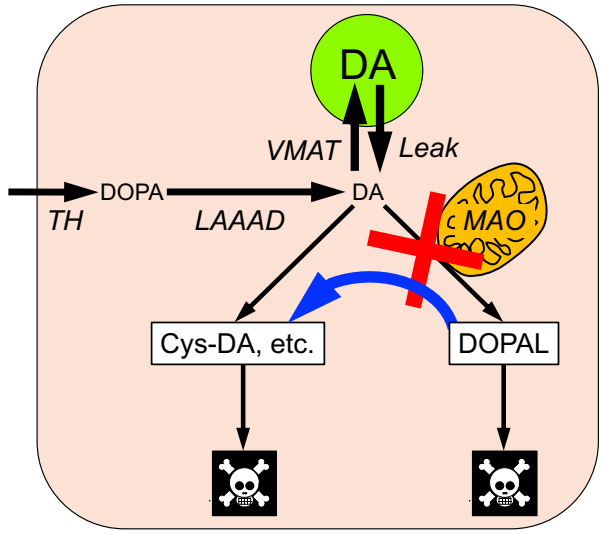
Large clinical trials of monoamine oxidase (MAO) inhibitors, or MAOIs, have failed to show clear benefit in terms of slowing the symptomatic progression of Parkinson's disease. A potential explanation for the lack of efficacy is the "MAOI tradeoff." MAO inhibition decreases DOPAL-mediated toxicity but at the expense of increasing toxicity from spontaneous oxidation products of cytoplasmic dopamine, such as 5S-cysteinyldopamine (Cys-DA). We were the first to measure DOPAL and Cys-DA simultaneously, demonstrating the shift from enzymatic to spontaneous oxidation of DA in catecholaminergic cells.



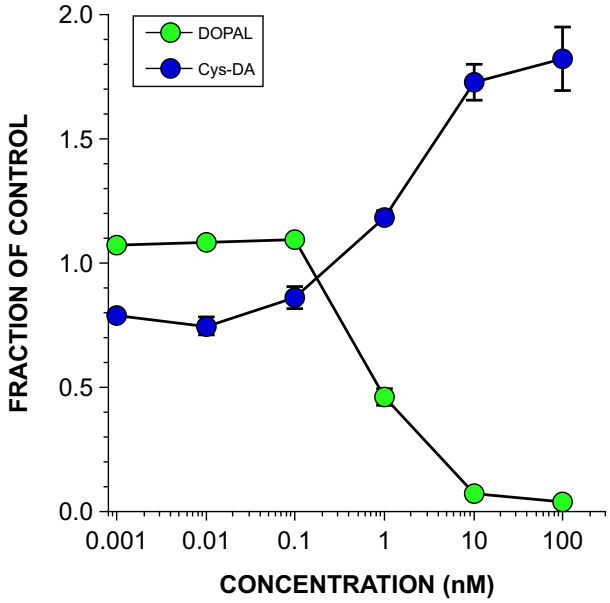
The getaway car analogy. Catecholamine neurons are like a getaway car's engine—always in idle.

The MAOI tradeoff probably was only a minor cause of the failure of MAO inhibition to slow the symptomatic progression of PD. The likely main cause was that by the time a patient has symptoms of the movement disorder, the degeneration is already advanced. This highlights the

importance of identifying the disease process in a pre-symptomatic phase.



The MAOI tradeoff. Monoamine oxidase inhibition (MAOI) shifts the fate of cytoplasmic dopamine (DA) away from enzymatic oxidation catalyzed by MAO towards spontaneous oxidation. The spontaneous oxidation products are toxic, do MAOI trades off one form of toxicity for another.

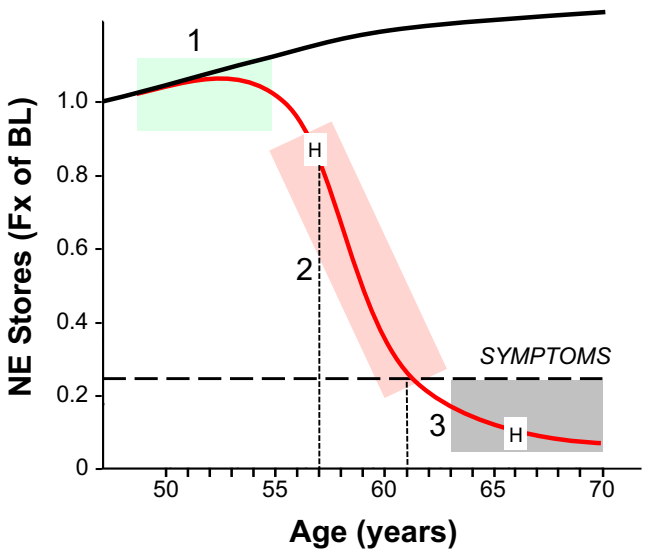


The MAOI tradeoff was confirmed by MAO inhibition by clorgyline decreasing endogenous DOPAL (green) while increasing endogenous 5-S-cysteinyldopamine (Cys-DA) (blue).

Modeling the progression of Lewy body diseases

An extension of the modeling project with Mark Pekker was to characterize the progression of the disease process in Lewy body diseases (LBDs). We expanded the project to predict the progression of catecholamine deficiency, based on the theories of homeostasis and catecholamine autotoxicity, which are discussed later in the section on stress.

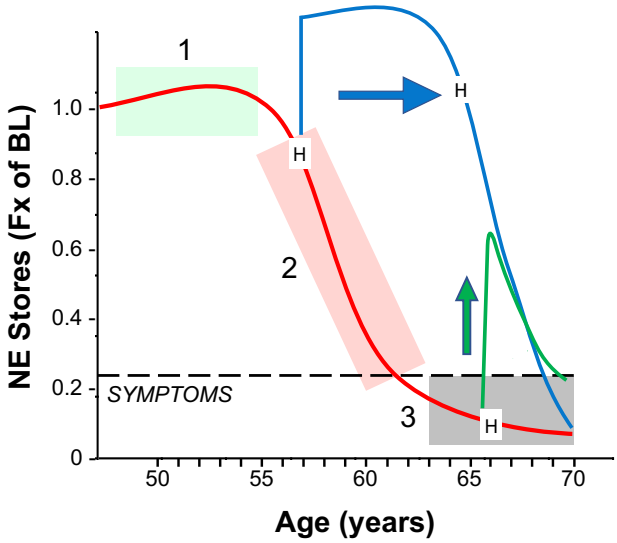
The model predicted a triphasic progression of catecholaminergic neurodegeneration in LBDs, from homeostasis in Phase 1 to dyshomeostasis and rapid loss of neurotransmitter in Phase 2 to advanced disease with a further slow loss in neurotransmitter in symptomatic disease in Phase 3.



Computational modeling predicts triphasic progression of catecholamine deficiency (in this case norepinephrine (NE) stores) in Lewy body diseases (red curve). Taking DOPAL-mediated toxicity out of the model eliminates the progression of NE deficiency (black curve).

According to the model, treatment that decreases DOPAL production and toxicity would exert only a transient benefit if the patient were already symptomatic. The same treatment, but begun in the pre-symptomatic phase after the transition from homeostasis to dyshomeostasis, would

substantially delay the onset of symptomatic disease.



Predicted effects of decreasing DOPAL production and effects by 30% in symptomatic patients (green curve) and at the transition from homeostasis in Phase 1 to dyshomeostasis in Phase 2.

The PDRisk study: Neurochemical aspects

In diseases such as Parkinson's disease (PD) that involve catecholaminergic neurodegeneration in the brain, the disease process begins long before the onset of clinical manifestations. The intramural NINDS PDRisk study was designed to test whether in individuals at increased risk of PD (based on genetic predisposition, olfactory dysfunction, dream enactment behavior, and orthostatic intolerance or orthostatic hypotension) biomarkers of catecholamine deficiency in the brain or heart predict a clinical diagnosis of PD.

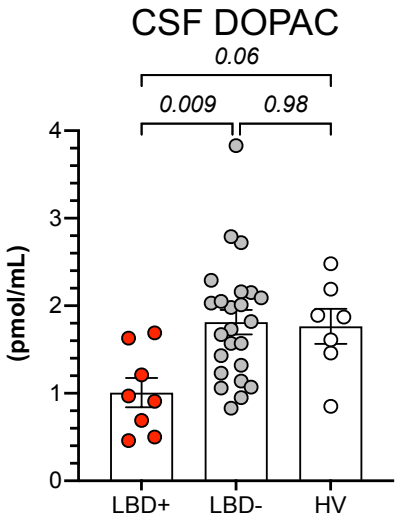
Given that CSF catechols provide a neurochemical window on central catecholaminergic systems, an important aspect of the PDRisk study was to assay CSF obtained upon initial evaluation of the participants, to see if the data could separate the group that developed a central Lewy body disease during long-term follow-up (LBD+ group) from the group that did not (LBD- group).

Advertisement for the intramural NINDS PDRisk study. The woman in the ad was one of our patients.

CSF levels of DOPAC, the main neuronal metabolite of dopamine, separated the LBD+ from the LBD- group. S CSF levels of endogenous DOPA, which is the product of the rate-limiting enzymatic step in catecholamine biosynthesis, and levels of endogenous DHPG, which is the main neuronal metabolite of norepinephrine, were not significantly decreased. Taken together, the neurochemical results pointed to central dopamine deficiency being a predictor of who among at-risk individuals would go on to develop a central LBD.

Preventing catecholaminergic neurodegeneration

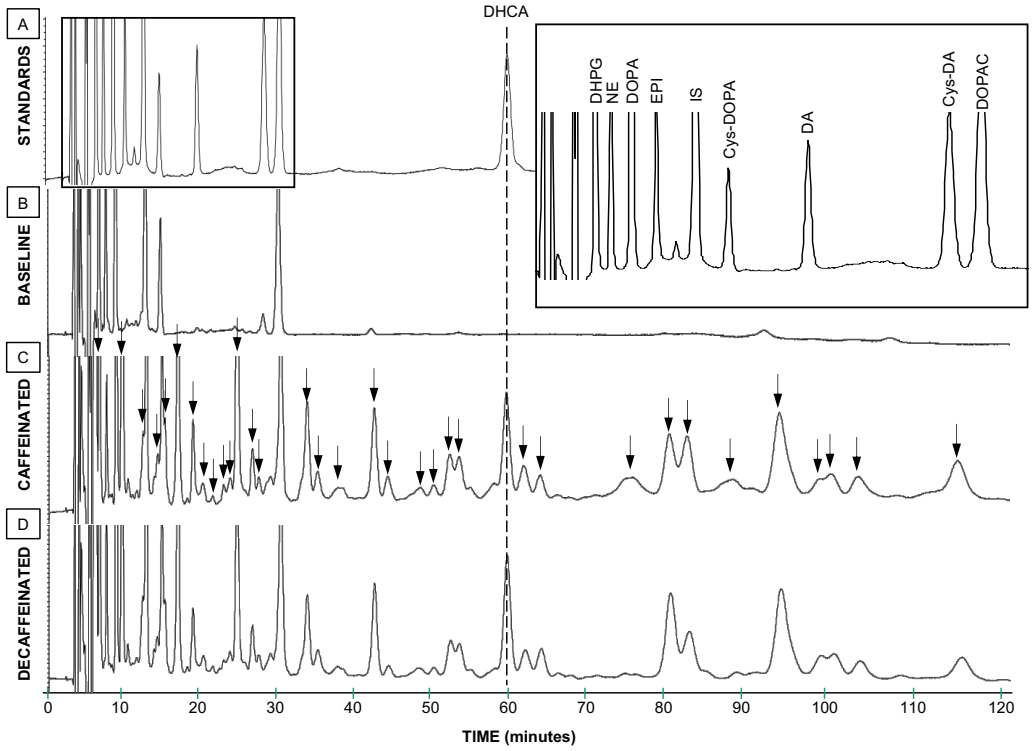
Catechols readily spontaneously oxidize and so in general exert antioxidant properties. Catechols are abundant in many dietary foodstuffs. Examples are 3,4-dihydroxyphenylethanol (DOPET, synonymous with hydroxytyrosol), which is the main antioxidant phenol in olives, and caffeic acid and dihydrocaffeic acid (DHCA), which are prominent antioxidant catechols in coffee. Purified dietary catechols could be novel nutraceuticals in clinical trials to prevent central neurodegenerative diseases in at-risk individuals. In particular, hydroxytyrosol decreases levels of the endogenous autotoxin 3,4-dihydroxyphenylacetaldehyde (DOPAL) and protects against alpha-synuclein-induced cytotoxicity.



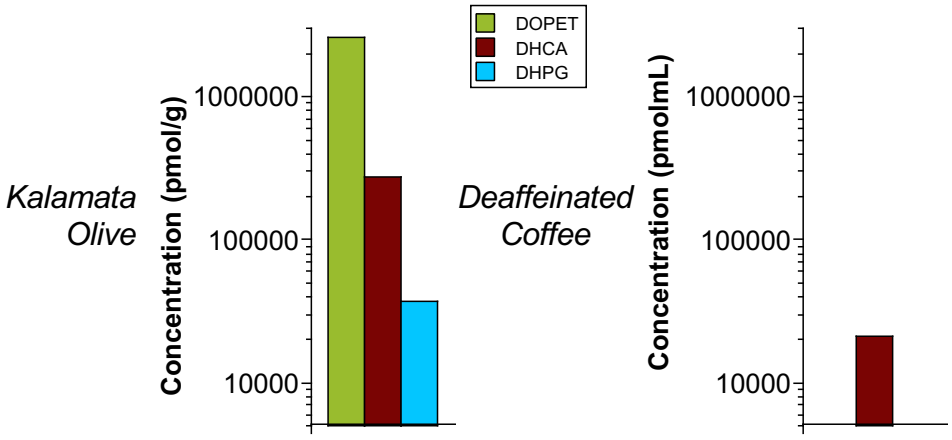
A neurochemical index of central dopamine deficiency, low CSF DOPAC, distinguished the group of at-risk individuals who developed a central Lewy body disease during long-term follow-up (LBD+) from the group that did not (LBD-) and from healthy volunteers (HV).

Batch alumina extraction followed by liquid chromatography with series electrochemical detection (LC-ED) is a well-established method for assaying levels of catechols in human plasma. We extended on the alumina extraction procedure to purify the catechols in olives and coffee. Olives of different types and caffeinated and decaffeinated coffee were subjected to alumina extraction. The catechol contents were quantified by LC-ED and compared among these foodstuffs. In olives, DOPET and 3,4-dihydroxyphenylglycol were the main catechols, and in coffee (whether caffeinated or decaffeinated), caffeic acid and DHCA were the main catechols. Olives and coffee also contained many other catechols that were not identified. Both olives and coffee contained substantial amounts of DHCA.

The same venerable alumina extraction procedure we've used for decades is a relatively straightforward method for purifying the catechols in dietary foodstuffs. The purified catechols could be the basis for novel nutraceuticals to treat or prevent diseases involving catecholaminergic neurodegeneration.



After people drink 2 cups of coffee, whether decaffeinated or caffeinated, the plasma contains numerous catechols.



Catechols in Kalamata olives and in decaffeinated coffee. Olives are rich in hydroxytyrosol (DOPET), dihydrocaffeic acid (DHCA), and 3,4-dihydroxyphenylglycol (DHPG). All these catechols are potent antioxidants.

The future of clinical catecholamine neurochemistry

Our group validated, refined, and for many years has applied liquid chromatography with electrochemical detection (LC-ED) for measuring plasma levels of catechols in a variety of biofluids and tissues. This technology has led to literally hundreds of publications over four decades.

Nevertheless, I think the era of LC-ED is coming to an end. I predict that liquid chromatography with tandem mass spectroscopy (LC-MS/MS) will supplant LC-ED, for a few reasons. First, LC-ED is time-consuming. A chromatographic run can take 120 minutes, depending on the analytes of interest, while a sample can be assayed by LC-MS/MS in a few minutes, so an obvious advantage of LC-MS/MS is much faster throughput. Second, there is decreasing availability of the type of post-column electrodes required for series electrochemical detection, and the electrodes that are commercially available are becoming increasingly expensive. Third, LC-MS/MS along with time-of-flight technology enables identification of a larger repertoire of compounds of interest. Fourth, a long-term theoretical advantage is versatility. For instance, by using ^{13}C-labeled, non-radioactive tracers, where each carbon on the benzene ring of the catechol is ^{13}C instead of ^{12}C, one can track comprehensively the neuronal and extraneuronal fate of catecholamines and their metabolites such as DOPAL. LC-ED cannot separate ^{13}C-catechols from ^{12}C-catechols, whereas with a difference of 6 atomic mass units, for LC-MS/MS this should be relatively straightforward. This technology could prove extremely powerful for identifying and quantifying alpha-synuclein oligomerization, quinonization, and aggregation by DOPAL.

The switch from LC-ED to LC-MS/MS may take years because of inertia, ignorance, and money. LC-MS/MS entails a substantial capital investment; however, the principle at play is the same for LC-MS/MS as for LC-ED: separate, then detect, just like the paper electrophoresis and ninhydrin spraying I did as a summer student at Andover 60 years ago.

CATECHOLAMINERGIC NEUROIMAGING

"What is this, 2001?"

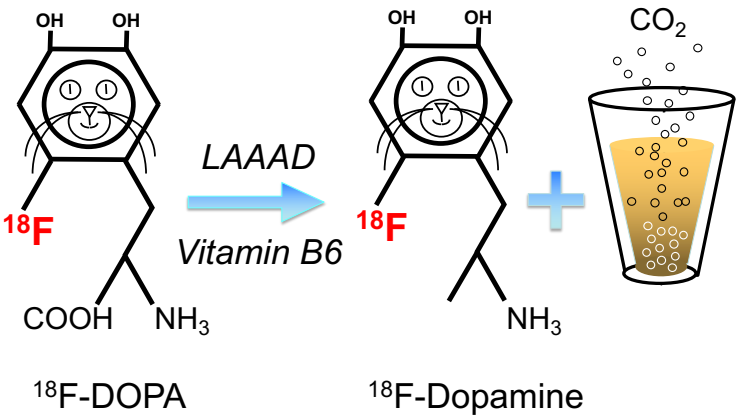
In the mid-1980s, Graeme Eisenhofer and I shared a cubbyhole in the lab of the Hypertension-Endocrine Branch in the NHLBI and shared an interest in autonomic and neuroendocrine systems. One day I received a phone call from Irv Kopin, who was then the Scientific Director of the NINDS. Irv related that a new technology, positron emission tomographic (PET) scanning, had potential for visualizing neurotransmitter systems in the brain. ^{18}F is a positron-emitting isotope of fluorine (the native, non-radioactive form is ^{19}F), and ^{18}F-DOPA was being developed to visualize central dopaminergic innervation in psychiatric and neurologic disorders such as schizophrenia and Parkinson's disease.

As an authority on catecholamine biochemistry, Irv knew well that DOPA is efficiently converted to dopamine (DA) by the enzyme L-aromatic-amino-acid decarboxylase (LAAAD, also known as DOPA decarboxylase). The same enzyme should convert ^{18}F-DOPA to ^{18}F-DA. ^{18}F-DA in turn might be an imaging agent to visualize sympathetic nerves.

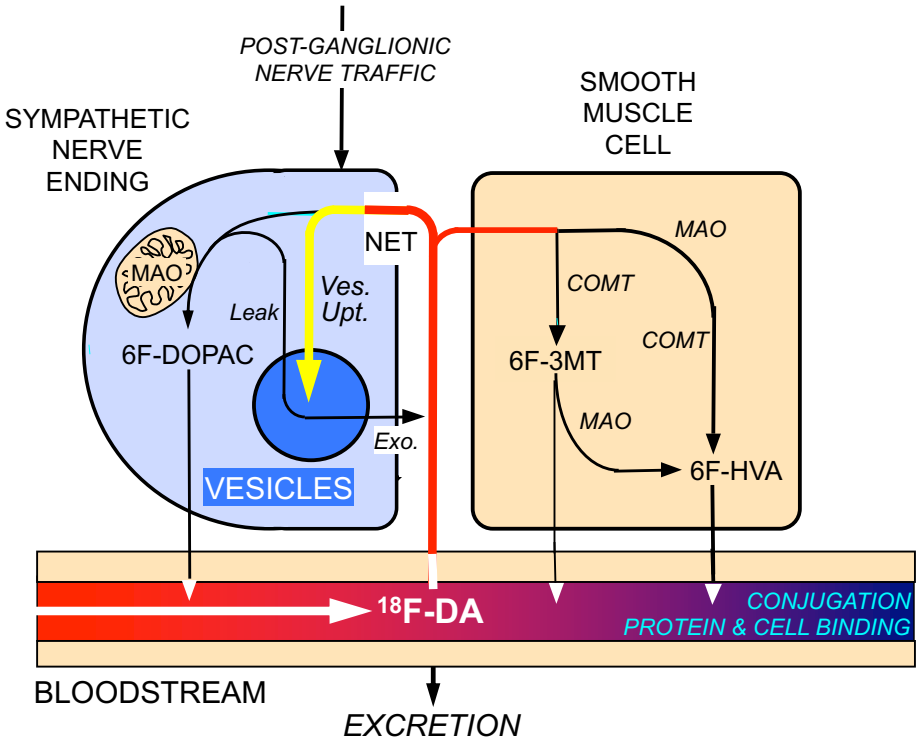
Stanley Kubrick's 1968 movie, *2001: A Space Odyssey,* is a classic of science fiction and one of the most influential films of all time. Its highly creative, futuristic technologies led to Kubrick's Academy Award for special effects. When Graeme heard about the suggestion that we look into developing ^{18}F-DA as a sympathetic imaging agent, he cracked, "What is this, *2001?*" In other words, this was science fiction.

Graeme got to work on the project and soon found out that there was no commercial source of LAAAD. Knowing that the kidney contains a tremendous amount of the enzyme, he followed a recipe to partially purify LAAAD from homogenizing hog kidneys.

You can imagine the stench in the lab!



Irv Kopin's idea about how to produce ^{18}F-dopamine (^{18}F-DA) by reacting ^{18}F-DOPA with the enzyme L-aromatic-amino-acid decarboxylase (LAAAD) and its co-factor vitamin B-6 (pyridoxal phosphate). A byproduct would be carbon dioxide. The solution is colored tan to indicate that oxidized catecholamine has a tannish color.



Concept diagram showing the fate of injected ^{18}F-DA in cardiac sympathetic nerves. Cardiac sympathetic neuroimaging by ^{18}F-DA PET is based on radiolabeling the vesicles in sympathetic nerves.

Neurology researchers at the NIH were interested specifically in 6-^{18}F-DOPA, but the synthesis yielded a mixture of 2-, 5-, and 6-^{18}F-DOPA, representing the different positions where ^{18}F was located on the benzene ring. An HPLC cleanup step was used to separate the compounds. This meant that 2- and 5-^{18}F-DOPA were trash. Graeme found that by reacting 2-^{18}F-DOPA with LAAAD, 2-^{18}F-DA was produced within several minutes.

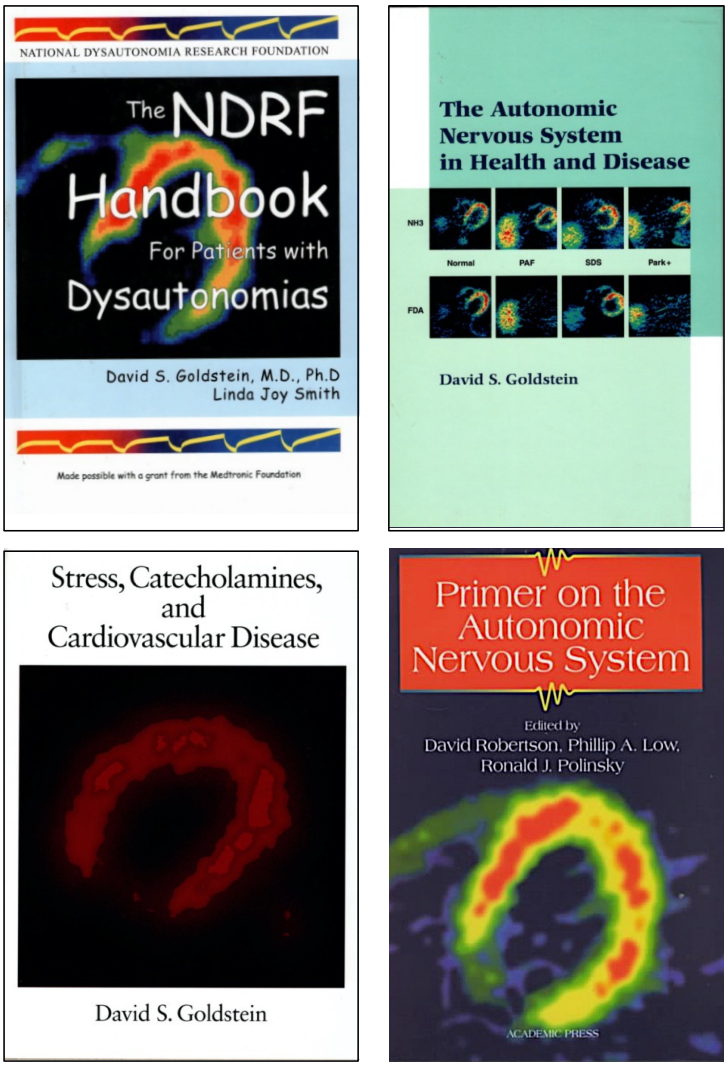
A PET scanner looks like a large doughnut; the subject is in the hole. The PosiCam was a prototype body PET scanner that had a large hole. The scanner was rarely used, because it was unreliable. We were allowed to use the PosiCam to image dogs. The superior cervical ganglion is the source of sympathetic innervation in the head. We found that in dogs with the superior cervical ganglion removed on one side in the neck, there was less ^{18}F-DA-derived radioactivity in salivary glands on the lesioned side—the first inkling that we were on the right track.

Because of visa issues Graeme had to leave the NIH. He obtained a position working with a colleague, Murray Esler, in Australia. Just before Graeme left, after scanning a dog with its head in the PosiCam we stuffed the animal further into the hole so that the chest was in the field of view. There was little radioactivity left (the half-life of ^{18}F is 1.83 hours), so we scanned the dog's chest for about 20 minutes. On the monitor we could see the dog's heart, like a white comet streaking through a black night sky. It was the first time anyone had seen the sympathetic innervation of the heart by PET scanning.

By the time Graeme returned from Australia 3 years later we were doing ^{18}F-DA PET scanning in healthy volunteers. Our initial report about visualizing sympathetic innervation by ^{18}F-DA PET scanning, published in *Circulation* in 1990, became a citation classic. So did our 1993 report in *Journal of the American College of Cardiology* about the initial findings in humans.

In the late 1970s radioiodinated metaiodobenzylguanidine (MIBG) was developed by William Beierwaltes at the University of Michigan as an imaging agent for visualizing adrenal tumors. Subsequently ^{123}I-MIBG

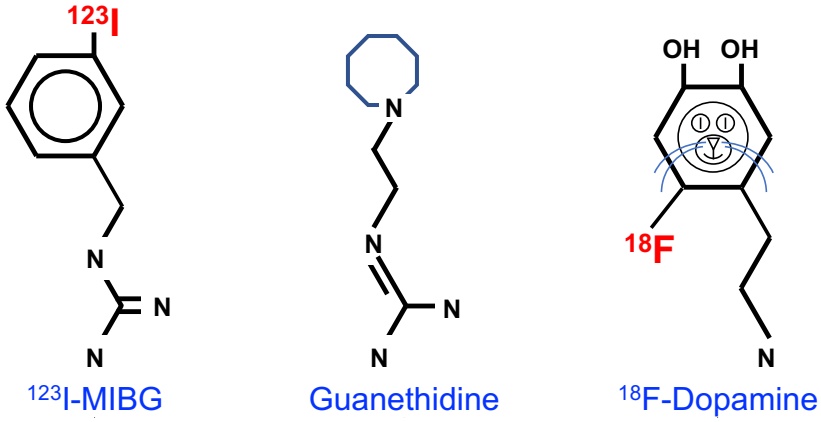
single photon emission tomographic (SPECT) was introduced for cardiac sympathetic neuroimaging. ^{123}I-MIBG SPECT is by far the predominant approach used.



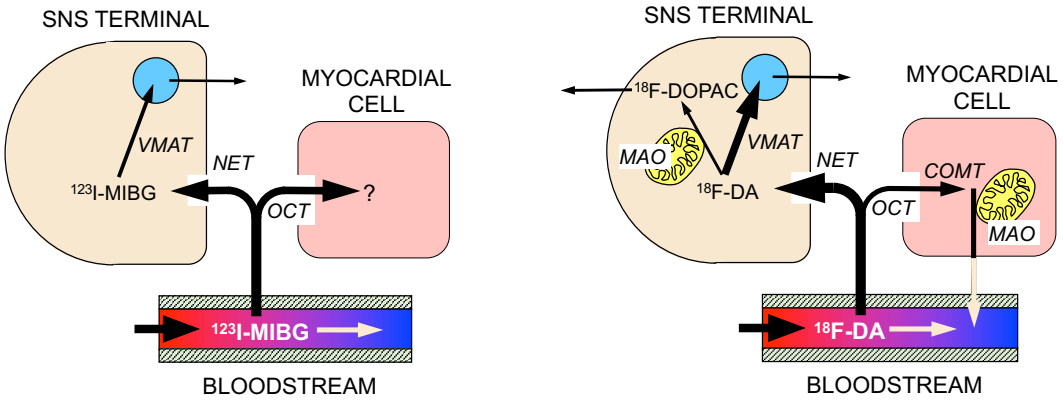
Cardiac ^{18}F-dopamine PET scans have been on the covers of several books.

PET scanning offers several advantages over SPECT scanning. These include better spatial resolution, a smaller amount of injected radioactivity, the possibility of measuring radioactivity concentrations in tissues in absolute terms, and, importantly, the analysis of curves relating to tissue

radioactivity over time (time-activity curves), which provides valuable information about not only innervation but also functional abnormalities in residual nerves, such as the vesicular storage defect referred to in the section on catecholamine neurochemistry. The time frames involved with PET and SPECT scanning differ. Injected cardiac sympathetic neuroimaging agents exit the bloodstream almost instantly and are taken up by sympathetic nerves extremely rapidly. Although the initial ^{123}I-MIBG SPECT scan, at about 15-30 minutes, is called “uptake,” peak ^{18}F-DA-derived radioactivity normally occurs by about 5 minutes after the injection.



^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) is neither a catechol nor a catecholamine. It is an analog of guanethidine.



Different fates of the non-catecholamine ^{123}I-MIBG and the catecholamine ^{18}F-DA. Note that ^{18}F-DA is a substrate for monoamine oxidase (MAO). ^{123}I-MIBG is not.

A key advantage of catecholamines over MIBG is the ability to examine specific aspects of function within sympathetic nerves. This is because catecholamines such as ^{18}F-DA are substrates for monoamine oxidase (MAO), whereas MIBG is not. ^{18}F-DA taken up into sympathetic nerves has 2 alternative fates. The main fate is uptake into vesicles via the vesicular monoamine transporter (VMAT). A minor but scientifically important fate is metabolism by MAO to form ^{18}F-DOPAC, which rapidly exits the terminals. If there were a vesicular storage defect, then for a given amount of ^{18}F-DA uptake there would be less radioactivity retained in the tissue and greater entry of ^{18}F-DOPAC into the extracellular fluid. By combining ^{18}F-DA PET data with neurochemical data about plasma levels of ^{18}F-DOPAC, we obtained the first evidence for a vesicular storage defect in Lewy body diseases. The report was published in the *Journal of Clinical Investigation* in 2011.

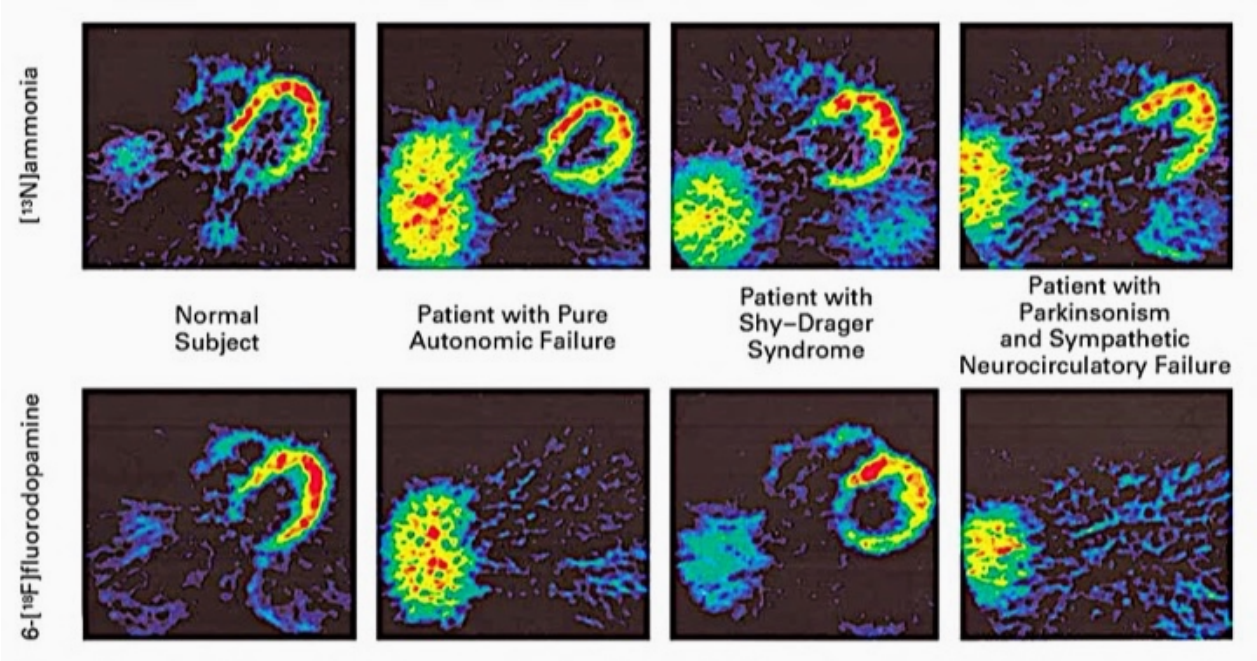
Cardiac noradrenergic deficiency in PAF and PD

Probably my most important discovery was that of cardiac sympathetic denervation in Parkinson's disease (PD) and pure autonomic failure (PAF) and normal innervation in most patients with multiple system atrophy. The report was published in the *New England Journal of Medicine* in 1997 and is a citation classic.

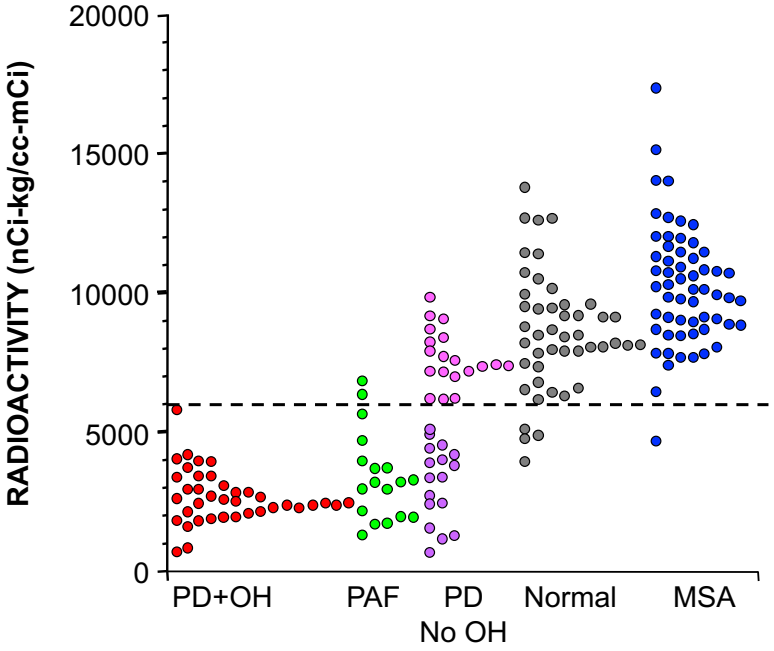
Ignorance isn't biased.

One of the motivations for developing ^{18}F-DA was to test the hypothesis that hypertensives are "hyper-tense" in that they have excessive sympathetic innervation or activity. Before testing this hypothesis, I had to validate the method. It was known that patients with idiopathic orthostatic hypotension (IOH, later designated pure autonomic failure, PAF) have low plasma norepinephrine levels during supine rest, whereas patients with OH from the Shy-Drager syndrome (SDS, later designated multiple system atrophy, MSA) have normal plasma norepinephrine levels. As predicted, PAF patients had abnormal cardiac ^{18}F-DA scans; indeed, the left ventricular myocardium couldn't be resolved from the chamber, so that there appeared to be no heart at all in the scan. Then we tested a patient

who had been referred for SDS. To our surprise, we found the same abnormal ^{18}F-DA scan as in PAF. MSA can be difficult to distinguish clinically from Parkinson's disease with orthostatic hypotension (PD+OH), and we asked whether the patient, who was quite parkinsonian, had ever been treated with levodopa/carbidopa. He hadn't, because his physicians knew that SDS usually doesn't respond to this treatment, and they were concerned that levodopa/carbidopa would worsen his OH. When the patient was tried on levodopa/carbidopa, not only was his OH no worse, but his movement disorder improved so much that he was able to transfer from assisted to independent living. He didn't have SDS; he had PD+OH. It didn't take long to confirm that PD+OH features the same abnormal cardiac ^{18}F-DA scans as in PAF. This was the first identified cause of autonomic failure in PD. I decided to pursue cardiac sympathetic neuroimaging in chronic autonomic failure syndromes. I never did find out whether hypertensives are hyper-tense.



Our 1997 New England Journal of Medicine report noted diffusely decreased ^{18}F-DA-derived radioactivity in the left ventricular myocardium in PAF and PD with sympathetic neurocirculatory failure and normal radioactivity in the Shy-Drager syndrome (subsequently re-named multiple system atrophy, MSA). All the patients had normal myocardial perfusion based on ^{13}N-ammonia PET scanning.



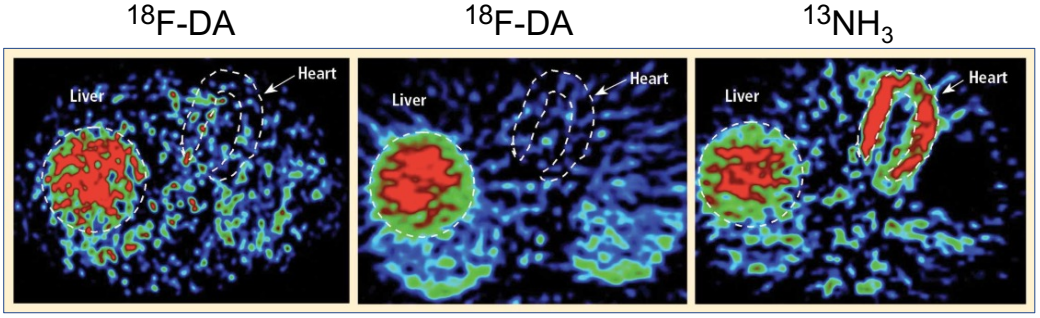
By the time of our 2011 Journal of Clinical Investigation article it was clear that Lewy body forms of autonomic failure are associated with low ^{18}F-DA-derived radioactivity and multiple system atrophy (MSA) usually is not.

The findings in our report were the beginning of a chain of discoveries, new insights, and concepts that continues to this day. The data indicated that PD isn't just a brain disease and movement disorder but involves an abnormality of the sympathetic nerves in the heart. Although there have been exceptions, most patients with MSA have intact cardiac sympathetic innervation.

A moral of this story is that ignorance isn't biased. If you have a hypothesis you want to test, chances are you'll come up with a positive finding, because you're biased. Journals are mainly interested in positive results, because there are two explanations for negative results. One is that there's nothing there. The other is that there is something there, but you didn't do the study right. But if you make a discovery that you didn't anticipate at all, and you confirm it by replication of the phenomenon, then you have to stop everything, because you've put your finger on the truth. Ignorance isn't biased. You should shift gears and pursue the discovery.

C-Threepio

We were the first to report that cardiac noradrenergic deficiency can precede by years the motor onset of Parkinson's disease (PD). Here is how this discovery came about. A patient underwent a workup including ^{18}F-DA PET scanning for a possible pheochromocytoma. The workup was negative, and he was given a diagnosis of "pseudopheochromocytoma."

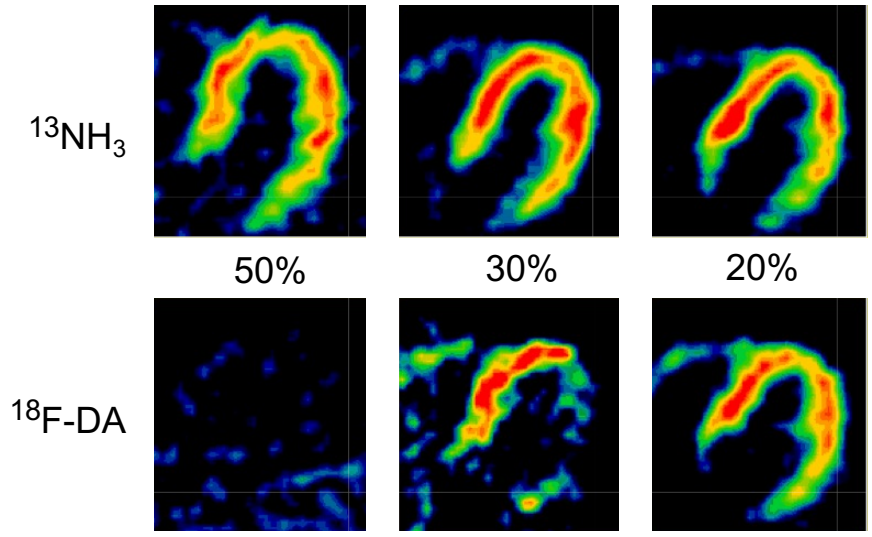


Initial Eval. | At Onset of PD 4 Years Later | At Onset of PD 4 Years Later

The first report of cardiac noradrenergic deficiency preceding the motor onset of PD.

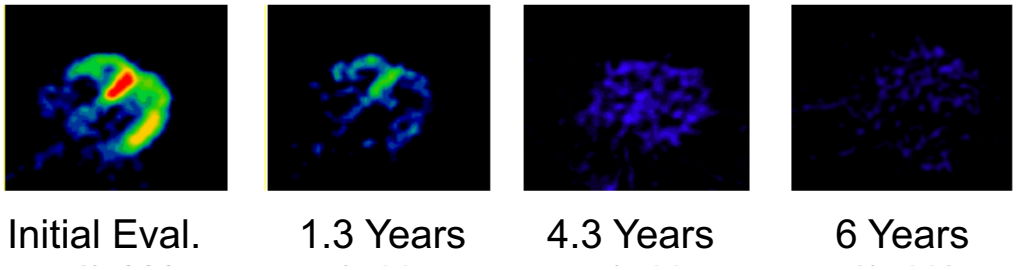
About 4 years later he returned for testing in a study about pseudopheochromocytoma. He reported the gradual onset of slow movement, limb rigidity, a shuffling gait, and decreased facial expression. He said he felt and looked like a robot, like the android C-Threepio in *Star Wars.* A neurology consultant diagnosed PD. ^{18}F-DA PET scanning showed a loss of sympathetic noradrenergic innervation, as is typical of PD. In retrospect, the PET scan from 4 years previously had shown the same loss of sympathetic innervation.

We found that only about one-half of PD patients without OH (PD No OH) have diffuse loss. Instead, about 30% have partial loss in the inferolateral wall, and about 20% have normal innervation.

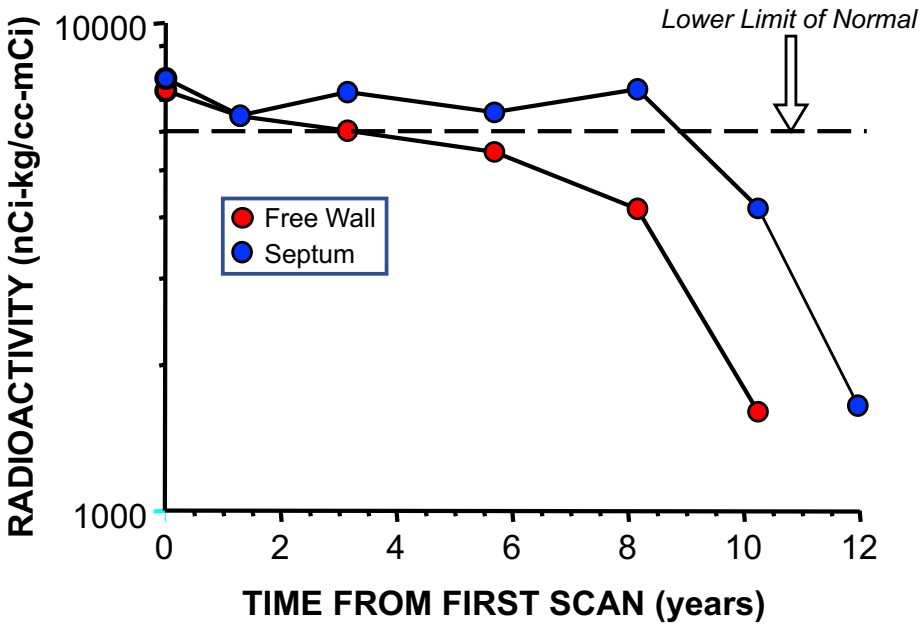


About one-half of patients with Parkinson's disease and no orthostatic hypotension have diffuse loss of ^{18}F-DA-derived radioactivity in the left ventricular myocardium.

By following PD No OH patients over years, it became clear that the localized loss was from catching the disease progression in a window in time. In one patient with PD No OH, upon initial evaluation only interventricular septal ^{18}F-DA-derived radioactivity was seen; within 2 years there was diffuse loss. In another patient with PD No OH, cardiac ^{18}F-DA-derived radioactivity was normal for about 8 years; then there was localized loss in the left ventricular free wall, followed in a couple of years by loss in the septum.



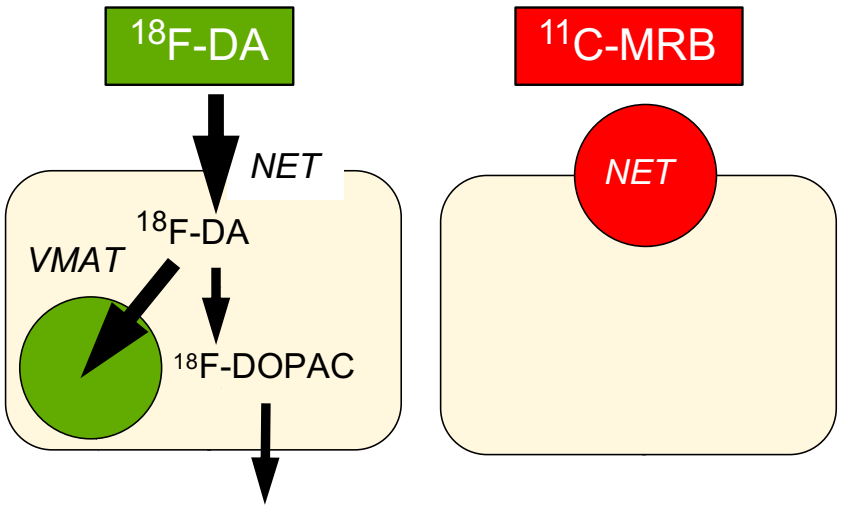
This patient with PD No OH had a rapid progression from localized to diffuse loss of ^{18}F-DA-derived radioactivity.



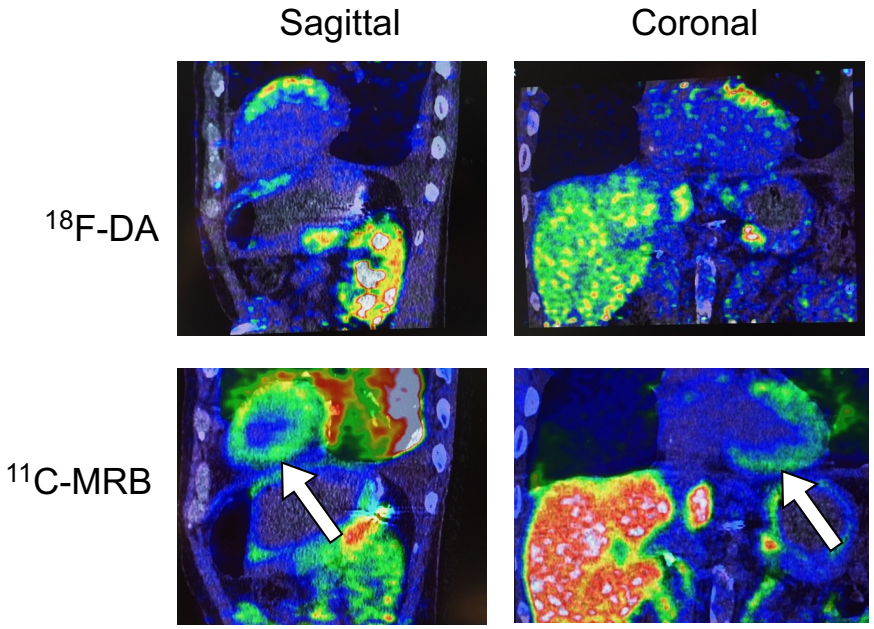
In this patient with PD No OH, cardiac ^{18}F-DA-derived radioactivity was normal range for about 8 years. Decreased radioactivity in the left ventricular free wall preceded decreased radioactivity in the septum. Progression of the cardiac sympathetic lesion within the heart

The loss of sympathetic innervation in PD therefore progresses over years from the bottom of the heart to the anteroseptal base of the heart.

We developed ^{11}C-methylreboxetine (^{11}C-MRB) as an imaging agent to visualize sites of expression of the cell membrane norepinephrine transporter (NET). ^{11}C-MRB binds to the NET, which is expressed on sympathetic nerves, but ^{11}C-MRB doesn't get into the nerves. ^{18}F-DA is also a ligand for the NET but does get into the nerves. By combining ^{11}C-MRB with ^{18}F-DA PET scanning in a patient who had undergone a heart transplant about 3 years previously, we discovered that at the top of the heart there was both ^{11}C-MRB- and ^{18}F-DA-derived radioactivity, reflecting new growth of sympathetic nerves.



^{18}F-Dopamine (^{18}F-DA) and ^{11}C-methylreboxetine (^{11}C-MRB) both are ligands for the cell membrane norepinephrine transporter (NET). ^{18}F-DA gets taken up into sympathetic nerves; ^{11}C-MRB does not.



^{18}F-Dopamine (^{18}F-DA) and ^{11}C-methylreboxetine (^{11}C-MRB) PET scans in a patient who had undergone a heart transplant about 3 years previously. The white arrows indicate that at the bottom of the heart there is ^{11}C-MRB- but not ^{18}F-DA-derived radioactivity.

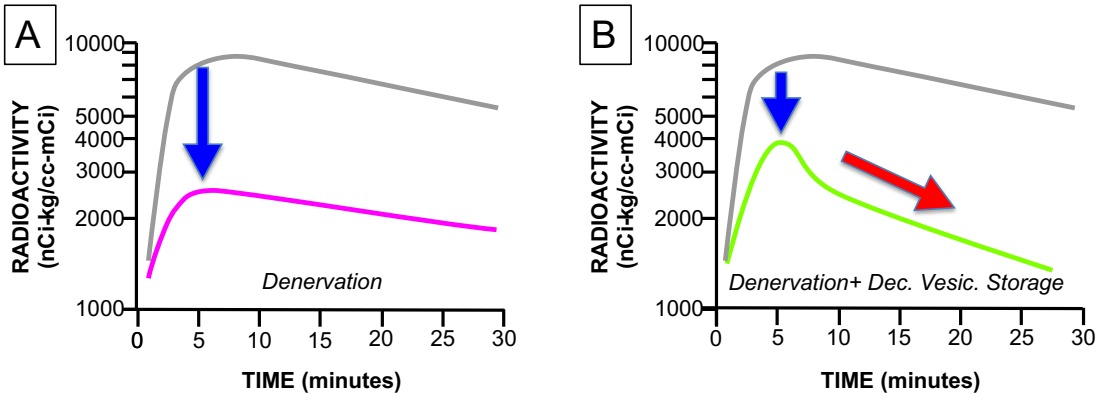
It seems that the progression of loss of cardiac sympathetic innervation in PD reflects a “die-back,” a mirror image of the growth of new nerves in heart transplant recipients.

We noted that at the bottom of the heart in the heart transplant recipient there was only ^{11}C-MRB-derived radioactivity (white arrows in the Figure). This observation suggests that the reinnervation process involves expression of the NET before the nerve terminals have developed the ability to store cytoplasmic catecholamines in vesicles.

Decreased vesicular storage: A common theme in synucleinopathies

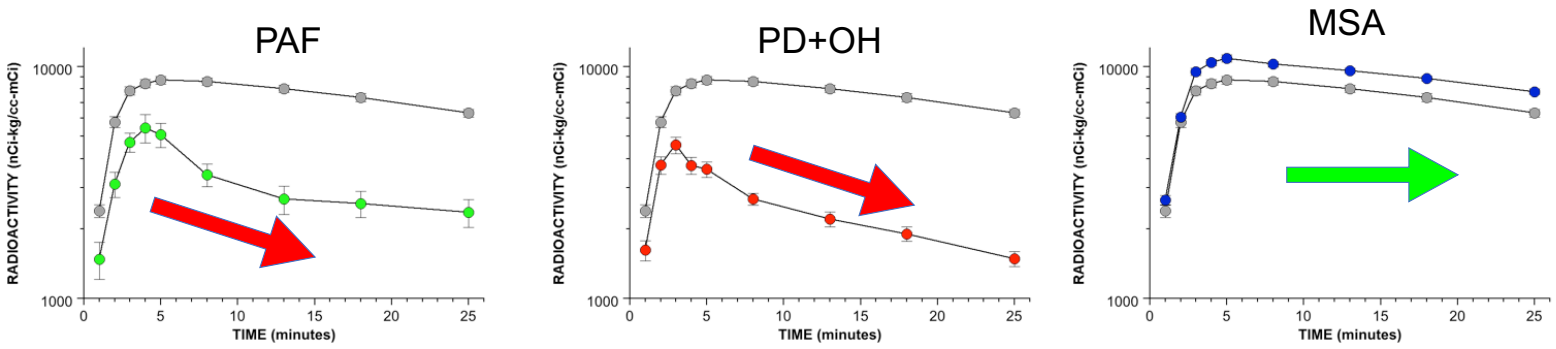
The section on clinical catecholamine neurochemistry describes evidence that Lewy body diseases (LBDs) entail functional abnormalities in residual catecholaminergic neurons (the sick-but-not-dead phenomenon) and that one of these abnormalities is a vesicular storage defect. Findings based on cardiac sympathetic neuroimaging have also indicated decreased vesicular sequestration in LBDs.

If there were denervation alone, then the curve relating ^{18}F-DA-derived radioactivity vs. time would be shifted downward, but without a change in the slope of decline, as shown in the Figure.



(A) Expected effects of denervation and (B) denervation with a vesicular storage defect on cardiac ^{18}F-DA-derived radioactivity. Denervation alone would shift downward the curve of radioactivity vs. time, without a change in slope (blue arrows). A vesicular storage defect would increase the slope (red arrow).

If in addition there were a vesicular storage defect in the residual nerves, there would be accelerated loss of the ^{18}F-DA-derived radioactivity.



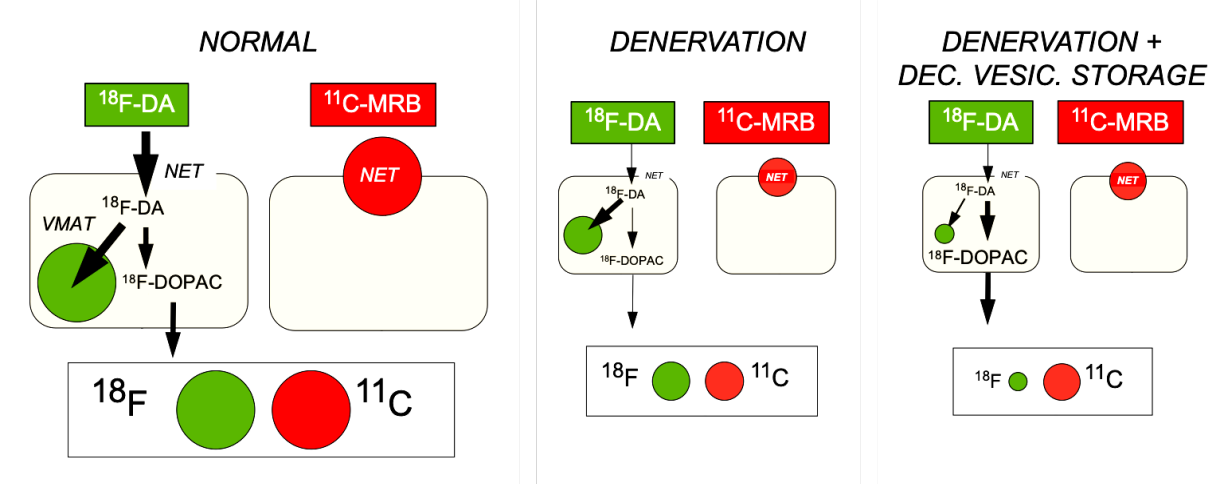
Accelerated loss of cardiac ^{18}F-DA-derived radioactivity in Lewy body forms of autonomic synucleinopathy (pure autonomic failure (PAF) and Parkinson's disease with orthostatic hypotension (PD+OH)) but not in the non-Lewy body form of autonomic synucleinopathy multiple system atrophy (MSA).

Multi-tracer neuroimaging to assess to identify cardiac sympathetic denervation separately from a vesicular storage defect

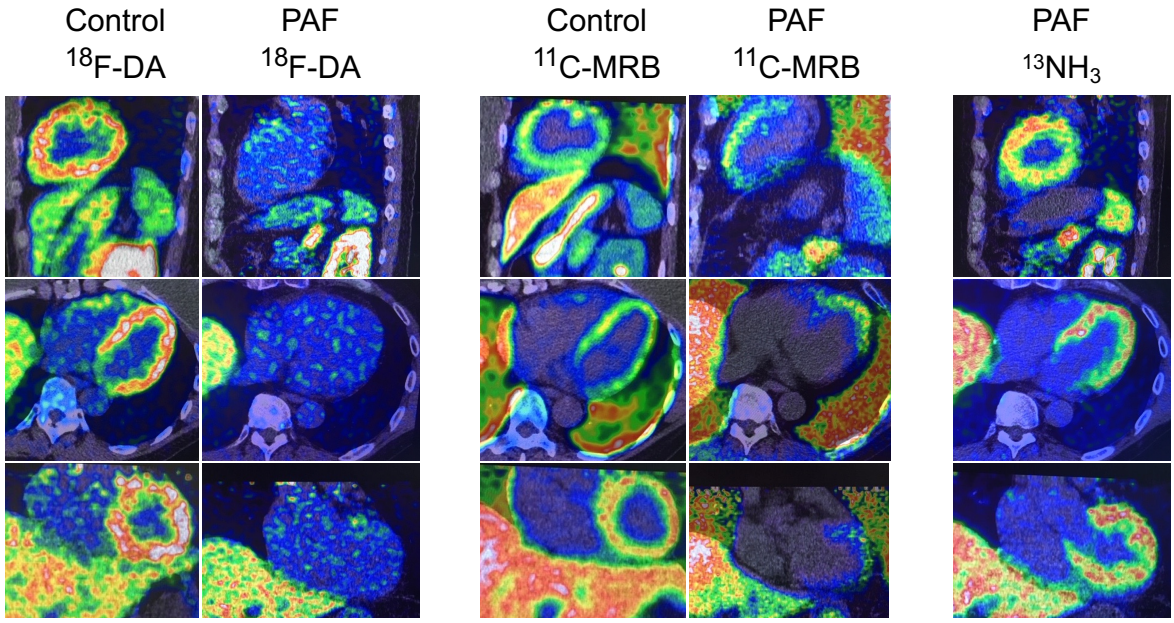
^{18}F-DA scanning alone cannot easily separate denervation from decreased vesicular storage as a cause of noradrenergic deficiency. To make this distinction, we combined ^{18}F-DA with ^{11}C-methylreboxetine (^{11}C-MRB) PET scanning. As noted above, ^{11}C-MRB is a ligand for the cell membrane norepinephrine transporter (NET) that does not enter the nerves, while ^{18}F-DA is a NET ligand that does enter the nerves. If there were denervation alone, ^{18}F-DA-derived radioactivity and ^{11}C-MRB-derived radioactivity would be decreased equivalently. If there were also a vesicular storage defect, there would be a greater loss of ^{18}F-DA-derived than of ^{11}C-MRB-derived radioactivity.

This is what we found in a group of PAF patients. ^{11}C-MRB-derived radioactivity was decreased by about 40% in the PAF group, indicating a moderate decrease in cardiac sympathetic innervation. Meanwhile, the rate

of loss of ^{18}F-DA-derived radioactivity was about 5 times faster in the PAF patients, indicating a substantial vesicular storage defect.

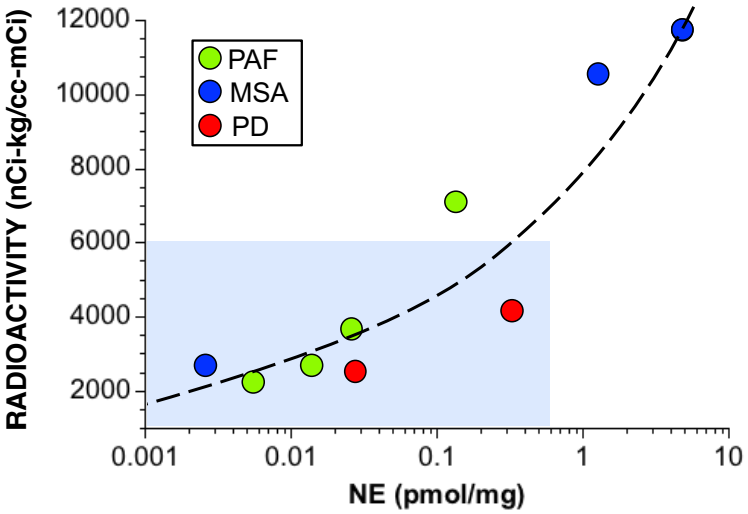


Concept diagram showing that if there were cardiac sympathetic denervation and decreased vesicular storage in residual sympathetic nerves, there would be a greater loss of ^{18}F-DA- than of ^{11}C-MRB-derived radioactivity.



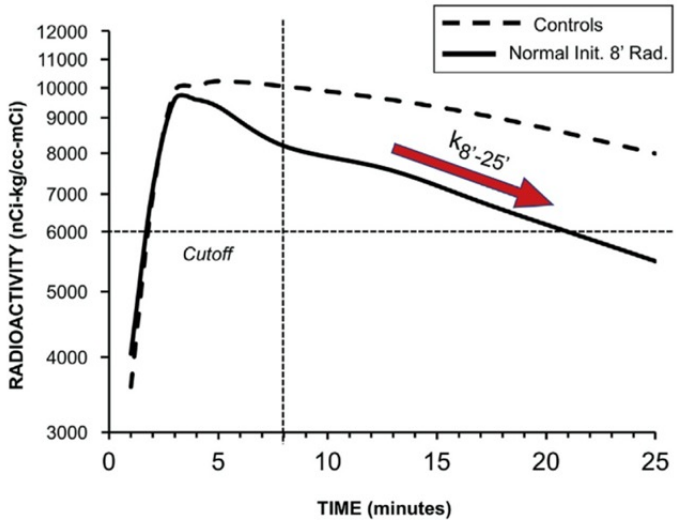
In PAF there is greater loss of ^{18}F-DA- than of ^{11}C-MRB-derived radioactivity, indicating a vesicular storage defect in residual cardiac sympathetic nerves.

To validate low cardiac ^{18}F-DA-derived radioactivity as an in vivo index of myocardial noradrenergic deficiency, whether from denervation or from functional abnormalities in residual nerves, we related ^{18}F-DA-derived radioactivity to post-mortem apical myocardial norepinephrine (NE) content in patients who had tissues harvested at autopsy.



Post-mortem validation of cardiac ^{18}F-DA-derived radioactivity as an index of myocardial norepinephrine (NE) content. The blue rectangle shows radioactivity and NE below the range of controls. Note that all the patients with a Lewy body disease (pure autonomic failure, PAF; Parkinson's disease, PD) had low ^{18}F-DA-derived radioactivity and low myocardial NE content. Two of the 3 patients with multiple system atrophy (MSA) had normal radioactivity and normal NE content.

In Lewy body diseases (LBDs), when does the vesicular storage defect develop with respect to the denervation? In a subgroup of patients with LBDs who initially had normal cardiac ^{18}F-DA-derived radioactivity and subsequently had decreased radioactivity during follow-up, the initial slope of decline in radioactivity during the scanning session was accelerated (red arrow in the Figure, suggesting that decreased vesicular storage can precede overt neuronal loss.

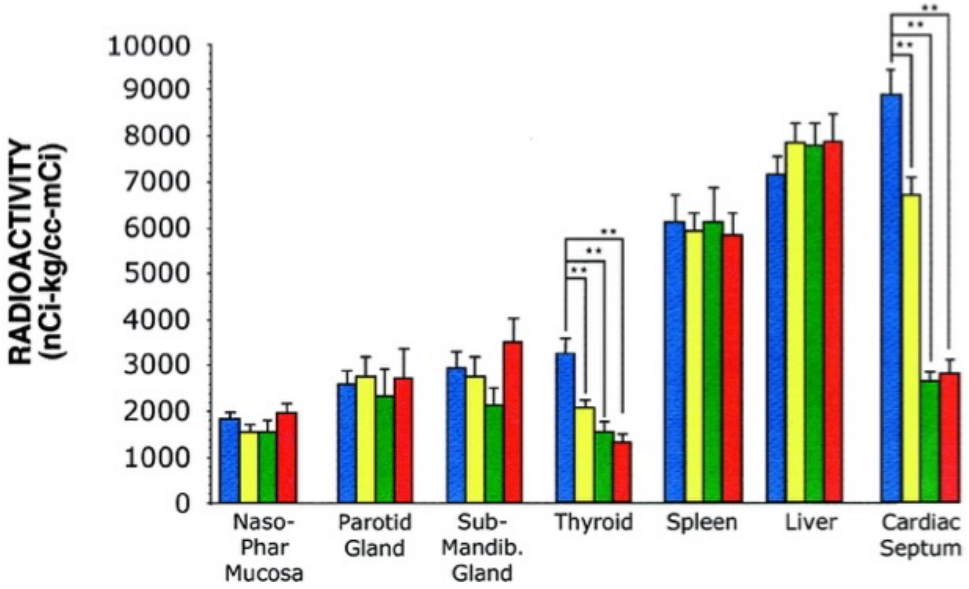


Upon initial evaluation a subgroup of patients with Lewy body diseases had normal cardiac ^{18}F-DA-derived radioactivity that decreased during follow-up, but the slope of decline in radioactivity during the scanning session was already accelerated (red arrow), suggesting that decreased vesicular storage can precede overt neuronal loss.

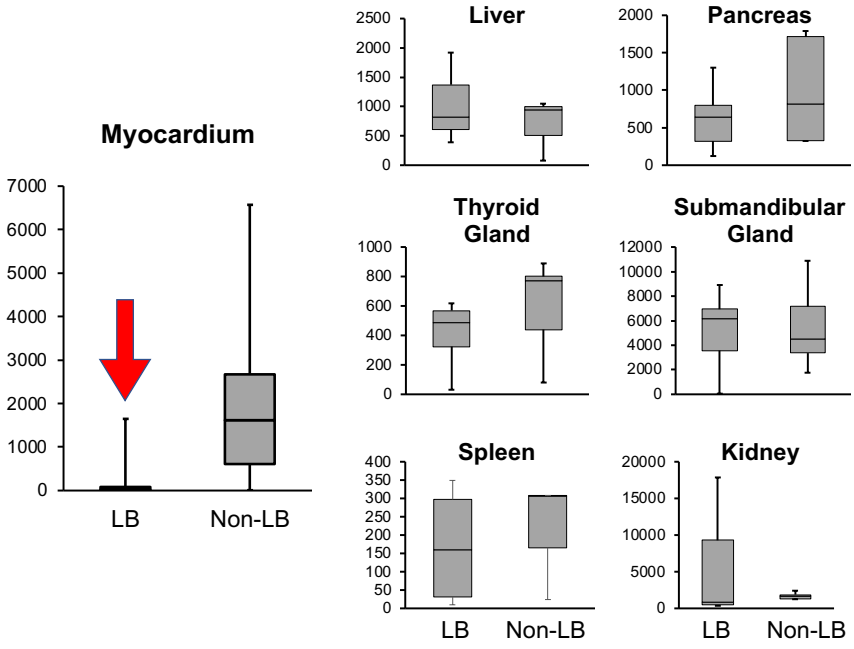
Cardioselectivity

We found long ago that in Lewy body forms of autonomic synucleinopathy the decrease in ^{18}F-DA-derived is far more prominent in the heart than in other organs, but until relatively recently there was no post-mortem evidence for cardioselectivity of norepinephrine deficiency in LBDs. Guillaume Lamotte, when he was working in our Section as part of the MEDSTAR/Georgetown-NIH residency in neurology, conducted a post-mortem organ survey and confirmed that in LBDs tissue noradrenergic deficiency is indeed cardioselective. Risa Isonaka's post-mortem immunofluorescence data, discussed later, also fit with this view.

If we knew why sympathetic nerves in the heart are especially vulnerable in Lewy body diseases (LBDs), we might gain insights about mechanisms of catecholaminergic neurodegeneration that could apply also to central LBDs and incite novel treatment or prevention strategies.



Tissue concentrations (means ± SEM) of ^{18}F-DA-derived radioactivity in organs of patients with PD and healthy control subjects. Blue=normal; yellow=PD without orthostatic hypotension, local loss of myocardial ^{18}F-DA-derived radioactivity; green=PD without orthostatic hypotension, diffuse loss of myocardial radioactivity; red=PD with orthostatic hypotension. (**) Significantly below control, p<0.01.



Post-mortem neurochemical evidence for cardioselectivity of loss of norepinephrine (NE) in Lewy body diseases.

The most important autonomic function test

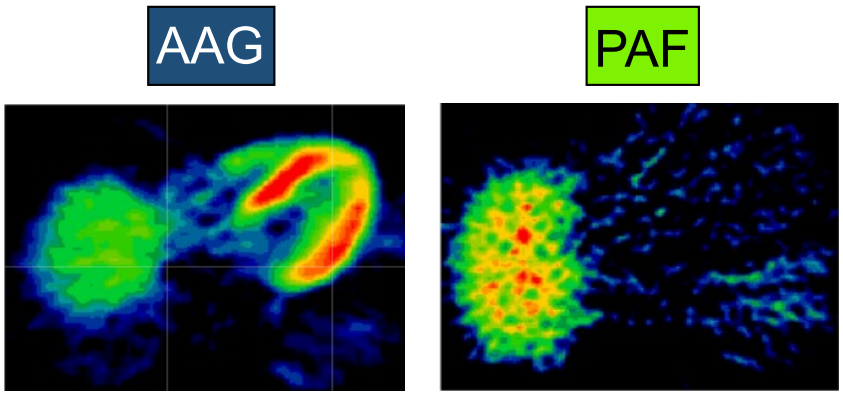
With Steve Vernino of the University of Texas we described the first case of autoimmune autonomic ganglionopathy (AAG). This rare form of autonomic failure manifests with pandysautonomia, meaning signs and symptoms of failure of all the components of the autonomic nervous system.

The case teaches a lesson about autonomic function testing. The most important autonomic function test is the history. The patient, a grandmother from Washington, DC, was referred for orthostatic hypotension without a known secondary cause and without symptoms or signs of central neurodegeneration—that is, pure autonomic failure (PAF). Since orthostatic hypotension is a cardinal manifestation of PAF, I was expecting that her chief complaint would be symptoms of orthostatic intolerance such as lightheadedness while standing. Instead, her chief complaint was that she couldn't make spit. She also was severely constipated. Because of her severely dry mouth and constipation she'd stopped eating, and indeed she looked like a patient with cachexia from end-stage cancer. She also complained of dry skin.

The autonomic nervous system has parts. Dry mouth and constipation are symptoms of failure of the parasympathetic cholinergic component. Orthostatic intolerance is a symptom of failure of the sympathetic noradrenergic component. Decreased sweating is a symptom of failure of the sympathetic cholinergic component. From the history it appeared that she had failure of all the components of the autonomic nervous system---pandysautonomia. When we tested her with trimethaphan, which blocks ganglionic neurotransmission, she noted no particular change. From these findings I conceptualized that her pandysautonomia was from a problem with ganglionic neurotransmission.

Steve Vernino at the Mayo Clinic had recently published in the *New England Journal Medicine* about autoimmune autonomic neuropathy identified by circulating antibodies to the neuronal nicotinic receptor that mediates ganglionic neurotransmission. I sent a sample of the patient's

plasma to him, and it proved positive. He had not previously found this abnormality in patients with PAF. This was the first reported case of what has come to be called autoimmune autonomic ganglionopathy (AAG).



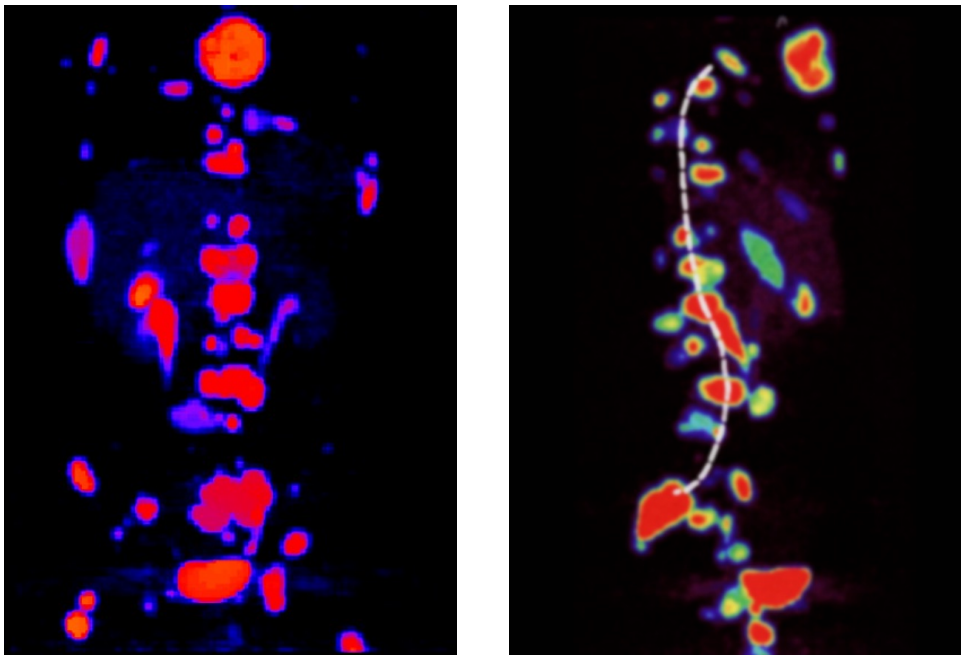
Cardiac ^{18}F-DA-derived radioactivity is normal in autoimmune autonomic ganglionopathy (AAG), in contrast with decreased radioactivity in pure autonomic failure (PAF).

In AAG the post-ganglionic sympathetic nerves should be intact; it's just that the message isn't being transmitted to them. In contrast with PAF, which involves a post-ganglionic lesion, our patient with AAG had normal cardiac ^{18}F-DA-derived radioactivity. AAG is extremely rare, but in all the patients with AAG whom we've studied since then cardiac ^{18}F-DA-derived radioactivity has been normal.

Pheos

As noted previously in the section on catecholamine neurochemistry, pheochromocytomas (pheos) are tumors of catecholamine-producing cells. Most (but not all) pheos express the cell membrane norepinephrine transporter. This means that ^{18}F-DA PET scanning can be used to visualize pheos.

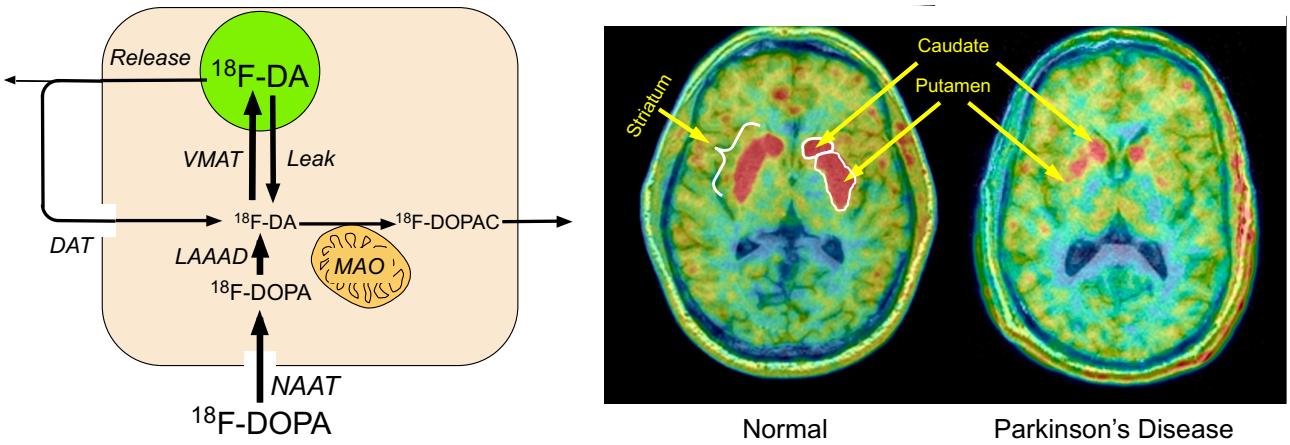
In patients with metastatic pheochromocytoma axial vertebrae are often involved, resulting in impressive ^{18}F-DA scans. In the example depicted in the Figure, virtually every bone in the axial skeleton has ^{18}F-DA-derived radioactivity, indicating widespread metastatic disease.



Coronal (left) and sagittal (right) torso ^{18}F-DA PET scans in a patient with metastatic malignant pheochromocytoma. Virtually every bone has tumor as indicated by ^{18}F-DA-derived radioactivity.

Brain ^{18}F-DOPA PET scanning

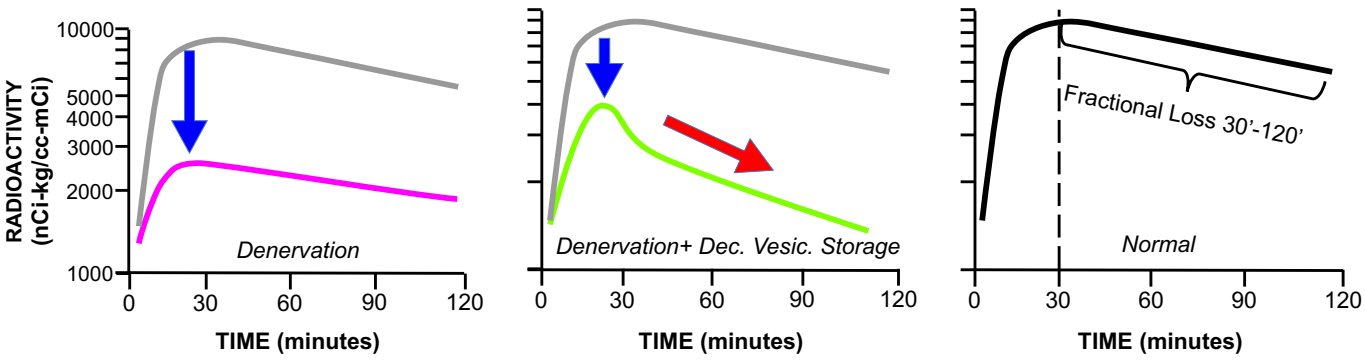
Since ^{18}F-DA is a catecholamine it cannot penetrate the blood-brain barrier. ^{18}F-DOPA is a catechol amino acid that can enter the brain from the bloodstream. Once inside the brain, all cells take up ^{18}F-DOPA, because it is a neutral amino acid, and all cells express a neutral amino acid transporter. In cells that express L-aromatic-amino-acid decarboxylase (LAAAD), the ^{18}F-DOPA is converted to ^{18}F-DA, and in dopaminergic, noradrenergic, and serotonergic neurons, which express the vesicular monoamine transporter (VMAT), the ^{18}F-DA derived from ^{18}F-DOPA is taken up in the vesicles. Brain ^{18}F-DOPA PET scanning therefore can be used to visualize sites of monoaminergic innervation—especially the striatum (the putamen and caudate), which has a high concentration of dopaminergic terminals derived from the substantia nigra in the midbrain.



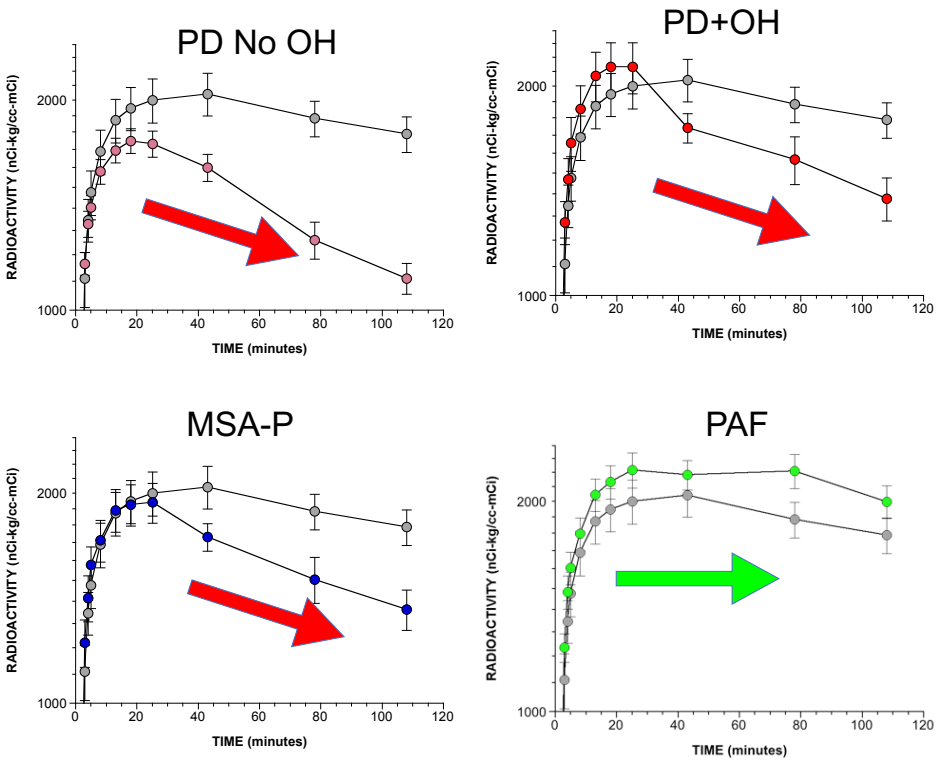
(Left) Concept diagram of the fate of ^{18}F-DOPA. (Right) ^{18}F-DOPA PET scans superimposed on the MRIs of a healthy subject and a Parkinson's disease (PD) patient. The PD patient has decreased striatal ^{18}F-DOPA-derived radioactivity, especially in the putamen.

Just as the slope of decline of cardiac ^{18}F-DA-derived radioactivity can be used to assess vesicular storage in sympathetic nerve terminals, the slope of decline in putamen ^{18}F-DOPA-derived radioactivity can be used to assess vesicular storage in nigrostriatal dopaminergic terminals. Denervation alone would be expected to shift downward the curve relating putamen radioactivity vs. time during a 120-minute scanning session, without a change in slope. A vesicular storage defect would be expected to accelerate the decline in radioactivity ("washout"). Between 30 and 120 minutes after administration of the PET imaging agent, putamen ^{18}F-DOPA washout normally is less than 20%.

We found that in groups of patients with parkinsonism (Parkinson's disease without or with orthostatic hypotension (PD No OH, PD+OH) and the parkinsonian form of multiple system atrophy (MSA-P)), there was increased putamen washout of ^{18}F-DOPA-derived radioactivity, whereas in pure autonomic failure (PAF) there was normal washout.



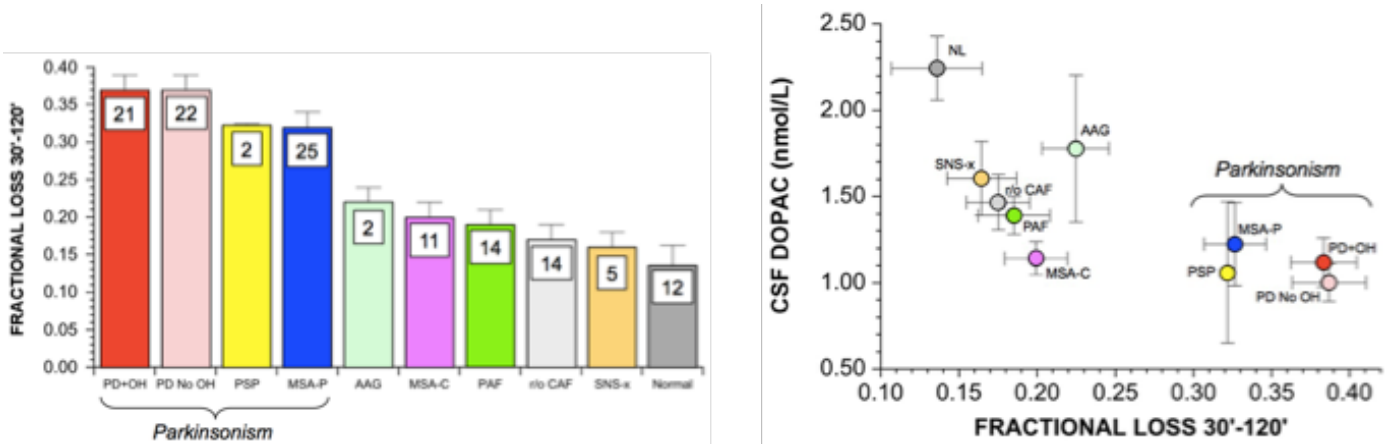
If there were a vesicular storage defect in nigrostriatal dopaminergic terminals, then there would be accelerated "washout" of putamen ^{18}F-DOPA-derived radioactivity.



Parkinsonism is associated with increased washout of putamen ^{18}F-DOPA-derived radioactivity.

Across a wide range of disorders, accelerated putamen ^{18}F-DOPA washout was associated specifically with parkinsonism. The parkinsonian groups also had decreased cerebrospinal fluid levels of DOPAC. The neuroimaging and neurochemical findings cross-validated each other.

Decreased vesicular storage—an example of the “sick-but-not-dead” phenomenon— therefore seems to be a common theme in catecholaminergic neurodegeneration in both the brain and heart.



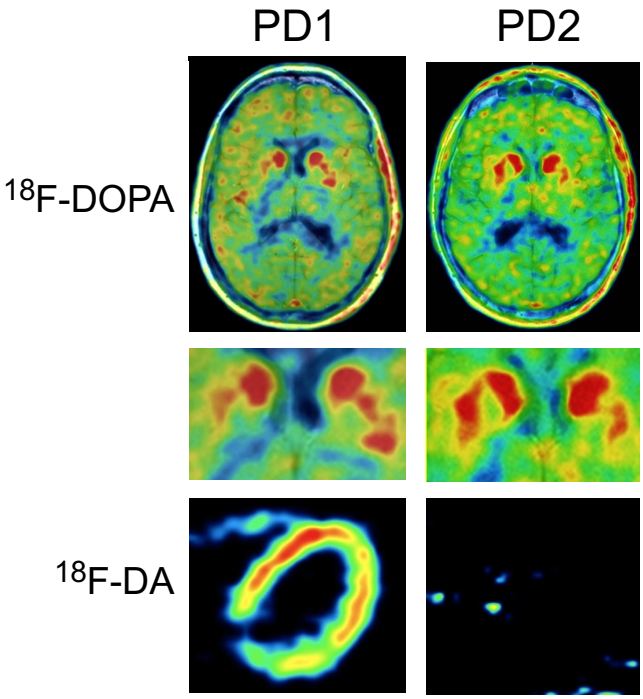
(Left) The fractional loss (“washout”) of putamen ^{18}F-DOPA-derived radioactivity is increased in patient groups with parkinsonism. The numbers in rectangles are the numbers of subjects. (Right) Parkinsonism is associated with both decreased cerebrospinal fluid (CSF) DOPAC levels and increased putamen washout of ^{18}F-DOPA-derived radioactivity.

Although cardiac noradrenergic deficiency can precede motor signs of PD, across individual patients abnormalities of cardiac ^{18}F-DA- and putamen ^{18}F-DOPA-derived radioactivity occur independently. The images in the Figure show extreme examples of this independence.

This independence could mean different “brain-first” vs. “body-first” pathogenetic sequences or even a “spotted banana” process where there is no specific spatio-temporal sequence in PD No OH.

The “smoking gun”

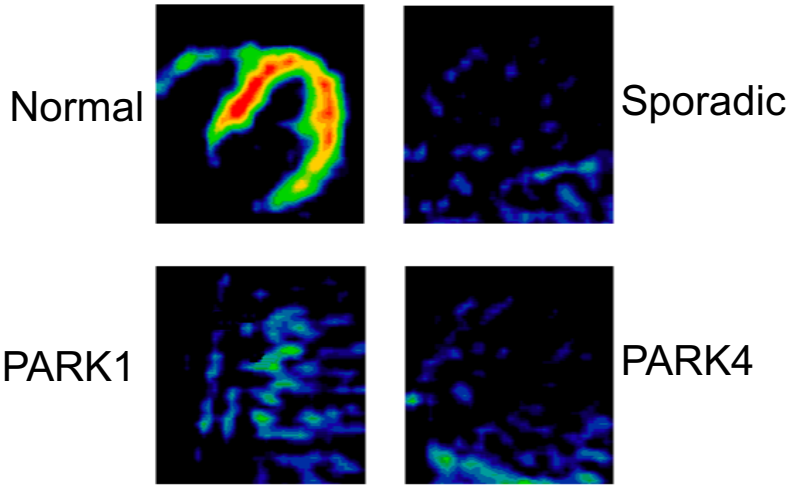
In 1997 the first identified genetic cause of Parkinson’s disease (PD) was reported—mutation of the gene that encodes the protein alpha-synuclein (αS)—PARK1. In the same year, Lewy bodies, a histopathologic hallmark of PD, were found to contain αS.



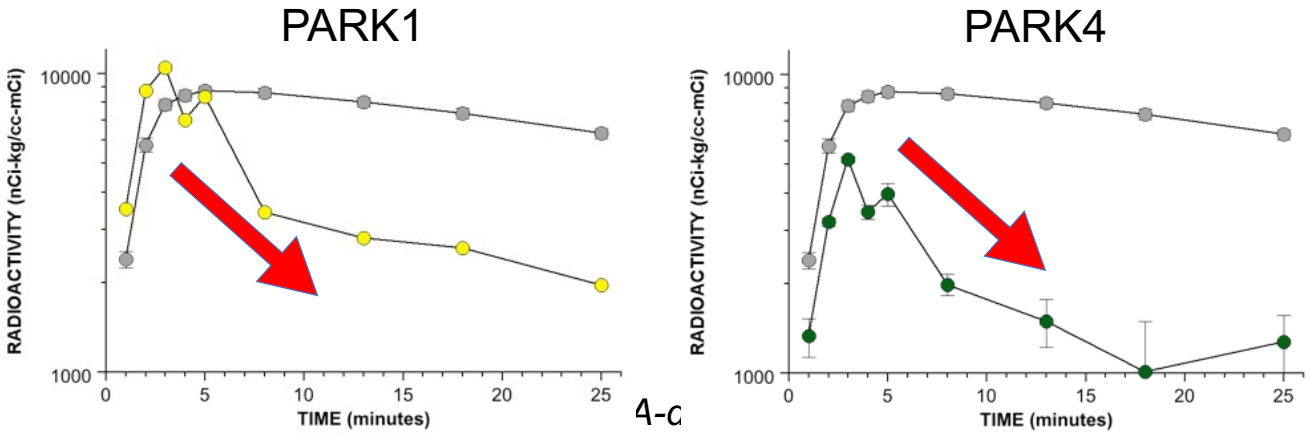
In these 2 PD patients with similarly abnormal striatal ^{18}F-DOPA-derived radioactivity, 1 has intact cardiac sympathetic innervation and the other markedly decreased ^{18}F-DA-derived radioactivity. The cardiac and striatal catecholaminergic abnormalities occur independently of each other.

In 2001 we provided the first evidence for a link between αS and cardiac noradrenergic deficiency, when we reported the case of a patient with familial PD from αS mutation who had profoundly decreased myocardial ^{18}F-DA-derived radioactivity. Subsequently we reported that patients with PD from triplication of the normal αS gene (PARK4) also had low ^{18}F-DA-derived radioactivity.

The finding of low ^{18}F-DA-derived radioactivity does not imply cardiac sympathetic denervation. The low radioactivity could also reflect a vesicular storage defect in residual nerves. In both PARK1 and PARK4 we found accelerated loss of ^{18}F-DA-derived radioactivity, indicating a vesicular storage defect in these forms of genetic PD. This was the first evidence that synucleinopathy produces a vesicular storage defect in cardiac sympathetic nerves.



PET scans showing low cardiac ^{18}F-DA-derived radioactivity in familial PD from mutation of the gene encoding alpha-synuclein (PARK1) and from triplication of the normal gene (PARK2)—the first reported evidence for a link between synucleinopathy and cardiac noradrenergic deficiency.



Both PARK1 and PARK4 entail accelerated loss of ^{18}F-DA-derived radioactivity, indicating that synucleinopathy can cause a vesicular storage defect in cardiac sympathetic nerves.

Microscopic sympathetic neuroimaging by immunofluorescence confocal microscopy

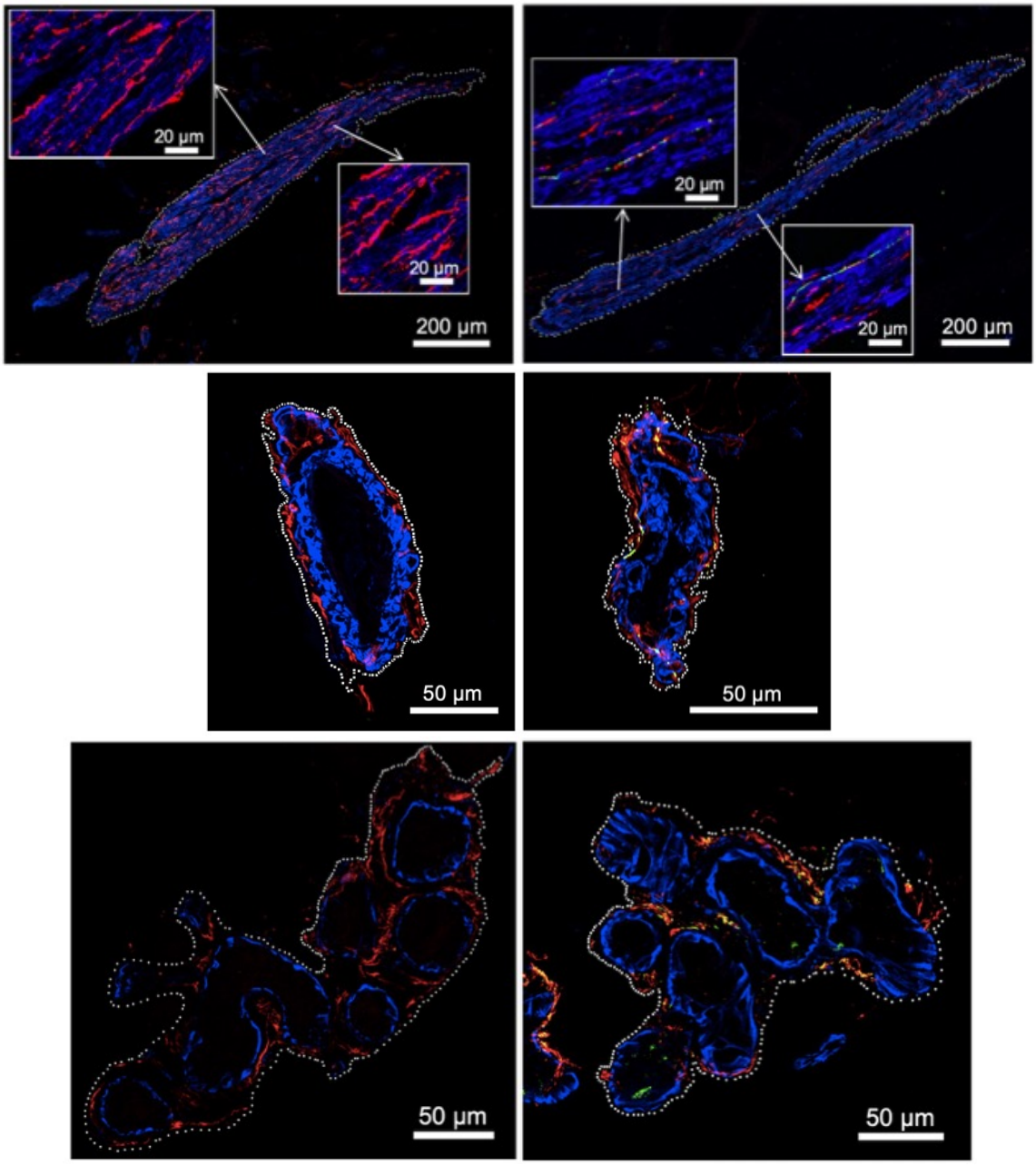
In 2017 Risa Isonaka, then under sponsorship by the Japan Society for the Promotion of Science (JSPS), introduced clinical microscopic

sympathetic neuroimaging by immunofluorescence confocal microscopy in our Section.

Risa Isonaka received the NIH Fellows Award for Research Excellence and 2 NINDS Clinical Research Excellence Awards, based on her mastery of clinical immunofluorescence microscopy for visualizing deposition of alpha-synuclein in catecholaminergic neurons and nerve fibers.

Risa visualized sympathetic noradrenergic nerve fibers by immunostaining skin biopsies for tyrosine hydroxylase (TH), a marker of catecholaminergic neurons. After almost 4 decades of studying the sympathetic nervous system, this was the first time I had actually seen sympathetic nerve fibers.

Risa demonstrated sympathetic noradrenergic fibers in arrector pili (pilomotor) muscles. Every hair that you have has an arrector pili muscle. Contraction of the muscle causes the hair to stand up, such as during distress. Arrector pili muscles are special in that they receive purely sympathetic noradrenergic innervation. In contrast, most of the sympathetic nerves supplying sweat glands use acetylcholine as the chemical messenger and are cholinergic. In arrector pili muscles the sympathetic noradrenergic fibers run parallel to smooth muscle fibers that express smooth muscle actin (SMA). Because of this, Risa could identify sympathetic noradrenergic fibers even in small pieces of arrector pili muscle.

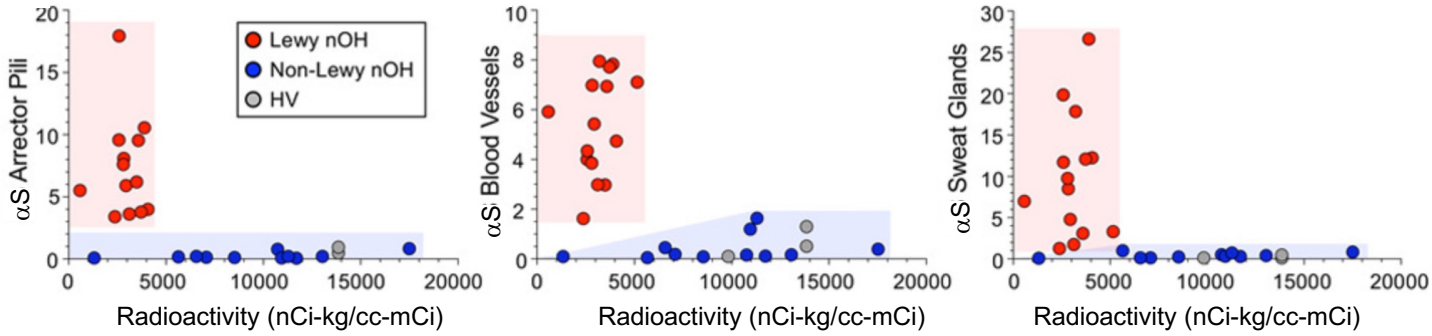


Immunoreactive alpha-synuclein (αS, green), tyrosine hydroxylase (TH, red), and smooth muscle actin (blue) in (top) arrector pili, (middle) blood vessels, and (bottom) sweat glands in (left) non-Lewy body and (right) Lewy body forms of neurogenic orthostatic hypotension (nOH). The yellow indicates αS colocalization with TH in sympathetic noradrenergic nerves in Lewy body nOH.

When Risa stained simultaneously for immunoreactive TH, SMA, and

alpha-synuclein (αS), she found that αS was deposited inside sympathetic noradrenergic fibers in skin biopsies from patients with Lewy body forms of neurogenic orthostatic hypotension (nOH)—i.e., pure autonomic failure (PAF) and Parkinson's disease with orthostatic hypotension (PD+OH). In the images, SMA is blue, TH is red, and αS is green. αS that is colocalized with TH is yellow.

Risa then found that the extent of buildup of αS in each of the 3 sympathetic noradrenergically innervated skin constituents was associated with neuroimaging evidence of cardiac noradrenergic deficiency based on ^{18}F-DA PET scanning. This means that analysis of skin biopsies for αS in sympathetic nerves could provide a biomarker of cardiac noradrenergic deficiency in Lewy body nOH.

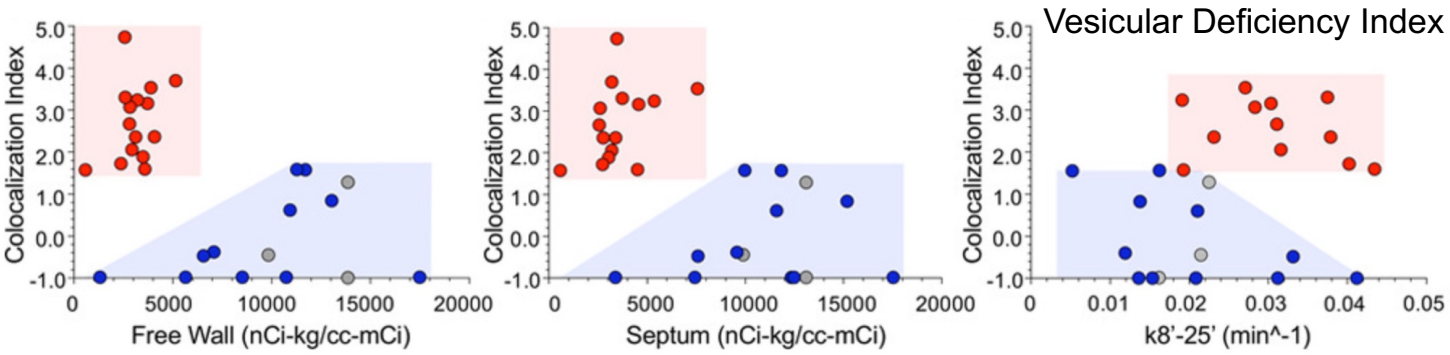


Alpha-synuclein (αS) in noradrenergically innervated skin constituents in skin biopsies in patients with Lewy body neurogenic orthostatic hypotension (nOH, red), patients with non-Lewy body nOH and with healthy volunteers (HV, gray).

I asked Risa to come up with a way to quantify the amount of "yellow," the amount of intra-neuronal deposition of αS. We devised an αS-TH "colocalization index" and applying it found that high αS-TH colocalization indexes were also related to neuroimaging evidence of cardiac noradrenergic deficiency.

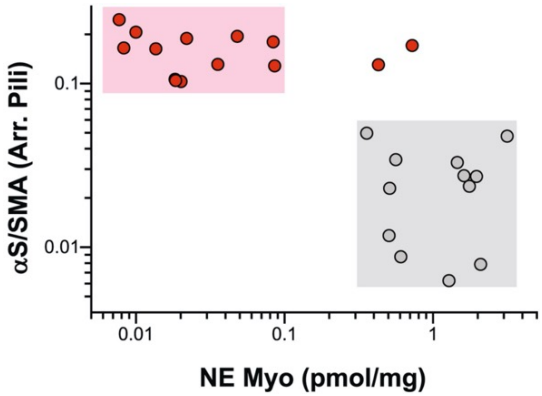
Importantly, patients with Lewy body nOH and elevated colocalization indexes also had evidence for a vesicular storage defect in cardiac sympathetic nerves. Risa's findings from patients with Lewy body nOH fit

beautifully with those from patients with inherited synucleinopathy and were consistent with a pathophysiological role of intra-neuronal αS deposition in cardiac noradrenergic deficiency.



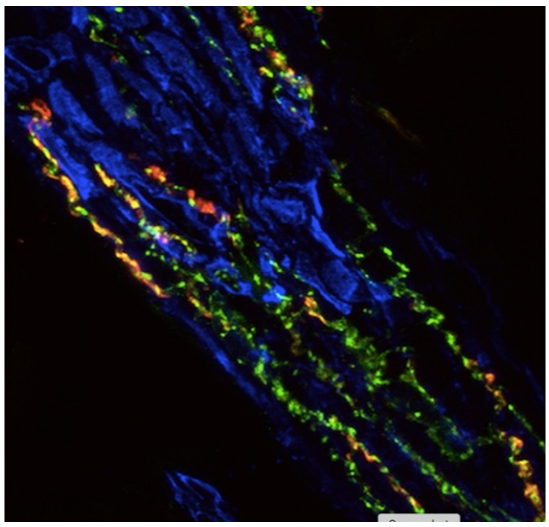
αS-TH colocalization indexes are associated with values for cardiac ^{18}F-DA-derived radioactivity, including an index of a vesicular storage defect.

In an autopsy study we asked whether increased deposition of αS in arrector pili muscles in scalp skin is related to myocardial norepinephrine (NE) deficiency. When Individual values for αS/SMA ratios in arrector pili muscles were expressed as a function of myocardial norepinephrine (NE) content in patients with Lewy body diseases (LBDs) and control subjects, all the LBD patients with myocardial NE depletion had elevated αS /SMA ratios in arrector pili muscles. These results provided post-mortem validation of skin intra-neuronal αS as a sensitive index of myocardial NE deficiency in LBDs.



αS/SMA ratios in arrector pili (Arr. Pili) muscles and myocardial norepinephrine (NE Myo) content in patients with Lewy body diseases (LBDs, red) and control subjects (gray). LBD patients with myocardial NE depletion have elevated αS/SMA ratios in Arr. Pili.

smoking Risa's striking images demonstrating intra-neuronal αS in patients with Lewy body diseases seemed like a "smoking gun" implicating αS in the death of catecholaminergic neurons; however, an unaffected elderly individual with duplication of the αS gene had normal ^{18}F-DA and ^{18}F-DOPA PET scanning despite obvious αS-TH colocalization.

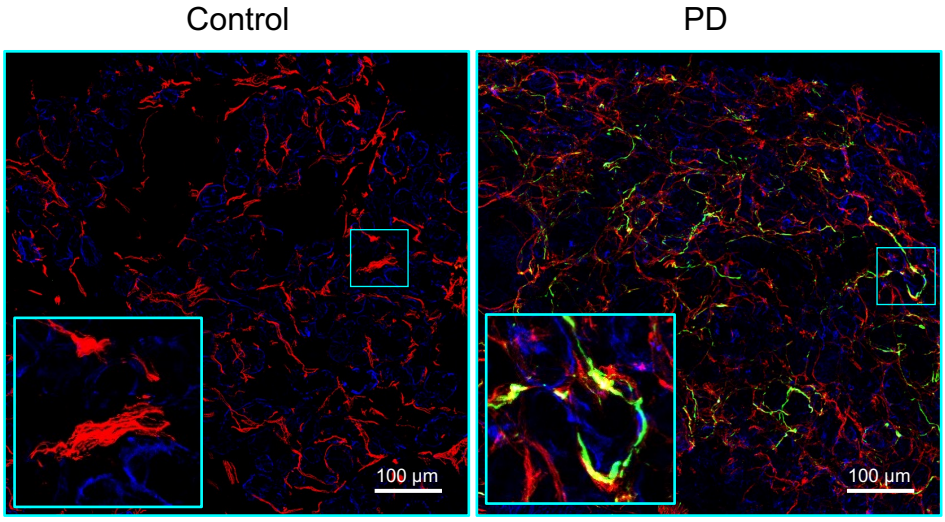


The "smoking gun," αS deposition in sympathetic nerve fibers in arrector pili muscle from an unaffected person with duplication of the gene encoding αS.

If increased αS deposition in sympathetic noradrenergic nerves were pathogenic, as opposed to being a non-pathogenic biomarker, then one would expect associations of intra-neuronal αS deposition with catecholaminergic denervation and with decreased norepinephrine (NE) contents in the same samples. We therefore assayed immunoreactive αS and TH concurrently with catecholamines in post-mortem skin, submandibular gland (SMG), and apical left ventricular myocardial tissue samples from patients with autopsy-proven PD and age-matched control subjects who did not have a neurodegenerative disease.

As expected, PD patients had increased αS in sympathetic noradrenergically innervated arrector pili muscles, SMG, and myocardium. Myocardial immunoreactive TH in the PD group was decreased from the

control group by 65%, but the groups did not differ in TH in either arrector pili muscles or SMG. Similarly, myocardial NE was drastically decreased (by 92%) in the PD group, but the groups did not differ in NE in either scalp skin or SMG.



Immunofluorescence confocal microscopic images of smooth muscle actin (blue), TH (red), αS (green), and αS within sympathetic noradrenergic nerves (yellow) in post-mortem submandibular gland tissue from a control subject (CTRL) and a patient with Parkinson's disease (PD). Despite obvious αS within sympathetic noradrenergic nerves in the PD patient, there was no decrease in total immunoreactive TH or in tissue norepinephrine content.

From these results we concluded that in skin and SMG augmented αS deposition in sympathetic nerves does not seem to be pathogenic.

The possibility remains that intra-neuronal αS deposition is pathogenic specifically in the heart, although why this would be the case remains mysterious.

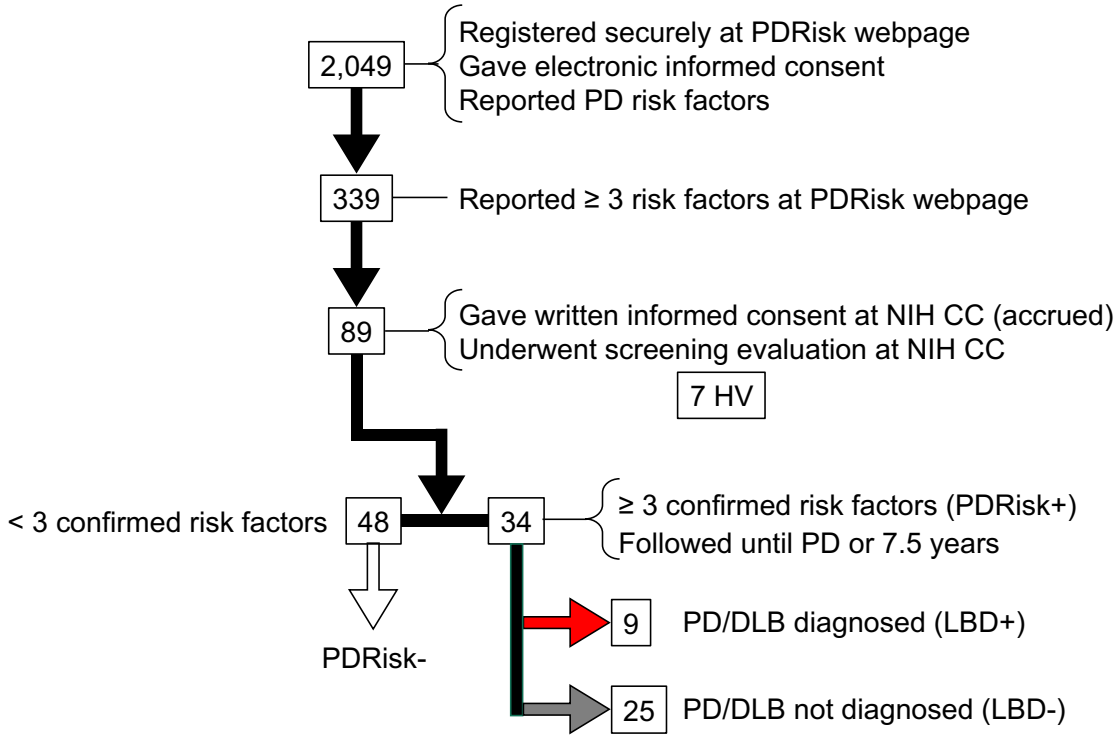
The PDRisk study (again): Neuroimaging aspects

By the time a person develops the motor manifestations in Parkinson's disease PD) or cognitive dysfunction in the related Lewy body disease

(LBD) dementia with Lewy bodies (DLB), substantial neurodegeneration has already occurred. There is great interest in identifying biomarkers that can detect pre-clinical LBDs.

Given that both diseases entail severe depletion of catecholamines in the heart, and that ^{18}F-dopamine (^{18}F-DA) provides a valid in vivo biomarker of myocardial norepinephrine (NE) stores, in the PDRisk study we asked whether in individuals at risk for developing PD biomarkers of cardiac catecholamine deficiency predict a diagnosis of PD during long-term follow-up.

In the PDRisk study people registered their risk factor information at a protocol-specific website about whether they had a family history of PD, loss of sense of smell, dream enactment behavior, or orthostatic intolerance. To be eligible the person must have reported at least 3 of the 4 risk factors.

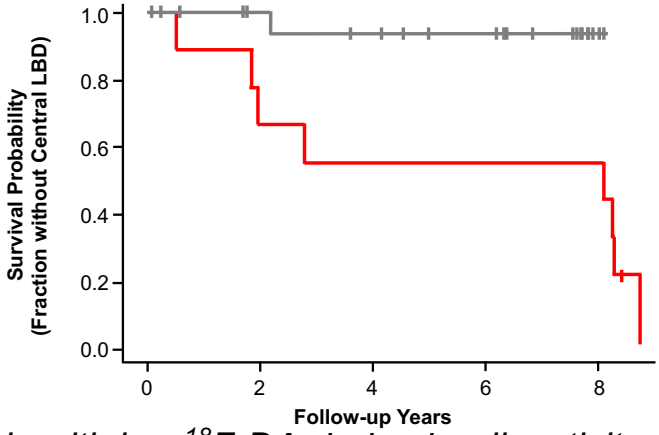


Flow diagram for the PDRisk study.

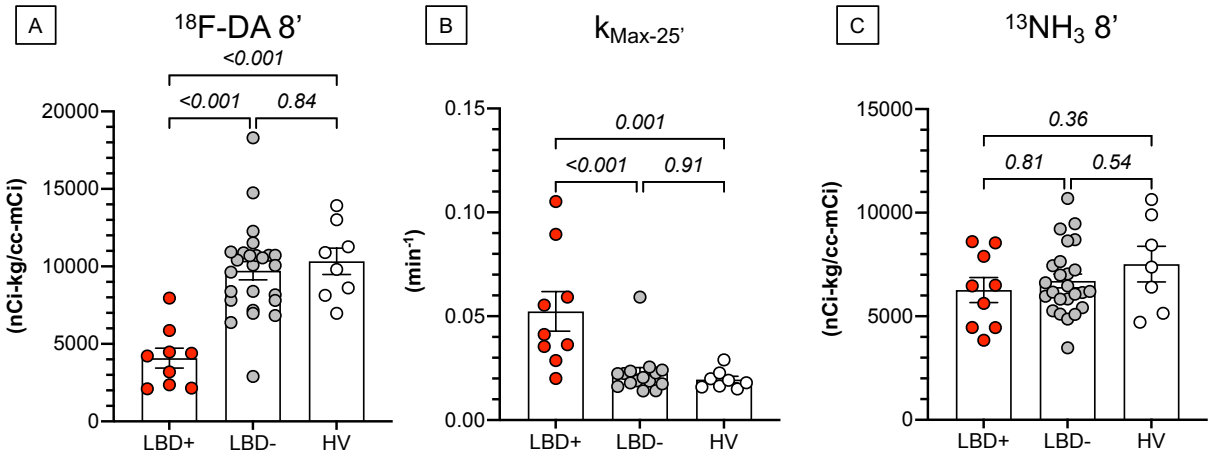
After on-site screening examination to confirm ≥3 risk factors, a total of 34 participants underwent ^{18}F-DA PET scanning as part of comprehensive

inpatient testing and were followed at 1.5-year intervals for up to 7.5 years or until PD was diagnosed by a blinded neurologist. Of the 34, 9 were diagnosed with a central LBD during follow-up (LBD+ group) and 25 were not (LBD- group).

The results were remarkable. Almost all the participants who had low ^{18}F-DA-derived radioactivity initially developed a central LBD during follow-up, whereas almost all the participants with normal radioactivity did not.

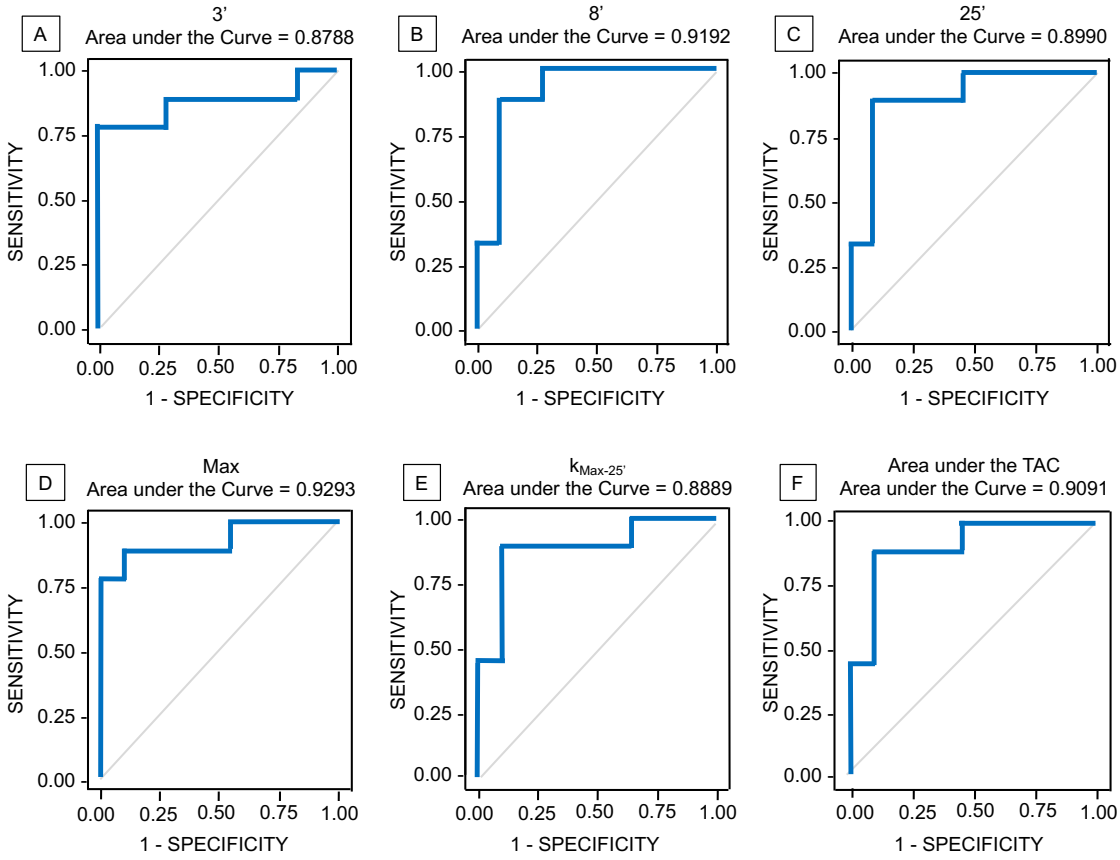


At-risk individuals with low ^{18}F-DA-derived radioactivity (cutoff 6,000 nCi-kg/cc-mCi) had a high likelihood of developing a central Lewy body disease (LBD) during follow-up, whereas almost all individuals with the same risk factors but normal ^{18}F-DA-derived radioactivity were not diagnosed with a central LBD during follow-up.



The LBD+ and LBD- groups differed in indices of cardiac sympathetic innervation (A) and a vesicular storage defect in cardiac sympathetic nerves (B), whereas the groups did not differ in an index of myocardial perfusion (C).

Conversely, the LBD+ group differed from the LBD- group in terms of a variety of indices of cardiac noradrenergic deficiency when they were initially evaluated.

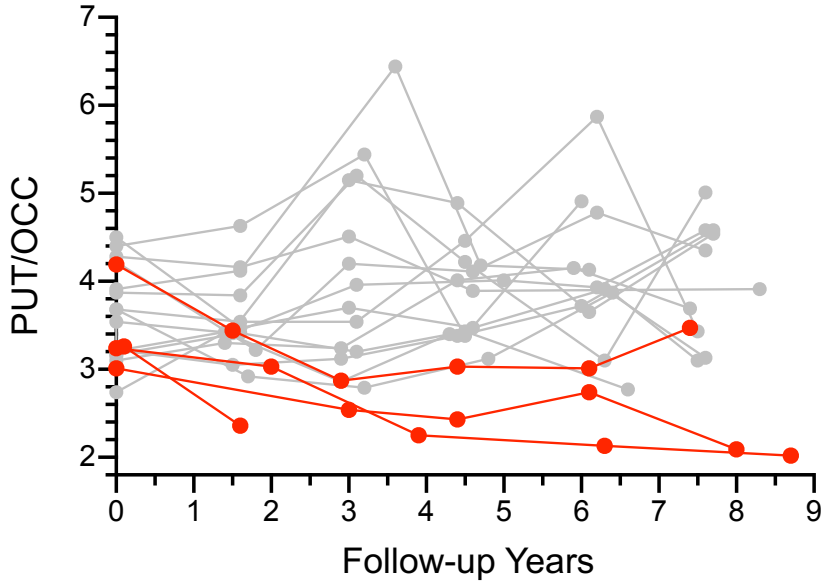


Kaplan-Meier curves showing that a variety of indices of cardiac sympathetic innervation sensitively and specifically separated the LBD+ from the LBD- group.

A sophisticated statistical modeling procedures showed that the low ^{18}F-DA-derived radioactivity in the LBD+ group reflected a combination of cardiac sympathetic denervation and a vesicular storage defect (an example of the sick-but-not-dead phenomenon) in residual nerves.

Among the LBD+ group, a substantial subgroup had initially normal putamen/occipital cortex (PUT/OCC) ratios of ^{18}F-DOPA-derived radioactivity that became abnormal by the time of diagnosis of a central LBD. Since the LBD+ group had low initial cardiac ^{18}F-DA-derived radioactivity, this meant that the cardiac sympathetic lesion preceded the

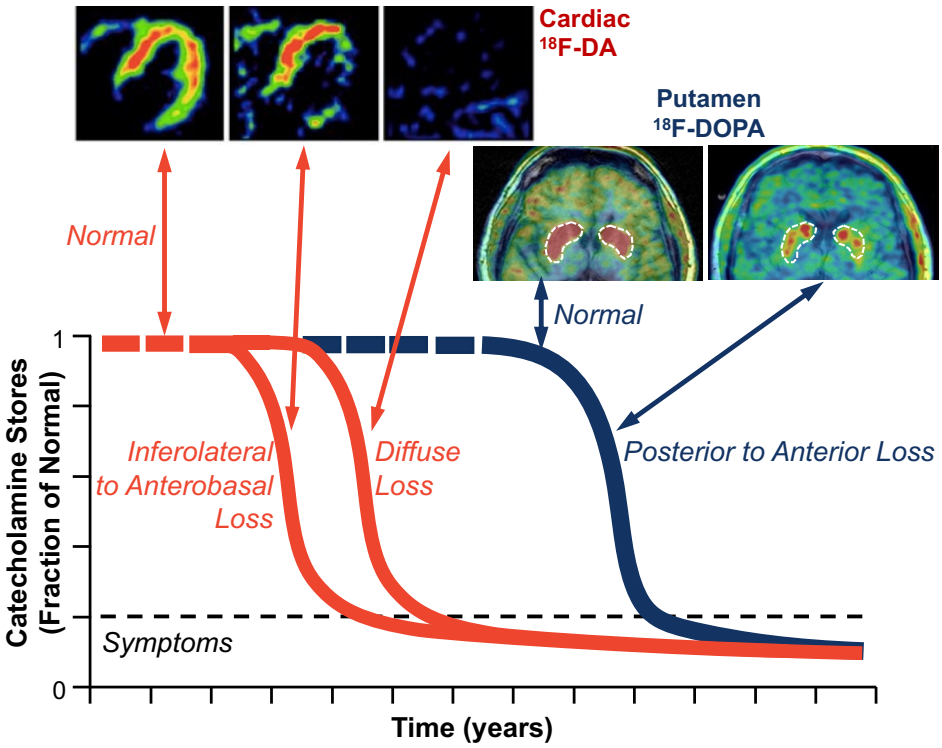
nigrostriatal dopaminergic lesion. That is, the disease process appeared to begin outside the brain, with early involvement of the autonomic nervous system.



Putamen/occipital cortex ratios of ^{18}F-DOPA-derived radioactivity in the PDRisk study. Highlighted in red are individuals in the LBD+ who had normal initial PUT/OCC ratios (cutoff 2.7) that became subnormal by the time of diagnosis of PD.

The results of the PDRisk study supported the concept of a tri-phasic loss of cardiac norepinephrine stores years before the loss of putamen dopamine stores, exactly in line with our case report many years previously about the patient with pseudopheochromocytoma who had low cardiac ^{18}F-DA-derived radioactivity 4 years before the motor onset of PD.

There are three key take-home messages from the PDRisk study. First, among individuals with multiple PD risk factors, low cardiac ^{18}F-DA-derived radioactivity predicts subsequent development of a central LBD. Second, LBD+ participants differ from LBD- participants in the occurrence of low ^{18}F-DA-derived radioactivity upon initial evaluation. Third, and most important, the ability to identify preclinical LBDs in at-risk individuals enables experimental trials to treat or even prevent a major class of neurodegenerative diseases.



Graphical Abstract from the PDRisk study. A tri-phasic loss of cardiac catecholamine stores revealed by ^{18}F-dopamine PET scanning precedes a tri-phasic loss of striatal catecholamine stores revealed by ^{18}F-DOPA PET scanning.

The future of catecholaminergic neuroimaging

Our article in the *Journal of Clinical Investigation* indicated the power of ^{18}F-DA PET for identifying preclinical central LBDs in at-risk individuals. Until now, ^{18}F-DA PET has been available only at the NIH Clinical Center. I hope the results documenting the ability to identify preclinical central LBDs will induce researchers at other institutions to join the Investigational New Drug (IND) application for ^{18}F-DA or apply for an IND, about which of course I'd be happy to advise or collaborate. In Brian Buffini's analogy of the three bowls, stability, success, and significance, I'd be filling the bottom bowl.

CARDIOVASCULAR AUTONOMIC PHYSIOLOGY AND PATHOPHYSIOLOGY

Using Brian Buffini's analogy of the 3 bowls, on the topics of autonomic physiology and pathophysiology I began pouring into the top bowl when as a college student at Yale I studied heart rate changes associated with the experience of emotion, as a medical student at Johns Hopkins I learned about the Oxford technique for measuring baroreflex sensitivity, and as a medical resident at the University of Washington I described the electrocardiogram in stroke. Throughout the decades since then of conducting patient-oriented research in intramural NIH I've continued to pour into the top bowl. As predicted from the analogy of the 3 bowls, by continuously pouring into the top bowl I attained success; now I feel the bottom bowl, the bowl of significance is beginning to fill up.

Different methods to assess baroreflex function in humans

By the late 1970s several techniques had been introduced to examine baroreflex function—especially baroreflex-cardiovagal function—in humans. By the Oxford technique one could inject a vasoconstrictor (e.g., phenylephrine) or vasodilator (e.g., nitroglycerine) and calculate the slope of the relationship between cardiac interbeat interval and systolic blood pressure. By application of external suction or pressure at the neck one could stimulate or inhibit carotid sinus baroreceptors and measure heart rate or blood pressure responses for given amounts of suction or pressure. During performance of the Valsalva maneuver, one could measure the extent of decrease in interbeat interval for a given decrease in systolic pressure in Phase II and the extent of increase in interbeat interval for a given amount of overshoot of pressure above baseline in Phase IV. When I compared these measures in the same subjects, the different indices were correlated with each other, but only weakly so. My report on the comparison of techniques to measure baroreflex sensitivity, published in *Circulation* in 1982, became a citation classic.

Comparison of Techniques for Measuring Baroreflex Sensitivity in Man

DAVID S. GOLDSTEIN, M.D., PH.D., DAVID HORWITZ, M.D., AND HARRY R. KEISER, M.D.

SUMMARY Because discrepancies about baroreflex sensitivity in essential hypertension may have resulted from the use of different measurement techniques, we assessed the extent to which the results of different techniques agree in the same subjects. The eight techniques studied were the change in RR interval per unit change in systolic pressure during the Valsalva maneuver, upon release of the Valsalva maneuver, after injection of phenylephrine and after injection of nitroglycerin; the changes in RR interval and in systolic pressure per mm Hg externally applied neck suction; and the changes in RR interval and systolic pressure per mm Hg externally applied neck pressure.

The average intercorrelation among these measures in 30 subjects was statistically significant ($r = 0.36$, $p < 0.01$), but suggests that variance in one measure accounted for an average of about 13% of the variance in other measures. Standard deviations among subjects were often as large as the mean, indicating important interindividual variability as well.

These findings demonstrate that baroreflex sensitivity varies widely among subjects and that different techniques for measuring baroreflex sensitivity probably measure different aspects of baroreflex function.

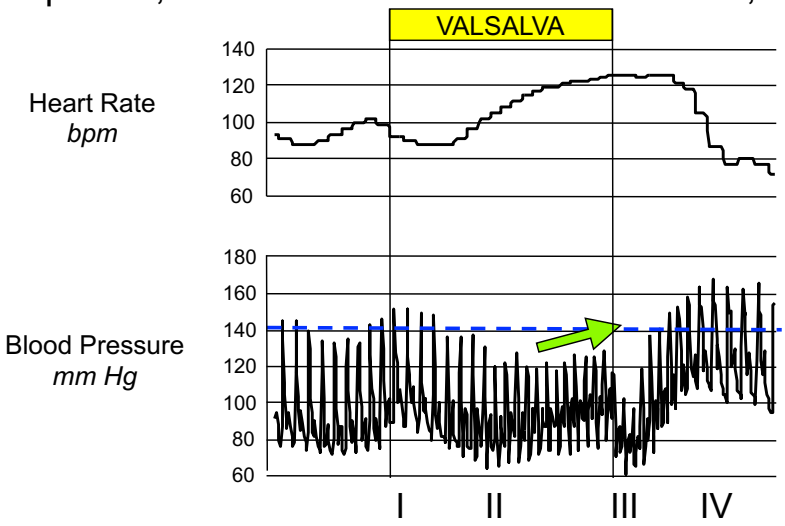
Abstract of the first report comparing clinical techniques for measuring baroreflex sensitivity.

Non-invasive detection of sympathetic neurocirculatory failure

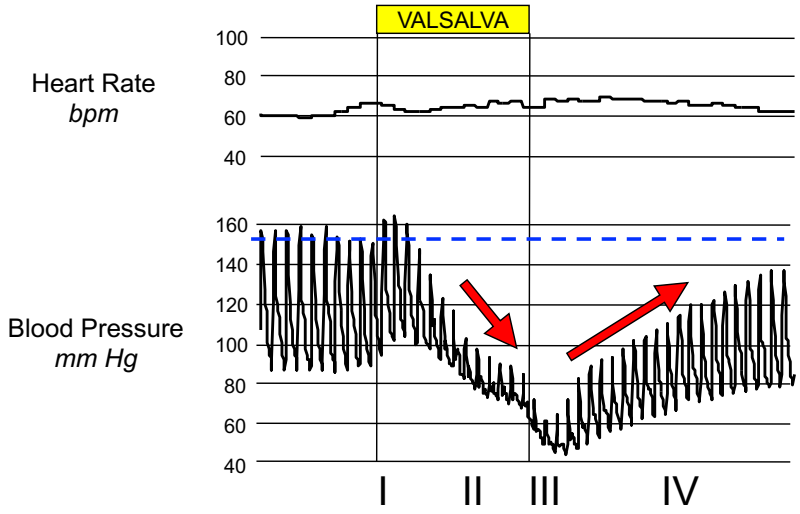
A cardinal manifestation of chronic autonomic failure is orthostatic hypotension (OH), a fall in blood pressure each time the person stands up. There are many potential causes of OH, including dehydration, prolonged recumbency, gastrointestinal bleeding, and drugs. How can one identify that OH in an individual patient is neurogenic (nOH) and reflects failure to increase sympathetic noradrenergic outflows reflexively in response to a decrease in venous return to the heart—i.e., sympathetic neurocirculatory failure?

One way to do so, using catecholamine neurochemistry, would be to measure the extent of orthostatic increase in plasma levels of norepinephrine (NE), the main chemical messenger of the sympathetic nervous system in cardiovascular regulation. Plasma NE levels normally approximately double within 5 minutes of transition from supine rest to upright posture. Another way, using a physiological approach, would be to track the blood pressure continuously when the patient performs the Valsalva maneuver.

When a person blows against a resistance or strains with a closed glottis, the blood pressure (BP) changes in 4 phases. In Phase I, just after starting to squeeze, the blood is forced out of the chest, and the BP



Normal heart rate and blood pressure responses to the Valsalva maneuver.



Abnormal heart rate and blood pressure responses to the Valsalva maneuver, indicating baroreflex-sympathoneural and baroreflex-cardiovagal failure.

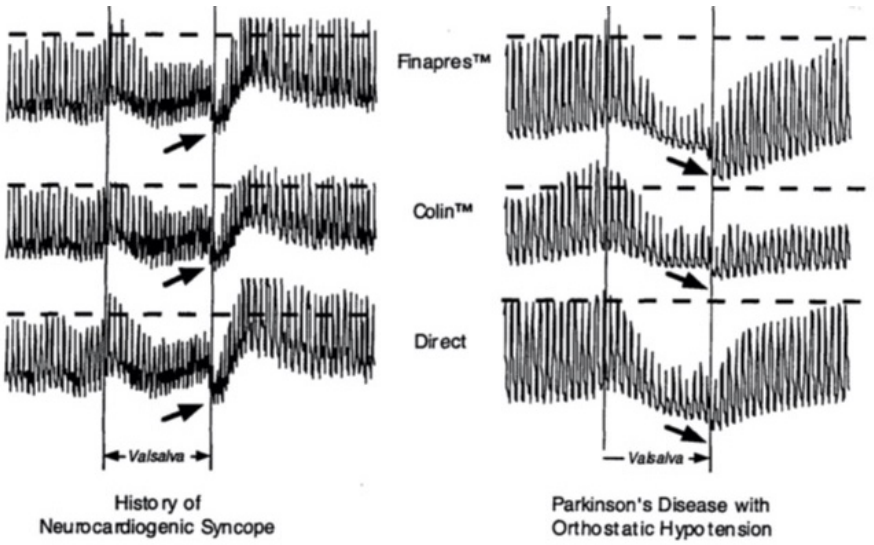
increases briefly. This is mechanical and has nothing to do with reflexes. As you continue to strain, the high pressure in the chest and abdomen results in less blood reaching the heart, and the heart pumps less blood, so normally in Phase II the BP falls. The brain rapidly directs a reflex in which

outflows in the sympathetic noradrenergic system (SNS) increase, norepinephrine (NE) is released, the NE binds to its receptors in the heart and blood vessel walls, and the blood vessels constrict. As a result, at the end of Phase II the BP increases, even though the heart is still pumping out less blood. Also during Phase II, the heart rate normally goes up, due to withdrawal of parasympathetic nervous system outflow to the heart via the vagus nerve. Then the patient relaxes. The BP immediately falls (Phase III)—a kind of mirror image of the increase in Phase I. The decrease in pressure in Phase III has nothing to do with reflexes. Finally, in Phase IV the patient is relaxed, and now there is no impediment in blood returning to the heart. The heart pumps the blood, but it pumps the blood into the reflexively constricted vasculature, and so the BP rapidly increases and overshoots the baseline value. Because of the overshoot in BP, in Phase IV the heart rate rapidly reflexively falls back to baseline.

In a patient with failure of this reflex, in Phase II the BP decreases progressively, and in Phase IV the BP returns slowly to and does not overshoot the baseline value. These are signs of baroreflex-sympathoneural failure. I've defined the combination of OH with baroreflex-sympathoneural failure as sympathetic neurocirculatory failure. In most (but not all) patients with baroreflex-sympathoneural failure there is also baroreflex-cardiovagal failure, with a blunted increase in heart rate during Phase II despite the large fall in BP.

Until the introduction of non-invasive means to track BP continuously via an autonomic finger cuff device or radial artery tonometer, identifying sympathetic neurocirculatory failure required intra-arterial cannulation. With non-invasive methods for recording BP continuously one can detect sympathetic neurocirculatory failure non-invasively.

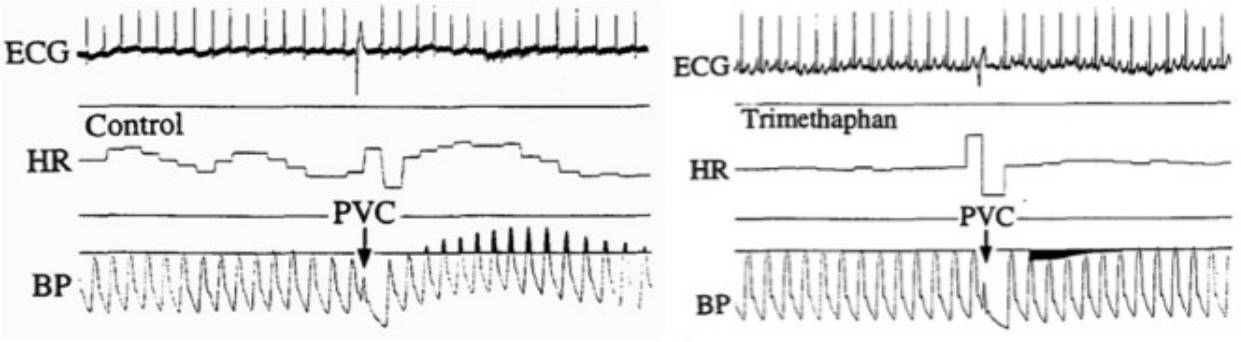
I was the first to report non-invasive detection of sympathetic neurocirculatory failure, with validation of both the finger cuff and radial artery tonometric systems vs. intra-arterial BP.



Validation of finger cuff (Finapres) and radial artery tonometric (Colin) systems for detecting baroreflex-sympathoneural failure

The one-beat Valsalva maneuver

Some patients cannot perform a technically adequate Valsalva maneuver. Patients with chronic autonomic failure often after cardiac ectopy. I discovered that in patients with sympathetic neurocirculatory failure related to pure autonomic failure, multiple system atrophy, or Parkinson's disease with orthostatic hypotension, after premature beats the pattern of blood pressure (BP) is abnormal. In healthy people, after a premature beat, the BP rapidly but briefly overshoots the baseline value, whereas in baroreflex-sympathoneural failure there is no BP overshoot after the premature beats. To confirm the altered BP pattern was the result of baroreflex failure, in a healthy subject who happened to have frequent premature ventricular contractions (PVCs) I reviewed physiological recordings from before and during ganglion blockade with trimethaphan. Before ganglion blockade, after each PVC the BP would rapidly overshoot the baseline value. During ganglion blockade, after each PVC the BP failure to overshoot the baseline value. These data demonstrated that the pattern of BP after a premature beat can provide a "one-beat Valsalva maneuver" for detecting baroreflex-sympathoneural failure.

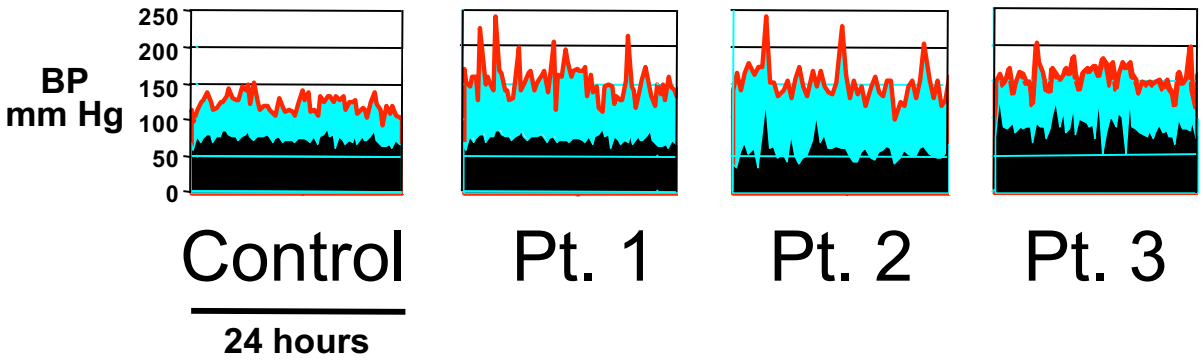


The one-beat Valsalva maneuver. In this healthy subject who had frequent premature ventricular contractions (PVCs), after each PVC the blood pressure (BP) would rapidly overshoot the baseline value. When the subject was ganglion blocked with trimethaphan, which eliminates baroreflex-sympathoneural and baroreflex-cardiovagal functions, after each PVC the BP failure to overshoot the baseline value.

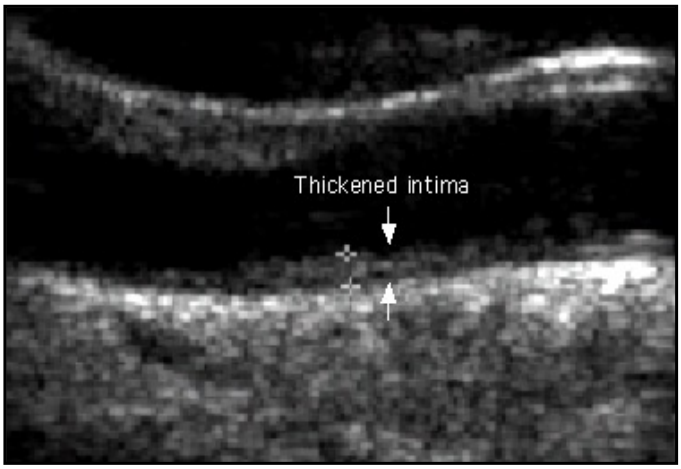
Baroreflex failure as a remote sequela of irradiation of the neck

When Yehonatan ("Yoni") Sharabi was a Clinical Fellow in our Section he noted that a group of patients with labile blood pressure had a remote history of neck radiation therapy, such as to treat a lymphoma. The disease itself was gone. He hypothesized—correctly—that baroreflex failure linked neck irradiation in the distant past with cardiovascular instability years later.

Radiation therapy tends to accelerate hardening of the arteries (arteriosclerosis) in the irradiated area. The baroreceptors are distortion receptors. They could become encased in a rigidified carotid sinus

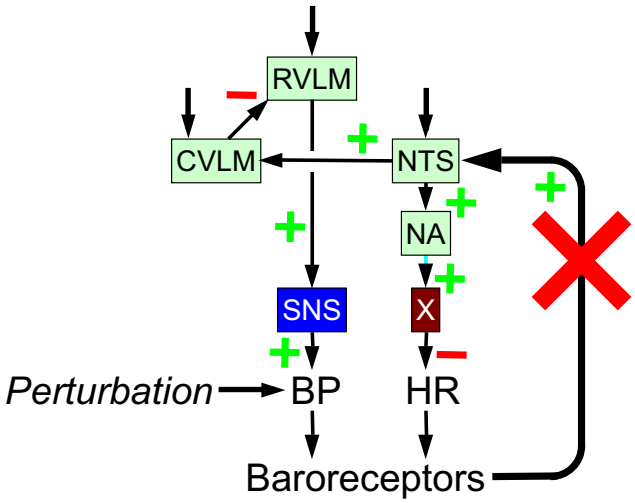


Labile blood pressure (BP) in 3 patients with a history of neck irradiation.



Thickened carotid artery wall in a patient with labile hypertension and a remote history of neck irradiation.

The result would be afferent arterial baroreflex failure. In afferent baroreflex failure, any perturbation altering BP is not buffered by way of the arterial baroreflex.



Afferent baroreflex failure.

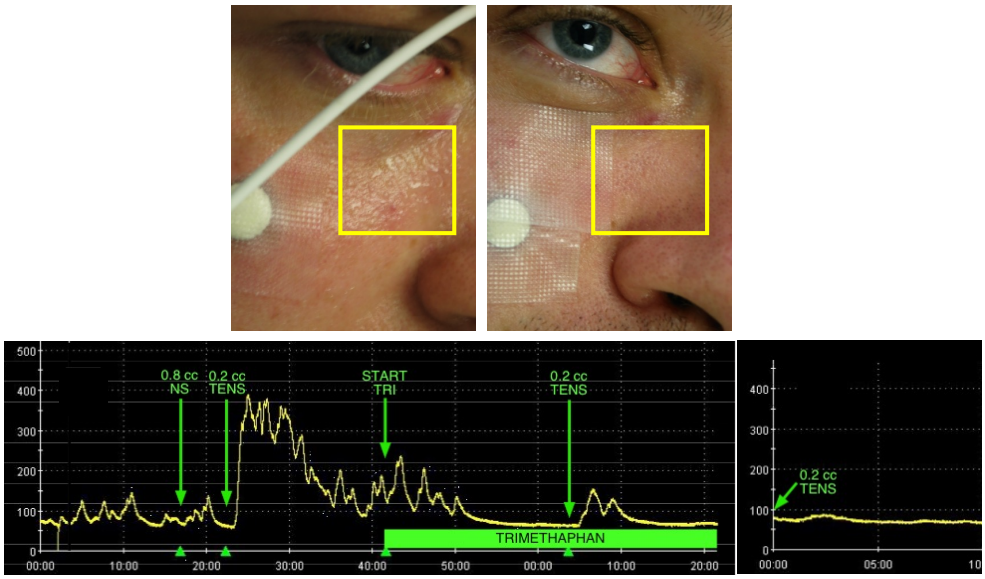
Painful sweating

Here is another illustration of the teaching that the most important autonomic function test is the history. A young adult man in Switzerland called about frequent episodes of pain, sweating, and flushing bilaterally in the face and scalp. Gustatory stimuli (e.g., orange juice, pickled onions) reliably evoked episodes, but episodes also frequently came on

spontaneously. The problem had begun during adolescence, about the time of topical treatment and then electrocauteries for facial warts. The patient reported benefit from tricyclic antidepressants, guanethidine, and trospium chloride (an anti-cholinergic for urinary urgency). There was no pain or excessive sweating in other body areas, nor pain with exercise.

The history of improvement of the painful sweating by drugs that work via the autonomic nervous system induced me to have the patient come to the NIH for testing. Our protocol called for the ganglion blocker trimethaphan, which decreases sympathetic and parasympathetic outflows, and edrophonium (Tensilon), which increases the outflows. Tensilon injection evoked the painful sweating, and trimethaphan prevented this effect. The patient brought trospium with him, and this also prevented the Tensilon-induced pain and sweating. Whereas bicycle exercise produced the same increment in forehead humidity as in a spontaneous episode, this did not evoke pain. Neither did infusion with tyramine, which increases norepinephrine release from sympathetic nerves. These findings pointed to the painful sweating being associated with parasympathetic cholinergic activation. As predicted from this concept, local iontophoretic acetylcholine administration to one cheek evoked pain and sweating—bilaterally. This suggested some form of central sensitization.

I presented the case on a Tuesday morning at the NINDS Grand Rounds, the old fashioned way with the patient actually there. A questioner in the audience asked whether an anti-cholinergic cream applied locally would be as effective as an oral anti-cholinergic with fewer systemic side effects. We had the NIH Pharmacy compound glycopyrrolate cream. By the end of the week the patient was cured. The patient’s case was reported in *Neurology.* We proposed that after destruction of cutaneous nerves, aberrant regenerant sprouting innervates sweat glands, producing gustatory sweating, and innervates nociceptors, producing pain. The patient’s chronic depression was alleviated, he returned to college and finished his degree. He then went on to obtain a doctor degree in molecular biology and as of this writing is a post-doctoral fellow.

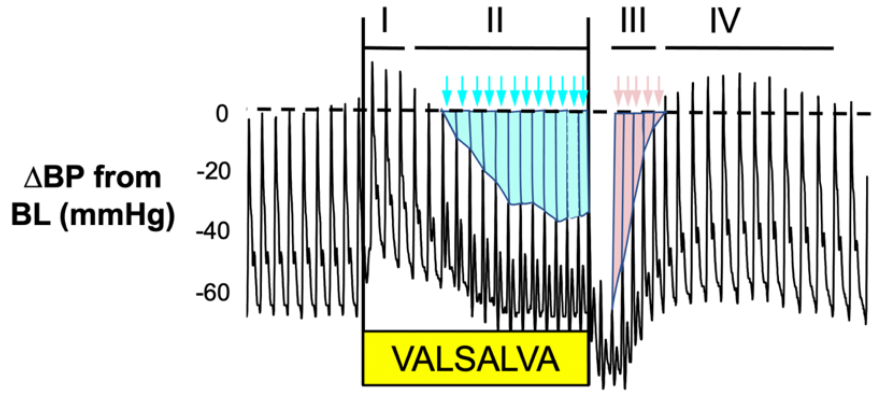


Painful sweating. Since edrophonium (TENS) evoked painful sweating and trimethaphan prevented this effect, the pain was autonomically mediated.

Quantifying baroreflex-sympathoneural function

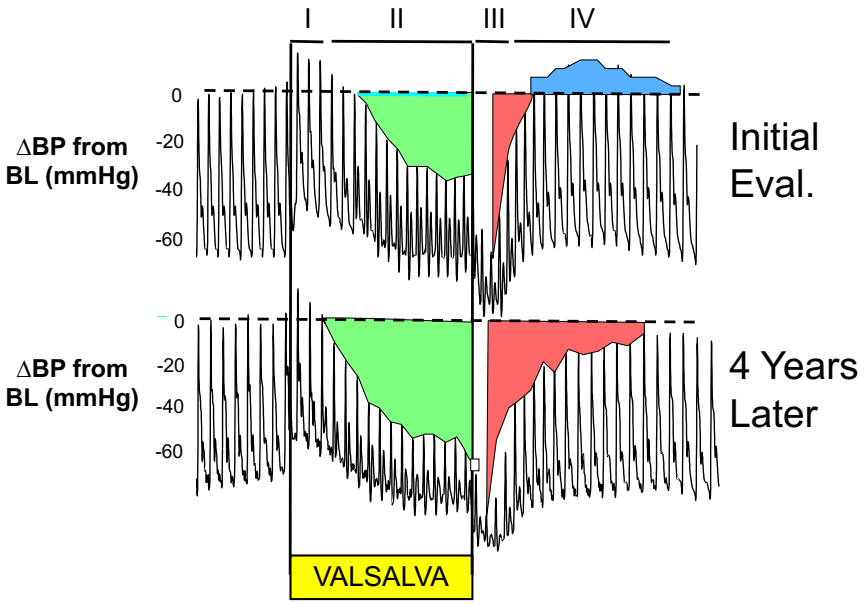
In 1971 I spent a summer at Oxford in an elective in cardiovascular physiology. Almost 40 years later, in 2010 Faisal Rahman was a medical student at Oxford when he did an elective in our Clinical Neurocardiology Section. Within 3 months he had not only initiated but had completed two projects, both of which were published with him as the first author. One of these was on his developing and applying a means to quantify baroreflex-sympathoneural function by analyzing beat-to-beat blood pressure (BP) responses associated with the Valsalva maneuver.

As noted above, in baroreflex-sympathoneural failure the BP falls progressively in Phase II, and in Phase III-IV the BP increases slowly back to the baseline value without an overshoot. This meant that if one integrated the area under the baseline BP during Phase II, the area would be large because of the lack of increase in BP at the end of Phase II; and if one integrated the area under the baseline BP during Phase III-IV, the area would be large because of the prolonged pressure recovery time. Faisal developed a method to carry out these integrations by applying the trapezoid rule.



Faisal Rahman developed a method using the trapezoid rule to integrate areas under the baseline blood pressure (BP) in Phase II (aqua) and Phase III (pink).

We used Faisal's method to document that in a patient who developed symptomatic Parkinson's disease between the time of initial evaluation and 4 years later, baroreflex-sympathoneural dysfunction developed.

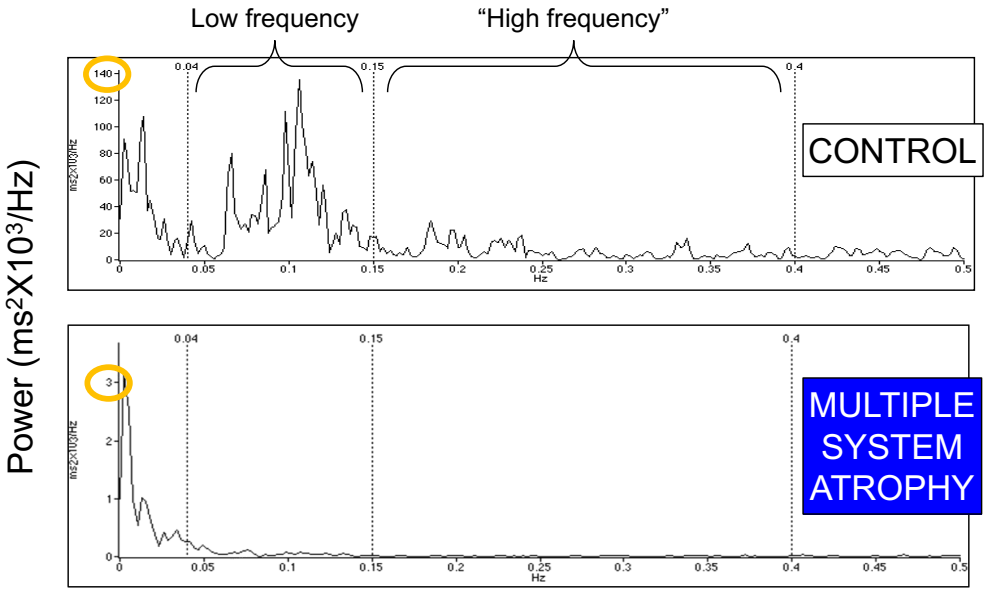


In this patient who developed symptomatic Parkinson's disease between the time of initial evaluation and 4 years later, baroreflex-sympathoneural dysfunction developed, as measured by applying the trapezoid rule to calculate baroreflex areas (green and red). There also was no post-Valsalva overshoot of pressure (blue).

The meaning of LF power

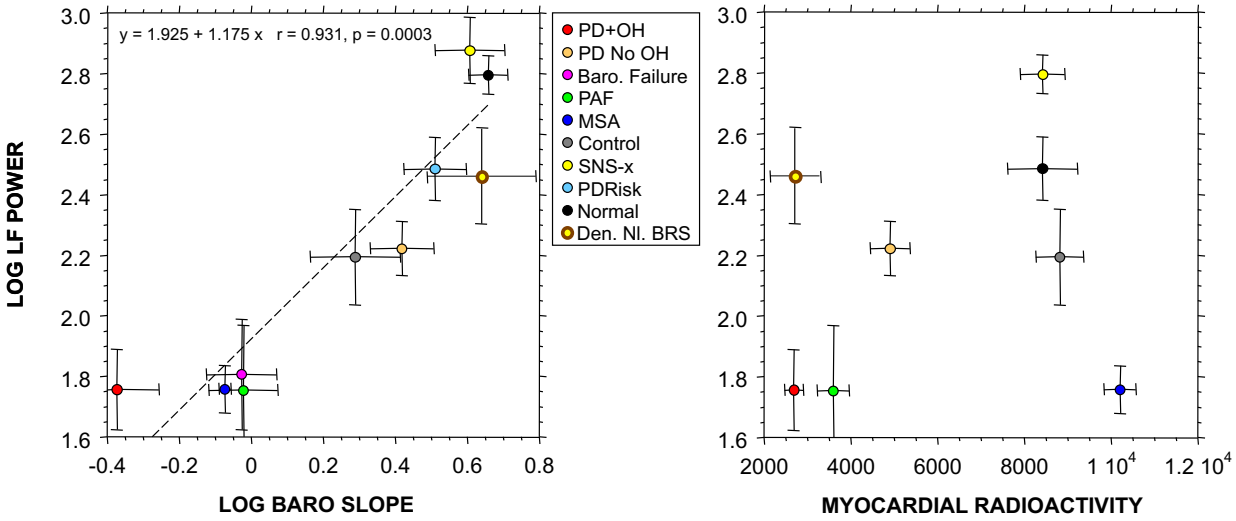
Faisal Rahman and Jeff Moak (a pediatric cardiologist who spent a year in our Section under an Intergovernment Personnel Act (IPA) agreement) were both instrumental in evaluating the meaning of low frequency (LF) power of heart rate variability.

Normally the heart rate increases with inhalation and decreases with exhalation—a phenomenon known as respiratory sinus arrhythmia, although it isn't an arrhythmia at all. The beat-to-beat heart rate oscillates in a wave-like pattern. If one graphed the size of the oscillation as a function of the frequency of the heartbeats, then at the frequency of breathing there would be a peak of "power." In people who have failure of the parasympathetic nervous system (PNS), there is little or no respiratory sinus arrhythmia, and so there is little or no peak of power at the frequency of breathing. This sort of analysis typically reveals a second peak of power, at a lower frequency than the frequency of breathing.



In this patient with baroreflex-cardiovagal and baroreflex-sympathoneural failure, at all frequencies of heart rate variability the power was extremely low (note different y-axis scales denoted by the tan ovals).

Power spectral analysis of heart rate variability offers the advantages of being safe, technically easy, and fast. The main disadvantage is that the meanings of LF power (and of the low/high frequency ratio, proposed to reflect “sympathovagal balance”) remain unsettled.



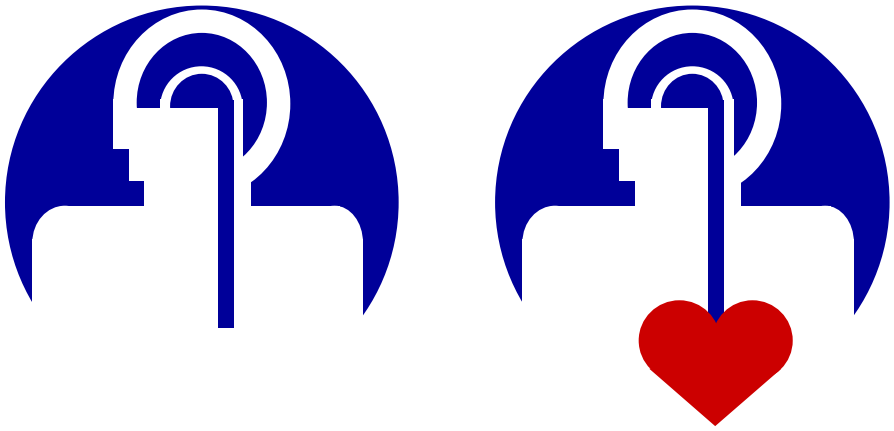
Meaning of low frequency (LF) power of heart rate variability. Across a variety of dysautonomias the log of LF power is related to the log of he baroreflex-cardiovagal gain but not to sympathetic innervation of the heart as indicated by myocardial ^{18}F-dopamine-derived radioactivity. LF power seems not to indicate cardiac autonomic “tone” so much as the ability to modulate that tone via baroreflexes.

Faisal went on to become a cardiology Fellow at Johns Hopkins, my medical alma mater and is now an Assistant Professor at Baylor as an interventional structural cardiologist. Jeff is the Director of the Electrophysiology and Pacing Program in the Children’s National Hospital Division of Cardiology in Washington, DC, and a Professor at the George Washington School of Medicine & Health Sciences.

STRESS AND HOMEOSTASIS

The logo of the NINDS

From the design of a previous logo of the NINDS, one would infer that the subject matter of NINDS research is the brain *qua* the brain—e.g., epilepsy, stroke, brain tumors, diseases of brain development, nerve networks, learning, memory, aphasia, encephalitis, or degenerative brain diseases. The bottom of the spinal cord is a dead end. When I founded the Clinical Neurocardiology Section in 1999, for the Section logo I tacked a heart onto the Institute logo. The message was straightforward: The brain does "stuff." It regulates functions of all body organs, one of which is the heart.



(Left) A previous logo of the NINDS. (Right) The logo I designed for the Clinical Neurocardiology Section, now the Autonomic Medicine Section.

This integrative function has been relatively ignored. To this day there is no organizational entity in NINDS—or NIH as a whole for that matter—that deals with disorders of regulation of body organs by the brain. In neurological circles autonomic medicine has been tolerated but never emphasized.

Selye revisited

Stress and homeostasis are well established as medical scientific ideas. Selye's theory of stress and Cannon's of homeostasis were

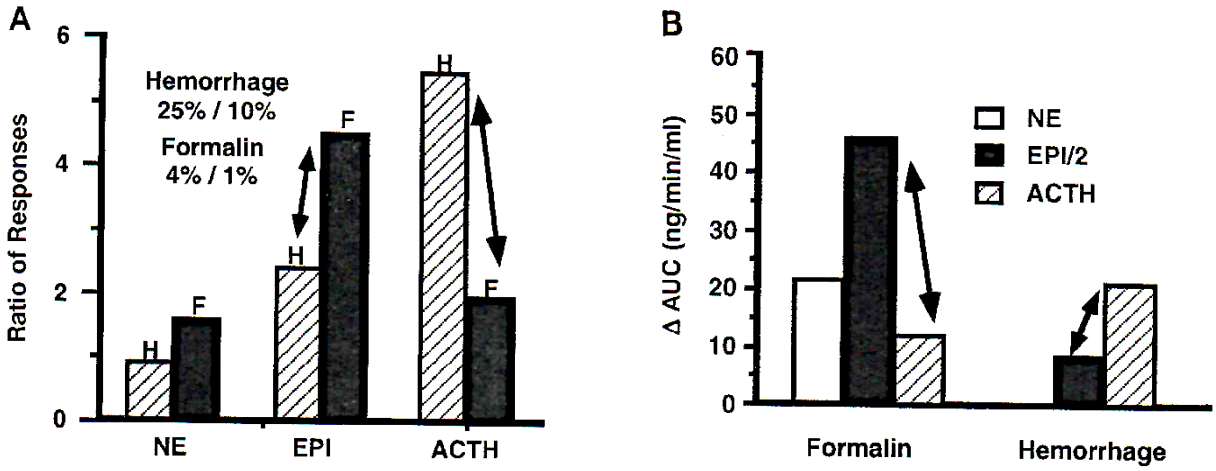
promulgated about a century ago. Hardly a day goes by without references in the media to adverse health consequences of stress or to the “fight-or-flight” response. Nevertheless, both theories have required updating, as I’ll explain.

Hans Selye introduced and popularized the stress concept. He defined stress variously as a stereotyped response pattern (the General Adaptation Syndrome, with three sequential stages of alarm, adaptation, and exhaustion), a condition or state that evokes this response pattern, or a stimulus that evokes the state. This definitional ambiguity weakened the theory as a scientific concept and incited the remark by Ffrangcon Roberts that according to Selye “…stress, in addition to being itself and the result of itself, is also the cause of itself.” Relatively late in Selye’s career, Selye defined distress as a form of stress that is unpleasant or harmful, in contrast with eustress, which he defined as pleasant and unharmful. These definitions are popular but are useless scientifically because they are circular.

Selye’s doctrine of non-specificity states that stress is the non-specific response of the body to any demand imposed upon it. About a half century went by before the doctrine of non-specificity underwent experimental evaluation, by our group.

The testing was based on stressors eliciting hypothalamic-pituitary-adrenocortical (HPA), sympathetic noradrenergic system (SNS), and sympathetic adrenergic system (SAS) responses. The doctrine of non-specificity predicts that at different stress intensities ratios of increments in neuroendocrine responses should be the same; however, when arterial plasma corticotropin (ACTH), norepinephrine (NE), and epinephrine (EPI) were measured simultaneously in conscious rats exposed to cold, intravenous insulin, hemorrhage, or immobilization, all of which increased ACTH levels, cold evoked large NE responses, insulin large EPI responses, and hemorrhage small NE and EPI responses, while immobilization elicited large increases in levels of both NE and EPI. These results were inconsistent with Selye's doctrine of non-specificity and the existence of a unitary “stress syndrome” and were more consistent with the

concept that each stressor has a particular HPA, SNS, and SAS "signature." Our report, published in the *American Journal of Physiology* in 1998, is a citation classic.



Testing Selye's doctrine of non-specificity. Selye's theory (with simplifying assumptions in order to render it testable) predicts that in each of the panels the arrows are parallel and the same length.

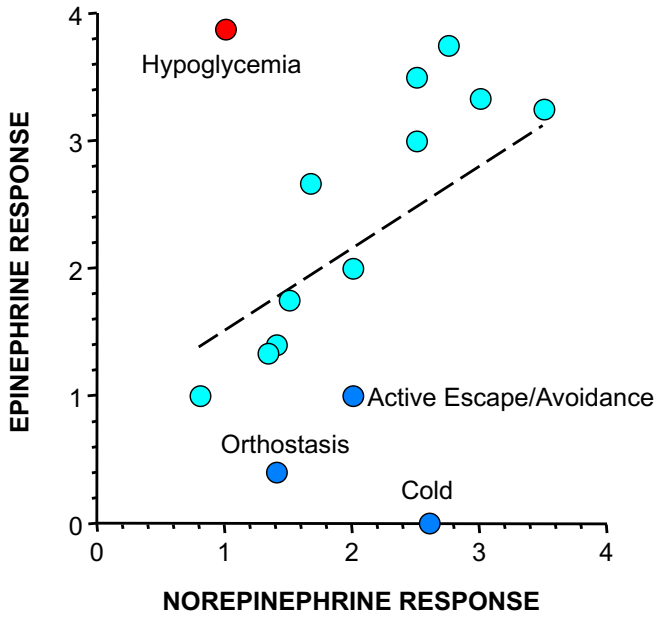
The term, "dishabituation," is used to refer to a return to the initial magnitude of response after habituation has taken place. A related phenomenon is exaggerated responsiveness of adapted organisms to a novel ("heterotypic") stressor. Individuals who have habituated to one form of stress exhibit exaggerated SNS, SAS, and HPA responses to heterotypic stressors. The doctrine of non-specificity also does not account for this phenomenon.

Cannon revisited

Walter B. Cannon, who coined the term, "homeostasis," was one of the first critics of Selye's theory. Cannon's critique centered on the assumption that a stereotyped response pattern would be adaptive regardless of the character of the stressor. Since a non-specific stress response would not have provided a natural selective advantage, a stereotyped stress response would not have evolved.

This criticism was ironic, because Cannon himself taught that the body responds to all emergencies the same way, increased secretion of adrenaline. How could the same response help maintain homeostasis in very different situations? Cannon's answer was that the body's response to emergencies, with adrenaline dominating that response, enhance long-term survival, even if in the short-term aspects of the response moved some of the levels for key variables from the ideal values.

This view is still widely held, although by now there is abundant evidence that the neuronal component, the SNS, and the hormonal component, the SAS, are constitutively active, responsive to activities of daily life, and can be activated differentially in response to different stressors. Activity of the SNS as indicated by plasma norepinephrine is more responsive than the SAS as indicated by plasma epinephrine to cold, orthostasis, and active escape/avoidance, while the SAS is more responsive than the SNS to hypoglycemia.



The sympathetic noradrenergic system (SNS) activity as indicated by plasma norepinephrine is more responsive than the sympathetic adrenergic system (SAS) as indicated by plasma epinephrine to cold, orthostasis, and active escape/avoidance. The SAS is more responsive than the SNS to hypoglycemia.

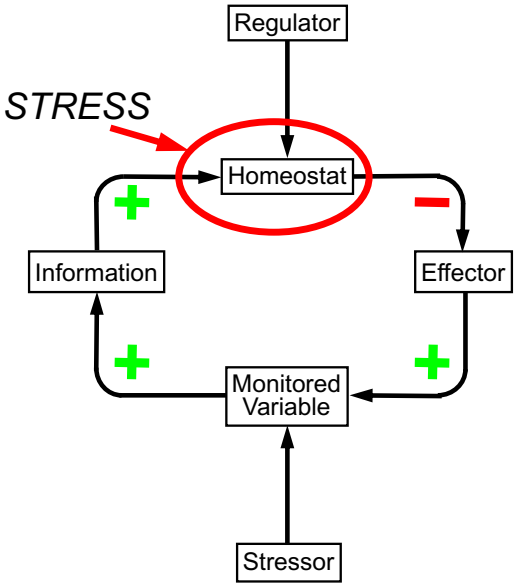
Cannon introduced the notion of “fight or flight.” By this term he meant that a rather stereotyped pattern of activation prepares the organism for extreme exertion in emergencies posed by antagonistic encounters. The state of knowledge at the time did not allow for the possibility of different patterns of physiological and biochemical responses that would be coupled to the behavioral and emotional experiences.

Experimentally, active avoidance behavior is associated with greater noradrenergic activation and passive freezing behavior with greater adrenergic activation, although central neural mechanisms underlying differential responses of the SNS and AHS are poorly understood.

The plasma catecholamine pattern in fainting reactions provides a clinically relevant example of differential SAS vs. SNS responses. We discovered that “sympathoadrenal imbalance” accompanies and often precedes these reactions. In sympathoadrenal imbalance there is a greater increase in plasma epinephrine than in norepinephrine levels.

A homeostatic theory of stress

As an alternative to Selye’s stress theory I’ve offered what I call the homeostatic theory. According to the homeostatic theory, stress is a condition or state in which a comparator “homeostat” senses a discrepancy between perceived or anticipated afferent input to the brain and a set-point or other algorithm for responding, and the integrated error signal results in altered effector outflows that reduce the discrepancy. Because of the negative feedback loop (an odd number of “-“ signs in the loop), the level of the monitored variable reaches a steady state.



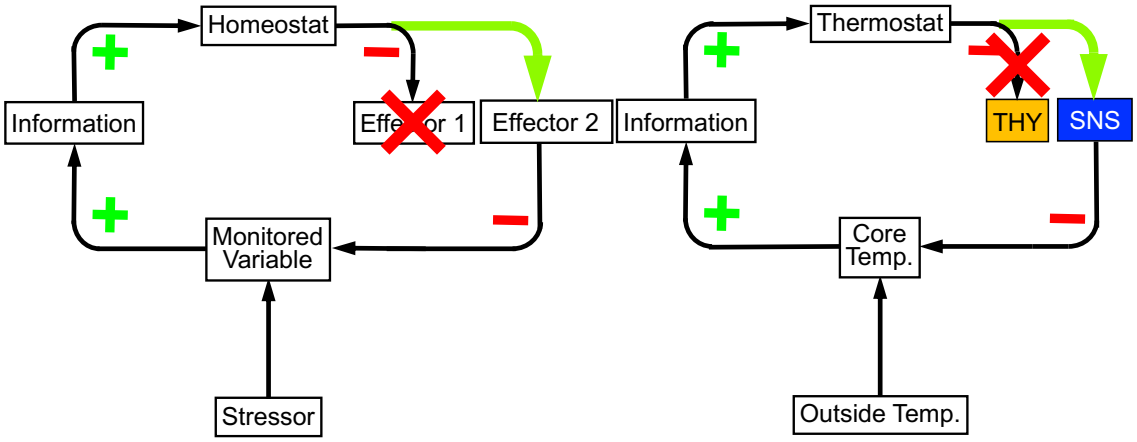
Homeostatic definition of stress.

Two corollaries of the homeostatic theory that lead to testable hypotheses are multiple effectors and effector sharing.

Multiple effectors

All the key monitored variables of the body are regulated by more than one effector. For instance, blood glucose levels are determined by insulin, glucagon, adrenaline, and cortisol. Such redundancy comes at relatively little cost. Meanwhile, having multiple effectors offers clear survival advantages. First, having multiple effectors allows at least some degree of control of the monitored variable if one effector is disabled. This is called “compensatory activation.”

Compensatory activation helps explain why, for instance, patients who are hypothyroid have increased sympathetic noradrenergic system (SNS) activity. Both the hypothalamic-pituitary-thyroid axis and the SNS are effectors that help maintain the homeostasis of core temperature. When the thyroid effector is disabled, compensatory activation of the SNS allows for at least some thermoregulation.



Compensatory activation explains sympathetic noradrenergic system (SNS) hyperactivity in hypothyroidism.

Second, having multiple effectors extends the range of control of the monitored variable. Consider how adding an air conditioner to a furnace extends the range of control of the temperature inside your house. There can be different effectors for decreases and for increases in core temperature, just as there can be different effectors for decreases and increases in blood glucose.

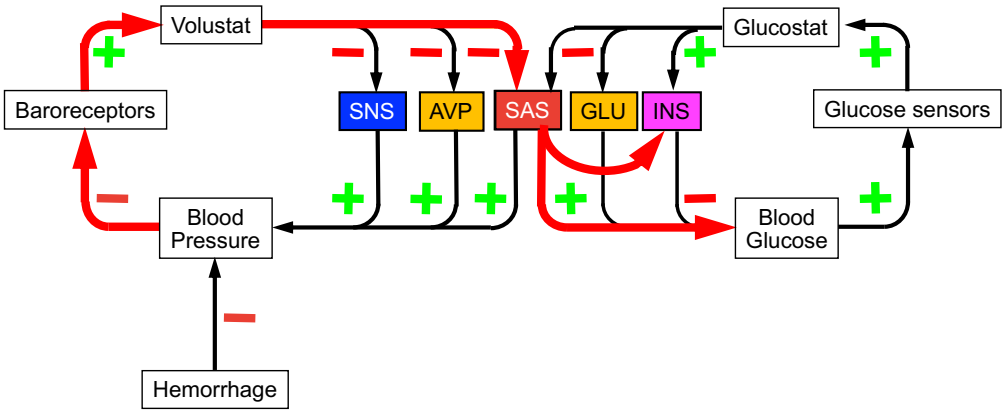
Third, having multiple effectors permits the evolution or learning of relatively specific patterns of response that are most adaptive for particular stressors.

Effector sharing

Different homeostatic systems can share effectors and by this means interact. Sharing of the sympathetic adrenergic system (SAS) effector can explain clinical phenomena such as hyperglycemia in patients who are in hemorrhagic shock (or any situation threatening organismic integrity). One may propose that medical or surgical emergencies in general tend to raise glucose levels because of sharing of the SAS effector.

Analogously, sharing of the arginine vasopressin (AVP, synonymous with anti-diuretic hormone) effector can explain hyponatremia in patients with congestive heart failure.

The homeostat theory is relatively simple to understand, responses to various perturbations (stressors) can be modeled mathematically, some phenomena such as distress-induced hyperglycemia.



Effector sharing. Sharing of the sympathetic adrenergic system (SAS) effector by the "volustat" and "glucostat" can explain hyperglycemia as a clinical sign in hemorrhagic shock.

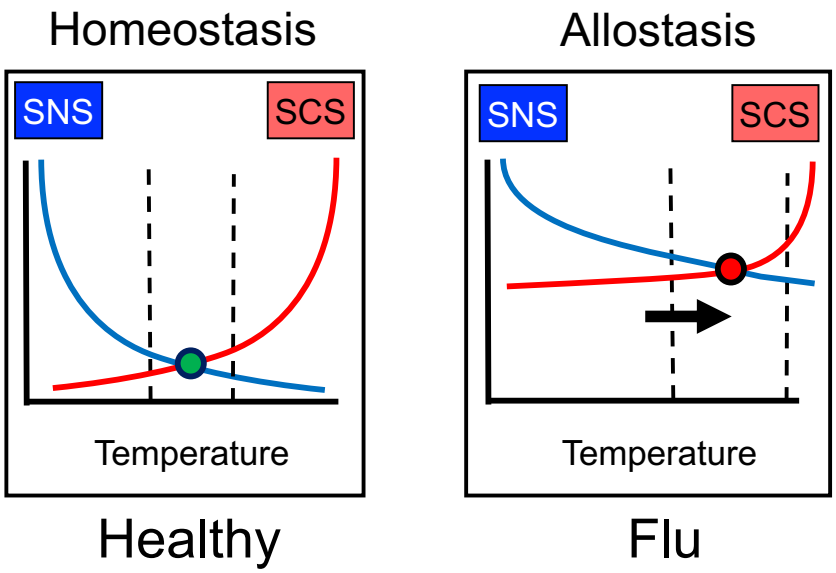
I've proposed a non-circular definition that holds that distress is a form of stress that is consciously experienced, associated with a perceived inability to cope, aversive (i.e., motivates escape or avoidance), produces instinctively communicated signs, and involves adrenocortical and adrenomedullary activation and homeostatic resetting.

From homeostasis to allostasis to dyshomeostasis

Allostasis refers to a temporary shift in an input-output curve. A low-grade fever when you have the flu is an example of allostasis. Once you recover and are back to your "old self," the homeostatic settings return to those before the acute illness. The homeostat theory relatively easily incorporates the concept of allostasis. In the homeostat model, allostasis is a change in the algorithm determined by the Regulator.

For instance, both the sympathetic noradrenergic system (SNS) and sympathetic cholinergic system (SCS) are effectors in regulation of core

temperature by evaporative heat loss. Activation of the SNS in response to cold results in cutaneous vasoconstriction and decreases evaporative heat loss. Activation of the SCS in response to heat results in perspiration and increases evaporative heat loss. Core temperature is kept within bounds. When you have the flu, the input-output curves of the effectors are shifted. Core temperature is maintained but within different bounds.



Homeostasis and allostasis. In allostasis there is a shift in input-output curves for oppositely acting effectors), resulting in regulation of the monitored variable (in this case body temperature) at a different level. The acceptable bounds are the vertical dashed lines. A low grade fever associated with a flu-like illness is an example of an allostatic state.

Irv Kopin and I conceptualized three ways homeostasis is maintained. The first is by negative feedback regulation—the stuff of myriad original research reports over decades. The second is by anticipatory allostatic adjustments in advance of the stimulus. The third—Irv's original contribution—is buffering.

In the model, the effectors are both autonomic and non-autonomic. Effector responses to a disturbance are determined by three forms of regulation.

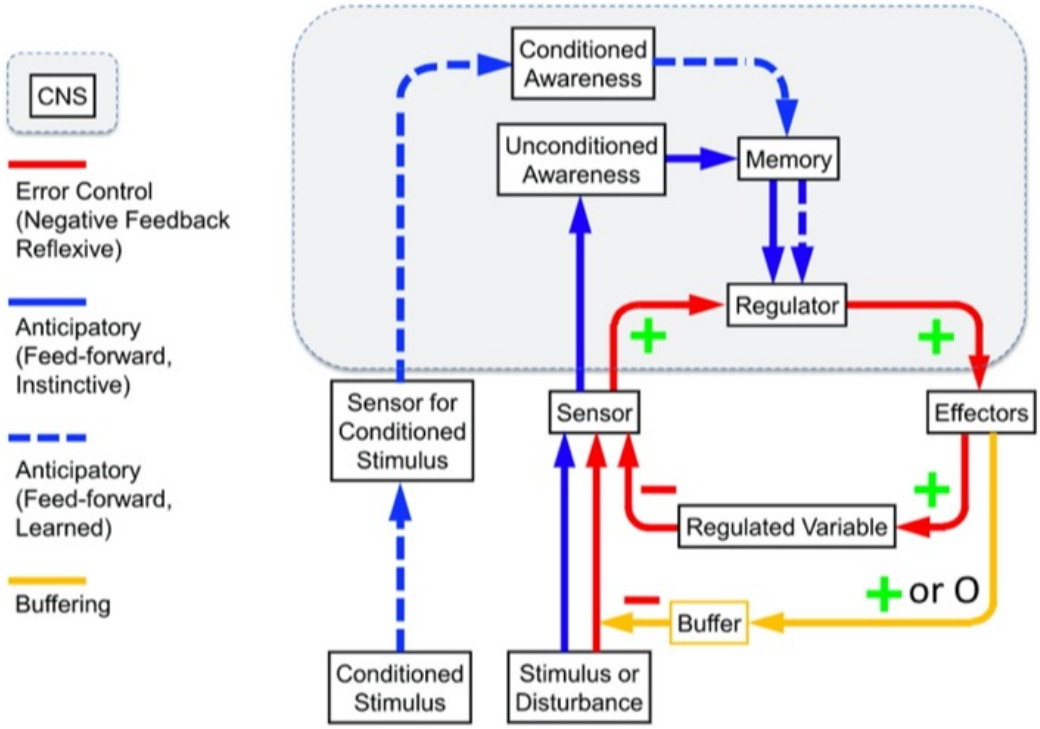
First, effector activities are determined from input-output (afferent-efferent) curves relating sensory input to effector output (error control by negative feedback).

Second, via exteroceptive or interoceptive input, effector activities are altered by instinct or imprinting, in advance of a change in the level of the regulated variable.

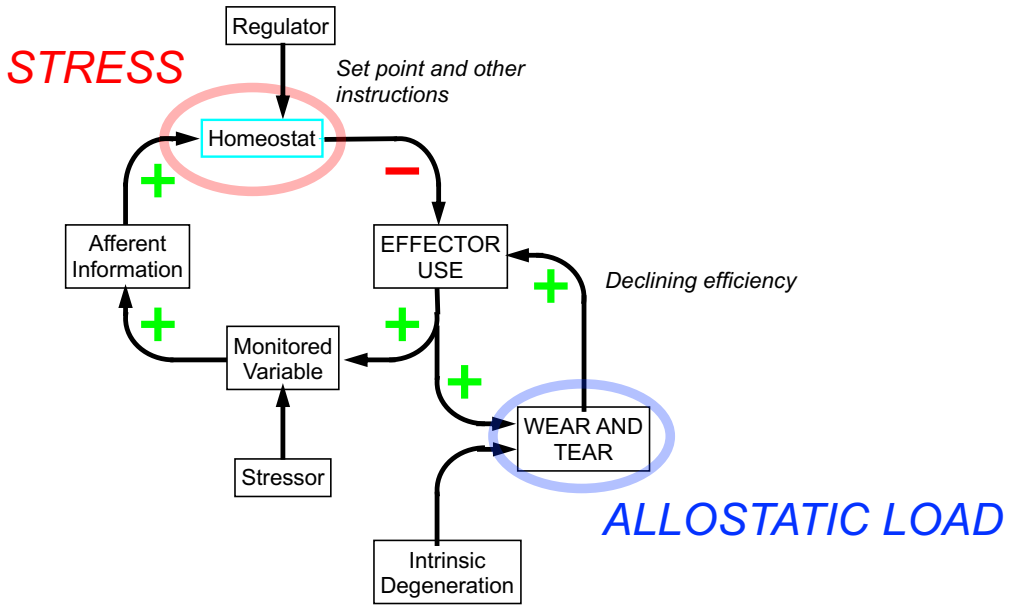
Third, via exteroceptive input, effector activities are altered by associative learning (classical or instrumental conditioning), also in advance of a change in the level of the regulated variable. The extent of sensor activation in response to a disturbance is modulated by buffering. Buffering is a means of diminishing the intensity of an external disturbance, thereby reducing the required use of reflexive homeostatic mechanisms.

Effector responses can also modify buffering in advance of a disturbance, via instinct or learned behaviors (e.g., piloerection during cold exposure, donning a jacket before entering a cold environment).

Allostatic states increase wear and tear, on both the effectors and the target organs—allostatic load. Declining homeostatic efficiencies (dyshomeostasis) associated with aging and chronic disorders can prolong or intensify the accumulation of allostatic load and eventually decrease thresholds for a variety of vicious cycles (positive feedback loops) that can be lethal.

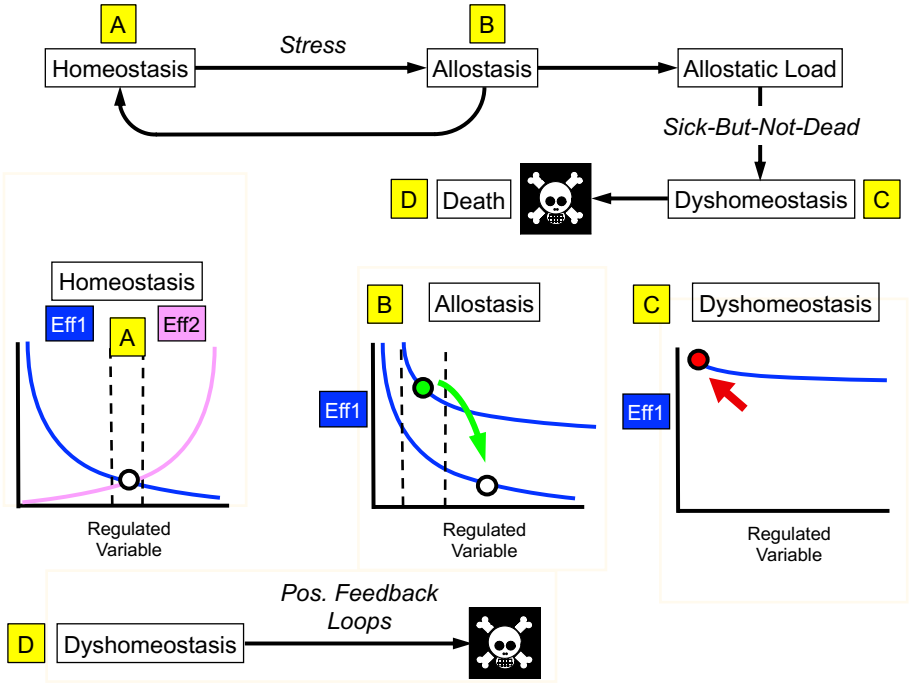


Conceptual model of homeostasis being maintained by negative feedback regulation (error control), anticipatory (feed-forward) control, and buffering.



Relationship between stress and allostatic load. Allostatic load can decrease effector efficiency, which increases effector use, which increases allostatic load—an inherently unstable positive feedback loop.

Stress-related allostatic states that are temporary may not exert a perceptible adverse long-term consequence (A and B in the Figure). Accumulation of allostatic load, however, results in effector dysfunctions—the “sick-but-not-dead” phenomenon. Progression of a disease process would be associated with dyshomeostasis (C) and a decreased threshold for induction of lethal positive feedback loops (D).



From homeostasis to allostasis to allostatic load to dyshomeostasis and death.

Homeostasis is a founding principle of integrative physiology. In current systems biology, however, homeostasis seems almost invisible. Is homeostasis a key goal driving body processes, or is it an emergent mechanistic fact? I’ve proposed that the integrative physiological and systems biological viewpoints about homeostasis reflect different epistemologies, different philosophies of knowledge. Integrative physiology is concept driven. It attempts to explain biological phenomena by continuous formation of theories that experimentation or observation can test. In integrative physiology, "function" refers to goals or purposes. Systems biology is data driven. It explains biological phenomena in terms of "omics", it depicts the data in computer models of complex cascades or

networks, and it makes predictions from the models. In systems biology, "function" refers more to mechanisms than to goals. The integrative physiologist emphasizes homeostasis of internal variables such as Pco_2 and blood pressure. The systems biologist views these emphases as teleological and unparsimonious in that the "regulated variable" (e.g., arterial Pco_2 and blood pressure) and the "regulator" (e.g., the "carbistat" and "barostat") are unobservable constructs. The integrative physiologist views systems biological explanations as not really explanations but descriptions that cannot account for phenomena we humans believe exist, although they cannot be observed directly, such as feelings and, ultimately, the conscious mind. I predict rapprochement of integrative physiology with systems biology. The resolution will avoid teleological purposiveness, transcend pure mechanism, and incorporate adaptiveness in evolution, i.e., "Darwinian medicine."

According to the homeostatic theory, stress is a condition or state, analogous to what in psychology is called an “intervening variables.” Examples of intervening variables are emotions and motivational states; they are not externally observable. Homeostats, monitored variables, and regulators are also not externally observable. The homeostat is a metaphor. No one has ever seen a homeostat, although it is possible that this could happen someday.

If homeostats are unnecessary and simplistic, what good are they? First, lumping the complex networks that make up the metaphorical regulators and conceptualizing purposes in controlling the monitored variables enable definitions of otherwise difficult ideas—such as stress. Second, thinking in terms of homeostats seems fruitful for bringing worthwhile conceptual questions to mind. For instance, in human evolution, were genetic changes fostering evaporative heat loss via the naked skin more adaptive than those fostering countercurrent temperature regulation via specialized vascular structures? This sort of question might be asked by an integrative or evolutionary physiologist but not by a systems biologist whose focus is on “how” rather than “why.” Third, the homeostat idea helps explain a variety of clinical phenomena, such as hyperglycemia signifying a poor prognosis in stroke.

Richard Kvetnansky

Richard Kvetnansky carried out pioneering experiments on catecholamines in stress based on sampling blood from indwelling arterial catheters in conscious rats. His visits to our Section led to several important discoveries published in 19 articles. Among the findings were that even gentle handling increases plasma levels of epinephrine, increments in plasma DOPA levels during immobilization reflect increased tyrosine hydroxylase activity, glucocorticoids restrain increments in catecholamine release and synthesis during immobilization, and activation of catecholaminergic systems is stressor-specific.

For researchers on catecholamines and other neurotransmitters in stress a highlight has been the quadrennial symposium Richard Kvetnansky organized on this topic at the castle in Smolenice, in his beloved country of Slovakia. He did this for 40 years. The architecture, musical performances, food, foliage, and setting, the trumpet fanfares at the opening ceremonies, and the lectures by the likes of Julie Axelrod and Irv Kopin are etched in my memory. This series saw the remarkable transition from the communist, authoritarian USSR to the free Slovak Republic. International attendees would travel by bus from the Vienna airport and cross the border near Bratislava. The first time I did this, the bus was stopped for a long time as soldiers with submachine guns inspected passports. The last time I did this, one hardly realized there was a border at all.

Mostly I remember the lecture room in the castle, where some of the world's leading authorities about catecholamines and other neurotransmitters in stress would sit, listen, question, and learn, all in one room, for a few precious, packed days at a time. As Bob Dylan sang, "I wish, I wish, I wish in vain, that we could sit simply in that room again."

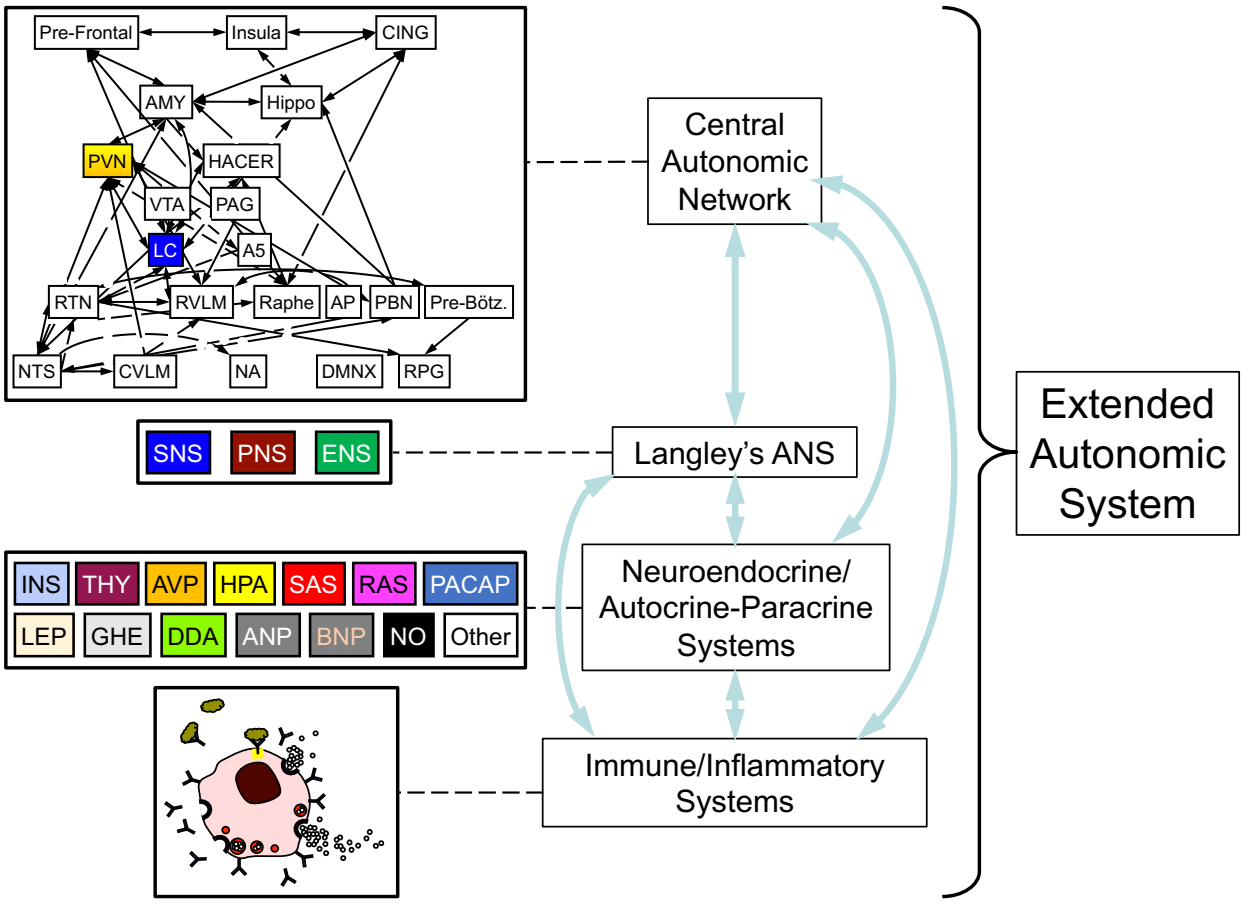
Smolenice castle in Slovakia was the site of a series of international meetings organized by Richard Kvetnansky on catecholamines and other neurotransmitters in stress.

The extended autonomic system (EAS)

Scientific advances since Langley defined the autonomic nervous system (ANS) have incited my proposal of an “extended autonomic system,” or EAS. There are 3 general reasons for this proposal. First, several neuroendocrine systems are bound inextricably to Langley’s ANS. The first to be described, by Cannon in the early 1900s, involves the hormone epinephrine, the main effector chemical of the sympathetic adrenergic system (SAS). Other neuroendocrine systems are the hypothalamic-pituitary-adrenocortical (HPA) system, the arginine vasopressin (AVP) system, and the renin-angiotensin-aldosterone system (RAS). Second, an evolving body of research links the ANS complexly with inflammatory/immune systems, including vagal anti-inflammatory and catecholamine-related inflammasomal components. Third, a hierarchical network of brain centers (the central autonomic network, CAN) regulates ANS outflows. Embedded within the CAN is the “central stress system” conceptualized by Chrousos and Gold.

Reciprocal influences among the four components of the EAS (6 combinations of interactions) set the stage for feedback loops that maintain

homeostasis and for feed-forward alterations in input-output curves that underlie allostasis.

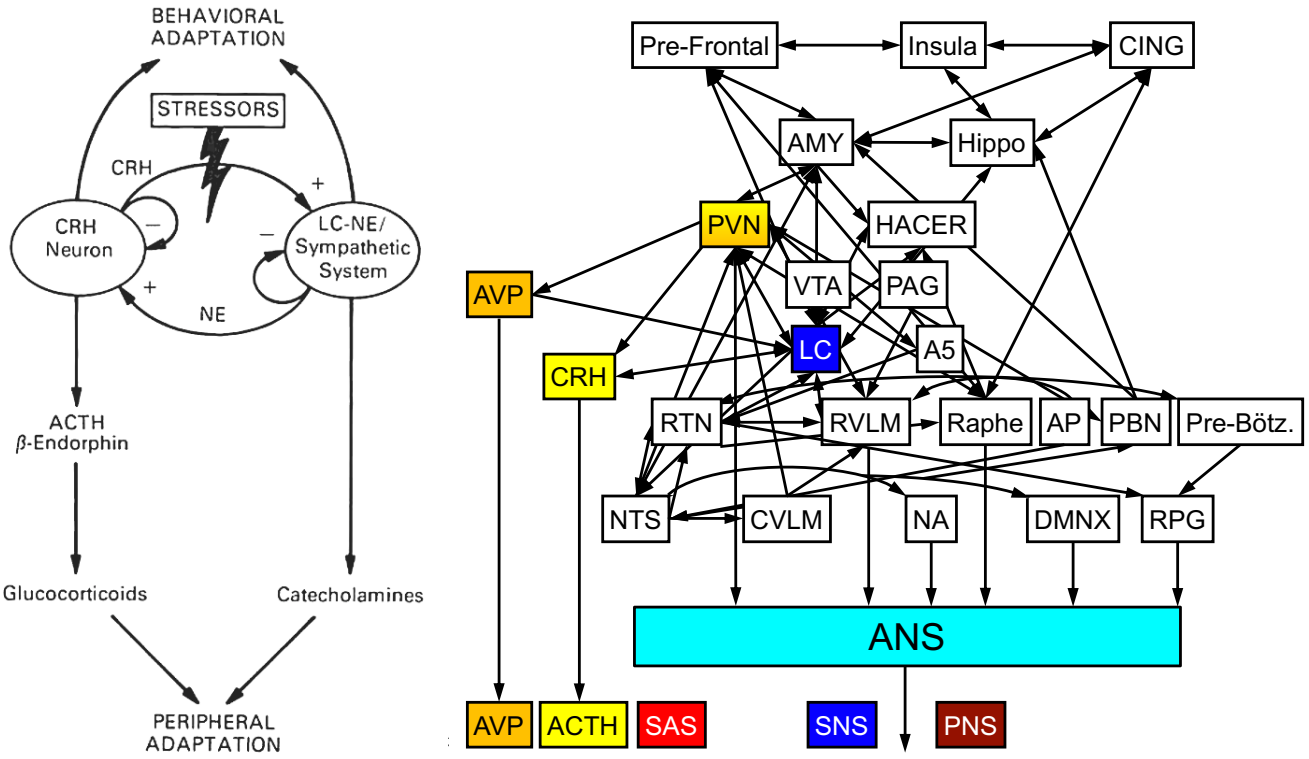


Overview of the extended autonomic system (EAS).

The EAS concept provides a framework for generating testable hypotheses related to the pathophysiology and pathophysiology-based treatment of a wide variety of complex, multi-system disorders of regulation.

The future of stress and homeostasis as scientific ideas

The last paper Irv Kopin and I wrote was about homeostatic systems, biocybernetics, and autonomic neuroscience. I understand he was still working on it during his last conscious moments. The Abstract seems appropriate as a conclusion to this section. It is reproduced verbatim here.

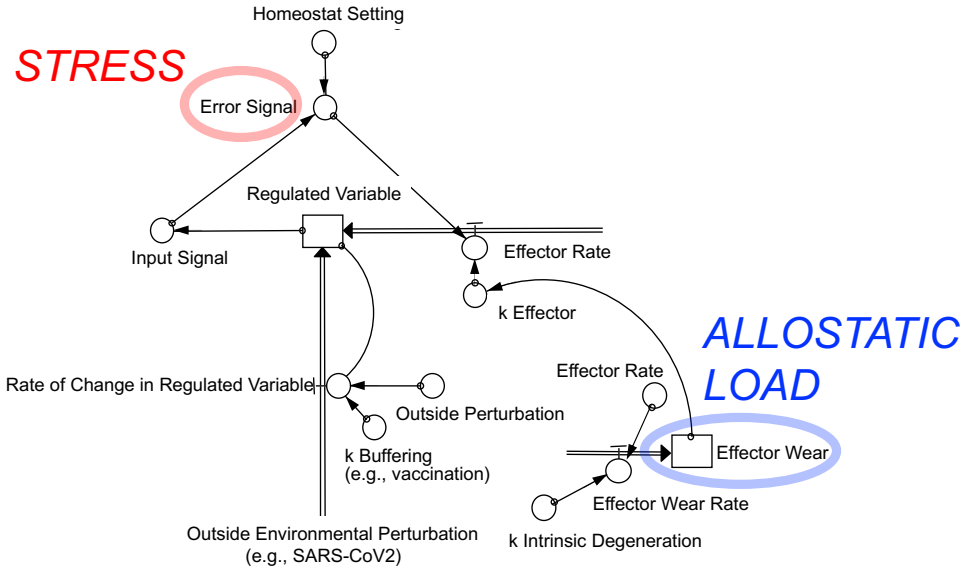


The "central stress system" (left) is part of the central autonomic network (right).

In this review we describe a series of major concepts introduced during the past 150 years that have contributed to our current understanding about how physiological processes required for well-being and survival are regulated. One can theorize that hierarchical networks involving input-output relationships continuously orchestrate and learn adaptive patterns of observable behaviors, cognition, memory, mood, and autonomic systems. Taken together, these networks function as "good regulators" determining levels of internal variables and act as if there were homeostatic comparators ("homeostats"). The consequences of models with vs. without homeostats remain the same in terms of allostatic load and the eventual switch from stabilizing negative feedback loops to destabilizing, pathogenic positive feedback loops. Understanding this switch seems important for comprehending senescence-related, neurodegenerative disorders that involve the autonomic nervous system. Our general proposal was that disintegration of homeostatic systems causes disorders of regulation in degenerative diseases and that medical cybernetics can inspire and

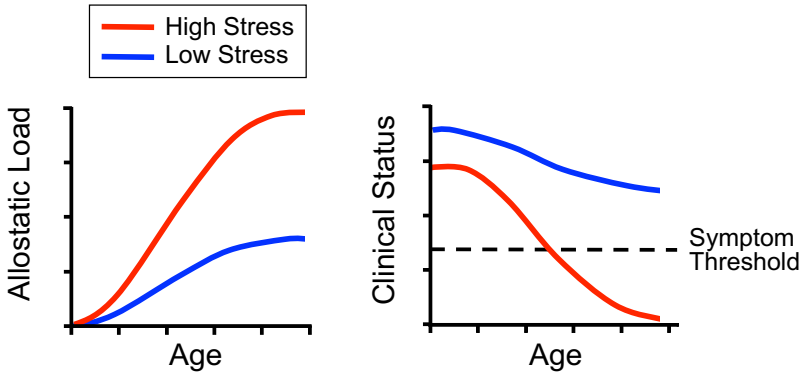
rationalize new approaches to treatment and prevention.

The homeostat theory of stress and allostasis lends itself well to computational modeling. For instance, here is a kinetic model based on the application, Stella™. Stress is defined by the error signal, and allostatic load by effector wear and tear.



A kinetic model of stress and allostatic load.

From this model one can generate predictions about chronic effects of stress and the development of symptomatic degeneration.



Model-predicted effects of stress and allostatic load on aging-related diseases.

The best essay I ever wrote was entitled, “How does homeostasis happen? Integrative physiologic, systems biologic, and evolutionary

perspectives." Here is the Abstract, which conveys what I foresee as the future of stress and homeostasis as scientific ideas.

Homeostasis is a founding principle of integrative physiology. In current systems biology, however, homeostasis seems almost invisible. Is homeostasis a key goal driving body processes, or is it an emergent mechanistic fact? In this perspective piece I propose that the integrative physiologic and systems biologic viewpoints about homeostasis reflect different epistemologies—different philosophies of knowledge. Integrative physiology is concept-driven. It attempts to explain biological phenomena by continuous formation of theories that experimentation or observation can test. In integrative physiology, "function" refers to goals or purposes. Systems biology is data-driven. It explains biological phenomena in terms of "omics"—genomics, gene expression, epigenomics, proteomics, metabolomics—depicts the data in computer models of complex cascades or networks, and makes predictions from the models. In systems biology "function" refers more to mechanisms than to goals. The integrative physiologist emphasizes homeostasis of internal variables such as pCO2 and blood pressure. The systems biologist views these emphases as teleologic and unparsimonious in that the "regulated variable" (e.g., arterial pCO2 and blood pressure) and the "regulator" (e.g., the "carbistat" and "barostat") are unobservable constructs. The integrative physiologist views systems biologic explanations as not really explanations but descriptions that cannot account for phenomena we humans believe exist although they cannot be observed directly, such as feelings and, ultimately, the conscious mind. This essay reviews the history of the two epistemologies, emphasizing autonomic neuroscience. I predict rapprochement of integrative physiology with systems biology. The resolution will avoid teleological purposiveness, transcend pure mechanism, and incorporate adaptiveness in evolution—"Darwinian medicine."

TAKEAWAYS

Always pour into the top bowl.

Find your talent, hone skills, learn to expand those skills, and apply them. Success will follow and then significance.

You have to measure something.

Science is about measurements that test ideas or inspire ideas more than the ideas themselves. If you can measure something that nobody else can measure, or you have developed a skillset for carrying out a procedure that no one else can conduct, then the world will beat a path to your door, and the ideas that others bring for applying your technology may be even better than yours. Ideas are easy, cheap, and quick, but data are hard, expensive, and take a long time.

Find a chevrusah.

Making observations and analyzing data with a partner and bouncing ideas off each other improve the quality and quantity of the research output. There are several examples of two people who have worked together receiving Nobel Prizes.

Ignorance isn’t biased.

If you make a discovery, you’ve put your finger on the truth. Stop what you’re doing and pursue the discovery. The business of science is testing hypotheses. The thrill is from making discoveries.

Patients are a unique scientific resource.

Patients tell us, and from their laboratory test results they show us, what is wrong. Our job is to learn what they teach.

Autonomic medicine is the future.

In June of 2022 I received from the Acting Scientific Director a memo in which she wrote, “I want to begin by reiterating how proud we all are of everything you have accomplished over a truly distinguished 40 years of research and service at the NIH. I want to recognize your myriad achievements and pioneering role in the field of autonomic medicine and in the study of catecholamine-related disorders, the impact of the many methodologies you have developed, your talent for and efforts in service of teaching and inspiring trainees, your contribution to the developing of your early-career colleagues through collaborations, and your dedication to NINDS and to harnessing the full and unique potential of the Intramural program for the maximal benefit of patients. I admire the depth and prolificacy of your scholarship, as well as your creativity and tenacity. You’ve been a credit to the NINDS Intramural Research Program, and for that I thank you.”

I was offered the opportunity to propose a clinical autonomic resource facility, “…to make sure that expertise in these concepts, frameworks, and methodologies remain strongly represented within the NINDS DIR Clinical program and available for the evaluation of our patient cohorts.”

Because of budgetary concerns, institutional priorities favoring basic reductionist over clinical integrative thinking, and anti- or pseudo-scientific politics, I doubt such a resource facility at the NIH will ever happen. Nevertheless, I predict that autonomic medicine, which I view as synonymous with systems medicine, scientific integrative medicine, and homeostatic medicine, will be at the forefront of future progress in understanding mechanisms of and managing multi-system, multi-disciplinary disorders of regulation. These include post-infectious, encephalomyelitis/chronic fatigue syndrome, postural tachycardia syndrome, irritable bowel syndrome, fibromyalgia, metabolic syndrome, Gulf War Illness, Lewy body diseases, and post-COVID syndrome. Perhaps another lesson from this book is never to give up your passion. To quote the mythologist Joseph Campbell, “Follow your bliss.”

For most of my life I've traveled an autonomic path. Many precious people have shared my journey—colleagues, friends, patients, caregivers, my wife Minka and our ever-growing family, and now you the reader of this memoir.

In line with Brian Buffini's analogy of the three bowls, for as long as I can I will continue to pour into the top bowl, the bowl of learning and practice. This is how the middle bowl of success fills. Eventually the middle bowl overflows to the bottom bowl, the bowl of significance.

Closing

Chances are we've never met and never will. Nevertheless, I can assert with confidence that, at this moment in time, we are sharing a miracle. The miracle is our sentient lives. My composing and your reading this are both products of our conscious minds. We will be gone in a flash, but we will have shared the gift of consciousness during our scintillations of existence. A Hebrew prayer states: "Blessed are you, God, King of the universe, who has given us life, and has sustained us, and has brought us to this day."