Krešimir Krnjević

Born:

Zagreb, Croatia (Yugoslavia)
September 7, 1927

Education:

Edinburgh University, M.B., Ch.B. (1949)
Edinburgh University, B.Sc. (Hons) (1951)
Edinburgh University, Ph.D. (1953)

Appointments:

Postdoctoral fellow,and Assistant Professor, University of Washington (1954–1956)
Postdoctoral fellow, Australian National University (1956–1958)
Principal, then Senior Principal Scientific Officer, Babraham Institute, Cambridge, UK (1959–1964)
Visiting Professor, Physiology Department, McGill University, Montréal (1964–1965)
Director, Anaesthesia Research Department, McGill University (1965–1999)
Chairman, Department of Physiology, McGill University (1978–1987)
Joseph Morley Drake Professor of Physiology, McGill University (1978–2001)
Emeritus Professor of Physiology, McGill University (2001–present)

Honors and Awards (Selected):

Beit Memorial Fellow (1952–1954)
Chief Editor: Canadian Journal of Physiology and Pharmacology (1972–1978)
Fellow Royal Society of Canada (1975)
Alexander Forbes Lecturer Marine Biology Labs, Woods Hole, Mass. (1978)
President Canadian Physiological Society (1979)
Council Member, International Union of Physiological Sciences (IUPS) (1983–1993)
Honorary Member, Croatian Pharmacological Association (1983)
Gairdner International Award (1984)
Officer of the Order of Canada (1987)
Jasper Lecturer Canadian Association for Neuroscience (1989)
Honorary President, Almae Matris Croaticae Alumni, Québec (1990-present)
Corresponding Member, Croatian Academy of Arts & Sciences (1992)
Wilder Penfield Prize, Government of Québec (1997)
Kershman Lecturer, Eastern EEG Association (1998)
Spiridion Brusina Prize, Croatian Natural Science Association (2001)

Krešimir Krnjević was a pioneer in the identification of brain neurotransmitters, notably such previously unsuspected endogenous amino acids as glutamate for excitation and GABA for inhibition, and a slow muscarinic excitation by acetylcholine (ACh). Also in showing that cholinergic fibers to cortex originate from the basal forebrain; and that ACh enhances cortical activity by reducing K conductance (G_K), an unprecedented mechanism of modulation, which could be counteracted by an equally novel hyperpolarizing action of cytoplasmic Ca ions, mediated by increased G_K. Resulting opposite shifts in cortical activity were proposed as possible mechanisms of consciousness and anesthesia.

Krešimir Krnjević

The Past Is a Foreign Country*

For me, this is both metaphorically and literally true: though I was born in Croatia, most of my life I have resided elsewhere. Albeit far from unique—numerous thousands of Europeans of the pre–World War II generations were uprooted—my situation had its own special features, which governed the course of my life and scientific career. In a sense, I was a precursor. Most of the millions who were "displaced" during that disastrous first half-century moved after 1933 when the Nazis got into power in Germany. My first exile began earlier, in 1930, when, as an infant, I was smuggled out of Yugoslavia.

My father, Juraj, was a great patriot, who devoted his life to his Croatian people and the Croatian Peasant Party—founded by an inspiring leader, Stjepan Radić, whose teaching he never ceased to promote through many decades of exile. As a child, I saw my father as a stern and demanding figure; but later, he earned my unqualified love and respect for his outstanding qualities: a strong will, tempered by an open mind and unselfish integrity, and total lack of interest in money. My doting mother, Nada, was an unending source of love and tenderness. She had a sunny and affectionate nature, as well as boundless energy, only partly expended in skiing and mountain climbing in neighboring Savoie (including Mont-Blanc). From my parents, I inherited both energy and optimism, which have served me well throughout my life.

I was born in Zagreb on September 7, 1927. By 1930, after the assassination of Stjepan Radić in the parliament in Belgrade and the advent of King Alexander's dictatorship, some leaders of the now outlawed Croatian Peasant Party (including my father) left the country. They wanted to convince major European powers of the need to restore democracy in Yugoslavia. So he went to Geneva, the seat of the League of Nations (the prewar equivalent of the United Nations). At the age of 2½, I was taken by my aunts from Zagreb to the Italian border—then at Rijeka—across which I ran, unhindered by the border guards, to my mother, waiting for me on the other side. We lived in Geneva for the next 10 years, and there I had my first education, up to the end of primary school. At home my parents always talked to us in Croatian, but my sister Biserka (5 years older) and I grew up speaking

*David Lowenthal, Cambridge University Press, 1985

French. This remained our standard language of communication, long after we'd left Geneva.

Even in Switzerland, life was far from easy for a political exile: with so many unemployed during those depression years, we were allowed to stay in Geneva on the condition that my father take up no gainful employment. But access to the lake or mountains was free. My parents were enthusiastic hikers. So every Sunday, we all walked 3 miles to the French border and then up the 4000-foot-high Mt. Salève. As a child, I had mixed feelings about the obligatory Sunday hike, but I grew to love mountains. We led a very quiet life and my schooling was uneventful. Summers I spent at an inexpensive holiday camp, run by our Catholic parish; neither father nor mother went to church, but I attended the catechism classes every Saturday. The Swiss phase of my life, generally calm and happy, like that blessed country, ended just as the European war was about to start. Alarmed by the quickly deteriorating European situation, in the summer of 1939 the Belgrade government reluctantly granted Croatia a near-autonomous status as the Croatian Banovina. We could now return to Zagreb. Having to leave Geneva and my friends was hard enough for me: far more upsetting was leaving my mother behind. My parents' separation (and ultimate divorce) was the deepest psychological trauma of my childhood.

Our arrival in Zagreb, on September 1, was an astounding event. At the railway station, the returning exile was welcomed by thousands—an occasion for many bouquets, speeches, and ovations, all in amazing contrast to our very quiet life in Geneva. We also discovered a large family of numerous uncles, aunts, and cousins, all making us feel warmly welcome. But at school, the early weeks were stressful, my rudimentary spoken Croatian being of little help when trying to cope with a rich and complex grammar. Within a few months, however, my sister and I were settling in this new, privileged existence. Though we missed Geneva's lake and mountains, Croatia offered an admirable, largely unspoiled, long Adriatic coast and islands, perhaps the finest in Europe. Sometimes I wonder, how different would my life have been if we had remained there? Would I have become an engineer, my greatest wish at the time? More likely, we would not have survived the takeover of Croatia by the Ustashi (extreme nationalists) after the German invasion of April 1941. As a prominent politician, strongly favoring the Western Allies, my father (and his children) would be early targets for liquidation. As it was, a bomb thrown into our house by Ustashi in December 1940 blew up within a few feet of me. I was seriously shaken but physically unharmed.

Another page was turned as we again went into exile in April 1941. When the German army invaded the country, my father agreed to be the Croatian vice-premier in the new Yugoslav government, getting ready to leave for Greece. A long and hazardous drive took us to Sarajevo (where I first experienced aerial bombing by the much feared Stukas); then over the mountains of Bosnia and Hercegovina to Nikšić in Montenegro. Nikšić had

an airfield from which we were to fly to Athens. Though we had to hug uncomfortably close to the Albanian mountains to avoid Italian fighters, our flight—in a bomber of the Yugoslav air force—should have been straightforward; it was cut short when, needing to refuel, we crash landed at a British air base in Agrinion, in northern Greece. Our luck held: albeit shaken, we were unhurt; but the journey had to continue overland, very slowly, first to Patras and then Athens. After the intense cold and snow-covered mountains of Bosnia and Albania, we marveled at the warmth and the blossoming orange trees in the streets of still peaceful Athens—the Germans were yet to reach Greece. A few days later, a slow flight took us from the Pireus to Crete, Alexandria, and then Cairo, where the unwelcoming Egyptian authorities immediately sent us off by the night train to Port Said, in the British-controlled Canal Zone. Our stay there, for a week or so, was followed by a long train ride to Jerusalem, where the Yugoslav government-in-exile finally reconvened, in an old monastery on the road to Bethlehem. In spite of the war and other hazards in Palestine, there was much to enthrall a young boy. Visiting the Holy sights or floating in the Dead Sea; and later, back in Cairo, riding a camel and climbing the great pyramids (as one could in those days) seemed like a great adventure.

But then, Biserka and I had to part from our father. Only the most senior members of the government could be flown to England; so all the other Yugoslavs went to Suez, to board the *Strathaird,* about to sail for South Africa. After a pleasant 2-week journey, we reached Capetown, where boarding schools were found for Biserka and myself. Thus began my third "incarnation" (as it were), now in the English-speaking world. Thanks to rapid progress, I finished my accelerated high school education before the end of 1943: with a very mixed bag of knowledge—in history for example, where 17th-century Dutch Cape settlers floated above medieval Croatian kings and an even deeper layer featuring William Tell's crossbow and apple. Apart from my introduction to English, that school's most significant and lasting impact was in the realm of music, for which I must thank a wonderfully empathic teacher, Valerie Norman, whose infectious enthusiasm and inspiring approach to piano playing aroused a passion for music that has never flagged. Life in Capetown was an interlude of peace and comfort. White people were privileged in South Africa, especially in Cape Province, with its temperate climate, rugged mountains, and splendid beaches: they enjoyed a high standard of living based, of course, on a shameless exploitation of the black majority. The few who thought about it seriously knew this could not go on forever. But most people accepted it as a gift from heaven. In any case, surely it would outlast their lives. . . .

Early in 1944, we left for England. Our mainly cargo boat (SS *Sarpedon*) reached Freetown (in Sierra Leone) without hindrance from the U-boats infesting the approaches to the Cape of Good Hope. We continued on to Liverpool, traveling very slowly but more safely in the middle of a large convoy.

Altogether, the voyage lasted 6 weeks. Nowadays, this would seem absurdly long and, surely, excessively boring. Not so. A surprisingly well-stocked library was available (where I first came across the *Origin of the Species*). Free to roam about the ship, I always had something to do or look at: the never-ending play of sun (or moon)-light on waves, the flying fish; at night the fluorescent spray and the ghostly sight of other ships gliding silently alongside. All males took turns at watching for enemy aircraft; and everyone attended the frequent lifeboat drills.

Arriving in a much battered Liverpool felt as if we were landing on another planet. The cold damp weather of early spring, the bomb-blasted ruins all about the landing area and Queen Street station, and the lean undernourished look of the local people could hardly present a greater contrast to the Land of Cockaigne we had left behind in Southern Africa. The train ride to London was interrupted several times by a vicious air raid, one of the last of the "conventional" bombardments. In London, we were issued gas masks, as well as coupons for food and clothing that were strictly rationed, and from then on, whenever I traveled anywhere in Britain, I had to report to the police at a backdoor entrance prominently labeled "Aliens, Firearms, and Dangerous Drugs."

As a child, I wanted to become an engineer. By this time, however, my interests had broadened: indeed, everything seemed exciting. But my father, remembering my long interest in engineering, had made arrangements to see people at a Cambridge college, Peterhouse. Not long after our arrival, we all went to Cambridge. It was agreed that Biserka—who had finished 1 year of university in Capetown—would go to Newnham, a college for women. Having no Latin—at that time an absolute requirement for admission to Cambridge—and being rather young (16), I was advised to learn some Latin and return the following year. To wait a whole year seemed to me a dreadful imposition. Instead, I opted for medical studies in Edinburgh, where the separate Scottish system had no Latin requirement for admission. After some additional coaching in chemistry and physics during the summer, I passed the first MB exam in the autumn and began my medical studies in October 1944.

Though far from secure—those marvels of the latest German technology, the V1 and V2 bombs kept up round-the-clock suspense—life in London was amazingly rich in cultural events, in spite of wartime restrictions. That summer, I seldom missed any of the lunch-hour concerts at the National Gallery. There was plenty more chamber music at the Wigmore Hall. And, after the concerts, as soon as some paintings returned to 2-3 undamaged rooms at the National Gallery, visual arts came as another revelation. Because they were so few, one could get to know the pictures really well, undistracted by the thought of dozens more rooms to visit. My father had rented a house in Chelsea—during the war, plenty were available and inexpensive—so I had a base from which to explore London, to which I was to return regularly during vacations.

Life in Edinburgh was rather different. Getting away from the V1's and V2's was a relief, but there was a sobering dourness about the city. Long before the festivals ushered in a cultural renaissance, there was little in the way of music, modern art, or theater, and no cinemas were open on Sundays. On the other hand, the town was always there, offering a wonderful variety of stone buildings and entrancing prospects. Throughout the 10 years that I spent in Edinburgh, I never tired of exploring the crooked streets below the Castle and the classically laid-out squares and crescents of the New Town. Edinburgh is an extraordinary architectural success—surely the loveliest city in Britain, and among the finest in Europe.

The first 2 years of our studies were mainly devoted to anatomy: especially the systematic dissection of the human cadaver to which I was assigned, together with 4–5 other students. Our focus was sharpened by frequent "spot" exams: what sequence of many structures would be penetrated by a needle inserted at this or that point? We were kept on our toes also by the frequent appearance of the already venerable E. B. Jamieson—the grand old man of Edinburgh anatomy—who, wearing a little black cap, shuffled about the dissecting room, asking pointed questions. By contrast, physiology was much harder to grasp. Unlike anatomy, it seemed to lack firmly established facts. The physiology teachers failed to inspire much enthusiasm, and they had some difficulty in controlling a very unruly crowd of young students, only recently freed from school discipline.

The clinical subjects, and especially regular exposure to patients, soon had a sobering effect. Unlike any other, medical training offers an accelerated schooling in life and many of its problems, as well as an exceptional variety of highly regarded and monetarily well-rewarded employment. Though I worked quite seriously, much time went into exploring music, including moderately successful attempts at playing the piano. Mozart was a particular favorite. In view of Mozart's prodigious output, Sacheverell Sitwell (1936) had commented that no one could hope to hear even half the works listed in Köchel's catalogue. This spurred me to listen to every (for me) new piece by Mozart, played mostly on the BBC's Third Programme: before I left Edinburgh, I could boast of having crossed out over two-thirds of the 626 items in the catalogue.

I also greatly enjoyed weekend climbing trips, as a member of the University Mountaineering Club, to the Highlands and, less often, the Lake District—the rudiments of rock climbing I'd picked up during summer visits to my mother in Geneva (from my step-father, Francis Marullaz, a great alpinist, who had conquered at least once every 4000 meter peak in Europe). My studies proceeded satisfactorily to their conclusion in 1949, by which time I was just old enough to become a registered medical practitioner. In those less exacting days, there was no requirement for further hospital experience (internship). So I could have joined some practice or even gone

off as a ship's doctor—as some of my colleagues did. When my closest friends enrolled in residency training, I was at first tempted to continue in a clinical career but then decided that I would concentrate on research.

Why did I make that choice? I enjoyed working with patients. That part of medicine very much appealed to me. What I did not like so much was the medical hierarchy, the deference to the established chiefs, having to conform to certain patterns of behavior and accepting the power games involved in climbing up the professional ladder. By contrast, in research I could contribute to new knowledge as a member of a relatively small, but worldwide community of scholars. This was better suited to my temperament, as well as my situation: having been uprooted several times from several countries and cultures, I felt a greater natural affinity for a career where one's performance counted more than one's provenance. Where could I train as a researcher? I went to the Physiology Department (in Edinburgh) and spoke to Philip Eggleton, the acting Head (to the Eggletons we owe the discovery of phosphocreatine, when both husband and wife were studying muscle metabolism in A. V. Hill's laboratory at University College). As always, he was very helpful. Though he mentioned the limited number of academic jobs then available, he did not try to discourage me. When he asked what field of research I was interested in, I replied that studies of the brain, especially how to bridge the chasm between brain physiology and psychology, seemed of particular interest. He thought both fields were becoming ripe for serious moves in that direction. As it turned out, we were somewhat overly optimistic. That happened to be the year (1949) when Donald Hebb's *The Organization of Behavior* was published (a book now much-cited); only several decades later did neurophysiologists begin to take a serious interest in cognitive processes and most psychologists see the relevance of synaptic events.

Before I could start my Ph.D. research, I had to complete 2 years of an Honors B.Sc. program. Apart from physiology lectures and labs, my other major topic was psychology, taught mainly (and rather effectively) by the head of the department, James Drever. This introduced me to such topics as motivation, learning, gestalt, and statistics that I hadn't thought about much before. It was a stimulating experience. For a lab project, David Whitteridge—the new Head of Physiology who had just come from Oxford—suggested that I try to record from vagal relay cells inthe cat's nodose ganglion. He had spent much time recording afferent signals from the lungs and heart, as "unitary" responses in fine strands laboriously dissected from the vagus. Microelectrodes might be a useful alternative. With acid-sharpened sewing needles—insulated except at the tip—I did manage to record some single units from the nodose. Albeit feasible, this method was probably not much less trouble than the alternative, and as far as I know, was not widely adopted. Nevertheless, when written up, this project qualified me for a Goodsir Memorial Bursary.

First Steps in Research

David Whitteridge had been present at the 1948 CNRS conference (in France) at which Hodgkin and Huxley's ground-breaking analysis of the squid nerve action potential was first presented to an international audience. The notion that Na ions played a crucial role was fiercely challenged by the Spanish-American neurophysiologist Rafael Lorente de Nó, emphasizing that peripheral nerves kept in Na free solutions conducted action potentials for many hours in the absence of any Na ions. In reply to the objection that desheathed nerves rapidly become inexcitable when Na ions are removed, Lorente de Nó contended that such desheathed nerves are not in a physiological state. It occurred to Whitteridge that the issue might be resolved by perfusing the nerve through its blood supply, thus bypassing the sheath. He proposed that I make this the topic of my Ph.D. research.

Before starting experiments, I spent several months putting together electrophysiological equipment. Apart from a Cossor oscilloscope, no commercial equipment was available. So, with cheap army-surplus material—resistors, capacitors, and vacuum tubes—circuits from C. J. Dickinson's indispensable manual (1949), a soldering iron, and, especially, the inestimable advice of Whitteridge's electronics technician ("Jock" Austin), even a raw student could produce the needed items in a relatively short time. Perfusing a frog's leg via the descending aorta posed no major problem. Within a few weeks, Whitteridge's idea proved correct: action potentials in the sciatic nerve disappeared after only a few minutes of Na-free perfusion. High concentrations of K^+ also rapidly blocked conduction, whereas uncharged molecules s such as acetone, acted about equally quickly when applied to the intact nerve or by arterial perfusion. Clearly, a lipid-permeable barrier prevented rapid diffusion of ions into or out of the nerve trunk. One possible objection remained. Could perfusion be more effective because a high density of capillaries greatly reduced diffusion distances *within* the nerve trunk? Indeed, when stained with a dye, the capillary supply proved far richer than might be expected in a simple nerve. Knowing the mean intercapillary distance and the rate of action potential block during perfusion, my calculations showed that faster internal diffusion could not account for the very rapid block seen when nerves were perfused with Na-free solutions. Having only a high-school knowledge of mathematics, I spent a great deal of time over the complex Bessel functions that describe diffusion in cylindrical coordinates. I was somewhat put out when, at my PhD oral, Phillip Eggleton, jotting a few numbers on the proverbial back of an envelope, in few minutes confirmed the general validity of my elaborate calculations—much to Whitteridege's amusement.

The preparation and results were first presented in a Demonstration at a Physiological Society meeting in Edinburgh (July 1952)—owing to my

habitual procrastination, too late to be cited by Hodgkin at the July 1952 Cold Spring Harbor Conference (he had shown interest in my preliminary results, which countered Lorente de Nó's objections). These findings were published in my first article, in the *Journal of Physiology* (1954). In a complementary study, I found that the diffusion barrier is probably the perineurium, which forms a continuous layer of epithelium (and not as previously thought the much looser epineuriuim), Indeed, the perineurium seemed analogous to, and possibly of similar origin as, the arachnoid cover of the central nervous system (CNS). These studies were written up for my Ph.D. thesis. The external examiner William Rushton (of Cambridge University), commented that this was not the way he would have tackled the problem but was otherwise quite positive. Thus ended my experiments on frog nerves. But my expertise came in handy some 50 years later, at McGill, when supervising undergraduates measuring conduction velocity in frog sciatic nerves; which I still do this regularly—my only postretirement teaching.

Two other related projects were completed in Edinburgh. One was a follow-up on Na^+ fluxes out of mammalian nerve trunks, in collaboration with Jack Dainty (of the Biology Department). Having worked during the war as a physicist at the Chalk River Nuclear station in Canada, Dainty was now using radioisotopes to measure ion fluxes from seaweed. Sending the resulting paper to the *Journal of Physiology* was a salutary experience. It came back with a searching critique, signed by Andrew Huxley, who pointed out that, because extracellular Na^+ is so abundant, in our calculations we needed to take into account a significant reverse influx of labeled Na^+. The other project also dealt with effects of Na^+ deficiency on neural function. For this, I teamed up with two old friends, colleagues from medical school, Peter Aungle (training to becoming a psychiatrist) and Robert Kilpatrick (who went on from internal medicine to the General Medical Council and ultimately the House of Lords). More ambitiously, we now had humans as subjects. We wanted to see whether salt deprivation could lower the concentration of Na^+ [Na^+] sufficiently to induce detectable changes in nerve and muscle function. Both our heroic volunteers (who remained good friends, even after their ordeal) put up with a punishing regime of a virtually Na-free diet and regular sessions in a sauna—to maximize Na loss by sweating. Thanks to remarkably effective Na homeostasis, however, plasma [Na^+] dropped only minimally (the greatest reduction in one subject was by only 10%), but the large loss of body water resulted in a substantial rise in plasma [K^+] and, even more alarmingly, in blood viscosity. A battery of tests of nerve and muscle function—including central reaction time and electroencephalogram (EEG)—showed no significant changes except, in the subject with the largest drop in plasma [Na], a significantly longer nerve refractory period. A dramatic highlight of these experiments came on the last day: having thoughtlessly taken an anchovy sandwich at a party, our subject had returned for an additional session in the sauna before undergoing the

usual intensive tests. Sitting in a chair, in my lab, when the high-frequency nerve stimulation approached its peak, he suddenly collapsed to the floor. For a minute, we thought we had killed him (and deprived his children of a loving father). Very fortunately, he soon recovered. Such brash, minimally safe-guarded experiments could not be done so easily now. There is much to be said for ethics committees.

Following recommendations from Whitteridge and Eggleton, I was awarded a Beit Memorial Fellowship. It was a relief to be no longer in need of any financial help from my father. With no possibility of free political activity under Tito, in spite of the offer of a good pension, he refused to return to Yugoslavia and remained in London. Having no steady income, he lived very modestly in Clapham, writing articles for a Croatian emigrant newspaper in Canada. Ever an optimist, he was convinced that the communist regime would end, sooner or later. For over 40 years, apart from regular visits to Canada, he lived in London, but not quite long enough to see his dream of a free Croatia come true. He died in 1987, aged 92, on the eve of the collapse of the Soviet system.

Departure from Edinburgh

At the 1953 Montreal International Physiological Congress, Whitteridge had been much impressed by scientists from Seattle. Albeit loath to leave Edinburgh, acting on his advice that I would benefit from exposure to a different research environment, I wrote to Ted Ruch, head of Physiology and Biophysics at the new medical school in Seattle. With the dynamic Vahé Amassian (a Cambridge and Middlesex graduate) already on his staff, Yale- and Oxford-trained Ruch thought highly of British education. He offered me a position as Assistant Professor. After spending a full decade in Edinburgh, before leaving for Seattle in September 1954, there were many things to settle. Foremost was getting married to Jeanne, at the Dominican chapel in George Square. Jeanne Bowyer had a checkered history, somewhat like mine. Born in Penang, Malaya, of an English physician and an Irish mother, she went to school in Scotland. In December 1941, with mother and sister, she had to flee Singapore as Japanese troops moved rapidly down the Malaysian peninsula. Their Dutch ship took them to Batavia and then Sydney, where they stayed several years. When she'd completed her schooling, they traveled to Durban, to join other members of her father's family, resident in Natal. Meanwhile her father, who had been superintendant of the main hospital in Singapore, was interned by the Japanese and died as a result of maltreatment. A few years later, Jeanne came to Edinburgh to study social work. After the wedding, we flew to Dublin for a brief honeymoon (and a memorable dinner at Jammet's). An immigrant visa that would allow Jeanne to work in the United States required a last-minute visit to the consulate in Glasgow (she benefited from her birth in Penang, the subquota

for non-Malays from Malaya being undersubscribed). And then we were on our way to London and Southampton, where we boarded the *Queen Elizabeth*. As the ship was about to sail, I posted my sciatic nerve histology paper to John Passmore, the editor of the *Quarterly Journal of Experimental Physiology*.

In New York, we were welcomed by my father's Croatian friend, Bogdan Raditsa (writer and former diplomat, then teaching European history at Fairleigh Dickinson University) and his wife Nina, the daughter of the Italian liberal and antifascist, Gugliermo Ferrero). Once again I felt as if we'd landed on another planet. New York in October was far warmer than it had been at mid-summer in Edinburgh—the strange sight of steam issuing from manhole covers made the heat seem even more oppressive. But the highlight of our first evening was a party at the Hamburgers (Phillip Hamburger was prominent writer for the New Yorker), It was a noisy affair, loud with argumentative New Yorkers going on about the forthcoming November elections. Much was shouted about "Charlie" Wilson's alleged "What's good for General Motors is good for the country" and "that bum Bender. . . ." What could we say when we were asked what people in Scotland thought about the elections. We could only try to be polite, and were probably thought to be rather stupid.

After a brief detour to Washington, to greet old friends from Zagreb, Dr. Maček—the exiled president of the Croatian peasant party and his family, whom I had last seen in March 1941 before driving off to Sarajevo—we boarded the train for Chicago for the first leg of our 4-dayrailway journey to Seattle. The air-conditioned train was welcome after hot and humid Washington, waiting for a tornado that also caused major flooding at Union station in Chicago. Having a few hours to spare before the departure of the train for Seattle, we wandered off along the Lakeshore and into the Art Institute We were thrilled to see, quite unexpectedly, Seurat's *Afternoon at La Grande Jatte*, well-known to us from illustrations in R. Wilenski's splendid book (my introduction to modern art). Our 'roomette' on the Milwaukee Road train provided both comfort and privacy. Spectacular scenery could be viewed from the "scenic dome" or the glass-surrounded end-compartment. Watching the moon reflected in the Mississippi or the moonscape of copper mining at Butte (Montana) was both exciting and disorienting. Not much trace of old Europe here.

At the end of the long transcontinental journey (soon superseded by much faster but far less interesting flying), we were greeted by Vahé Amassian, smoking a fat cigar and driving a large, bright red Oldsmobile convertible—all in sharp contrast to his very English accent and manner (St Paul's school and Cambridge). We enjoyed his company, but not for long. Though of British nationality, as a single man, at the peak of the Korean War he could not escape the draft. From the station, he drove us to the Meany Hotel ("every room a corner room"!) in the University district, near the University

of Washington campus. At the Medical School, we were introduced to Ted Ruch. He had brought to Seattle several colleagues from Yale, as well as the Yale teaching system: once a week, each staff member headed a "discussion" group of 10–12 students, so everyone had to attend all the lectures to be able to deal with all aspects of physiology. Another rule was that no one lectured on a topic related to his or her own research. I was assigned digestion. This was all very sensible, but it was unduly time consuming and certainly very different from physiology teaching in Edinburgh, where all the lectures were given by Whitteridge himself. I wonder how long the Yale system survived as the department's contingent of dynamic researchers grew by leaps and bounds.

There were other curious features. Before taking up my appointment as assistant professor, I had to sign a statement that I would not take part in activities that might undermine the government of the United States. Each month, when picking up our checks, everyone had to sign a sheet listing all the staff members and their respective salaries—displayed for all to see in the departmental office; this was to ensure that everyone was physically present in the department. The students were neatly dressed and well-behaved, males with crew cuts, girls in skirts and bobby socks; they were attentive in class and often asked questions—quite unlike the rowdy young Scots. The University Bookstore sold mainly T-shirts and sports equipment, with only a few shelves of books on display at the back, mainly standard textbooks. The central role of sports in the life of the university became even more blatant when Robert Oppenheimer—invited to give a series of lectures to the physicists—was refused access to the campus by the president of the university, on the grounds that Oppenheimer had lied to the U.S. government. Everyone (including the city) being passionately involved in the far more important question whether the coach of the poorly performing Huskies should be given the boot, there was little concern on the campus (the football coach was fired by the Board of Regents; but, soon after ran successfully for the position of Vice-Governor of the state of Washington. . .). Such was academic life in the mid-1950s. Of course, student uprisings during the next decade led to radical changes. When I visited Seattle in the 1970s, the students wore tattered jeans, and had long and disheveled hair and beards; several bookstores actually full of books had sprouted, and I felt sure no university president could safely indulge in arbitrary decisions.

Seattle is splendidly situated: between Puget Sound and Lake Washington with mountains ranging on both east and west sides. It was like a return to Geneva, with the added bonus of the beautiful cone of Mt. Rainier glistening in the fall sunshine—though everyone assured us that once the rain began, it would be invisible for 6 months. In some ways, this was better than Switzerland with its overcrowded trails. We hiked or snowshoed—in winter the only way of getting around mountains covered by 30–40 feet of snow. If more than half-mile from the highway, one had the vast expanse of

mountain all to oneself. We also skied, at Snoqualmie, Stevens Pass, Mt. Baker, or Mt. Rainier (at Paradise Valley). In these ventures we were often accompanied by friends such as Eric Stein—a biochemist from Geneva, working with another friend, Eddy Fischer (also with a Swiss background)—or Goran Bauer (orthopedic surgeon and bone researcher from Lund) and their wives. During the summer, we swam or sailed on Lake Washington; or tried (unsuccessfully!) to dig for clams on a Pacific beach; and ventured further afield to the Blue Mountains of eastern Oregon. The crowning achievement was to climb Mt. Rainier, leading on my rope Eric Stein and the enterprising Dean of the Medical School, George Agaard—by the regular route, not difficult but a long slog up the snowfields. We duly signed our names in the book kept at the summit near the edge of the crater. A longer drive took us to Montana and Wyoming (including Yellowstone). Another journey, to Canada—to celebrate Christmas with Croatian friends on Vancouver Island—was less successful. At Vancouver airport, the official pointed out that my student visa was good for only one entry to the United States: he'd be happy to let us into Canada, but I would not be able to return to Seattle without getting a new U.S. visa—which usually required at least several weeks. If the cost of the return flight posed a problem, he could have us "deported" from Canada, but this would feature permanently in the official record. Somewhat dejected, we flew back to Seattle on the same plane.

All this activity was not conducive to research. The 2 years in Seattle were the least productive of my career. Ted Ruch's expectations were disappointed. Working with my own rig, I shared a large lab space with Dexter Easton, who was trying very hard to isolate a single muscle fiber with its innervation intact. He wanted to confirm decisively his belief that muscle action potentials were smaller at the end-plate owing to interference by action potentials in adjacent fibers, rather than shunting of membrane currents by transmitter action—as proposed by Katz and Fatt. Bernard Katz was so incensed that soon after, he wrote a paper for the *Journal of Physiology* devoted solely to a systematic demolition of Dexter's thesis.

For my own research, I decided to record from the cat's cerebral cortex. First, I tried to make intracellular recording easier by eliminating vascular pulsations: the blood supply to the brain was detoured into a glass chamber containing air that would absorb the pulsations before the blood was returned to the carotids. This very bloody procedure, described only in an abstract, yielded a few, not very impressive intracellular recordings. Of greater interest were marked ontogenetic changes in the cortical response evoked by paw stimulation. In very young kittens, the large primary response was reversed, being positive instead of negative as in mature animals. These data, confirmed by later observers, were never submitted for publication.

We Travel across Another Ocean

How did I come to leave Seattle for Canberra? In 1955, Stanley Bennett (head of Anatomy at the University of Washington) organized a symposium on synaptic mechanisms. Bennett and Eduardo de Robertis (in exile from Peron's regime in Argentina) had recently discovered synaptic vesicles. With Eccles, Frank, Fuortes, Kuffler, Hunt and Lloyd attending, this was a particularly successful event—though it was sad to see David Lloyd so obdurate in refusing to accept Eccles' new findings about spinal inhibition. Jack Eccles was in top form, presenting also the new data on Renshaw cells and the evidently cholinergic nature of recurrent inhibition. I had met Eccles when he visited Edinburgh, but we got better acquainted in Seattle where gave him some penicillin injections to treat a bad cold. Much impressed by his talks, I was happy to accept his offer of a visiting fellowship. So, in September 1956, we sailed from Vancouver on the Orient line's *Orcades*. A thoroughly enjoyable voyage—including delightful 1-day stops in Honolulu, Fiji, and New Zealand—got us to Sydney by early October. We arrived on a Saturday morning, too late to have all the luggage off the ship before all the stevedores departed for the Randwick Park races. We took the train for Canberra on Monday. At Canberra station, we were greeted by Jack Eccles, who drove us to our residence at the Australian National University.

There wasn't much to Canberra in 1956. After some minutes, I asked our host when we would reach downtown. His reply was: "We've just passed it." The main institution in Canberra was the Parliament. Members of parliament would come for the parliamentary sessions, staying at the only hotel, and return to Sydney or Melbourne as soon as the sessions were over. The Australian Institute for Advanced Studies was the pet project of the Prime Minister, Robert Menzies, who established its statutes and financial basis. It had started only a decade or so earlier. Eccles, who was Australian, had gone to Oxford to work with Sherrington and remained there till the end of the 1930s. He returned to his homeland, to the Kanematsu Institute in Sydney, to which he brought B. Katz and S. Kuffler to form a celebrated team; but then went to New Zealand to become the head of the Physiology Department at Dunedin. His pioneering intracellular findings in spinal motoneurons overturned his long and vehement opposition to chemical transmission, especially at central synapses. For a philosophical underpinning to his conversion, he was much indebted to the Austrian logical positivist Karl Popper—also in Dunedin at that time—who believed that science advances by the refutation of wrong hypotheses. Eccles remained in Dunedin until he was offered the position at the new universityn in Canberra. For the first few years, he and the visiting Paul Fatt worked in temporary huts—by no means unproductively—while the new John Curtin School of Medical Research was being constructed. Soon after our arrival,

Ricardo and Mela Miledi also came, from Mexico. As Eccles was busy recording from chromatolysed motoneurons (with Ben Libet), Ricardo and I joined David Curtis in a study of short-latency excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) in sacral motoneurons. According to David Lloyd, short-latency contralateral inhibition could be demonstrated in sacral segments even if the inhibitory signal was elicited well after the excitatory one. This he took as clear evidence that the inhibitory pathway was monosynaptic, not disynaptic as postulated by Eccles. Our results were clear. The mean latency of IPSPs was significantly longer, by some 0.75 ms, in keeping with Eccles' findings in lumbar motoneurons. These experiments were completed within a month or two. At this point, lacking the equipment needed to continue with the spinal cord, Ricardo and I were invited by Bill Liley to use his lab and equipment: he had finished his work on neuromuscular transmission in the diaphragm and was now writing his Ph.D. thesis. Moreover, he showed us how to dissect the phrenic nerve and diaphragm from rats, anesthetized with ether. Invariably, having mounted the preparation in the recording chamber, Bill would sit back, light a cigarette, and, unthinkingly, drop the match into the nearest bucket—into which he had earlier disposed the large wad of ether-soaked cotton-wool. The fireworks were impressive.

Neither Ricardo nor I had worked on the neuromuscular junction. Taking turns at doing experiments, our collaboration proved most enjoyable and productive. Though we worked together for less than a year, it resulted in seven publications. There were several highlights. By stimulating the phrenic nerve after cutting all but one axon, we were able to show (for the first time) that a single motor unit is scattered quite widely over the diaphragm. We obtained direct evidence that failure of neuromuscular transmission can occur *presynaptically*, probably at points of axonal branching, and that in fatigued muscle, adrenaline exerts its beneficial actions at several, pre- and postsynaptic sites. By careful placement of a microiontophoretic electrode, and releasing only minute amounts of acetylcholine, we could evoke responses comparable in time course to end-plate potentials. Extracurricular adventures included skiing between eucalyptus trees in the Snowy Mountains and a vividly memorable inland excursion via Hunter, Booligal, and Wilcania to Cobar, and then over the Blue Mountains to Sydney and back to Canberra. Owing to the severe shaking induced by endless miles of corrugated tracks, by the time we reached Cobar, my ancient Standard Tourer was coming apart. The radiator was leaking badly, the front wheels had nearly come loose, and the chain brakes were minimally functional. The front axle, held by only one remaining screw, had to be fixed before we left Cobar. The brakes were less of a concern, the landscape being utterly flat and the roads free of traffic most of the way. But we had some narrow escapes when we had to cross the Blue Mountains and later,

near Sydney, at intersections with traffic lights. Sleeping out under the wonderfully starry Australian sky and seeing clouds of budgerigars, parrots, and cockatoos, as well as many small and large lizards, emus, and of course kangaroos, more than made up for the hazards.

During the second year in Canberra, apart from some experiments on salivary glands with Anders Lundberg, I worked mainly with Eccles on intra-axonal recordings of dorsal root potentials. Several components of these large responses had been reported by various authors, including Matthews and Lloyd. Our detailed analysis laid the basis for Eccles' subsequent studies on presynaptic inhibition. Another interesting finding was presynaptic axonal hyperpolarization following tetanic stimulation: in keeping with the idea that a larger action potential releases more transmitter from the nerve endings, this could explain the well-known posttetanic potentiation.

Working with Eccles was a most refreshing experience. Always a full participant in experiments, though he let us do the bulk of the dissection, he insisted on preparing himself the fine filaments of dorsal and ventral roots for stimulation. He sat at the controls at the stimulator and the camera throughout experiments that often went into the early hours of the morning. Blessed with a remarkable memory, and drawing on his wide knowledge of neuroscience, he was ever ready to discuss new findings and ideas. His enthusiasm when embarking on a new experiment was like that of a child with a new toy. Though not outstanding as an original thinker, his vast energy, industry, and uncanny ability in grasping the significance of new concepts or techniques, and using them in his own thinking and investigations, were the foundations of an exceptionally successful research career, crowned by the well-deserved awards of a knighthood in 1958 and a Nobel Prize in 1963.

Both our sons were born in Canberra, as was the Miledis' only son Rico. When Eccles heard us worrying about the babies, he commented: "Wait till they grow up. Then you'll have something to worry about." Having nine children of his own, he spoke with the voice of experience. In most respects, life in Canberra, with little to distract one from work, was ideal for research. But one felt very far from the really interesting parts of the world. So rather than take up some possible posts in Australia, after meeting with John Gaddum during his visit to Canberra, I was happy to accept his offer of a job at Babraham—where he had recently moved from Edinburgh, to become the new Director of the ARC Institute. So we turned yet another page.

Another Long Sea Voyage

Our departure, late November 1958, didn't run smoothly. Till the last hour, I was closeted with Eccles, selecting material for figures for the paper on posttetanic hyperpolarization, which I had insisted on writing. When I got home, I found my wife in tears, with so much remaining unpacked, and the

babies howling. So we were too late to catch the afternoon train to Sydney—to the dismay of our colleagues assembled at the station to say goodbye. The delay was of no other consequence as our ship, the Blue Funnel line's *Helenus*, carrying mainly cargo and some two dozen passengers, was coming to Sydney only 2–3 days later. Sailing along the Great Australian Bight, accompanied by an albatross, went smoothly. So did the journey across the Indian Ocean, enlivened by flying fish and dolphins and achingly lovely sunsets. Our first port of call was Aden. The pleasantly warm conditions persisted along the Red Sea; but ceased sharply as we entered the Mediterranean—we could no longer wander about the decks in shorts and sandals. When we saw Etna glimmering at sunrise and sailed through the straits of Messina, we knew we were back in Europe. How could I also not be excited seeing Stromboli erupt at regular intervals as we passed the Ionian Islands? Stromboli had featured so prominently at the end of one of my favorite Jules Verne books, *Journey to the Centre of the Earth*. At our next stop, in Genoa, my mother was at the dock, eagerly waiting to see us and the very new grandchildren. Albeit under continual rain, treading the old paving stones of the ancient republic felt like a return to my true home. Though the Mediterranean had been chilly, much worse was to come after Gibraltar. The notorious Bay of Biscay fully lived up to its reputation. Buffeted by fierce wind and mighty waves, we rolled and pitched until even hardened travelers—such as we thought ourselves—were unwontedly queasy.In London, my father had arranged some accommodation for us in Putney. We didn't know that we were in a flat owned by the parents of Alec Bangham—who was going to be a helpful colleague at Babraham. Alec's father, an expert on coal, prophetically condemned the burning of coal as a mere source of energy. Unluckily for us who had just arrived from Australia, it was a bitterly cold winter. In Babraham, the house that had been allotted to us on the Close was so cold that the pipes froze and we had no running water. The landscape around Babraham was very beautiful, all the trees glistening with hoar frost in the bright sunshine. But, for the first few days, with two babies to look after, we all suffered.

Though in principle devoted to research on general animal physiology, Babraham was in effect becoming a thriving center of brain research. Gaddum, was especially interested in neuropharmacology. He brought down from Edinburgh Marthe Vogt, a fine scientist, who had collaborated with Dale and Feldberg in ground-breaking studies of cholinergic transmission, but whose learned, but somewhat dryly delivered lectures were largely-wasted on the raw Edinburgh students. She was now probing energetically monoaminergic mechanisms in the brain. Even more familiar was Catherine Hebb, who had been one of my teachers in the Edinburgh Physiology Honors Course. The younger sister of Donald Hebb, she had left McGill to work with De Burgh Daly in Edinburgh. Her particular interest was the role of choline acetyltransferase in the synthesis of acetylcholine (ACh). New to

me were Victor Whittaker—whose pioneering isolation of synaptosomes revolutionized neurochemistry—and Rex Dawson, an expert on phospholipids, at that time considered very stable constituents of cell membranes, little involved in ongoing cell function. Dawson's work on inositol phosphates contributed to Berridge and Irvine's discovery, 20 years later, of IP3's pivotal role as second messenger. Not mincing his words, he told me that physiology had run its course and that the future belonged to biochemistry. – which of course I denied, unprophetically.

Acetylcholine in Muscle

At first, I continued with experiments on the phrenic-diaphragm preparation. In Canberra, we had shown that EPP-like responses can be evoked with minute quantities of ACh; but these were still much greater than previous estimates of ACh release in ganglia. Perhaps the gap might be narrowed by measuring ACh released in the diaphragm by stimulation at a low frequency. In collaboration with Jim Mitchell—who did the bioassays—we observed a mean release of 0.12 pmole of ACh per impulse, corresponding to only 10^{-17} mole per phrenic nerve ending. This was only marginally less than the amounts of ACh with which we had produced EPP-like responses in Canberra, in the same muscle. We also measured ACh diffusion in agar gels and used those data when analyzing the time course of ACh diffusion out of the diaphragm; and also in a study of ACh release from microeletrodes to get an estimate of the transport number of ACh during iontophoresis.

Transmitters in the Cerebral Cortex

In late February 1961, John Phillis and his wife arrived from Australia with their two infant boys (one only 6 weeks old). Here he was, full of bounce and vigor, eager to get on with experiments. Eccles had recommended that he should stay with the frog spinal cord—on which he had been working in Canberra. My proposal that we continue iontophoretic experiments that I had started in the cat's cortex was much more to his liking. We were both young and energetic, and had excellent technical support from Keith Legge as well as good workshop facilities. Three experiments (usually ending in the early hours of the morning) per week, alternated with days when data were analyzed and fresh electrodes were prepared for the next experiment. Working with five-barrelled micropipettes, of the type developed in Canberra, we soon began an exhaustive survey of the cerebral cortex, looking for transamitter-like actions. Having four channels available for testing different substances, we were able to move ahead quite fast. Over the next 2 years, we applied a very wide range of compounds—but especially those occurring naturally in the brain—on some 4000 units, recorded in the cerebral and cerebellar cortex, mostly in anesthetized cats, as well as some

rabbits and monkeys, and some unanesthetized " cerveaux isolés." By far the most impressive effects were the fast excitation and inhibition produced by amino acids and the slower excitation by ACh.

ACh: A Transmitter in the Cerebral Cortex?

Within 4 months of John's arrival, we had tested ACh on 1374 cortical neurons. Of these, 200 showed excitation, characterized by a slow onset and prolonged after-discharge. Responsive neurons were found in all areas of cortex, mainly in deeper layers. A subliminal action was often revealed by superimposing ACh release on weak excitation by glutamate. As initial tests of atropine seemed to block equally effectively glutamate-evoked firing, initially we thought that, albeit not nicotinic (nicotine had no consistent effect), this was not a muscarinic action. When we discussed these results with Gaddum, he questioned this conclusion. Were we applying too much atropine and so eliciting a nonspecific block of firing? Repeating the tests with lower concentrations of atropine (as well as other agents) indeed confirmed a muscarinic mechanism. Its slow time course of action was very much in keeping with the classical descriptions of peripheral (parasympathetic) muscarinic mechanisms. Bearing in mind previous evidence that ACh is released in the cerebral cortex by K.A.C Elliott, F.C. MacIntosh, and H.H. Jasper (all working independently at McGill), our ACh-sensitive neurons were likely to have a physiological role in cortical function. But where did the cholinergic innervation come from? The cortex itself, or some deeper region? With my colleagues Catherine Hebb and Ann Silver, we found that choline acetyltransferase, normally plentiful in cortex, disappeared from a gyrus isolated in situ by undercutting. So the cholinergic axons must have a subcortical origin.

Ascending Cholinergic Pathways

To locate cholinergic pathways and cells, Ann Silver and I made a detailed analysis of the distribution of acetylcholinesterase (AChE) in the cat's brain. The results were unequivocal. Large fiber tracts such as the callosum or the internal capsule showed no AChE staining; but numerous fine fibers traveling toward the cortex did, and so did large groups of neurons situated in the deepest part of the forebrain. In the large brain of mature cats, however, we could not follow stained fibers all the way from the base of the forebrain. But in cat embryos and foetuses, we could readily identify ChE-stained cells migrating from the germinal epithelium to cluster in the deeper part of the pallidum and the substantia inominata; and, from these cells, sharply stained fibers growing toward, and ultimately invading, the previously quite unstained cortex. Along the medial wall of the hemisphere, a similar projection of fibers originated from the strongly stained medial septal region.

These findings were fully in agreement with those of Shute and Lewis's in the rat's brain—pursued independently in the Cambridge Anatomy department. In view of the known fluctuations in ACh release according to sleep and wakefulness, we boldly speculated that the observed ascending pathway, by facilitating neuronal firing in the cortex, might play a role in generating "consciousness." The characteristic ACh-induced after-discharges seemed particularly significant in the light of Libet's observation on patients undergoing neurosurgery: when their thalamus was stimulated electrically with single shocks, they felt nothing, however strong the stimulation; but they became conscious of even weak shocks applied repetitively for at least 0.5 second. Many scientists—including Adrian, Morrison, Dempsey, Bremer, and Jasper—had noted a correspondence between after-discharges in sensory cortex and wakefulness. But why would such repetitive activity lead to "consciousness"? A simple (no doubt simplistic) explanation could be that repetitive firing elicits the changes in synaptic efficacy needed for learning. If consciousness required the continual formation and retrieval of memories, it might well depend on the activity of ascending cholinergic pathways.

Amino Acid Transmitters

Unlike ACh, long suspected of playing a major role as transmitter in brain, amino acids were not viewed as candidates for such function. They did not fit the then current expectations. For example, the high concentration of glutamate and related amino acids in the brain seemed to argue against this notion. In any case, glutamate was a major constituent of proteins; it was involved in metabolic reactions related to the Krebs cycle and removed potentially toxic ammonium produced by metabolism. Clearly, amino acids belonged to biochemistry. For pharmacologists, another important negative feature was the absence of any relevant bioassays.

L-Glutamate

Albeit present throughout the animal kingdom, glutamates's many-fold signaling functions came to light only *after* the discovery of its potent action in the mammalian CNS (in 1960). In a vast study of seizures induced by various chemicals, Hayashi (with some 100 collaborators) found that glutamate was one of the most potent. But he concluded that glutamate could not be the "excitatory transmitter" (Hayashi, 1959). On the other side of the Pacific, at Caltech, Van Harreveld was comparing the effects of various agents extracted from cerebral cortex on spreading depression in rabbit cortex and on muscle contraction in the crayfish. In both preparations, L-glutamate had the strongest action, though aspartate and D-glutamate were also very potent in cortex (but not crayfish). So Van Harreveld (1959) proposed that L-glutamate, released from depolarized cells, could be a major factor in the

propagation of cortical spreading depression but made no suggestion as to its possible function in crayfish muscle. Meanwhile, on yet another side of the Pacific, in Canberra, Curtis, Phillis, and Watkins were recording the effects of various amino acids applied to spinal neurons by iontophoresis from microelectrodes. In 1960, they reported a marked, but "nonspecific" excitatory action of L- and D-glutamate and aspartate. Thus, by the end of the 1950s, there was a consensus that glutamate strongly excites many central neurons but was unlikely to be a synaptic transmitter.

Within a few weeks after starting our experiments , it became obvious that glutamate was outstanding as excitant of cortical neurons. As we first reported to the Physiological Society (in September 1961), L-glutamate strongly excited virtually all neurons in the cerebral (and cerebellar) cortex—in good agreement with Van Harreveld—much as in the spinal cord, except that D-glutamate was far less effective. Apart from some non-endogenous sulphonated derivatives, none of the numerous other agents tested (including monoamines, which were mostly depressant) had such a fast, quickly reversible and easily reproducible action. With optimal electrode positioning, a brief burst of firing could be elicited by very short pulses of iontophoretic current (lasting only 20 ms), which would release only some 50 fmole of glutamate, much less than the average amount of glutamate present in brain cells. From the time course of evoked firing, the calculated effective glutamate concentration was about 100 μM—in good agreement with threshold values of 100–200 μM later observed in olfactory cortex (by Misell and Richards, in 1979) and not much higher than Van Harreveld's estimate of 35 μM for crayfish muscle.

In all respects, L-glutamate's action was very much like the fast nicotinic action of ACh in muscle and sympathetic ganglia. The unique combination of a very high concentration in the brain—ensuring a ready supply for release—and its high excitatory potency made L-glutamate an obvious candidate for the role of fast synaptic transmitter. One counterargument, the absence of an extracellular catabolic enzyme—analogous to AChE at cholinergic junctions—was irrelevant, because glutamate was known to be avidly taken up by brain cells, a mechanism of removal that has proved to be the most common for other transmitters. Another supposed negative feature, that glutamate strongly excites spinal Renshaw cells, which are activated by recurrent cholinergic axons, ignored their possible innervation by glutamatergic fibers; or, what could not then be suspected, that cholinergic axons might also release glutamate as a cotransmitter. At any rate, I was convinced that L-glutamate was probably the main excitatory transmitter in the CNS.

One aspect of glutamate's action, which later proved of great clinical importance, completely eluded us: its "excitotoxic" action. Indeed, we emphasized as an attractive features of glutamate the apparent absence of any noxious effect: neurons could be excited either repeatedly or continually

for many minutes without causing any obviouse harm—which seemed in keeping with its proposed role as physiological transmitter. So when Olney began reporting (in 1967) that the ingestion of mono sodium glutamate'(MSG') caused neuronal degeneration, we at first greeted his papers with disbelief. That an excess of transmitter might be deleterious seemed paradoxical. This lesson taught me the importance of controlling for long-term effects of bioactive agents.

GABA and Inhibition

The arrival of Ernst Florey, a young Austrian zoologist, in California, in 1953, was a crucial event in the history of synaptic inhibition. He came to work in the laboratory of the Dutch zoologist Wiersma, who had been studying the properties of a sensory organ in crayfish muscle, recently discovered by a Polish zoologist (Alexandrowicz) working in Plymouth (England). This multinational research took an unexpected turn when Florey found that an extract from bovine brain ("Factor I") strongly inhibited the firing of this sensory neuron. In the next act, the scene shifted to Montreal, to KAC Elliott's neurochemical laboratory at the Neurological Institute. After testing on the crayfish preparation all agents known (at the time) to be present in the brain, Bazemore, Elliott, and Florey found that GABA (identified as a major constituent of brain in 1950 by Roberts and Frankel and two other groups) was the most effective. This finding established GABA as possible inhibitory agent in crustacean muscle. Could it also be an inhibitor in the CNS of vertebrates? Spurred by Florey and MacLennan's report that topically applied Factor I depressed spinal transmission, Curtis's group in Canberra tested GABA on single spinal neurons. Indeed, GABA strongly inhibited all neurons, but this action, like that of glutamate, was dismissed as nonspecific. This was the general opinion at the first *Inhibition and GABA* Symposium (organized by Eugene Roberts in1959), when John Phillis and I began our experiments on cortical neurons.

Of the many agents we tested, only GABA and a few related omega amino acids (such as beta-alanine) consistently had a powerful, but quickly reversible inhibitory effect on cellular firing throughout the cortex and cerebellum. Even very brief applications of GABA could inhibit firing. In contrast to the spinal cord, however, where Curtis et al. had observed comparable effects of glycine and GABA, glycine was much less potent in the cortex. Bearing in mind that GABA is normally present in the brain in high concentration, we concluded that GABA was likely to be the natural transmitter at inhibitory synapses in the cortex and cerebellum, and probably other parts of the CNS.

After John Phillis's return to Australia in September 1961, Mirjana Randić (a visitor from Zagreb), Donald Straughan, and I began to explore inhibition in the cortex, comparing GABA's action with natural inhibition.

In these extracellular recordings, inhibition was elicited on a background of controlled unit firing induced by glutamate. This enabled us to observe the inhibitory action of various inputs, both quickly and reproducibly, and on many more cells than could be recorded intracellularly. Our most striking finding was that inhibition was the predominant responses to activation of every pathway tested, including the afferent thalamo-cortical, recurrent pyramidal (evoked by antidromic stimulation)and transcallosal. With stronger stimulation, firing could be interrupted for up to 200 ms. When stimulating the cortical surface, inhibition was evident throughout all cortical layers, over a horizontal distance of 1 cm, albeit restricted to the stimulated gyrus. Such powerful and widespread inhibition ran counter to a long held belief that synaptic inhibition was absent from the cortex. Though its long duration was compatible with a long-lasting chemical transmitter action—comparable to that of GABA—we could not detect a selective block of inhibition by various convulsants. Our failure to recognize picrotoxin- or metrazol-induced intense excitation as due to block of inhibition was a glaring mistake. Knowing that picrotoxin suppresses both inhibition and the effects of GABA in crustacean muscle, we should have tested it more carefully. As when initially testing atropine on ACh's action, we misjudged the effects of large doses of antagonists and strong stimulation. Picrotoxin's anti-GABA action in the vertebrate CNS was to be shown only a few years later by my first graduate student at McGill. Which raises the question: how did I come to McGill?

Why Did We Leave Babraham?

At Babraham, my research was progressing well. Life was quiet, but Cambridge with its Arts Theater and Cinema was only a few miles away. London was also easily accessible for weekend visits. In many ways, this was an ideal situation for getting on with one's work. Scientifically we were far from isolated. I went to most of the near-monthly meetings of the Physiological Society, often driving to outlying parts of the country with my old Canberra friend, Ricardo Miledi (by then well into his long and productive collaboration with Bernard Katz at UCL). I also regularly attended the Hardy club meetings in Cambridge, at one of which Francis Crick presented his great DNA story. In 1964, I was promoted to a more senior position at Babraham and also became a U.K. citizen—up to that time my status as "Displaced Person" had not obviously hindered my career. I suspect that few (if any) other countries would be as open-minded in this respect. So why did we leave for Canada? Quite by chance. At a meeting in London, Ben Delisle Burns proposed that I spend a year at McGill as Visiting Professor during his impending sabbatical leave. I could use his office and lab and do a bit of teaching. When I discussed this idea with Jeanne, she agreed that, after nearly 6 years at Babraham, it could be a pleasant "sabbatical" change.

My employers raised no objections. By September 1964, we were on our way, sailing on the *Empress of Britain*, to Montreal. Awaiting us at the dock were the Head of Physiology at McGill (Frank Campbell MacIntosh, generally known as "Hank") and Andrě Grolimond, my earliest friend, from the very first schooldays in Geneva. Andrě had emigrated to Canada, first to British Columbia, where he'd met his wife Joan, and then gradually moved east: he was now in Montreal, working for Hoffmann-La Roche.

Things had started well. We were renting Ben Burns' house on Victoria Avenue in Westmount, conveniently situated within walking distance from a school for the boys and a short drive from the McGill campus downtown. Life in Montreal was an altogether new and exciting experience. Neither Jeanne nor I had ever lived in a really large city. We knew London quite well, but only as visitors. In Montreal, we were in a vibrant metropolis, with many theaters, cinemas, galleries, and plenty s of music—so much that one could not see or hear more than a small fraction of what was available (the mark of a rich cultural center). For me especially, the mixture of French, English, and American influences was exhilarating. Though we were not fully aware of it, Quebec was in the middle of its "Quiet Revolution," which utterly changed a previously conservative, inward-looking, and church-dominated society. More evident were preparations for the world exhibition, EXPO 67, for which highways, a metro system, and even a new island were being built. The winter was cold but often sunny, and summer really hot. Montreal is unusually well situated, with numerous skiable hills in winter and warm lakes in summer, mostly within an hour's driving. My particular favorite was Mont St. Hilaire, rising from the flat St. Laurent's valley, one the seven Monteregian hills produced by lava outflows and the only one still preserving the ancient forest that originally covered the whole region. It had been bequeathed to McGill to preserve its character as a pristine nature reserve. Only 20 km away, it offered many fine trails for short or long hikes as well as an attractive lake.

The Physiology Department was on the main campus, in the Biology Building—opened in the1930s by Sherrington. No goats and sheep about, as at Babraham but rather a bustling city. From the window in Ben's lab, looking south over the trees of the campus, in late afternoon I was dazzled by the sight of a very handsome skyscraper (Place Ville Marie), its glass and aluminum surface illuminated by the setting sun. In spite of so many distractions, I kept busy. There was much to write up from experiments at Babraham and some teaching. There was also a rich fare of seminars on many topics—especially at the Neurological Institute (where Penfield was still active). GABA was a major focus of activity, with Elliot, Jasper, and van Gelder still trying to make sense of conflicting evidence. The year passed quickly.

Wanting me to stay at McGill, Hank MacIntosh maneuvered the faculty into offering me the Research Chair in Anesthesia—which had remained

open since the departure of Gordon Robson. I was attracted by this position, independent of either Anesthesia or Physiology and having no teaching obligations. With it came the prospect of substantial laboratory space in the new Medical Sciences Building, nearing completion on th the lower slope of Mt. Royal. Some disturbing news from Babraham decided the issue. Concerned by the proliferation of basic neuroscience at Babraham, the authorities advised everyone that research must focus on farm animals. With the choice of directed research on one hand and a research group of my own on the other, I accepted the post in Anesthesia Research.

We had to return to Babraham for a few months to sort out the formalities and pack our belongings. By the end of the summer, Jeanne and the boys were again sailing on to Montreal, while I flew to Budapest to attend a meeting. For my wife, moving into the newly purchased house in Westmount was a challenging experience. In the empty rooms, water had to be boiled in an electric frying pan borrowed from new friends. Things were not made easier by my having to travel again, this time to Japan for the 1965 International Physiological Congress.

Visiting Japan was both exciting and disconcerting. On my first day, heavily jet-lagged, I wandered off to see the sights. Needing a meal I stopped at a restaurant with outdoor seating in the park near the Imperial Palace. With the menu displayed concretely in the window, as very life-like plastic models in full color, ordering food was easy enough. Loudspeakers relayed a Brahms symphony as I struggled with chopsticks, picking at what I thought would be an appropriate local dish. Then I noticed that the Japanese at neighboring tables were all eating spaghettis and risottos with forks and knives. But they were too polite to pay overt attention to the foreigner. Looking forward to an admirable collection of Japanese art, I walked to the museum in Ueno Park. Instead of numerous beautiful screens, or at least plenty of fine woodcuts, the large rooms were virtually empty, except for the odd vase—the very antithesis of the great European collections, with minimal wall visible between the paintings. By contrast, the Kabuki theater more than lived up to expectation: an engrossing auditory and visual experience, combining grand costumes, very stylized acting, and wonderful music. I was mesmerized and could have stayed another hour beyond the regular 4-hour performance. Equally fascinating, but on much larger scale, were the temples and gardens of Kyoto. Perhaps the most unsettling event in Japan occurred on my last night in Tokyo, when I stayed in a traditional inn—recommended by guidebooks as a uniquely Japanese experience. After an early meal in my sparsely furnished room, sitting on a tatami mat, and facing the prospect of a long evening with nothing to read, I turned on the not at all traditional looking television set. To my amazement, before the screen came on, I heard someone speaking quite unmistakable Croatian. Was I hallucinating in these strange surroundings, or was it the sake that I had drunk

with my meal? The explanation was much simpler. Lovro Matešić, the conductor of the Hamburg opera and on tour in Japan, was being interviewed during the intermission. A most curious coincidence.

Scientifically, the Congress was very satisfying. The cerebellum was coming into its own: Masao Ito's masterly demonstration that Purkinje cells are inhibitory revolutionized people's thinking about the cerebellum and inhibitory neurons—which could no longer be considered as exclusively local interneurons. The impressive research performed by Ito and his collaborator over the next decades fully deserved recognition by a Nobel award—as I proposed in the 1980s when asked to make a recommendation. This never came. The workings of the Nobel committee remain unfathomable. It was interesting to see in the flesh such venerable Japanese scientists as Hayashi and Kato—the author of the monograph *The Microphysiology of Nerves*, in which he described, in colorful English, his intricate studies on single nerve fibers and incidentally stated that he would not hire as technician anyone unable to master within 20 minutes the technique of single fiber dissection. My main involvement at the Congress was in a symposium on synaptic transmitters at which I stressed the importance of amino acids as presumptive transmitters throughout the CNS. I never wavered from this belief.

In Montreal, I was joined by my first postdoctoral fellow. Susan Schwartz had just completed her graduate studies at Albert Einstein under the supervision of Vahé Amassian. She had been analyzing patterns of firing of various types of sensory afferents and wished to follow up by looking at transmitter actions on the second-order neurons in the cuneate neucleus. Before doing any experiments, we had to obtain and assemble a minimum of electrophysiological equipment. Albeit impressively shielded, the recording rooms on the 12th floor in the new McIntyre Centre, were quite empty. With the help of Keith Legge—my excellent technician; he had come from Babraham to join us—we scrounged around and borrowed odd items. Within a few weeks, we were able to start experiments.

First on my priority list was to try to get more direct evidence about GABA's inhibitory action. Though I was convinced that GABA was the most likely inhibitory transmitter in the cortex, we needed to show that the synaptic potentials (IPSPs) and GABA had similar effects on the cell membrane, independently of the membrane potential. We therefore had to record intracellularly long enough to compare IPSPs and the effects of several GABA applications. Two microelectrodes were needed, one with a tip fine enough to penetrate cells and another for GABA release. I had tried a concentric arrangement, with the finer-tipped electrode inserted inside a wider, GABA-containing barrel; but iontophoretic currents generated huge recording artifacts. A possible solution (suggested by Bob Werman, who had visited us in Babraham and now worked in Indianapolis) was to use two pipettes, cemented together but with separate tips. He was kind enough to give me

a Narishige micromanipulator for the preparation of such double electrodes—which indeed proved very effective.

In spite of many technical problems—our intracellular recordings in the cortex in situ were most often transient—several aspects of inhibition worked in our favor. Prominent IPSPs could be evoked in all neurons by surface stimulation; they were associated with large increases in membrane conductance; and hyperpolarizations became more prominent as the membrane potential deteriorated. The results were clear. GABA raised the membrane conductance, thus occluding IPSPs, in a dose-dependent manner. Current injections reversed GABA's effect and IPSPs at the same membrane potential, and both were also reversed by injecting Cl ions into a cell (such reversed, depolarizing IPSPs could even generate action potentials!). But intracellular injections of GABA did not affect the membrane conductance. These findings, which strongly reinforced the idea that GABA is the main inhibitory transmitter in cortex, were first reported in *Nature* in September 1966. In contrast to these marked effects on neurons, when tested on inexcitable cells (presumed glia) GABA caused only a mild depolarization and no increase in conductance. We suggested that this depolarization might reflect electrogenic uptake, in keeping with previous evidence that brain slices take up amino acids.

Before Susan Schwartz returned to New York, we had a good look at neurons in the cuneate—working together with my first graduate student Anibal Galindo (a Columbian anesthetist) and George Yim, visiting from Purdue University. Like spinal neurons, cuneate cells were equally sensitive to glycine and GABA and were readily excited by glutamate. We also tested ATP, which had been proposed as the transmitter released by primary afferents: ATP did excite some cuneate cells, but far less consistently than glutamate. From this we concluded that the sensory pathway was probably also glutamatergic. A surprising feature of cuneate neurons was the pattern of firing in response to glutamate, which closely matched the characteristic discharge of different types of sensory axons. Fast adapting axons evidently formed synapses on fast adapting cells (and so on). Such matching of pre- and postsynaptic properties was unexpected—though it made s sense functionally.

A few more pages were added to the GABA story. With new collaborators, including John Kelly (Wellcome Fellow from Edinburgh, where he obtained a Ph.D. in pharmacology under Bernard Ginsburg's supervision), Jean-Pierre Dreifuss (visiting from Geneva), and Mary Morris (another anesthetist, starting her Ph.D.), we obtained more data on IPSP-associated and GABA-induced conductance changes and compared the rates of reversal of IPSPs seen when different anions were injected into neurons. For inorganic univalent anions, the apparent rates of permeation through the IPSP channels were linearly related to their limiting conductance in water, but polyvalent anions, such as sulphate and citrate, were virtually impermeant.

From these data, we concluded that the width of the inhibitory channel was approximately twice that of Cl^-. The more surprising finding that some large organic anions also reversed IPSPs seemed to indicate an interaction with the hydrophobic component of the channels but may have been caused by some indirect effect. In these experiments, we measured only the early component of IPSPs, which reversed most rapidly. If we had examined the later, less reversal-prone component, we might have discovered another inhibitory action of GABA, that mediated by K ions and $GABA_B$-receptors—but that came to light only a decade later, in Norman Bowery's seminal paper of 1980.

Home Life in the 1960s

During the 1960s, Montreal was moving toward a peak of excitement and optimism generated by EXPO 67. This great event proved enormously successful. From all over Canada and the adjoining United States, visitors poured in to enjoy the happy "ambiance." National pavilions catered to every taste, ranging from the very traditional Soviet—displaying impressive machinery—to the U.S.'s geodesic dome, containing both the recent lunar module and a tongue-in-cheek collection of Hollywood relics. Long lines formed outside the Czechoslovak *Laterna Magika*, a clever melding of stage and film and the futuristic cinema at the Canadian *Labyrinth*. Great opera companies and a fine collection of paintings were imported, and much else. It was a golden year, when Canada also celebrated the centenary of its constitution: "one country, one people" seemed a reality. It did not endure. The success of the "quiet revolution" led to a resurgence of Quebec nationalism, further stimulated by de Gaulle's celebrated call "*Vive le Québec libre*!" The new slogan was "*Maîtres chez nous*!" By the late 1960s, the student agitation—that had started in Paris and California—reached Montreal. Here, it took a special form: part Marcusian/Marxist, part Québécois/nationalist. At McGill, students occupied the Principal's office and held long palavers. Young radicals, unexpectedly led by taxi drivers, wanted to reform society; but also loudly voiced long-suppressed resentment against the privileged status of Anglo-Canadians and the English language. Thus, McGill was attacked both internally (by its own leftist students) and externally, as both a bastion and a symbol of "Anglo" supremacy. In time, the student disturbances subsided, as they did everywhere else. In Montreal, many of the newly conscious "Québécois"—no longer subservient "French Canadians"—became increasingly militant. At first, there were single terrorist acts (bombs in mail boxes). Then, after the formation of the National Liberation Front, some prominent individuals were kidnapped (one even executed). The situation in Montreal became critical; the provincial government seeming to lose control, the federal government stepped in, sending in armored cars to police the streets. The city was soon back to normal; but the presence of troops in Montreal exacerbated nationalist feeling. The sense of being a nation apart grew ever stronger. The provincial political scene became

sharply polarized between ostensibly federalist Liberals and the avowedly separatist Parti Quebecois.

The Opposite Effects of Acetylcholine and Cytoplasmic Ca^{2+} on Excitability Are Mediated by Opposite Changes in K^+ Conductance

Perched in our McIntyre tower, little disturbed by the momentous events of the period, our research went on unabated. A major shift of focus took us back to cholinergic mechanisms in the cortex. In experiments (with Ann Silver and Rod Reiffenstein) on cortical slabs, chronically isolated from the rest of the brain by undercutting in situ, we found no evidence of denervation supersensitvity to glutamate and GABA, but the cells failed to respond to ACh. The unusual features of ACh's action in the cortex—its slow time course and the absence of a clear conductance increase—suggested some novel mechanism of excitation. Could ACh produce a slow depolarization by activating electrogenic Na/K pumping? There was a precedent: the Hokins had shown that ACh stimulates Na secretion by the salt gland in birds. Clearly, we needed to test metabolic blockers that should interfere with the postulated pump-mediated action.

Mechanism of ACh Action in the Cortex

In a joint study—with Hiroshi Kawamura, René Pumain (*'coopérant' from Paris*) and Jean-Marie Godfraind (from Louvain, then still a bilingual university), we looked at the effects of some metabolic inhibitors. By reducing the supply of ATP—needed for the operation of the Na/K pump—such inhibitors should interfere with the muscarinic action. Indeed, dinitrophenol (DNP), an uncoupler of mitochondrial function, very effectively suppressed the action of ACh. Though at first tempted to conclude that ACh acted by accelerating electrogenic Na/K pumping, closer examination of intracellular recordings revealed that DNP consistently hyperpolarized cells and reduced their membrane resistance. Because DNP's effect had a very negative reversal potential and was quite insensitive to intracellular injections of Cl^-, a more likely explanation was that DNP increased K^+ conductance.

In parallel experiments with René Pumain and Leo Renaud (M.D., but now a Ph.D. student; in spite of his name, Anglophone Canadian), we found that ACh's characteristic slow depolarizing action was associated with an *increase* in membrane resistance. In corresponding voltage-current plots, the depolarization disappeared at a very negative potential. These observations could be readily explained by suppression of an ongoing hyperpolarizing conductance (either G_K or G_{Cl}). But Cl^- injections, which greatly altered the Cl-mediated IPSPs, had no effect on ACh's action. So we concluded that ACh excited by suppressing G_K. This was a new idea, for which we knew of no parallel in the literature. But the data were too consistent to be ignored.

So here was a novel mechanism of transmitter action, not as a direct excitant but rather potentiating other excitatory inputs. It was the first example of what later came to be known as modulator actions. Such modulations of synaptic actions by ACh (and various moamines and peptides) were subsequently identified at many other sites and in many species. As it turned out, ACh blocked the voltage-sensitive *m*-type K current—later identified by Brown and Adams—and the A current, as well as less voltage-sensitive K channels, including some that maintain the resting potential or mediate the later phase of postspike hyperpolarizations. In this manner, ACh increased the general level of activity of cortical neurons; and perhaps most important, facilitated and prolonged their responses to afferent intputs. All these features supported the concept of a cholinergic regulation of cognitive function. which might account for the loss of cognitive function that follows-degeneration of the ascending cholinergic pathways in Alzheimer-type dementia.

Neuronal Ca Ions and Excitability

How would G_K be raised by DNP? One possibility, that DNP might be taken up by the cell membrane and act as a *selective* K channel, seemed unlikely. On the other hand, it was known that DNP, by short-circuiting the inner mitochondrial membrane, tends to release Ca ions normally accumulated by mitochondria. If a rise in cytoplasmic Ca^{2+} somehow activated K^+ channels in the plasma membrane, we would have an explanation for the hyperpolarizing action of DNP. This was an exciting new possibility. If true, cytoplasmic free Ca^{2+} could be an important modulator of excitability, whether caused by influx of external Ca^{2+} or by release from internal stores.

At that time, internal signaling by Ca^{2+} was virtually unknown. The only relevant information that I could find was a brief 1968 report (by R. Whittam) that metabolic inhibition makes red blood cells more permneable to K^+, probably by raising internal Ca^{2+}. In fact, this had been observed a decade earlier by G. Gardos: his discovery was later recognized by hematologists by referring to the "Gardos channel". Totally new findings are seldom made by a single individual or group. So it was in this case. Unknown to us, while we were proposing that DNP hyperpolarizes mammalian neurons by raising internal [Ca^{2+}], Bob Meech, then a visitor in Strumwasser's lab at Caltech, was busy injecting $CaCl_2$ directly into large molluscan neurons. His experiments showed quite unequivocally that internal Ca^{2+} activated K^+ channels. Although I had attended the1970 FASEB meeting in Atlantic City, where both Meech and I presented our respective stories—at different sessions—we knew nothing about each other's findings. Not until I visited Cambridge later that year (in September 1970) and gave a lecture attended by Bob Meech (who had returned to the Cambridge Zoology department), did I learn about his results.

Our hypothesis needed testing more directly by injecting Ca ions into CNS neurons. But this was not feasible in the cortex with the only micro electrodes then available, high-resistance "sharp" electrodes. The solution was to do the experiments on the much larger spinal motoneurons. Working "blind," we could not insert separate microelectrodes into a given cell, but some motoneurons can withstand the insertion of 3- (or even 4-) barreled electrodes having tips up to 2–3 μm in diameter. By passing currents from a $CaCl_2$ to a KCl-containing channel, Ca^{2+} could be released into cytoplasm while keeping current flow through the cell membrane to a minimum. But even this minimum was sufficient to mask most membrane potential changes. The clearest effects observed in these arduous experiments—performed with Anne Feltz and a graduate student (Adam Lisiewicz)—was a reversible drop in excitability and membrane resistance, probably caused by a rise in G_K—as indicated by the negative reversal level of the potential changes.

Apart from the relatively selective block of cholinergic excitation, we had little evidence that the postulated Ca^{2+}-dependent G_K was of much significance for motoneuron function. For the next few years, working with several collaborators—including Bob Werman (on hemisabbatical leave from the Hebrew Universtiy), Ernie Puil (then at the Universitě de Montréal), John MacDonald, Andrea Nistri, and Yvon Lamour (all post doctoral fellows)—our experiments focused on this problem. An obvious test was to try to prevent any $[Ca^{2+}]$-dependent changes by buffering internal $[Ca^{2+}]$. Injections of EGTA indeed sharply reduced AHPs, indicating that Ca^{2+} influx during the action potential was responsible for the AHP—which is an important determinant of the maximum firing frequency of many neurons. There was also some reduction of resting membrane resistance, suggesting that cytoplasmic Ca^{2+} helps to stabilize the resting potential. EGTA also suppressed the action of DNP, in keeping with a DNP-induced rise in cytoplasmic $[Ca^{2+}]$. We further showed that only only Sr^{2+} had a Ca^{2+}-like effect, whereas Mg^{2+}, Co^{2+} and Mn^{2+} suppressed AHPs and increased the membrane resistance—which we interpreted as probably owing to competition with Ca^{2+} for the relevant K^+ channels. On the other hand, when applied *outside* motoneurons, Co^{2+} and Mn^{2+}, well-known blockers of Ca currents, greatly depressed AHPs, confirming the external origin of the Ca^{2+} responsible for the AHP.

For me, this was an exciting time. One does not often come up with a really new idea, especially one that stood up to critical examination, Though at first greeted with much skepticism—AHPs were thought to be mediated by a voltage-dependent K current (Hodgkin and Huxley's 'delayed rectifier')—the new findings were soon confirmed by numerous groups. The discovery that Ca^{2+}-dependent channels regulate excitability opened up a vast area of research, as such channels are found in many central and peripheral cells, throughout the animal kingdom. A particularly interesting feature is

that they can be activated by either Ca^{2+} influx or Ca^{2+} release from internal stores—including endoplasmic reticulum, as described a decade later by Berridge and Irvine.

The release of Ca^{2+} from mitochondria, linked to changes in cellular respiration, might even play a role in the control of consciousness—insofar as this depended on a heightened degree of neuronal firing in the cortex, maintained by ongoing liberation of ACh. The opposite actions of ACh and cytoplasmic Ca^{2+} on K^+ channels seemed very suggestive A prominent theory held that narcosis is caused by depression of cellular respiration (Verworn, Quastel). It was tempting to propose that mitochondrial depression by anesthetics could lower the activity of central neurons by raising cytoplasmic [Ca^{2+}], thus suppressing consciousness. I did not resist the temptation. To me, it was an attractive idea; but few experts have agreed. One objection was that mitochondrial Ca^{2+} uptake occurs only when [Ca^{2+}] reaches very high, unphysiological levels. The main consensus, in this disputatious field, is that general anesthetics act by enhancing $GABA_A$ receptor-mediated inhibition. Curiously, albeit well aware of Pavlov's belief that sleep is produced by a spread of "inhibition" over the cortex, I had not considered increased inhibition as a likely mechanism of anesthesia. Bearing in mind more recent evidence that mitochondria do play a major role in Ca^{2+} homeostasis, even under normal conditions, I still like to believe that activation of Ca^{2+} sensitive G_K may contribute to general anesthesia.

Modulation of Synaptic Transmission in Some Afferent Pathways

After spending much time writing a comprehensive review of chemical mechanisms of synaptic transmission (Physiol Rev, 1974), my research interests returned to more peripheral synaptic mechanisms. Still outstanding was the question of the nature of the transmitter at primary afferent synapses. Our earlier observations in the cuneate nucleus suggested glutamate—obviously suitable because of its very strong and brief action. We had also tested another candidate, Substance P (SP), but the SP-containing tissue extract that we applied had no consistent effects. Only a few years later, a peptide extracted from salivary glands and purified by Susan Leeman proved to be SP. So we were now able to repeat iontophoretic tests in the cuneate and spinal cord with the pure compound. This time, there was a clear effect: a slow and prolonged excitation, comparable to ACh's action, and quite different from the sharp synaptic responses evoked by sensory stimulation. This looked more like a modulator action that could potentiate fast synaptic transmissions, perhaps those involved especially in nociception (as indicated by further observations by my colleague Jim Henry). According to some intracellular recordings, like ACh, SP induced a slow depolarization and rise in membrane resistance, consistent with a block

of K^+ channels. In related experiments, we found no evidence that baclofen was a selective antagonist of SP. In addition, we confirmed previous reports that baclofen strongly depresses monosynaptic transmission in the spinal cord; and also found that it lowered the excitability of motoneurons and raised their membrane conductance. But we failed to realize that baclofen was a selective agonist for the $GABA_B$ receptors identified by Norman Bowery 2 years later.

Prolonged after-potentials, a prominent feature of primary afferent synapses, reflect another form of modulation: presynaptic inhibition (originally named by Eccles), which operates by reducing the amount of transmitter released by afferent fibers. One possible physiological function would be to suppress painful inputs, as postulated by Melzack and Wall's Gate Theory (1965). But how did it operate? One mechanism, GABA-mediated terminal depolarization, had been proposed by Eccles. An alternative (or additional) mechanism was depolarization by K^+, released from unmyelinated afferent terminals. This intriguing problem led us (Mary Morris and I) to measure extracellular K^+ accumulations in the spinal cord and the cuneate, using the recently developed K^+-sensitive microelectrodes. At both sites, clear increases in extracellular $[K^+]$ followed afferent stimulation. We explored systematically such changes in $[K^+]$, at different depths in the tissue and as evoked by a variety of types and frequencies of stimulation. The observations could be fitted by a model combining fixed release of K^+ per impulse with its removal, partly by diffusion to inactive neighboring tissue and partly by uptake by the Na/K pump—the significant role of uptake was revealed more directly by blocking Na/K pump activity with strophantidin. For this modeling, I could indulge in playing with diffusion equations, something that had remained as a hobby from my calculations of diffusion in sciatic nerves, as graduate student. Albeit a satisfying occupation, the modeling took up much time and effort that perhaps could have been better spent in following up experimentally some suggestive leads. At any rate, these data showed a good agreement, over most of their time course, between changes in K^+ potential and the smaller, prolonged changes in local DC potential. This would be expected if the latter were caused by K^+-induced depolarization of neurons and/or glia. The fit was good, except for an early mismatch, indicative of another depolarizing mechanism, most likely that of GABA-mediated presynaptic inhibition.

Recordings in Hippocampus In Situ

We turned the page to a new chapter near the end of the 1970s when we started recordings in rats. Hitherto, our studies had always been on cats, but their rapidly increasing costs were becoming prohibitive. Going against the fast growing rush toward slices, with my new colleagues, Y. Ben-Ari (from Paris) and Wolfgang Reinhardt form Germany, we decided to continue

experiments on the brain *in situ*. Ben spent a good while working out how to reach the hippocampus with stimulating and recording electrodes held at sharp angles from the vertical—they had to be angled because the rat's brain is so small. I believe we were the first to obtain useful intracellular records from the rat's brain *in situ*. Focusing on IPSPs and GABA, we found large IPSP conductance increases and even better agreement between the effects of GABA and IPSPs than in our experiments on neocortical neurons. An unexpected feature, however, was that IPSPs rapidly diminished, or even disappeared during repetitive stimulation at frequencies above 5 Hz. This was not simply caused by the change in transmembrane Cl^- gradient resulting from Cl^- influx because the IPSP conductance change also diminished or vanished. Could GABA (or some other agent) released by stimulation act presynaptically to block further GABA release? Another possibility was suggested by some evidence that GABA applications became less effective—a postsynaptic change, such as receptor desensitization or more rapid removal by uptake. That IPSPs could be so labile was not just an epiphenomenon: it might help to explain why the hippocampus is notoriously susceptible to paroxysmal activity ("seizures"). This form of disinhibition ('DSI') long remained mysterious but in the end shown by Roger Nicoll to be mediated by cannabinoid release from the post-synaptic cell.

Acetylcholine also seemed to mediate disinhibition. Acting via both muscarinic and nicotinic receptors, ACh facilitated population spikes, evidently by depressing IPSPs. Could this play a role in the cholinergic component of the theta rhythm, driven by septal input (as reported by Vanderwolf)? When we stimulated repetitively the medial septum, hippocampal population spikes were facilitated—by a mechanism at least partly mediated by ACh, since it was diminished by applying hemicholinium, a blocker of choline uptake and thus of ACh release. Though suggestive, the results were rather variable, possibly owing to activation of the septohippocampal GABAergic fibers later found by Tamas Freund.

In all these experiments, we were joined by Nicole Ropert (recently graduated from Laval University, where she had worked under the supervision of Mircea Steriade). Every scientist is special in his or her own way. Mircea Steriade was more special than most. A native of Romania, his scientific orientation was set for life in Brussels, by visiting Frederic Bremer, whose lifelong interest in sleep physiology and thalamocortical function he inherited. In 1968, he was invited to Canada by Jean-Pierre Cordeau (who planted the seeds for the now flourishing neurosciences at the Université de Montréal). As on all later occasions, I was much impressed by Steriade's enthusiasm and the polemical vigor with which he spoke about his work. He took up a position at Laval University, in Quebec City, where he remained for the next 40 years, recording from the brain *in situ* to analyze cortical and thalamic activity and interactions—for decades, it had been one of the main topics of brain research, but it was going out of fashion by the late 1960s.

Albeit increasingly isolated, Steriade refused to give up. Deeply convinced that such complex events as sleep and waking could not be usefully studied in isolated tissues, he never wavered from his focus on brain mechanisms *in situ*. Only recently did Steriade's prolific research get its due recognition when consciousness (hitherto taboo) began to be viewed as serious topics of scientific investigation. Steriade's numerous contributions, and his expertise and deep knowledge, set him at the center activity in this rapidly evolving area of neuroscience—a very happy, satisfying outcome for an exceptionally focused career.

The hippocampus was full of surprises. Not the least were the large changes in extracellular K^+ and Ca^{2+} that accompany neuronal firing. My colleague Mary Morris having much experience with K^+- and Ca^{2+}-selective microelectrodes, together with our visitor from Edmonton (Rod Reiffenstein) we began a systematic study of changes in extracellular K^+ and Ca^{2+} concentrations in hippocampus ($[K^+]_o$ and $[Ca^{2+}]_o$, respectively) It was soon obvious that hippocampus differed greatly from the cuneate, where $[K^+]_o$ rose linearly with the frequency of stimulation. In the hippocampus, the changes were far greater and more complex, the rise in $[K^+]_o$ being linear only up to a frequency of 3–4 Hz. At this point cells started firing in bursts, the synaptic potential rapidly diminished, and $[K^+]_o$jumped to a much higher but stable level near10 mM. The sudden rise in $[K^+]_o$ was always accompanied by a sharp but only transient fall in $[Ca^{2+}]_o$. If stimulation was maintained at 5–8 Hz, periodic increases in $[K^+]_o$ and falls in $[Ca^{2+}]_o$ occurred at intervals of about 30 s. The large changes in $[Ca^{2+}]_o$ were sharply limited to the pyramidal stratum of CA1, in contrast to the widely distributed increases in $[K^+]_o$. The large reductions in $[Ca^{2+}]_o$, clearly the result of somatic Ca currents activated during busts of firing, were especially interesting. They would limit extracellular Ca^{2+} available for further influx into pyramidal cells and the inhibitory nerve endings, predominant at this level, thus reinforcing the disinhibition seen during repetitive stimulation. All these features of hippocampus indicated an exceptional ability to translate relatively low frequency inputs (in the theta range) into bursts of firing and Ca^{2+} influx that can trigger long-term changes in synaptic efficacy. A less desirable aspect is the limited range of safe operation of this hyperreactive organ, notoriously prone to seizures.

Nonresearch Activities

Thanks to my schooling in Geneva, speaking French came as easily as English. But as my French education stopped with primary school, my vocabulary was rather limited. In contrast, English was the language of all my formal education after the age of 14. Thus, in English, I have a wider vocabulary though somehat less efficient mechanics of speaking—my voice tends to give out toward the end of a lecture or after long conversations.

In French, the articulation comes more easily, but I cannot produce the elaborate sentences of the educated Frenchman. This was somewhat less of a problem in Quebec, where people do not speak like typical Frenchmen. Indeed, the problem—especially during the first years—was more in understanding the local dialect; but, in time, I learned to enjoy its lively, colourful and unpretentious qualities. Large or small, conventional or avant-garde, theaters abound in Montreal. So did new writers, including at least one, Michel Trenblay, as good as any contemporary in the world, as well as several gifted film directors. The Quiet Revolution released much pent-up psychic energy in a vibrant cultural renaissance. There was much to enjoy.

As a Croat, I was naturally sympathetic to the Francophones' determination to rise out of what they saw as a colonial status. Higher education and research began to flourish, especially in the sciences. Francophones were becoming major achievers even in business—something they'd been viewed as congenitally incapable of doing. I was not convinced, however, that separation from Canada was desirable. Unlike Croatia, Quebec was not subject to a ruthless, all-powerful central government. A great deal of power in Canada has devolved to the provinces—for Quebec, even that of collecting its own taxes. Indeed, had Yugoslavia been as half as decentralized as Canada, it might well still be in existence. At the federal level, the influence of Quebec is quite out of proportion to its population: since 1968, most Canadian prime ministers have come from Quebec. By definition, Canadians are North Americans who did not wish to be U.S. citizens. To survive as an independent nation they need to stay together. In this respect, Canadians are very much like the Swiss—who also wanted to remain separate from powerful neighbors: in spite of the differences in language, religion, and world outlook, the variegated cantons realized that they had to be united. Throughout their history, French Canadians saw the Protestant, English-speaking New Englanders as the greatest threat to their national survival. When, after their revolution, American forces (as well as Franklin) marched to Montreal and urged the French Canadians to join the new Union, they were driven away by the local inhabitants. Curiously, a surprising number of Québécois nationalists now proclaim a greater affinity for Americans than for other Canadians—seemingly oblivious of the fate of large francophone communities in Louisiana and New England.

The fast rise of nationalist feeling, and especially the advent of a separatist Parti Québécois government in 1976, was very disturbing for the "Anglos" of Quebec. Though having spent their lives here, few could converse in French. New rules making French the only official language, restricting the use of English in advertising, and especially forcing children to go to French schools, convinced many thousands of Anglos that there was no future for them in Quebec. Migration to Ontario (or further west, increasingly) soon became a flood. Major firms moved their headquarters to Toronto. Even at McGill, an English (or perhaps more precisely Scottish) bastion

when I came in 1964, many of the staff were now leaving. Although language was not a problem for me, I was concerned about my family's prospects in an independent Quebec—likely to be hyper-nationalist, at least initially (like the new republics born of ex-Yugoslavia). I therefore considered more seriously than usual offers of possible positions elsewhere. One particularly, from the University of Geneva, was very attractive as it might enable us to live near my aging, increasingly ailing mother. Outstandingly active all her life—much devoted to mountaineering and skiing—she was now seriously handicapped by vicious attacks of asthma (from which she was to die in February1980). Our close proximity would be a great comfort to her. But it didn't work out. While the terms of the Geneva position were under somewhat protracted discussion, Sam Freedman, the new McGill Dean of Medicine asked me to be a candidate for the Chair of Physiology. A distinguished clinician and researcher in oncology, Sam Freedman broke new ground as the first Jewish Dean of a faculty that severely limited the entry of Jewish students before World War II. It was a clear sign of how much McGill was changing after the departure of much of the Anglo establishment.

Why did I accept this heavy responsibility? Mainly because I saw it as a new kind of challenge, at a time when the morale at the University was undergoing a major crisis. The Physiology Department was a large concern. Though Physiology's budget came from the Dean of Medicine, its main teaching was for the faculty of science, largely because physiology was viewed as a good avenue into medical school. Indeed, so many were enrolled in our first-year course that, at one point, lectures had to be given in a downtown theater. And of course there were medical students, honors students, and over 70 graduate students. Caring for all these was a correspondingly large staff with good strength across the board, from neurophysiology to immunology. Though my own teaching was not very onerous, administration took up a good deal of time. But I protected myself by staying as much as possible on the 12th floor—in Anesthesia Research, where I could get on with my research—rather than in the chairman's office on the 10th floor. For the first 2–3 years, the Dean, Sam Freedman, was very supportive, accepting my argument that the basic sciences, unlike clinical departments, had no additional sources of funds from wealthy donors or charities. We were able to maintain our small library, seminar series and workshop facilities, and even recruit new staff. Of all my initiatives during those years, probably the most satisfying (and successful) was getting department members to sponsor a Vietnamese "boat people" family. Taking on the responsibility of providing for three adults and four small children meant having to find accommodation, employment for the father, schooling for the children, medical help when needed—all requiring a major collective effort. I like to think that a sense of common purpose helped to reinforce departmental morale at a time of serious political and economic uncertainty.

As university funding was cut deeply, a new dean, Dick Cruess, was appointed with a mandate to reduce spending. We had to further tighten our collective belt—over 7 years, the departmental budget was reduced by one third (allowing for inflation). This made life distinctly less pleasant and members of the department more restive. Nevertheless, in view of the department's strong performance in teaching and research, the Dean asked me to stay on for a second 5-year term. Convinced that Physiology was thriving, his main concern now was to build up Pharmacology—admittedly by far the smallest basic science department in the Faculty of Medicine.

By the ninth year of my tenure, I felt the need to take a break from the increasingly time-consuming administration. During this first sabbatical leave since my arrival at McGill, I renewed my collaboration with Y. Ben-Ari (ex-postdoc and now good friend), who had taken over an INSERM unit at Port-Royal, in Paris. Having studied hypoxic block of neuronal function in mature hippocampus (as described later), I wanted to repeat the experiments on slices from immature (newborn) rats: — by that time,we were all working on hippocampal slices. For some months, I joined Ben's group at Port-Royal, where we had access to pups of any age. The team included Enrico Cherubini (descendant of Laerzio Cherubini, the Roman lawyer for whom Caravaggio painted the Louvre's notorious *Death of the Virgin*). The experiments went very well. In keeping with the known resistance of human infants to anoxia, compared to mature hippocampus, early postnatal hippocampus was much less sensitive to anoxia. At the end of my sabbatical leave, I decided to go back to full-time research and stepped down from the Chair of Physiology.

What did I achieve as Physiology Chairman? One thing mainly (I like to believe). The department held together and on a more or less even keel through a period of great political uncertainty and financial duress. Despite unfavorable conditions, we attracted some dynamic young scientists—Al Shrier, Ellis Cooper, John Hanrahan, - who would fully live up to their promise. And we kept our best senior staff, including Geoffrey Melvill-Jones, a world leader in research on vestibular motor control who had been offered a Wayneflete Chair at Oxford. What I did *not* do was move the department toward molecular biology—as did my successor, David Goltzman. My own inclination was against an unduly reductionist approach, perhaps owing to an upbringing in medicine and weak grounding in hard science. Inevitably, the wondrous new possibilities offered by molecular and genetic techniques would prove irresistible, especially for younger scientists. But to make sense of the exponentially increasing outpouring and complexity of new information will surely require the more integrative approach favored by physiologists. For that reason, as a discipline, physiology has survived and will continue to thrive, especially in in areas such as neuroscience, where vast amounts of diverse data lend themselves to—indeed are crying out for—a broader synthesis.

Later Years

Though from the very beginning I wanted to bring closer together brain physiology and psychology, for the first decade my research had focused on peripheral structures, and then moved up from nerve and muscle to the spinal cord. Finally, I reached the cortex. I was now closer to the site of the mind-brain boundary—where consciousness is somehow generated. When we found that ACh makes cortical neurons more responsive to incoming signals, we proposed that this might be the cellular basis for consciousness. Though not a breakthrough, it was a useful advance. Another aspect of consciousness, perhaps even more fascinating, is that it is so easily but reversibly suppressed pharmacologically, notably by general anesthetics. Even if the "mind" can exist independently of the brain—a question for which one can foresee no definitive answer—its expression in consciousness is "mediated" by brain circuits that are highly susceptible to chemical manipulation. Some of our findings suggested interesting possibilities. If agents such as dinitrophenol can block the action of ACh in the cortex, apparently by raising cytoplasmic [Ca^{2+}] and activating K^+ channels, might not anesthetics operate in a comparable manner?

More recently, I tried to answer a related question: how does brain anoxia (or ischemia) so rapidly abolish consciousness? Humans subjected to temporary brain ischemia become unconscious within some 20 seconds. How is this reflected at the level of brain cells and synapses? An obvious target for searching study was the hippocampus, known to be one of the first portions of the brain to be affected by anoxia. Recording *in vitro*, from hippocampus in brain slices, under optimal conditions for stable intracellular recordings—with the possibility of analyzing membrane currents under voltage-clamp—surely we should soon find out precisely how anoxia produces such a rapid loss of function. It didn't work out quite that way.

My latest postdoctoral fellow Jean Leblond—a psychology Ph.D. from Université Laval (in Quebec City)—also happened to be my first francophone Quebecois collaborator. A bright, mildly pudgy young man, with a pawky sense of humor, he never felt quite at home in very cosmopolitan Montreal—on Monday mornings, he would come by bus from Quebec city (a 3–4 hour ride) and return home every Friday afternoon. But he cut good slices. Our main problem was making "sharp" microelectrodes, fine enough to penetrate the cells but not having such high resistance as to preclude voltage-clamping. Danish investigators had found that anoxia induced an early hyperpolarization,which they attributed to a rise in K^+ conductance, as well as block of synaptic transmission. We confirmed these observations, which supported the idea that block of respiration induces Ca^{2+} release from mitochondria and activation of Ca^{2+}-sensitive K^+ channels. But K^+ channels might be activated, equally plausibly, by another intracellular signal, a drop in cytoplasmic ATP level. This mechanism of G_K activation had been

discovered in the heart by Noma, 5–6 years earlier. Such K_{ATP} channels might well be present in hippocampal neurons. Over the next few years, we (and others) tested various pharmacological blockers of K_{ATP} channels, with conflicting results. After many experiments—in which I was joined by several excellent collaborators, including Gül Eredmli (from Ankara), Andrei Beloussov (from Moscow), Yao-Zhong Xu (from Hefei) and, Jean-Marie Godfraind (still a regular visitor from Brussels)—we concluded that the K^+ channels activated by hypoxia, being G protein dependent and blocked by both ACh and isoprenaline, were predominantly of the Ca^{2+}-activated slow AHP type; and most likely not adenosine-activated K^+ channels - though adenosine was largely responsible for the hypoxic block of synaptic transmission. As is so often the case, the simple picture I originally had in mind turned out to be far murkier than I expected; though overall in keeping with the idea that the cellular response to loss of energy supply is to minimize energy expenditure (as emphasized by Peter Hochachka). In the brain, this is achieved by a rapid block of neuronal firing and synaptic activity on which conscious perception depends.

China and Chinese Colleagues

By the mid 1980's, graduate students and postdocs began to come from China. The first, Liang Zhang (from Wuhan), was a prodigiously hard-working student. Of an unusually cheerful disposition, Liang was highly articulate in English, but almost impossible to understand. This was to be a recurrent problem with Chinese students who, in China, are practically never exposed to English spoken by non-Chinese. How different from most European countries. In Croatia, very impressed by the excellent English spoken by all my young relatives, I commented on the efficiency of their English teachers at school. In fact, from an early age they had learned English watching and hearing American and British programs on television. Here was a lesson for China, where such programs are invariably dubbed in Chinese.

YaoZhong Xu, and I met by chance in Dubrovnik. On leave in Italy (from the USTC, in Hefei), he had come from Trieste to attend the Summer school in Biophysics (superbly organized by Greta Pifat, of the Rudjer Bošković Institute). Quite unlike my other Chinese visitors, Xu was a bon-vivant. He had traveled to all parts of Italy and admired the sites and cities, noting the beauty of the women and the excellence of the cuisines—most of all he appreciated the unhurried lunches. Perhaps he was making up for his exile to Inner Mongolia during the Cultural Revolution though even in Mongolia, ever adapting to circumstance, he found a wife, and enjoyed riding on ponies. Xu spent a year with me in Montreal and later arranged my three visits to China (in 1991, 2001, and 2003). In 1991, the first 2 days Jeanne and I were hosted by T. P. Feng at the Shanghai Institute of Physiology.

China's leading neurobiologist, in prewar years Feng had worked in Chicago with R. W. Gerard and in London with A. V. Hill. He returned to China and set up a very productive laboratory in Shanghai. He survived the great upheavals of the war years, the Maoist takeover, and the Cultural Revolution but was unable to do much science until he was reestablished as Director of Research at the Academy in Shanghai. Being familiar with his many publications—first, on the nerve sheath, and later on end-plate potentials (in Canberra, Eccles had all Feng's reprints from the *Chinese Journal of Physiology*)—I was delighted to make his acquaintance when he visited North America in the early 1980s. In Shanghai, I met two other Chinese scientists who in their youth had also worked overseas, H. C. Chang at Mill Hill with John Gaddum (my mentor at Babraham), and H. T. Chang, who was a dynamic CNS researcher at Yale and the Rockefeller Institute until the mid-1950s, when he returned to China. At the time of my first visit (1991), the streets of Shanghai were clogged with cyclists, undisturbed by cars. Owing to power shortage, there was no electricity after 10 a.m. By 2001, Shanghai was almost unrecognizable. Cars had replaced the bicycles. Whole quarters were being razed to make room for large office and apartment buildings. And the unremarkable view across the Huang-Po River had turned into a forest of skyscrapers (as architecture, mostly uninspiring, but nonetheless eye-catching). China's stupendous growth is hardly news to anyone nowadays, but seeing it firsthand made a deep impression.

In 2003, I spent a month in Hefei, lecturing as visiting professor. Apart from the ever denser and more chaotic traffic, much was changing, even in a relatively small provincial town (by Chinese standards, but nonetheless with 1 million inhabitants). For example, a major intersection was blocked for several days by a "sit-down" strike of civil servants, calmly watched by policemen redirecting the traffic. A few years earlier, even such a peaceful demonstration would not have been tolerated. One weekend, I was driven to a much smaller town, in southernmost Anhui province to visit the new middle school opened by an enterprising Mrs. Wong. I was taken from class to class to address the bemused children who had never heard an English-speaking foreigner. So in communist China, just as in capitalist countries, parents were happy to pay high fees to have their children go to a private school. In spite of the apparently unyielding politics of its government, Chinese society (not just the economy) was rapidly evolving. Some other vivid memories from China come to mind: in Inner Mongolia, a Tibetan-style Buddhist monastery, Genghis Khan's putative tomb, a lavish feast (including camel hooves) in a yurt, and sliding down huge sand dunes. In Xian, an extraordinary mosque complex, architecturally in pure Chinese style; and much else.

My next visitor from China, Yanguo Hong was also old enough to have lived through the Cultural Revolution. He was exiled to a remote mountainous area where, as physician, he had an exhausting time traveling up and

down steep hillside tracks. Then came PingJun Zhu. Originally from Hangzhou, he was studying cardiac electrophysiology at the Université de Montréal, but decided he wanted to work with me on hippocampus. At first, I was very reluctant, not wishing to "steal" him from his PhD supervisor, but he was very insistent. Tall and cadaverous—despite vast quantities of dumplings prepared by himself and consumed at lunchtime—he was a really serious worker. His research on the protective actions of glucose and adenosine against irreversible brain damage was very productive. As a postdoctoral researcher, he (and his very lively wife and little daughter) moved to the United States. Curiously, we quite recently again became collaborators (at long distance, as mentioned in the next section). My last Chinese student, Yong-Tao Zhao, was untypically keen on athletics, including tennis. Albeit successful in some very difficult experiments, his heart was not in research. He left after only one year.

The Turkish Connection

Of all my "connections" the richest has been that with France. I was fortunate to have as visitors such gifted scientists as Anne and Paul Feltz, René Pumain, Yehezkel Ben-Ari, Yvon Lamour, Nicole Ropert, and Jean-Louis Bossu (in chronological order). Another connection, with Turkey, was more fortuitous. How did it come about? In a very roundabout manner, via an IBRO Workshop held in 1974 at the Pahlavi Medical School in Shiraz - a delightful town graced by the finest bazaar and most colorful domes imaginable, Participants were invited from all countries in the region (under the Shah's enlightened rule, even Israel!). Two came from Turkey, including the pharmacologist Ogűz Kayaalp from Ankara. Though I did not see Kayaalp again till some decades later, in 1981 he wrote to me from Hacettepe University (Ankara), strongly recommending for a postdoc position his ex-student Turgay Dalkara, a research-oriented neurologist**.** An ardent nationalist—like all his compatriots—Turgay was a most pleasant collaborator. Our studies (with Conrad Yim, a Canadian Chinese) on hippocampus *in situ* revealed striking ephaptic interactions between the closely apposed pyramidal cells. Such mutual reinforcement of synchronized excitation would facilitate network oscillations and seizures—both characteristic features of hippocampus.

Another 'Turkish' student, Nadia Agopyan, though born in Istanbul, was really Armenian. An exuberant personality, she later had an exciting postdoctoral career. First, one year in Japan, with N. Akaike (in Sendai); her uninhibited laughter, as well as disparaging comments about male-chauvinist treatment of women employees made a great impression. Her second postdoc year—now accompanied by her husband Peter Miu (also my graduate student)—in Sackmann's laboratory in Heidelberg, became equally memorable when she installed their very young baby in a corner of the lab.

After this they returned to North America. The next arrival from Turkey, Gűl Erdemli, was recommended by Turgay Dalkara. She took the torch from Jean Leblond, continuing the investigations on membrane currents induced or altered by hypoxia in hippocampal slices. My appreciation of Turkey became even stronger when Turgay organized a symposium on brain ischemia in Urgűb (Cappadocia). After the meeting, driving from Urgűb via Konya to the Mediterranean, and then, mainly along the coast to Izmir, I discovered a country immensely rich in different landscapes and ancient monuments, remnants of Greek and Roman Ionia, and the long-enduring Byzantine and Ottoman empires.

My Turkish connection ended with my last graduate student, Selva Tekkök, another medical graduate of Hacettepe University. Her experiments on the effects of glucose deprivation on synaptic transmission, also in hippocampal slices, took an unexpected turn when she discovered a curious, unsuspected manifestation of synaptic plasticity. Substituting glucose with 2-deoxyglucose (2-DG, a nonmetabolized derivative) for 10-20 min resulted in block of synaptic transmission—as expected—but when glucose was reapplied, the return of synaptic transmission was invariably followed by a pronounced and long-sustained rebound of EPSP amplitude. This intriguing, long-term potentiation (LTP)-like phenomenon was of interest, as the only form of LTP induced by blocking metabolism at a precisely known step in the glycolytic pathway. Though readily taken up by cells and phosphorylated, unlike glucose 2-DG is not further metabolized and just accumulates in the cells (hence its well-known use as an index of glucose uptake). For Selva, this topic had the advantage of being quite new and therefore wide open for all kinds of investigations, with no competition to worry about. Albeit having no obvious physiological relevance, 2-DG has been proposed for the treatment of a variety of disorders , including obesity, viral infections, malignant tumors and parkinsonism. Its potential effects on learning and memory may have a wider significance.

The Postretirement Years

At my retirement in 2000, I stepped down after heading Anesthesia Research for 35-years. Though sometimes referred to as “anesthetic research” or, more seriously, criticized for insufficient relevance to clinical anesthesia, it had been a thriving, highly productive operation. “Curiosity-driven” research, in neurosciences as in other fields, often throws new light on old issues and practices. In this manner, basic research supplies new tools for improvements in clinical practice. This truism cannot be overemphasized, especially now that “translational” research has become the latest fad, forcefully promoted by university administrations and granting agencies.

Two stressful events marked the first decade of my retirement. After 40 years in a relatively large house on Belmont Avenue, we moved to a much

smaller apartment, more appropriate for two "retirees." Having to deal with huge amounts of material (especially books), accumulated over four decades, was physically draining and psychologically traumatic. Parting with books is always hard; with so many, it was devastating. But we managed to keep a good number and survived.

Naturally, I had to vacate Anesthesia Research to make room for Fernando Cervero, my able successor. An expert on nociception, he established a busy center for basic research on pain. I was given an office nearby, on the 12th floor. But there was not enough space for long shelves full of scientific journal, as well as rows of filing cabinets, brimful with experimental protocols and data, reprints, and correspondence Having to throw out so many precious, handsomely bound journals was distressing, but only a temporary hindrance: journals becoming increasingly available on the Internet, regular visits to the library were no longer necessary. Neither was a secretary, as, at first tentatively and laboriously, I learnt how to use a word-processor. At any rate, as emeritus, I had an office. Conveniently next door, in Pharmacology, my Croatian colleague Ante Padjen had his office and laboratory. Also born in Zagreb, Ante was much involved in Croatian expatriate affairs. We were both members of an association of alumni of Zagreb University (in my case, as honorary member); and also an International Croatian Initiative (whose aim, during the Tudjman years, was to promote democracy in Croatia). We both also had a life-long interest in music (in his case expressed more tangibly by creating a medical faculty orchestra, *I Medici di McGill*). Ante allowed me to move some of my old equipment into a corner of his shielded room. This was the site of my last "hands-on" experiments and also of my first close encounter with transgenic mice. Jasna Križ, my only Croatian collaborator, was an ex-Ph.D. student of Ante's and was now working with Jean-Pierre Julien. They had produced mice overexpressing peripherin—an intermediate filament, apparently involved in some neurodegenerative conditions. In these mutants, could high levels of peripherin in the hippocampus be reflected in altered synaptic transmission? We recorded synaptic responses in hippocampal slices, from many mutants and control littermates, but found only some changes in the magnitude of LTP. Curiously, they were opposite in direction in CA1 and CA3.

My continued involvement in research, albeit still quite deep and time consuming, is no longer at first hand, but rather as advisor in planning experiments, interpreting data, and writing up papers or grant applications. My main collaborators, both leaders of very active groups, have kept me quite busy. The first was Jiang-Hong Ye (at the New Jersey Medical School)—a native of Guandong, whom I had met in Akaike's lab in Japan. His research has focused on synaptic transmission in the dopaminergic, reward pathway—generally believed to mediate drug addictions—in particular modulation of transmission by strychnine-sensitive actions of glycine and taurine and some unsuspected presynaptic effects of ethanol.

My second ongoing collaborator, Mauro Costa-Mattiol, an exceptionally gifted and dynamic young scientist, was born in Uruguay. Having obtained his PhD at the University of Nantes, he spent several years at McGill as postdoctoral fellow in the laboratory of Nahum Sonenberg (at McGill), a world expert on the hugely complex mechanisms of RNA translation. Further exploring the functional role of the translation initiation factor eIF2α, they studied transgenic mice deficient in GCN2—a kinase which phosphorylates eIF2α. To their surprise, these mice were able to learn and memorize more efficiently than control littermates. In hippocampal slices from the mutants, the threshold for sustained LTP was also lowered. These findings seemed paradoxical, because activation of eIF2α suppresses *general* translation, which would prevent the synthesis of new protein(s) known to be essential for sustained LTP and for behavioral memory. The explanation is that phosphorylated eIF2α activates ATF4, a *repressor* of CREB, a transcription factor involved in learning. Hence, in mutants lacking GCN2, reduced phosphorylation of eIF2α removes the inhibition of CREB by ATF4, thus facilitating learning. The pivotal role of eIF2α phosphorylation was confirmed in the second phase of the project in which increasing or reducing eIF2α phosphorylation elicited the expected bidirectional changes in learning. My own contribution to this vast project lay in almost daily discussions with Mauro over the interpretation and writing up of a great variety f data. Although Mauro is now in Houston, at the Baylor College of Medicine our ongoing discussions have continued by email or telephone. A series of exciting new findings by Mauro and my ex-student Ping Jin Zhu have revealed a quite unexpected function of PKR—another eIF2α-related kinase—as regulator of GABAergic inhibition, probably acting through interferon-γ. Perhaps most surprising is that mutants lacking PKR are prone to mild electrographic seizures; but they perform better than littermates in tests of learning and long-term memory. A fascinating example of the anything but simple interactions between molecular, cellular and cognitive function.These forays into large-team research—so different from my own life-long practice—have been hugely instructive. I learned that I was still capable of learning, which was personally satisfying but of no great consequence. More important was seeing, so to speak at first hand, both the extraordinary power of molecular genetics and the awe-inspiring, seemingly unfathomable complexity of living matter. It is encouraging to know that so much can be done at last to narrow the gap between cognition and brain physiology and biochemistry. However, to achieve this, large teams and abundant funds have become essential—a situation not unlike that of physics, to which biology has long been aspiring. As members of such teams, individual researchers tend to become narrowly specialized and have fewer opportunities for personal initiative. Can they have the all-consuming attachment to their work, characteristic of many researchers of my generation? If research becomes a 9-to-5 occupation, albeit beneficial for family life, it will be very different. The future, like the past, is a foreign country.

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