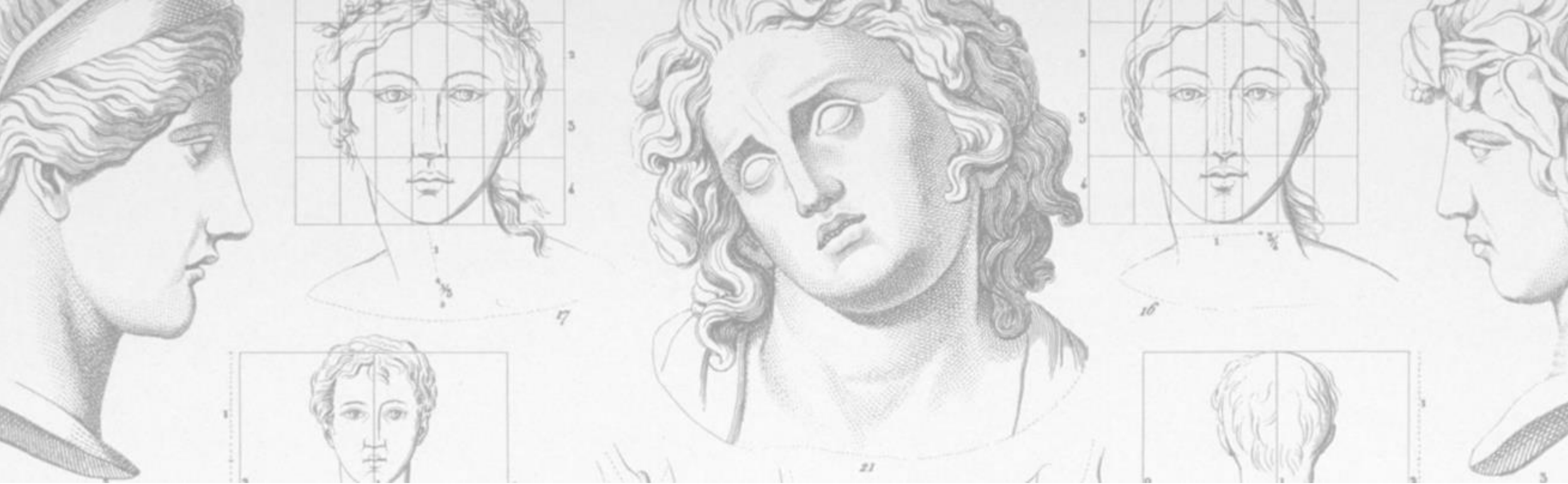

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# Augusto Claudio Guillermo Cuello

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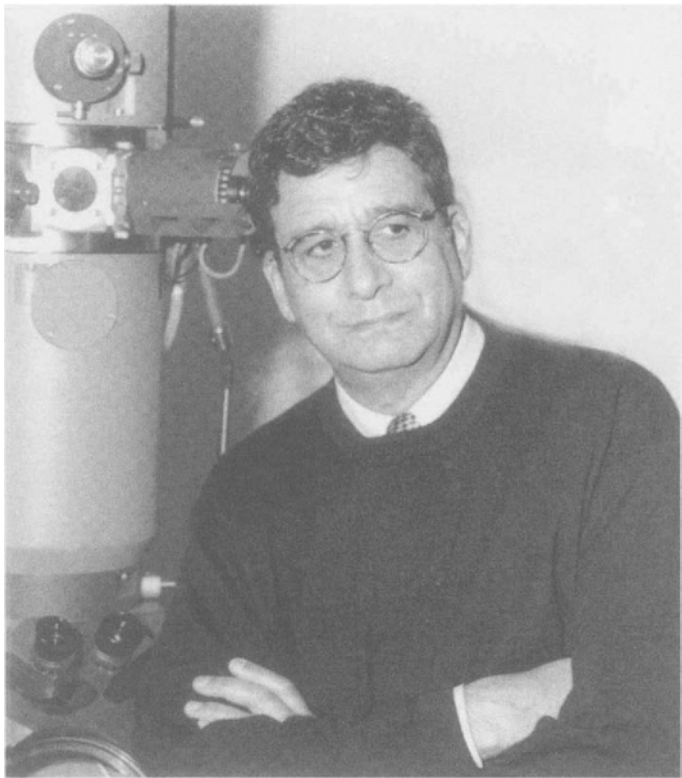

# *Augusto Claudio Guillermo Cuello*

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*Claudio Cuello has carried out fundamental neuropharmocological studies of neurotransmitters. His pioneering work helped establish the dendritic release of neurotransmitters, the application of monoclonal antibodies to neuroscience research, the localization and function of substance P and the endogenous peptides, and pharmacological approaches to neural repair.*

# Augusto Claudio Guillermo Cuello

## I Was Not Meant to Be a Scientist

How does one become a scientist? I guess there are thousands of routes. However, my path was rather unconventional. I was meant to become a soldier, a Catholic priest, an historian, or a lawyer; of all professions, science was the least likely. When I was a young child, my family was profoundly influenced by the Spanish cultural tradition of the 'sword and the cross.' Over my bed hung a portrait of the Liberator of Argentina, Chile, and Peru, General San Martin, wrapped in the Argentinean flag. I received a strong Catholic education and, along with it, I acquired many fears and prejudices, including that hell was very real—you could feel it—and Jews were not to be trusted.

My family background was not an inviting one for a scientific career. My father, a relatively successful journalist, was a rather bohemian and unrealistic individual for whom magical thinking, family, and country mythologies played an important role. The Cuellos, the Freyres, and the Basalduas apparently came from Spain during the first colonization of the Rio de la Plata. My father, Juan Andres Cuello Freyre, filled me with tales of incredibly brave gauchos (Argentine cowboys) and the life of patrician 'demigods.' I was made to feel my responsibility as a descendant of heroic and famous generals who fought the Spaniards across the Andes or who 'conquered' the desert. An image, which is indelibly printed in my mind, is that of my grandmother (Delfina Freyre-Basaldua de Cuello) and her sister (Mercedes Freyre-Basaldua de San Martin) donating to the Province of Buenos Aires the family table on which the 'Acuerdo de San Nicolas'—the first attempt at a national constitution for Argentina—was signed.

I did not know my paternal grandfather but he must have been quite a character. He was the son of a 'resero' (a gaucho who owned and transported herds from the pampas to the Andes) and a lady of Irish ancestry. His name was Juan Argentino Cuello, and one cannot be more Argentinean than that. He was the first of his line to dress in a European fashion, favored Milton above other authors, and was a liberal Freemason and the

grand master of the San Nicolas lodge. He was also the head of a major provincial post office and strongly believed in progress and education.

My mother, Rita Maria Sagarra Estorache, on the other hand, was born in Argentina from very Spanish parents. Her father was from Granada and her mother from Sevilla. They were the epitome of Spaniards from the end of the nineteenth century. They brought me the magic of the 'old country' which I still feel somewhat belongs to me. My grandfather, Augusto Magencio Sagarra took a great interest in me. He used to sing me fragments from 'Zarzuelas' while playing his guitar, and he told me half mystical stories of lost Moorish treasures in the family's 'cortijos.' He told me about his father, a rural medic from Zaragoza (he probably knew Ramon y Cajal), who received a Royal Order from the Queen Regent for his actions during the cholera epidemics in Almeria in 1885 (the diploma of which hangs in my study). He also gave me a vivid account of his own life from the comfort of a well-to-do family in the south of Spain to his passage through a monastic seminar and military school and the hardships that came with the death of his father that forced the family to emigrate to Argentina, a 'land of promise.' I clearly remember my great-grandmother, Rita Maria de las Mercedes Martinez Alvarez de Cienfuegos y Plasencia, sitting in her rocking chair in contemplative silence in a patio full of begonias, most likely wandering through beautiful Andalucian gardens, gatherings of the nobility, or playing with the Infanta Isabel. At home I keep a copy of the coat of arms of the Alvarez de Cienfuegos y Plasencia, painted by my great-grandfather, and the original royal charter by which the king granted the family rights to have soldiers under their command and a limited number of English prisoners at their service in Granada!

When I worked in Cambridge, I established contact with many of my Spanish relatives from the Sagarra side of the family in Granada, Zurgena, Vera, and Madrid. I have yet to meet my more distant relatives, the Alvarez de Cienfuegos and Plasencia, whose medieval ancestry my mother has patiently reconstructed over the decades. My mother, a retired schoolteacher, is in her eighties and is enthusiastically playing with computers. She was and still is an avid reader.

During my childhood, family life was somewhat chaotic and unpredictable. My father's journalistic and political engagements caused the family to move from Buenos Aires to a succession of cities, including Patagonia (Comodoro Rivadavia), La Plata, Cordoba, La Paz (Bolivia), and Santa Fe, before heading back to Buenos Aires without a penny. In his last political–entrepreneurial gamble in Bolivia, my father lost everything with the foundation of the newspaper *Hoy*.

With each move there was a new house and a new school. Sometimes, schools changed as we moved houses in the same city or because of my family's financial ups and downs. In total, I attended five different primary schools in 7 years. My favorite school was the majestic, very Catholic,

La Salle College in Cordoba, where the discipline was so strict that one was caned on the tips of one's fingers if one did not memorize the different tenses of French verbs properly. The La Salle Brothers ran a most efficient educational machine. I was fascinated with the impeccable order, the exactness in time keeping, the beauty of the surroundings, the ritual of the long-table lunches, and the camaraderie among the pupils, an interesting bunch, most of whom were children of the 'establishment' and of the historical Cordoba families. The La Salle period coincided with the peak of my religious fervor. I wanted to be a priest.

The most glorious part of my formal education was secondary school. My parents were on their way to Bolivia to launch a newspaper that eventually resulted in their economic ruin and nearly cost my father's life in a gun attack. They asked me if I would like to attend a boarding school, the San Martin Military Lyceum. I was fascinated with the idea. Imagine wearing a uniform, being trained to use real guns, the promise of sharing the glory of our distinguished ancestors, and modeling my life after General San Martin. No more lead soldiers, this was the real thing! However, there was a drawback—there were thousands of applicants per year. For present-day readers it may be difficult to imagine, but in the early 1950s the Argentine military was still highly respected. The Military Lyceum was considered to be one of the most desirable schools in the country, along with the National College of Buenos Aires. The school's role was to generate graduates who would move into civilian professions while maintaining a strong link to the military establishment. To secure a place I had to prepare for the admission examinations and was therefore given an eccentric and demanding private tutor in our beautiful colonial house in La Paz. This was my first serious intellectual challenge. I passed the examination and went to Buenos Aires to live with my Spanish grandparents. There were about 400 new cadets registered as freshmen in 1952. Of those, approximately 100 (about 1 in 4) were destined to graduate as 'Bachelors' and 'Second Lieutenants'; thus, the admission quota for the second and consecutive years was gradually reduced, until it was about 100 in the fifth year. Each of us had a ranking order number, per academic year, based on our scholastic achievements, behavior, and 'military aptitude.' By the second year (we were just kids!) we were initiated into classical military subjects, including forced marches, bayonet drill, and musketry training using the heavy German Model 1909 Mauser rifle. During those years our battle uniform evolved from that of the old Prussian style with gaiters and long jackets to the more contemporary American World War II style.

By the third year of schooling, cadets were granted 'status militaris,' which meant that technically we could be called upon to fight and that we were subject to the same code of military conduct as the professionals, including the court martial. This passage to 'manhood' was celebrated with

a formal ceremony in which, in a spectacular formation, every cadet advanced one by one to receive a small sword in a large plaza filled with sobbing mothers wrapped in expensive overcoats. My father gave me my sword and I felt that I was crossing the Andes to battle as my forefathers had done. I was going to be a military man.

The third to the fifth years at the Lyceum were extremely rich in experiences both within and outside the school. During those years the Peron regime fell and a new air of freedom burst into the country, even reaching into the military. We were encouraged to express our ideas, argue, and even elect representatives to the 'cadet-mess.' It was during this time that I started a magazine called *Ariel*, in which I was allowed to publish poems from Paul Elouard and Neruda or comments about Tennessee Williams' and Bertold Brecht's plays. I could even discuss classical Spanish literature with the school director, the late General Turolo. Argentina has never since seen the same quality of cultivated and liberal military men.

It was in this rather unlikely place that I discovered science and my vocation for brain research. Our civilian professors were excellent. They had a good rapport with the cadets and a large proportion of them were extremely inspiring individuals. My 'conversion' was achieved through the combined teachings of Professor Greenberg (who dared, in a Catholic military school, to talk about the 'Judeo-Christian' ethical traditions) on logic, Professor Binda on psychology, and Professor Tejero on anatomy. The latter was a practicing M.D. with a particular interest in the central nervous system (CNS). In his lectures he tried to correlate elements of psychology and behavior with the hardwiring of the brain, and he allowed us to see the real thing in carefully prepared and dissected human brain preparations. It was at this time that I was first exposed to the 'neuronal theory' and the name of Ramon y Cajal. I was awed and my destiny was sealed. I decided to apply to the Buenos Aires Medical School. My early military fantasies were abandoned. I was thirsty for scientific knowledge, and I wanted to understand the brain's function and human biology.

## University Years

My entrance into the university was a major cultural shock for me. I had left behind the wonderfully harmonious and predictable boarding school life and entered the chaos and imperfections of civilian life. To make matters worse, my parents had been seriously impoverished as a consequence of political changes and financial gambles. Both of my parents had to work extra hours simply to keep the house afloat. If I wanted to study medicine, I was on my own. I had to earn my living working late hours as a journalist on the local newspaper of the industrial Avellaneda district. While working, writing about odd social events, I started university with a few borrowed books and a surgical set given to me by Dr. Moshe

Sandbank, the father of a secondary school companion. Living outside the protective walls of my secondary school, I was appalled to learn about the flagrant social inequalities of Argentina. At the time, only state-run universities were allowed in Argentina. The administration of President Frondizi sent to congress legislation allowing the creation of private universities, but the majority of students, myself included, believed that state universities were a social–cultural fulcrum where people from diverse backgrounds were amalgamated. We were passionately against this proposed law. Soon after the start of classes there were massive demonstrations, and I joined the battle by mobilizing the student body in my district. I organized the occupation of secondary schools in protest and ran an elaborate protective network, greatly assisted by my military education. As the visible leader of the Avellaneda movement at the peak of the revolt, I was taken to police headquarters, where I was compelled to negotiate the release of public buildings in order to avoid violent confrontations. By midterm, the political battle was lost. The new law had been passed and I was not in a position to take examinations. I had wasted my time and energy on impossible political battles. I decided to quit university and wander around Latin America to 'find myself.' I left home with no more than 100 dollars, a tent, and a rucksack. I spent 3 months 'hitch-hiking' through the hard Chilean desert, spectacular Peruvian ruins (unforgettable Machu Pichu), and colorful Bolivian markets. I had incredible experiences. I met the most interesting people—poets, miners, indian peasants, jungle indians, mafia bosses, university professors, writers, economists, industrialists, priests—all of whom had their own unique message. I had plenty of opportunities to reflect on my aspirations. At the end of my travels, I accepted the fact that I could not simultaneously change the social realities of Argentina and study medicine. I opted for medicine.

The opportunity to become a full time student once again arose when I met my past instructor in the medical admission courses, Dr. Horacio Encabo, who facilitated, through the offices of Juan H. Tramezzani, a fellowship for me from the Roca Family Foundation. It was a modest stipend that permitted one meal per day (generally coffee and a sandwich); however, it kept me afloat until I obtained a fellowship from the University of Buenos Aires, sponsored by the late Professor Eduardo P. De Robertis.

Juan Tramezzani in those early years acted as a 'moral tutor' to me and lent me many books from his personal library. Unfortunately, later in life, we moved apart due to our divergent visions of the country and science. As a medical student, De Robertis became my role model. Like me, he had had to overcome great difficulties in order to study medicine (something that I discovered only after he passed away). In his lectures, he conveyed the excitement of research and discussed the latest developments in cell

biology. Nevertheless, the student body feared him. However, I was fascinated by his lectures and competed for a highly sought after teaching assistant position in his institute and department.

In the late 1950s and early 1960s, the university saw a renaissance in medicine. At the School of Medicine I particularly enjoyed the science of medicine. We had excellent teachers during this time, many of whom had returned to Argentina from the United States or from Europe after the reestablishment of democracy. These teachers also had very active research programs, which coincided with the creation of the National Research Council (Consejo Nacional de Investigaciones Cientificas y Tecnicas; CONICYT). At the time, the University of Buenos Aires had an established tradition in biomedical research since the names of Houssay (pituitary 'diabetogenic factor,' today's somatotropin), Braun Menendez (who discovered the angiotenisn–renin mechanism), and Federico Leloir (the pentose biosynthesis pathways) were fresh in everybody's mind. De Robertis' *Cell Biology* text was an international best-seller. By this point, his classical description of the ultrastructure of synapses was already made, he had proposed the exocytotic mechanism of hormonal release in the adrenal gland, and his team was competing with Whitaker's group at Cambridge for the isolation of synaptic vesicles. De Robertis' lab was bursting with activity. In 1960, I joined the Department of Histology and Embryology as a teaching assistant and had my first opportunity to witness real research in action. This was during the period when De Robertis' team obtained the purest possible subcellular fraction of synaptic vesicles by bursting nerve endings with a hypoosmostic shock (a fortuitous consequence of omitting the buffer!) (De Robertis *et al.*, 1961, 1962). While working in histology, I was given the task of generating the entire collection of microscopic preparations for a new neuroanatomy course that was to be launched by Fernando Orioli (the disciple of Mettler). To be part of the team, one was expected to produce large-scale drawings of every possible nucleus and fiber tract from Weigert's stained sections, from the lower spinal cord to the anterior commissure level of the human brain. It was an excellent discipline that left me with a profound understanding of the human brain.

There were many influential group leaders in the department, most notably David Sabatini, who latter became the head of cell biology at New York University, and Herns 'Coco' Gerschenfeld, who, as a Maitre de Recherche at L'Ecole Normale Superior in Paris, became a point of reference in the neurobiology of the 1970s and 1980s. Coco has been a paternalistic friend who has witnessed my career's evolution from a disoriented medical student to a mature scientist. There were other equally inspiring scientists at the Department of Histology and Embryology, such as Pellegrino de Iraldi, who interacted with Pio del Rio Hortega when he took refuge in Buenos Aires during the Spanish Civil War. All these professors

gave histology lectures with genuine passion and blended the past with the latest discoveries in cell biology, embryology, and neurobiology. I was in my element.

During my last year of medical school I joined Amanda Pellegrino de Iraldi's group at the De Robertis' Institute. She was part of the original team that had isolated and characterized synaptic vesicles. Amanda had then proceeded to define the 'dense cored vesicles' as sites of catecholamine storage. The model used in these studies was the sympathetic nerve terminals in the rat pineal gland. My project was to combine pharmacology with ultrastructural changes in these vesicles. We made some interesting observations on the effects of guanethidine and reserpine that never reached the printed stage because, soon after, I completed my M.D. (in 1965) and joined the Argentine Antarctic Campaign of 1966.

## Antarctica

The main reason I went to Antarctica was that I managed to persuade Martha Kacs, my wife and the mother of our two daughters Paula and Karina, to marry me. It was the beginning of a long-standing, very solid, and loving relationship. I was fascinated by her and by her family. Martha was (and remains) an avid reader of excellent literature. She impressed me with her exquisite taste and brought many new things to my life. Her father, Boris Kacs, was from Latvia. He was a man of few words whose family was nearly exterminated during the Holocaust. It has been one of my most important accomplishments and moving experiences to have recently found the remaining members of his family in Israel. Her mother, Rosa Feldman, was one of the most wonderful people I have ever known. I became extremely close to Martha's parents and they accepted me without reservations. A Catholic–Jewish marriage was not common in the Argentina of the 1960s. There were barriers to break. I knew Martha was the person for me, but I was unable to offer her even the most minimal economic stability. I thought that if I joined the Antarctic Institute as a scientist for a year I could save enough money to secure a flat and start a life together. There was a major problem to be overcome: How could I apply my nascent scientific background to an Antarctic campaign? The pineal gland provided the answer. At that time Julius Axelrod and Richard Wurtman had published a series of fascinating papers on the pineal synthesis of melatonin and the circadian rhythm for HIOMT (hydroxy-indol-methyl-transferase). I reasoned that if pineal activity was circadian and the cycles were controlled by daily light regimes, then the pineal glands of animals in extreme latitudes should be influenced by the long dark–light seasons resulting in alternative 'circaannual' pineal rhythms. I presented my project to Otto Schneider, the scientific director of the Argentinean Antarctic Institute, who supported it enthusiastically and

allowed my late incorporation into the Antarctic campaign to become a member of the 'Admiral Guillermo Brown' Scientific Station.

Within a few months I left for Antarctica from Tierra del Fuego, crossing the Drake Passage aboard the aging navy cargo ship Bahia Aguirre (now lying in the depths of the ocean). My first encounter with Antarctica was near mystical. I was not prepared for it. I was deeply moved by the exceptional beauty of snowy hills that appeared to have a luminosity that can only be seen at extreme latitudes, and I was certainly not prepared for the absolute silence. Being out of the omnipresent noise and lighting of our cities and towns was a wonderful experience.

The research station was located at the Antarctic Peninsula, between two impressive glaciers. It was built on a huge rock dominating Paradise Bay. It is perhaps the most beautiful spot in Antarctica. I can still recreate in my mind moments when I would sit alone on the top of the mountain that shielded our station, immersed in the most overwhelming silence broken only by the cracking of the ice from neighboring glaciers. The sky was pure and most of the time the stars were visible with unique splendor.

There were 12 members stationed at the institute, nearly half of whom were scientists or technicians, the rest being support personnel. The labs were equipped to the highest standard and I had a clear idea of what to do. After the summer was over and we were cut off from the mainland, the only means of communication with the outside world was by radio. The members of the station became increasingly irritable and intolerant as the winter, with its long nights, progressed. There was barely an hour of light in midwinter. When I became aware of these changes, well before winter, I altered my work routine so that I would only see my colleagues during dinner. I slept during the day and worked at night despite protestations from the head of the station. I established a rigid disciplined routine for myself, and many ritual activities, performed with monastic precision. These included walks, laboratory work, reading classical literature, listening to music, writing, and playing bridge after dinner. As a result of my self-imposed isolation and discipline, I managed to avoid the conflicts that were becoming frequent occurrences in midwinter.

From that particular campaign only two of us ended up with research publications in international journals: Graeme Wilson, a guest from the British Antarctic Service (now chair of optometry in Birmingham, Alabama), and myself. I had managed to obtain pineal specimens from Weddell seals periodically and to study their cytology, searching for signs of annual variations. As I had predicted, the pinealocytes changed dramatically during the long Antarctic winter. The cells were loaded with lipid inclusions, which were gradually depleted as the light cycles became longer. In addition, the pineal gland of Antarctic Weddell seals turned out to be enormous (possibly the largest in the animal kingdom) and displayed a peculiar layered organization.

Antarctica was an enriching experience. I enjoyed fighting against the odds, capturing seals in impossible locations, walking in furious blizzards, and melting snow for our running water. Spring and summer came. The sea ice shell broke into large panels that collided among themselves during sea storms, creating the most curious ice sculptures. The glacier sea front broke into monumental icebergs that left the bay circling menacingly before us, just outside the large window of our sitting room, barely 20 meters from the water's edge. The summer crews came back. I boarded a small navy boat, the Yrigoyen, and we were caught in one of the worst storms witnessed in the straight. I finally arrived in Buenos Aires by plane, where I embraced Martha on the middle of the tarmac. It seemed unreal to be together again. We married within 3 weeks, just enough time to prepare the necessary documents. Before tying the knot, however, I serenaded Martha at her balcony with a Scottish bagpiper in full regalia, courtesy of my new friend Graeme Wilson, not a very common sight in central Buenos Aires.

## Back to Buenos Aires

On my return from Antarctica, I joined the Institute of Neurobiology, which was then directed by Juan Tramezzani. This institute was in the same building as the Institute of Experimental Medicine, which was still directed by 'Don Bernardo' (the physiology Nobel laureate Bernardo Houssay), and the Institute of Biochemical Research, led by the Nobel-to-be Federico Leloir. Don Bernardo was a fascinating man of incredible memory for science and history. He occasionally invited me for coffee to discuss my scientific progress. These were exciting and intimidating experiences. There I completed my studies of the Weddell seal pineal that were published in *General Comparative Endocrinology* (Cuello and Tramezzani, 1969). This work on the neurobiology of the Weddell seal has stood the test of time. It was commented on generously in the classic textbook, *Seals of the World*, by Judith King (Oxford University Press, 1983). Many years later, while I was working on neuropeptides at Oxford, my collaborator John Priestley was asked at a college dinner if I was the well-known 'seal specialist.' John found the question hilarious and told the college guest zoologist that there was no relation whatsoever— 'Cuello is a neuropharmacologist–neuroanatomist working in neuropeptides,' he affirmed. The following morning he related the story to the lab, most amused by the episode. It was difficult to convince him that the 'seal man' and his laboratory director were one and the same person.

My findings on seal pineal gave me a great deal of satisfaction. On the one hand, it was subsequently shown by Wurtman and collaborators that the pineal gland of arctic seals displays a circaannual rhythm for the synthesis of melatonin, reinforcing my original proposition, based on

histological and histochemical observations. On the other hand, these studies resulted in an invitation to present my results at the Second International Conference on Antarctic Biology held at the Scott Polar Institute (Cuello, 1970). It was during this, my first international scientific presentation, that I was shaken by the magnificence of Cambridge. To say that I fell in love with Cambridge University and the city is not an exaggeration. I promised myself that I would return there. I thought, and I still think, that Cambridge was magic. A year after my first European exposure, our first daughter, Paula Marcela, was born in Argentina. At this time, I was working at the Institute of Neurobiology, under the direction of Juan H. Tramezzani, where I embarked on some comparative neuroendocrinology work. There, I developed an experimental model to study the physiology of pineal in birds (Cuello *et al.*, 1971, 1972; Cardinali *et al.*, 1971) and I became interested in neuroendocrinology. I decided that it was time to acquire experience abroad and I was delighted when William Ganong accepted me into his lab in San Francisco.

## San Francisco

I left for San Francisco with an Argentine fellowship although I soon found out that I had obtained a National Institutes of Health (NIH) international fellowship (Fogarty Program). We moved to California in April of 1970 with Paula, now a 9-month-old baby. These were traumatic moments in our family. Martha, who was an only child, lost her mother months before departing for the United States and then her father soon after our arrival in San Francisco. It was a blow from which we never fully recovered.

We went to the United States with a dose of distrust of American culture. However, all our reservations and prejudices soon disappeared. California suited us. We liked the people and made many friends. The Ganongs (Fran and Ruth), in particular, were most supportive at all stages of our American experience. Fran Ganong was interested in the role of catecholamines in neuroendocrinology. At that time catecholamines were a 'big thing.' The technique of Falck and Hillarp had allowed the young Swedes Annica Dahlstrom, Kjell Fuxe, Urban Ungertedt, and Tomas Hökfelt to identify dopaminergic and noradrenergic neurons in defined CNS subsets. The challenge was to find a function for them. I was supposed to examine the role of the noradrenergic component in the hypothalamic control of ACTH secretion. For this, I was expected to combine morphology, physiology, and biochemistry. Umberto Scapagnini, who was moving back to Naples, gave me technical instruction on the fluorimetric measurement of catecholamines, as did Richard Weiner, who was also changing labs. I was given a small lab in the University of California at San Francisco (UCSF) Department of Biochemistry and another lab in

anatomy for the electron microscopical work, a space granted by Jack De Groot. My desk was in physiology, where I had my scientific discussions and social interactions. Among the physiology gang, besides Ganong, Mary Dallman had an important intellectual influence on me. In order to advance on the project it was essential to derive reliable information on tissue catecholamines. It was not easy to obtain accurate measurement of catecholamines. Fluorimetric techniques were erratic and they could easily render false fluorimetric signals with the good old Aminco Bowman. I became fanatical about water purity (I constructed an elaborate system for the production of tridistilled water) and also became obsessed with glassware cleanliness. Finally, data started to be generated. By using false catecholamine metabolites we were able to show that noradrenaline was implicated in the hypothalamic control of ACTH (Cuello *et al.*, 1973c). We also postulated that the noradrenergic input to the lateral hypothalamus could further exert a direct influence in the median eminence. In the late 1960s and early 1970s, Thoenen, Tranzer, Algeri, Bloom, and others demonstrated that a false transmitter, 6-hydroxydopamine, could provoke the specific degeneration of noradrenergic axons in both the peripheral and the CNS. By using 6-hydroxydopamine in conjunction with electron microscopy, we detected degenerating axons in the median eminence suggesting the existence of a noradrenaline innervation in the area of liberation of 'releasing factors' (Cuello *et al.*, 1974). There was now a need for direct biochemical evidence for the noradrenaline detected in the median eminence, but for that I would have to wait until Cambridge.

The work done at UCSF provided me with my first entry into competitive international research. Equally important, our experiences in San Francisco allowed us to understand North America and the privilege of freedom of expression. We realized that in the United States we could say just about anything we wanted and people would listen with interest or argue in a friendly manner. We were given credit for our intrinsic values and the opportunity to develop as individuals. It was a great liberating feeling. While we were experiencing this personal development, our Argentinean friends were experiencing yet another cycle of military dictatorship, and unfortunately some of our colleagues and heads of research institutes were courting the military in power.

During these happy years in San Francisco our second daughter, Karina Rosa, was born. When the NIH fellowship expired, there was still the possibility that I could spend a third year abroad supported by the CONICYT (Consejo Nacional de Investigaciones y Tecnias). I went to Chicago for a Federation Proceedings meeting to present my work on hypothalamic catecholamines and ACTH control. There I met Les Iversen, with whom I discussed the possibility of a postdoctoral stint in his lab. He reacted positively to my proposition and in that year (1972) Les took two postdoctoral fellows: Richard Zigmond from Rockefeller University and me from UCSF.

With Leslie Iversen at the Cambridge MRC Neurochemical Pharmacology Unit (c. 1978).

## Cambridge, First Period

My mission in Cambridge was to develop a sensitive assay to measure catecholamines in minute samples of the CNS. I warned Les that I was not a biochemist, to which he responded, 'Anyone who has been able to measure catecholamines with the fluorescent technique can be regarded as a biochemist.' Then, to stress my point, I added 'but I have never worked with enzymes,' to which Les responded in his best phlegmatic style, 'As Julius (Axelrod) would have said, the only thing you have to do with enzymes is to keep them cold.' I ran out of arguments and went back to my station to plan how I would attack the problem. Following that exchange, Les gave me a copy of a protocol to purify COMT (catechol-*O*-methyltransferase) from liver and some notes derived from past attempts made by R. Hiley to develop the technique. I was shown my bench space (measured with great precision) and desk to be shared with Richard ('Ziggy') Zigmond.

I had to optimize conditions for COMT incubation to render a good yield of *O*-methylated products to allow detection of minute amounts of catecholamines. The main difficulty was the efficient chromatographic separation of the enzymatic products such that the sensitivity and reproducibility of the assay would allow work with micrograms of tissue sample. I played with a multitude of chromatography systems, and I was

initiated into the tricky business of loading milliliter samples neatly in tiny spots in chromatographic paper and carefully aligning the paper in huge glass tanks saturated with the most toxic combinations of solvents. I was lucky in finding a good combination of conditions that were standardized. The procedure resulted in one of the first effective and highly sensitive radioenzymatic methods to assay dopamine and, later, all major catecholamines. We published the method as a short communication in the *Journal of Neurochemistry* (Cuello *et al.*, 1973a) and later a more detailed account of it, with improvements, in chapter form in *The Neurobiology of Dopamine* edited by the late Alan Horn (Cuello, 1979). Access to such procedures allowed us to obtain the first direct biochemical evidence for a noradrenergic input in the rat median eminence. This study was groundbreaking not only in providing fresh evidence for another neuroendocrine loop for catecholamines but also for opening up the possibility of obtaining biochemical data from minute CNS samples. An example of this was the first characterization of dopaminergic mechanisms (catecholamine content and uptake and adenylate cyclase stimulation) in the olfactory tuberculum and nucleus accumbens (Horn *et al.*, 1974).

I greatly enjoyed working on the bench with Les and having daily discussions with him. I admired his capacity for extracting the essential aspects of complex scientific issues and the elegance of his scientific writing. Our report on the biochemical and pharmacological characterization of noradrenergic input into the median eminence was published in *Nature* (Cuello *et al.*, 1973b) and it was generously quoted at the time. The noradrenaline pathway to the median eminence was even made into a popular cartoon that was distributed in the form of a laboratory poster. I think, in retrospect, that the historical relevance of this paper and others that followed was that we were, along with other labs in the United States and Sweden, opening the way for integrating biochemistry with pharmacology and neuroanatomy, a multidisciplinary modality that made what modern neuroscience is today.

I spent 11 glorious months in Cambridge, from where I managed to learn a great deal of science and to learn about British idiosyncracies. Regarding the latter, I remember my amazement when the members of the joint Lunch-Journal Club of the MRC Neurochemical Pharmacology Unit and the Cambridge Department of Pharmacology spent an entire session pondering whether to maintain the price of the sandwiches by lowering their quality or to maintain quality and ask for an extra 5 pennies. Some of the best minds of the country were at this session.

We made many friends in Cambridge, including Cesar and Celia Milstein and Virgilio and Clarita Lew. I met Cesar while I was carrying my rats from the temporary animal house outside the main building. I might have been saying something to the rats in my best Argentinean–Spanish, such as 'you better behave and produce good data,' when someone asked

me, 'Are you Argentinean?' It was the beginning of a marvelous friendship and, later, an equally happy collaboration. The gatherings with the Milsteins and the Lews were scientific, musical, culinary, or a combination of these, but mostly culinary. Those were the days when 'fat' was not a dirty word. We discussed books, life, and the oddities of Britain for which we all share a common deep appreciation. Our girls adored their newly discovered adoptive uncles and aunts, who became an important factor on our return to Britain. Cambridge grew on us in a manner and to an extent that we had not anticipated. At the end of our stay in Cambridge, I was invited to give lectures in Sweden, France, and Italy. I took my family along on these trips in an old Austin 1100 that was literally welded together in one piece after having been broken into two rusty halves. We finally returned to Argentina, leaving Europe from Genoa on the Italian cruise ship Christosforo Columbus. It was a slow, bucolic sailing trip, which softened the impact of our return to a harsh South American reality.

## Return to Argentina

The story of my return to Argentina has little to do with science but much to do with the struggle of a scientist to survive in a politically charged environment. We arrived in Buenos Aires in October 1973 in the middle of a chaotic return to democracy. The Peronists were elected after decades of being banned from electoral processes. The elected president was forced to resign to give way to the aging General Peron, who was invited to return to Argentina from his golden exile in Spain. The Peronist movement was a continuous spectrum from the extreme Left to the extreme Right. These diverse factions exerted their influence in a democratic and undemocratic fashion, and the university became a political battlefield.

In this environment I joined the Department of Biology, School of Pharmacy and Biochemistry, University of Buenos Aires, where I was soon made an assistant professor. However, I was a 'suspect' professor. I had been out of the country when everybody was taking visible political positions. My interest was in science, research, and excellence. People on the Right assumed that I was a 'crypto-Communist' and those from the Left that I was a Fascist. Reason was not high on the agenda. To reach my lab I had to negotiate political posters, banners, and pamphlets. It was not unusual to examine a student who would try to intimidate me by hinting that he was part of an armed underground organization (and therefore capable of violence). Most of the 'montoneros' (left wing activists) students viewed me as part of the establishment that they should eliminate. I also knew that I was not in favor with the right wing activists with paramilitary links. Soon after my arrival, I had the bad idea of saying in a newspaper interview that I was in favor of rational, scientific medicine and against 'magical thinking' and 'healers.' Someone close to the Intelligence

Services alerted me that the Minister of Interior, a well-known enthusiast of the esoteric, took it as a personal attack and, as a consequence, I was included on the black list of the paramilitary group under his control. In Argentina at that time, to be under the suspicion of the ominous 'triple A' (Argentine Anti-Communist Alliance) was not very comforting. It was the beginning of the 'political disappearances.'

My time in Argentina was spent teaching, organizing labs with the few resources available, writing grants, meeting with influential officials in the Science Administration, and simply surviving. I only did well in the latter. I could not do much science, despite the fact that Salomon Langer (of the $\alpha 2$ adrenoceptors fame), my next door neighbor in the Department of Biology, offered me bench space where I could perform radioenzymatic assays. I also set up high-resolution radioautography in my lab and I had access to De Robertis' electron microscopes in the Faculty of Medicine. However, by the time everything was in place, including enthusiastic and talented collaborators, I had to leave the country once again.

When I was still trying to remain in Argentina, establishing a decent research program, I was called by the secretary of science and technology, Julio Olivera, a brilliant economist and true gentleman, to act as a member of a multidisciplinary 'think tank.' This advisory board was given the mandate of conceiving new plans for the future scientific and technological development of the country. We worked on concepts and programs to allow a more inclusive, flexible, fairer system for the distribution of resources. The plan would have facilitated the exchange of personnel within institutions and strategic regroupings. Such an idea was obviously against the very feudal organization of science in Argentina and Latin America in general which was prevalent at the time. Having in mind the instability of the country, we asked to work without official appointments. It was a wise decision. I enjoyed those meetings in which we dreamt about launching a new 'open plan' for grants (with international scrutiny) and creating a system for the easy mobility of scientists across provinces and between diverse research units. Both initiatives would have counterbalanced the terrible territorial and political influence of groups within the CONICYT and the control of lives and careers for reasons other than scientific merit. However, the political situation deteriorated seriously. After the death of President Peron, the vice president, his wife Isabel ('Isabelita') Peron, took over. A few months later there was a furious political clash. Olivera, a nonpolitical technocrat, resigned when the new regime was installed. He phoned me before tendering his resignation. Soon afterwards the university was in turmoil. A bomb was placed in the house of Raúl Laguzzi, the Dean of the School of Pharmacy and Biochemistry. The explosion killed his newborn baby, whose body ended up in the elevator's shaft, several floors below. It was pure madness. I decided I could not risk my family and that I had no professional future in

Argentina. That very night, I went to my Olivetti and wrote nonstop approximately 30–40 letters to the friends and scientific acquaintances that I had made during my experience abroad.

I received encouraging responses from many countries, including France, Switzerland, and Australia. Firm offers came from the United States and Britain. I have to say a few words of gratitude here. Fran Ganong sent me a telegram offering me funds if I needed to leave the country in a hurry. Floyd Bloom invited me to join him in his new enterprise at Scripps and Geoffrey Burnstock offered me a temporary position at University College. The handwritten letter from Les Iversen came last. He offered me a 1-year contract at the MRC Neurochemical Pharmacology Unit. I could not believe my luck. While in Buenos Aires I dreamed about working in a proper academic environment. We quietly sold everything we owned because access to cash was an invitation to kidnappers, and I asked for a leave of absence from both the university and CONICYT.

The last months in Argentina were a nightmare. The political assassinations did not stop and the university was in turmoil. The new minister of education declared that 'research funds are lost in the dark of night' and that 'allocating funds to research would be the wrong path for the university to take.' A new university rector was appointed, whose first move was to fire many 'suspicious' professors, myself included. My consolation was that the Nobel Laureate Federico Leloir and other very distinguished colleagues suffered the same treatment. We were reinstated after a month of uncertainty. Police officers were also stationed inside the faculty buildings.

To better understand the climate of intolerance at the university, I quote public declarations made at the time. The government delegate (acting dean) of the Faculty of Medicine, referring to the newly imposed order, said that 'activities took place under the protective and liberating image of our blue and white flag, the spiritual presence of our President Isabelita (Peron) and the crucifix, symbol of the religious faith.' For his part, the rector of the university made equally illuminating remarks, such as 'the university is with the fatherland, with the Church of Christ, with the Army of San Martín and our glorious police' and 'the Argentinean thing is to be with those who die and kill for the fatherland' (I remember thinking at the time that it was a difficult thing to achieve if one dies first).

A few months before leaving for Cambridge, my wife was walking along Plaza San Martín listening to angelic Christmas carols when undercover policemen shot dead a man walking 2 meters in front of her. We were counting the minutes until we left. We finally quietly departed Argentina in March 1975 with the clear notion that it was a voyage of no return. In the interim period we lost 50% of our assets in a sudden devaluation of the peso. We had to start all over again.

## Cambridge, Second Period

We promptly reorganized our life in Cambridge and forgot the chaos we had left behind. The wisdom of our move was dramatically illustrated to us with the Argentine military coup of 1976. The Military Junta took control, directly or indirectly, of all scientific and educational institutions, and in April 1976 I received notification that I was fired as a career scientist from the CONICYT (I later received an apology from the democratically elected government in 1984). I guess that in the minds of the Military Junta I was leading a subversive organization from Cambridge, but if that was the case I was not aware of it. We realized that, had we remained in Argentina, I could have easily joined the list of 'disappeared ones.' We pledged not to return to Argentina until democracy was restored. However, in 1980, I did make a 1-week visit at the invitation of Fernando Orioli, my old professor of neuroanatomy, to give a plenary lecture on the newly discovered neuropeptides at a Pan American Congress of Neurology held in Buenos Aires. When I was questioned at the Central Department of Police concerning my passport renewal, for a moment, as I was led through sinister corridors, I thought I was not going to see my family again.

We focused on a British future. Our priorities were to establish normalcy in our professional and personal lives. The girls adapted very quickly to their formal schooling in Cambridge because they had attended an English school while in Buenos Aires. Martha taught Spanish for adults in the Cambridge Polytechnic and I was back to science in familiar settings. Besides the Iversens, we had the privilege of socializing with bright and interesting people such as Max and Giselle Perutz, William Feldberg, Martha Vogt, Edith Bulbring, Mary and Hans Blaschko—friendships that started in Cambridge and continued after our move to Oxford.

With Les, we were developing a new procedure to study GABA uptake in discrete CNS nuclei from microdissected fresh brain slices. We focused on the hypothalamus, in which we noticed, among other things, an important GABA incorporation in the nucleus suprachiasmaticus. We speculated that GABA mechanisms could be at play in circadian rhythms. At that time Ichiro Kanazawa (now head of neurology at Tokyo University) was doing a postdoctoral stint at the MRC NCPU. Ichiro knew the by then well-standardized GAD enzymatic technique. Therefore, we examined the hypothalamus of rats at noon and midnight. Obtaining brain samples in darkness created some complications. We completed a study that we never published showing a GABAergic circadian rhythm in the nucleus suprachiasmaticus. I regret that this piece of interesting data did not find its way to publication. The main reasons were that I became involved with dendritic storage of dopamine and, like many of us in Les' unit, we also became carried away with the possibility that substance P could be a peptide transmitter. I recovered some of this investment in GABA later in

a series of studies with my first Cambridge Ph.D. student, Erika Jaffe (work done largely at Oxford), in which she most elegantly demonstrated the uptake and depolarization-stimulated release of $^{3}$H-GABA from dendrites of the granule cells of the olfactory bulb (Jaffe and Cuello, 1980). It was the second demonstration of a dendritic mechanism in liberating neurotransmitters in a synaptic-like fashion, following that of dopamine in the substantia nigra.

My Cambridge project on GABA was interrupted when Laurie Geffen from Flinders University spent a sabbatical in our Cambridge unit. Les suggested that he join my lab, in which he witnessed some of our techniques and protocols that were cutting edge in neurochemical pharmacology. Laurie thought that we could test the idea of a possible involvement of dendrites in the release of transmitters. He convinced Tom Jessel and myself to attempt it and Les approved the project. We managed, for the first time, to microdissect the rat substantia nigra from fresh tissue slices in a viable manner for transmitter uptake–release experiments. We compared the characteristics of the uptake and fractional release of potassium-stimulated tritiated dopamine from the substantia nigra tissue slices with that of the striatum. We established that there was a calcium-dependent release of dopamine from the somatodendritic region of the substantia nigra that was comparable with, but not identical to, that of the dopamine-rich axonal terminal areas. We sent a communication to *Nature* that was positively reviewed (Geffen *et al.*, 1976). It was the first direct evidence that dendrites, besides axonal nerve terminals, could release neurotransmitters upon stimulation. The results made sense because they could explain many electrophysiological observations previously made by Bunney and Aghajanian in serotonergic nuclei and by Groves and Wilson in the nigrostriatal pathway supporting the concept of local self-inhibition of these cells. We postulated that 'the coordination of excitability of dopaminergic neurons within the substantia nigra could be mediated by a DA receptor located either on the DA neurons or presynaptically on the axon terminals innervating them.' This prediction proved to be correct with the posterior elucidation of the existence of two types of dopamine receptors (DA 1 and DA 2, with other receptors types to follow later) by Kebabian and Calne (1979) and the fact that cyclic nucleotide phosphodiesterase was localized in nerve terminals presynaptic to dopaminergic dendrites (Minneman and Cuello, 1979). We also postulated, based on the *in vivo* application of false transmitters, that the storage site of dopamine in dendritic shafts should be short cisterns of the smooth endoplasmic reticulum (Cuello and Iversen, 1978), a case that has only been recently confirmed by Pickel and collaborators, who revealed 'tubulovesicular' sites displaying immunoreactivity to vesicular monoamine transporter (Nirenberg *et al.*, 1996). In addition to our publications the theme of dendritic release of dopamine *in vivo* was extensively developed in France

by Glowinsky and collaborators (Cheramy *et al.*, 1981), who elegantly characterized the pharmacological control of this release mechanism. While in Oxford, I extended the concept of dendritic release to amino acids and peptides (Cuello, 1982a). The idea that dendrites store and release neurotransmitters is today a widely accepted concept in neuroscience and pharmacology because it explains a wide number of local endogenous transmitter actions and pharmacological responses.

The environment of the MRC NPCU was extremely stimulating. There were brilliant students, some of whom are most distinguished leaders in present-day neuroscience such as Tom Jessell and Richard Miller, and equally talented heads of groups, such as John Kelly and Alan Horn. There was a particular emphasis on developing or improving methodologies. This, no doubt, gave us an edge. The COMT radioenzymatic assay of catecholamines played a pivotal role in these studies. Some of the projects were made possible by perfecting the small chamber superfusion system developed by Tom Jessell for *in vitro* studies of minute CNS pieces. Tom also mastered the radioimmunoassay of newly discovered peptides at the highest possible level of sensitivity. Les was still at the bench and produced the finest biochemical data with ligand-binding techniques or analysis of cyclases. Finally, at that time I had just developed a method to rapidly microdissect tissue samples under the microscope following their natural myelin landmarks by transillumination in a cold chamber. This method was a modification of the procedure described earlier by Zigmond and Ben Ari (1976) in which we eliminated the aniline staining step. The modification obviated a time-consuming step that could potentially interfere with tissue assays. To identify CNS nuclei or layers, we used instead the natural myelin landmarks that clearly delineate the smallest nuclei under transillumination simply by the differential optical density of myelinated fibers. This modification permitted rapid sampling of practically any CNS nucleus following its exact natural limits. A detailed description of the method was later published with an Oxford D.Phil. candidate, Susan Carlson, along with a collection of fresh tissue micrographs and diagrams from representative levels of the rat CNS (Cuello and Carson, 1983).

While at Cambridge we had a visit from Masanori Otsuka from Tokyo, who gave us 'tutorials' on the arguments supporting a transmitter function for the peptide substance P. Substance P occupied an important place in the collective memory of pharmacologists during a good part of the past century. The 'P' stands, as the story goes, for the sequential letter written in tubes with extracts obtained from nerve tissue by Gaddum and Von Euler in the early 1930s. In the 1950s, Lembeck demonstrated that this 'factor' was enriched several-fold in sensory nerves when compared to motor nerves. This was an extremely relevant observation because Sir Henry Dale, in his 1935 Dixon lecture (inspired by the finding of Lewis and Marvin that sensory nerves release antidromically a histamine-like

substance), formulated that 'the same transmitter should be present in all processes of the sensory neuron (central and peripheral)' (Dale, 1935). This statement was later wrongly interpreted as the 'Dale principle,' meaning ' one neuron, one transmitter,' a point that I tried to clarify in a couple of reviews (Cuello, 1983a, 1987). Therefore, this as yet undefined compound was in the right place to be considered a sensory transmitter by being enriched in peripheral branches of sensory nerves.

A proteinaceous material could be extracted from a variety of tissues that had the properties attributed to substance P: vasodilation, lacrimation, and salivation. The identification in 1973 by Leeman, Powell, and collaborators of an undecapeptide as the 'mythical' substance P factor allowed for the first time the study of a new and well-characterized peptide as a transmitter candidate in the CNS and peripheral nervous system (PNS). Ichiro Kanazawa went to Powell's lab in Ireland and soon raised in Cambridge a highly sensitive anti-substance P antibody in guinea pigs. Soon after, Ichiro generated his excellent antiserum against substance P and I managed to visualize the peptide by immunofluorescence. Looking through the microscope for the first time, the shining green fluorescent fibers in the spinal cord were a wonderful sight. I was moved by the beauty of the microscopy images and by the fact that, after Tomas Hökfelt, we were the second laboratory in a position to reveal the actual cellular sites of these peptides that were, until that time, hypothetical. I wondered whether to attempt a systematic study of its distribution throughout the CNS. I hesitated because I thought Tomas Hökfelt would do it anyway. Les, again, prompted me to do it. He said, 'Tomas is not God, you know.' Well, we did it. It was the first comprehensive neuroanatomical mapping of a neuroactive peptide with transmitter-like characteristics. Other neuroactive peptides were soon to emerge. The paper illustrated the fundamental concept that these peptides were not being stored at random or in any cell but, instead, in defined neuronal systems and therefore had their own unique properties. The publication was delayed by the journal for more than 6 months because 'one of the reviewers had a skiing accident.' The paper should have had a tag for the Year 1977; instead, it was published in March 1978 (Cuello and Kanazawa, 1978). We were not amused.

We later demonstrated through subcellular fractionation studies that substance P immunoreactive material was highly enriched in the synaptic vesicular fraction from CNS tissue, a basic requirement for a putative transmitter substance (Cuello *et al.*, 1977b). With Rainer Gamse and Fred Lembeck, we also provided the first evidence for a somatofugal axonal transport of substance P, as would be expected of a peptide requiring synthesis in the rough endoplasmic reticulum of sensory ganglia somata (Gamse *et al.*, 1979). The Cambridge group had by then produced a good compilation of the early results on the distribution and release of substance P and its possible roles (Cuello *et al.*, 1977a). At the time, this

provided the most compelling argument to consider substance P and, by extension, 'neuroactive peptides' as transmitter candidates or modulators. Nearly a quarter of a century later, the discussion on the true nature of these peptides continues.

At this point, I again discuss Tomas Hökfelt. He was clearly a shining light in the peptide saga. His papers have been of extreme clarity, foresight, and precision. Besides his scientific qualities, Tomas is a superb colleague and a family friend whom one can always count on. In the late 1970s, Tomas had clearly demonstrated the presence of substance P in the dorsal horn of the spinal cord and in several peripheral tissues. He proposed that these fibers were of sensory origin. However, there was no direct demonstration that this was the case. We were able to establish experimentally the sensory origin of peripheral substance P branches in the skin and substantia gelatinosa. In order to achieve this, we produced stereotaxic lesions of the trigeminal ganglia and cut the mental nerve, a purely sensory branch of the trigeminal nucleus supplying the skin of the lip (Cuello *et al.*, 1978a). With Julia Polak and A. G. R. Pearse, we demonstrated that the substance P sensory system is well represented in the human spinal cord (Cuello *et al.*, 1976). Further compelling evidence for the involvement of substance P in nociceptive functions in humans was provided by us while at Oxford with the finding that the peptide was selectively absent in the substantia gelatinosa of the spinal cord from post-mortem samples of patients who suffered familial disautonomia (Riley-Day syndrome), a very debilitating condition accompanied by lack of pain perception (Pearson *et al.*, 1982).

While at Cambridge, John Kelly drew my attention to the fact that there was a compound studied by the Hungarian researchers Jancsó and Knyihar that depleted fluoride-resistant acid phosphatases from the dorsal horn, an enzyme with a distribution resembling that observed for substance P. I injected capsaicin (8-methyl-*N*-vanillyl–6-nonemide) following Jancsó's protocol to find that it rapidly depleted not only phosphatases, as expected, but also the striking substance P immunofluorescence. Tom Jessell promptly provided accurate quantitative evidence of the depletion by radioimmunoassay and we published the results in a much-quoted short communication in *Brain Research* (Jessell *et al.*, 1978b). This was the first demonstration that a drug could alter tissue levels of a neuroactive peptide. Capsaicin has consequently been shown to deplete many peptides and has become an excellent tool for the investigation of sensory neuropeptides. Capsaicin has even defined specific receptor sites in nociceptors and is today an over-the-counter topical medication to control arthritic pain, shingles, and diabetic neuropathy.

One of my claims to fame is that I pushed George Paxinos in the direction of neurochemical anatomy, a fact he graciously acknowledged in the preface of his popular *Atlas of the Rat Brain* (Paxinos and Watson, 1986).

George was an experimental psychologist visiting Cambridge. He took an interest in the emerging research on neuroactive peptides. He had developed a modification of the so-called Halasz stereotaxic microknife. George's knife allowed the controlled release and retraction of a cutting edge with maximal stereotaxic precision. The instrument permitted us to judiciously interrupt the smallest fiber tract without damaging main vessels. We provided a detailed description of the microknife and its applications years later in our first immunohistochemistry book (Cuello, 1983b). We found the stereotaxic deafferentation procedure most valuable in combination with the immunohistochemical and biochemical analysis of deafferentated areas, permitting us to define the actual CNS pathways containing substance P. For these studies, we assembled a team composed of George Paxinos, Tom Jessell, Piers Emson, and myself. Our discussion gatherings were held in the MRC cafeteria or on the grass at the Addenbrooks site. Many groundbreaking papers on the neurochemical neuroanatomy of peptidergic neurons were thus generated at Cambridge (Jessell *et al.*, 1978a; Cuello *et al.*, 1978b; Emson *et al.*, 1978; Paxinos *et al.*, 1978a,b).

Overlapping our substance P research emerged the saga of endogenous opioid peptides. The excitement came from the labs of Avram Goldstein and Sol Snyder in the United States and of Hans Kosterlitz in the United Kingdom. Since we were in the United Kingdom, we followed the saga of the discovery of the endogenous opioids mostly from Kosterlitz (who also introduced me to the best malt whisky). Publication of the Hughes and Kosterlitz paper in *Nature* (Hughes *et al.*, 1975) that reported the isolation of the enkephalines (EKs) was like an explosion. The subject was discussed with much enthusiasm in research labs and the media. One of my early micrographs of EK immunoreactivity was displayed at a show at the London Museum of Natural History. Also, John Kelly showed our micrographs (never published) on the remarkably developed enkephalinergic system in fish in a House of Lords Committee dealing with angling.

We thought that we could contribute to the scientific discourse by identifying enkephalinergic pathways. Thus, in collaboration with Marina del Fiacco, we established a close correspondence between the territories occupied by enkephalin (an antinociceptive peptide) fibers and those occupied by substance P (a pronociceptive peptide) fibers in the substantia gelatinosa of the trigeminal nucleus. We found that while substance P was dependent on sensory integrity, the EK-immunoreactive neurons were local circuit neurons. These studies, along with the data on opiate receptors from LaMotte, Simantov, Kuhar, and others, prompted Jessell and Iversen to investigate their functional interactions in the trigeminal nucleus (Jessel and Iversen, 1977). I also provided the first evidence of their presence in the human CNS in locations analogous to those of experimental animals (Cuello, 1978).

At the start of the endogenous opioid saga, the general concept was that enkephalins were released exclusively from CNS interneurons. The presence of enkephalinergic cell bodies in the caudate putamen and EK immunoreactive (IR) fibers in the globus pallidus led us to believe that both components belonged to the same neurons. To test this hypothesis, I asked George Paxinos to perform a series of knife cuts to partially disconnect the caudate putamen from the globus pallidus. This he did brilliantly, producing the first evidence that endogenous opioids could also act at a distance from cell bodies in classical long neuronal pathways (Cuello and Paxinos, 1978).

I never thought that I would leave Cambridge. The family was happy in this tranquil and charming town. Our house was located meters from the MRC and Pharmacology, off Nightingale Park. I enjoyed my job and our friends, with whom we used to have bucolic punting picnics on the river Cam. I had by then a moderate share of recognition and I was often invited to present papers at society meetings in continental Europe and British universities, including Oxford. At this time Oxford was about to launch a new joint lectureship between the Departments of Pharmacology and Human Anatomy. It should be explained that a British lectureship, until very recently, covered the equivalent range of positions from assistant to full professorship in North America (the only professor was the chair of the department). In 1977, David Smith (currently the chair of pharmacology at Oxford) visited us in Cambridge and encouraged me to apply for this newly created position. I consulted with Martha (who resisted the idea) and with Les Iversen and Cesar Milstein, who both agreed it was a good opportunity, although I had my doubts. I feared the prospect of teaching demanding English students. Not without worries, I finally put my name forward. I said 'Martha don't worry, I will never get it.' I told her exactly the same thing when I was later invited to visit McGill.

## My Oxford Time

There were nearly 100 applicants for the Oxford lectureship in neuroanatomy and neuropharmacology. To my surprise, the position was offered to me. The selection committee was a panel of 12 distinguished professors and lecturers. At one point I was asked whether I was planning to return to Argentina (meaning that the university would not like to invest in someone who would not last). I vividly remember the moment when I said, 'The Argentina of my expectations no longer exists.' It was painfully true. The Argentina that I had been educated for and dreamt about was gone for good. In 1977, the country was in the midst of a sordid secret war.

We moved from Cambridge to Oxford in July 1978, when, in addition to my university lectureship, I obtained a fellowship at Lincoln College. I was given the Edward P. Abraham Senior Research Fellowship. The fellowship

was named after 'Ted' Abraham, who isolated cephalosporin and who also participated in the historical penicillin saga. Ted left a generous endowment to Oxford University and Lincoln College. I came to know and respect Ted, who I continued to visit even after the Oxford years. Both Ted Abraham and Bill Paton (Professor Sir William D. M. Paton) became family friends. They both endorsed my application for British citizenship. Abraham and Paton have left an important mark in Britain and cherished memories in our family.

Our time at Oxford was a wonderful experience for my family and me. The only difficult moment was during the Falklands–Malvinas conflict. We had escaped military dictatorship only to endure in Britain the pains of conflict between two countries to which we felt deeply attached. The event was not easier for my daughters because children can be cruel and cannot differentiate people's personal backgrounds. All this being said, at the peak of the crisis we received more invitations to dinners than we could accommodate and wonderful letters reiterating friendship. The letters from Les Iversen and Norman Heatley (of the penicillin team) were most moving.

When I thought I had already learnt the unwritten codes of Cambridge, I had to confront a brand new set of unwritten rules at Oxford. The two universities had very different cultures. To learn their codes was a fascinating game, mainly at college: How to pass the port wine, who walks in front of whom, and other more substantive and yet elusive behaviors. I came to Pharmacology full of energy and demanded logistic changes on all fronts. In order to bring me down to reality and invite me to be more patient and diplomatic, Professor Paton gave me a book titled *Microcosmografia Academica, Being a Guide for the Young Academic Politician*. It was a 1909 satirical guide written by F. M. Comford for new Oxford dons aspiring to rapid advance in academia (ironically, the same book was also given to me by my Cambridge colleagues at the MRC unit on my farewell party). I got the message but I continued my campaign to modernize the two departments to which I was serving. It paid off but I had to work very hard because I was also teaching full courses. For the pharmacology course, I had to demonstrate the classic Dale's experiments in cats to reveal muscarinic and nicotinic (ganglionic) effects. In human anatomy, a very practical course in neuroanatomy was given under the leadership of Tom Powell. It included microscopy and the dissection of the human brain following a very ingenious textbook. Finally, I had to take care of my college students. I used to offer 6 hours per week of one-to-one tutorials at college, where I was provided with a large and well-decorated room overlooking Lincoln, Jesus, and Exeter Colleges. Hands-on research could only be done in between terms.

Life at Oxford was bursting with experiences and amazing college feasts. I loved my daily walks through historical stone buildings from my

Discussing hybridoma technology with Cesar Milstein in Oxford's woods (c. 1980).

labs in the two departments to college. Lincoln was, and still is, a small, friendly college. The Rector and his wife, the late Lord Trend and Lady Trend, received us warmly. The college was founded in 1427 and, having been for centuries a relatively poor college, it escaped the disastrous effects of nineteenth-century modernization observed in other richer Oxford colleges. The college has a great historic legacy. Past members include John Wesley, the founder of the Methodist Church; Lord Florey, of penicillin fame; and Mark Pattison, who made the Oxford academic reform thus defining the organization of the modern university and, in the process, also allowing college fellows to marry (a gesture that generated a housing boom in Oxford with the legitimization of preexistent relationships). The Senior Common Room offered a wonderful opportunity to enjoy discussions with fellows on subjects unrelated to medicine, such as history, philosophy, English, economy, and classics, an aspect that I believe is unfortunately missing in North American universities. I participated intensively in college life, although, I have to confess, I failed to gain a place in the exclusive college wine committee. However, I managed to introduce the Rioja wines to high table.

To my surprise, I enjoyed teaching in college. Indeed, it was a pleasure to teach and have discussions with some of the brightest British students. I witnessed the uneventful transition, after 500 years, from single male college to coed (women were quite human, after all). With Eric Sidebottom, I shared the tutorial education of nearly all the basic science curriculum of

our medical students. The Lincoln medics did exceedingly well in their final examinations despite the fact that during my time they had an extra dose of neuroscience. I was called 'Dracuello' because the label on my college room read 'Dr. A. C. Cuello.' I used to push them hard, for which they also called me 'slave driver' and probably worse. The fact is that we (Martha and I) enjoyed their company greatly and, I think, they enjoyed our eccentricity. As part of my duties, we often entertained them at home, a practice that is alien to the North American academic culture. I kept in contact with some of my 'medics' and five of them visited us in Montreal. When I left college the students presented me with a beautiful lithograph of Lincoln College and a pen engraved 'To the best tutor in the world.' It was the sweetest possible lie, which I chose to believe.

While in Oxford I continued the successful collaboration with Cesar Milstein that had commenced in Cambridge. We applied monoclonal antibodies for the first time in neuroscience research. With Cesar, I also developed some new principles in hybridoma technology. During that period, I was regarded in some circles as an immunologist and was even invited to write reviews in immunology publications (Milstein and Cuello, 1984; Kenigsberg and Cuello, 1987). This collaborative work started at the time of the actual 'birth' of monoclonal antibodies, with the publication of the classical *Nature* paper of Köhler and Milstein (1975). I was fascinated by the possibilities of the technique that resulted from my immunobased work on neuropeptides. It was at a time—when declaring my intentions of going monoclonal—that a distinguished colleague said, 'Claudio, whatever you do with monoclonals can be done by a rabbit.' However, history has shown that this is not quite correct. Cesar's Nobel recognition was a great moment. I was very pleased for him and for what it represented. It was a great privilege to share with Cesar, Celia, and the Milstein family the great excitement of the elaborate celebrations in Stockholm, and I was most gratified when Cesar referred to our collaboration in his Nobel address.

My collaboration with Cesar Milstein was an extension of a profound friendship that permeated (and still does) all aspects of our lives. It primarily consisted of long discussions while walking for hours in Cambridge, Oxford, or forests between the two places. We imagined all sort of theoretical scenarios for the generation of better immunological probes for the cellular and subcellular detection of tissue antigens. At the closing of those discussions we used to draw schemes that guided the experiments to follow. To this day, we both regret that we did not take proper note of them or enter them into a dated protocol book. Had we done so, we could have successfully defended the patents for bispecific monoclonal antibodies. This was a difficult lesson. Before publishing our *Nature* paper (Milstein and Cuello, 1984) that reported the generation of bispecific monoclonal antibodies, we initiated a U.S. patent application with MRC

endorsement. We soon learned that there was an interfering patent based solely on the theoretical principle. The fact that we had produced the first bifunctional monoclonal antibodies was of no value to secure intellectual property. In consequence, the first scientific demonstration of hybrid-hybridomas, as illustrated in our *Nature* paper, became merely a convenient 'proof of principle' in the eyes of the U.S. Patent Office.

I briefly describe our hybridoma work. With Cesar we provided the first applications of the new technology in neuroscience. We initially generated a monoclonal antibody against substance P illustrating that, against the popular credo, monoclonal antibodies could be used in radioimmunoassays (Cuello *et al.*, 1979). At that time it was thought that radioimmunoassays could only be done with the avidity offered by polyclonal antibodies. This particular antibody was very widely distributed and aided many fine studies in neuroscience throughout the world. With the emergence of monoclonal antibodies the concept of 'monospecific' antibodies became common currency. This idea implied that a monoclonal antibody would recognize a single epitope. However, with the generation of a monoclonal antibody against a small-molecular-weight compound (serotonin), Cesar and I demonstrated that there is a substantial difference in the antibody binding of haptens free in solution or fixed to proteins or presented in tissue preparations (Milstein *et al.*, 1983). A clear-cut example of 'intrinsic cross reactivity' of a single monoclonal antibody was thus presented. Because I was interested in the accurate quantification of immunoreactions and in revealing tissue antigenic sites with a single antibody, I experimented with radioactive antibodies. The standard procedure until then was the iodination of immunoglobulin fractions. We generated radioactive antibodies by incubating 'starving' hybridomas in culture media containing tritiated amino acids. Thus, monoclonal antibodies were made radioactive during biosynthesis and therefore applicable in one-step radiometric immunoassays or radioautography, thus avoiding troublesome cross reactivities derived from the application of developing antibodies or the denaturation of immunoglobulins resulting from iodination. We called them 'internally radiolabeled' monoclonal antibodies (Cuello *et al.*, 1982a). Ours and several other laboratories still apply this procedure when one-step antibody reaction is desirable for the identification of single or multiple sites (in combination with other techniques). A description of the protocol for the preparation of internally radiolabeled monoclonal antibodies has been incorporated in the popular Maniatis's *Manual of Molecular Biology Techniques* (Sambrook *et al.*, 1989) as well as in our immunohistochemistry books (Cuello, 1983b, 1993).

For many years, Cesar and I speculated on whether we could construct, biosynthetically, monoclonal antibodies in which each combining site could recognize a different epitope. As mentioned previously, in 1984 we finally managed to generate monoclonal antibodies capable of recognizing two

different epitopes at each of their combining sites by fusing lymphocytes from hyperimmunized rats with cells from an established hybridoma made to become HAT sensitive (as the original myeloma cell line). These antibodies were the result of a 'trioma.' We baptized these dual-binding capability antibodies as 'bispecific'(as they are called today) after brainstorming with the late Alan Williams, who at the time was the director of the MRC Cellular Immunology Unit at Oxford. The applications of bispecific antibodies obviously go beyond the field of neuroscience (Press *et al.*, 1995) in which they were initiated. Years later, Suresh managed to generate bispecific antibodies by fusing two established hybridoma cell lines, thus generating 'quadromas' (Suresh *et al.*, 1986a). This, our first quadroma, was secreting bispecific antibodies against substance P and antihorseradish peroxidase. A detailed report of practical and theoretical aspects of the hybrid-hybridoma technology was later published in *Methods in Enzymology* (Suresh *et al.*, 1986b). Other hybridomas, triomas, and quadromas followed at McGill (Kenigsberg and Cuello, 1987, 1990; Semenenko *et al.*, 1988; Kenigsberg *et al.*, 1990).

My work with monoclonal antibodies was my first exposure to biotechnology. Although my first and overriding interest was (and still is) pursuing scientific undertakings, I became keenly aware of the industrial possibilities of this technology. In 1978, I contacted a couple of financiers to propose the launching of a monoclonal-based company. I was told that there were no real industrial possibilities based on my initiative, and I did not persist. It should be said that monoclonal antibodies today generate business amounting to billions of dollars per year and that the first hybridoma-based company was created a decade after my initial push. However, I later founded an immunodiagnostic company in Montreal to which I initially merely acted as a consultant. Recently, the management could not handle a financial crisis and I was forced to take a more prominent role and became the chairman of the board of directors. To make a long story short, there was a happy ending. I managed to reorganize financing and operations and to protect the long-term employment prospects of many people. In the process I learned a great deal from lawyers, businessmen, distinguished scientists, and industrialists and, throughout the years, I enjoyed working with very special people such as Phil Gold, Mark Rosenstein, and Miguel Madanes. It is ironic that my first attempts to organize biotechnology initiatives in the early 1980s were viewed with suspicion from some important university quarters. Nearly two decades later most universities, including McGill, encourage scientists to go that route with the secret hope that it will cure the financial shortcomings of ever-contracting governmental institutional grants.

While at Oxford I continued studies on sensory peptidergic neurons. It was the first time in the neurosciences that we were able to study sensory

transmitter candidates. Our antibodies allowed us and others to dissect out the rich geography of central and peripheral peptidergic nerve terminations. Thus, with John Priestley (now professor and head of cell biology at St. Barts, London University), one of my Cambridge students, and consecutively D.Phil. graduate student and Beit Memorial postdoctoral in Oxford, we focused on the fine ultrastructural detail of enkephalinergic and substance P-ergic endings at their termination site in the dorsal horn of the trigeminal nucleus. Some of that work was done in collaboration with a dynamic Hungarian visiting scholar, Peter Somogyi (currently director of the Oxford MRC Neuroanatomical Pharmacology Unit), whose first experience in immunohistochemistry was with us. This interaction was of relevance because it contributed to clarifying the information regarding the modality of termination of presumptive sensory peptidergic fibers and the relationship with intrinsic enkephalinergic boutons (Priestley *et al.*, 1982; Priestley and Cuello, 1989). This last aspect was of particular importance because we thought that this relationship held the key to understanding the 'gate control' mechanisms modulating nociceptive information from incoming sensory fibers. Ronald Melzack (McGill) and Patrick Wall (UCL) postulated the gate control theory on the basis of electrophysiological studies. In Cambridge, Tom Jessell and Les Iversen had demonstrated in tissue slices that opiates inhibit the release of substance P from trigeminal nucleus tissue slices (Jessell and Iversen, 1977). This was a seminal paper in the sense that it gave the first indication of a functional interaction of the two peptidergic neuronal systems. It was interpreted that the 'sensory gating' took the form of a hypothetical axo-axonic synapse and was quickly portrayed as a fact in numerous textbooks. We deemed it essential to know the actual microanatomical substrate of these synaptic relationships. In order to do this we conducted ultrastructural investigations utilizing an internally radiolabeled substance P monoclonal antibody combined with conventional immunoenzyme procedures for enkephalin IR sites (Cuello *et al.*, 1979). The study revealed substance P and EK IR boutons establishing synapses in a common dendrite rather than in an axo-axonic configuration, suggesting that the dendrites of second-order neurons were ultimately responsible for enkephalinergic gating. This was in our view the primary synaptic gating. However, because the presence of opiate receptors in primary sensory fibers was undeniable, we also supposed that any enkephalinergic inhibitory action on incoming sensory axons should be of nonsynaptic nature (Cuello, 1983c). This particular synaptic relationship between substance P and enkephalin immunoreactive synaptic sites was thoroughly revisited at McGill with Alfredo Ribeiro da Silva *et al.* (1991), who elegantly and convincingly confirmed the axodendritic nature of these terminations by simultaneously applying internally radiolabeled and bispecific monoclonal antibodies.

Margaret Matthews and I worked together to establish the origin of substance P-containing fibers in the sensory ganglia. We used the guinea pig as a model in which Margaret, with unparalleled surgical skills, separately eliminated every possible input to the inferior mesenteric ganglia. In correlative light and electron microscopical studies, we provided direct evidence that peripheral branches of sensory neurons (i.e., their 'dendritic' ends) establish synaptic contacts with effector noradrenergic neurons in sympathetic ganglia (Matthews and Cuello, 1982, 1984). This was an unexpected finding because these processes were classically expected to terminate as free endings. The existence of such connections opened up the possibility of long-suspected sensory-autonomic visceral circuits without spinal cord participation. The concept had acceptance in autonomic physiology, and our scheme has been reproduced in Ganong's *Medical Physiology* (Lange). Our views on the organization of substance P pathways in the CNS and PNS were summarized in a CIBA symposium publication (Cuello *et al.*, 1982b).

I had two excellent Canadian collaborators in Oxford. One was Rejean Couture (currently professor of physiology at the Universite de Montreal), who took an interest in the trigemal sensory system model. He produced some of the most elegant demonstrations of the effects of antidromically released substance P from purely sensory branches of the mental nerve (Couture and Cuello, 1984). The other was Eric Pioro, who came to Oxford as a Rhodes Scholar. With Eric, we extended to the human species much of the knowledge gathered in experimental animals regarding the existence of substance P and enkephalin-containing neuronal pathways. Eric took advantage of postmortem material from specimens gathered by Trevor Hughes that displayed focalized CNS infarctions or other lesions. These preparations with such pathologies mimicked experimental electrolitic lesions (Pioro *et al.*, 1984a,b, 1985). This work was essentially Eric's thesis. We also later investigated neuropeptides in the human brain from specimens that had been archived for more than 50 years in the Vogt's collection (Brain Research Institute, Dusseldorf) (Mai *et al.*, 1986). The ensemble of observations in the human brain formed a solid reference chapter published in Paxinos' *The Human Nervous System* (Pioro *et al.*, 1990). Eric also joined me later at McGill, where he made the most comprehensive analysis of p75 low-affinity neurotrophin receptor sites in the rat CNS (Pioro and Cuello, 1990a,b).

During my Oxford time the 50th anniversary meeting of the British Pharmacological Society took place at our university. I proposed organizing a symposium on the emerging issue of colocalization of neurotransmitters that resulted largely from the emergence of neuroactive peptides. The symposium was a resounding success and included the participation of Tomas Hökfelt, Erminio Costa, Vicky Chan Palay, and Humberto Viveros, among others. I edited a book titled *Co-Transmission*, which emphasized

the functional aspect of the problem (Cuello, 1982b). It was probably the first time that the term 'cotransmission' entered into circulation. The meeting was closed with a memorable dinner at Lincoln College.

In Oxford I presented evidence for a dissociation, at the ultrastructural level, of the presence of peptide ligands (enkephalinergic synapses) and the location of sites known to possess the corresponding opiate receptors in the substantia gelatinosa of the trigeminal nucleus. I brought attention to this problem in a special issue of the *British Medical Bulletin* (Cuello, 1983c). We were confronted with an analogous situation at the cellular level in the striatum in which a high concentration of EK-containing terminations were found in the globus pallidus (Del Fiacco *et al.*, 1982), whereas the highest abundance of opiate receptors was known to be in the neostriatum. I emphasized this lack of correspondence between endogenous peptides and their cognate receptors, defining the phenomenon as a 'mismatch' in two reviews (Cuello, 1983c,d). The term mismatch caught on and it was widely applied to describe analogous situations and was also used to support so-called 'volume transmission.' I was pleased to see that in the scholarly review of the problem made by Herkenham, he acknowledges the origin of word and concept by stating, 'The term mismatch, as it applies to the lack of register between informational substances and their receptors, was first introduced into the literature by Cuello' (Herkenham, 1991).

The identity of the CNS cholinergic neurons remained largely speculative until the 1980s. There was strong evidence provided by Feldberg, Vogt, McIntosh, and others that it was a major central neurotransmitter. In Cambridge, Shute and Lewis, and also Krnejvic and others, attempted the application of acetylcholinesterase (AChE) histochemistry as a neuroanatomical tool. Butcher brought it to an even more refined art, combining histochemistry with the enzymatic inhibition of AChE by applying organophosphorous compounds (pharmacohistochemistry) allowing the detection of 'cholinergic' neuronal somata. However, the hard evidence had yet to come from the direct cellular visualization of the acetylcholine biosynthetic enzyme (choline acetyltransferase; ChAT). The excellent attempts at enzyme purification by Jean Rossier provided the hope of raising specific antibodies against the enzyme for direct immunohistochemical detection. A clear demonstration came from Thoenen's lab, where Felix Eckenstein developed the first monoclonal antibody against ChAT (Eckenstein and Thoenen, 1982). Levey, Wainer, Salvaterra, and others later developed equally good antibodies.

Fortunately, we had early access to Eckenstein's antibody. It was central to the excellent D.Phil. thesis written by Michael Sofroniew (now professor of anatomy and neurosciences at UCLA). With Michael, we produced some of the earliest descriptions of CNS cholinergic nuclei, including some brain stem pathways. In the 1970s Davies and Maloney (1976) and Bowen and collaborators (1976) produced strong evidence for a

rather selective loss of cholinergic markers in the cerebral cortex in postmortem brain of Alzheimer's patients. Mesulam and coworkers (1983) demonstrated that the bulk of the cortical cholinergic input originated from the so-called nucleus basalis magnocellularis (NBM) (of Meynert, in primates). 'Magnocellular' Nissl-stained neurons were reported by Whitehouse and collaborators (1982) to partially disappear in Alzheimer's disease. These observations, along with the extensive pharmacological and psychiatric evidence for a relationship between cholinergic function and loss of memory, were extended to generate the 'cholinergic hypothesis of Alzheimer's disease.' The hypothesis had some parallelism with the paradigm of dopamine deficits in Parkinson's disease in that the transmitter was central and probably primary to the pathology. Tom Powell and Carl Pearson (Pearson *et al.*, 1983) had demonstrated at Oxford that cortical lesions in the cat result in the 'disappearance' of Nissl-stained magnocellular cells of the NBM. Michael Sofroniew and Carl Pearson reinvestigated the issue by applying the ChAT monoclonal antibody in the rat. To our surprise, extensive cortical lesions did not eliminate ChAT IR cells but rather they developed a marked and long-lasting atrophy (Sofroniew *et al.*, 1983). We carried out a single-case Alzheimer's disease postmortem study in which we observed an analogous phenomenon (Pearson *et al.*, 1983). These studies raised the possibility that the cholinergic involvement was secondary to cortical lesions and not the other way around, a concept that is generally accepted today. We summarized our views on the organization of CNS cholinergic neurons—in which we included a note on the proposed secondary cholinergic involvement in Alzheimer's disease in a widely quoted *TINS* review (Cuello and Sofroniew, 1984).

One day in December, while entering the lobby of the Department of Anatomy during my routine commute from Pharmacology, I was told that the dean of the McGill Faculty of Medicine was on the phone and wished to talk to me. He plainly asked if I knew that the chair of pharmacology was available, to which I answered 'yes.' Then he directly and without hesitation asked me, 'Are you movable,' which provoked contained laughter in me. Accustomed, as I now was in Britain, to reading messages in between lines, this was a style for which I was not prepared.

## McGill

Dean Cruess sold me on McGill, at which William Osler and Ernest Rutherford had been faculty members. He was full of enthusiasm and love for his job and the faculty he represented. The move to Canada and McGill made sense. My experimental work in Oxford was split between three departments—pharmacology, anatomy, and later pathology for the hybridoma component. I had had to give up my aspiration of leading an MRC unit and I was given notice that there was no room for further

expansion of my labs or for acquiring my own tissue culture lab. The teaching, although enjoyable, was very taxing. I had never, until then, wanted to be head of an academic unit. However, I found the prospect of reorganizing an entire university department very exciting. My mandate was to reinvigorate the Department of Pharmacology and Therapeutics. The department had a long tradition. It is perhaps the oldest department of pharmacology in North America, having been initiated by Andrew Holmes in 1824 as *Materia Medica* with the Foundation of the McGill College. It had among recent chairs Mark Nickerson, who introduced the concept of 'spare receptors' and the first irreversible α-adrenoceptor antagonist. McGill also had great resonance for me because the names of Penfield, Milner, Sourkes, Melzack, Quastel, and Hebb were already part of the foundations of neuroscience.

I was given carte blanche to upgrade the physical space and to recruit promising scientists. We made important steps in improving the logistics of the department and creating new services. My 15 years as chair were eventful. I managed to bring to McGill many very gifted academics, such as Paul Albert, Guillermina Almazan, Paul Clarke, Yves De Koninck, Dusica Maysinger, Alfredo Ribeiro-da-Silva, Moshe Szyf, and Uri Saragovi, some of whom have already completed the cycle from assistant to full professor. While the total number of full-time staff remained stable or moderately decreased, the number of publications in journals of high impact rose steadily to nearly 100 papers per year, outside funding grew nearly fivefold, and the graduate student body increased from 18 to 60–70. The department is currently graduating nearly 5% of all the pharmacology Ph.D.s in North America. These accomplishments were possible thanks to the high quality of the preexisting and newly recruited professors. It was for me a happy experience. The department has gained great international visibility and it was central to the organization of the XII IUPHAR (International Union of Pharmacology) held in Montreal in 1994 (Cuello and Collier, 1995).

The McGill academic environment is open and cooperative, and there is no shortage of inspiring personalities. Some of them have left important marks on the development of modern pharmacology. During my mandate I organized endowments for prizes to recognize excellence in medical and graduate students. These new prizes honor the names of eminent McGill scientists who made major contributions to pharmacology, such as Mark Nickerson; Hank McIntosh, who introduced the notion of a choline high-affinity uptake mechanism in cholinergic synapses; Melville, who pioneered studies in the peripheral sympathetic system; and Theodore L. Sourkes, who made crucial discoveries leading to L-DOPA replacement therapy in Parkinson's disease.

The quality and continuity of my research work at McGill was assured by the initial technical assistance of Philip Tagari and recently by the

dedicated work of Adriana Ducatenzeiler and Sylvain Cote. While at McGill, I sustained my interest in CNS forebrain cholinergic neurons as a model for studies in neuronal degeneration and repair. We were initially interested in pharmacological approaches to rescue the atrophic neurons of the NBM. Our interest in nerve growth factor (NGF) resulted from discussions with Rita Levi Montalcini when she visited our Oxford lab before I left for McGill. Her collaborator, Luigi Aloe, spent much time learning the immunohistochemical techniques that he put into good use in later years. These interactions boosted my interest in the NGF story. At the same time, Adriana Consolazione, who did a postdoctoral stint in the lab working during our brief interlude with serotonin, had joined FIDIA Pharmaceuticals in Abbano Terme, Italy. She interested Guido Toffano, from FIDIA, in our research and I was provoked to try the sialoganglioside GM1 in our lesion model. I was initially skeptical about its potential value as a neuroreparative agent, as ganglioside literature was very muddled. However, there were good *in vitro* data and persuasive papers by Gorio and collaborators regarding the sprouting of motor neurons (Gorio *et al.*, 1980). Induced by the enthusiasm of Adriana Consolazione, we initiated some studies at Oxford on the effects of gangliosides, but the comprehensive studies were performed at McGill, initiated at the time of my move. Our work on the GM1-induced rescue of degenerating NBM following experimental cortical strokes was coincidental with the findings of Franz Hefti, Larry Williams, SilvioVaron, and, later, others on the dramatic effects of NGF in rescuing seemingly 'lost' cholinergic neurons of the medial septum (Hefti, 1986; Williams *et al.*, 1986). We initially used the parenteral route with large amounts of the ganglioside injected daily. The experiments revealed that the lesion-induced atrophy of ChAT IR neurons could be prevented with this treatment and that the levels of ChAT enzymatic activity could be restored in microdissected samples of the NBM region (Cuello *et al.*, 1986). I think that there was a great degree of skepticism concerning these results from my colleagues in the neuroscience field, which probably still remains. However, the fact is that gangliosides did work. With Robert Leeden, we showed that the sialic acid residue was essential for the trophic-like properties of GM1 (Cannella *et al.*, 1990). We repeated the experiments on reparative effects of gangliosides, comparing them with those of NGF, in the same experimental model of unilateral cortical stroke. We found that the final morphological and biochemical effects on the recovery of the cholinergic phenotype were similar but that the dose range required for gangliosides to mimic NGF effects was several orders of magnitude different (Cuello *et al.*, 1994). Furthermore, we found that gangliosides potentiated the *in vitro* effects of NGF on embryonic cell survival and also in the rescue of cholinergic neurons *in vivo* (Cuello *et al.*, 1989). This potentiation of NGF effects by gangliosides was confirmed by others, and the work of Italo Mocchetti (Rabin and Mocchetti, 1995) and

Lloyd Greene (Ferrari *et al.*, 1993, 1995) has provided a mechanistic rational to it by demonstrating that GM1 gangliosides promote the phosphorylation of TrkA molecules, a possibility that was put forward in one of our reviews (Cuello *et al.*, 1994). The ganglioside scene was drastically reduced by the collapse of FIDIA Pharmaceuticals. Unfortunately, this brought on the closure of the FIDIA Neuroscience Center at Georgetown University, where Erminio Costa, Alessandro Guidotti, Hari Manev, and many others were carrying out very exciting research, including the development of ganglioside derivatives. Before the closing of this period, I managed to write a review on the putative neuroprotective effects of gangliosides in *Advances in Pharmacology* (Cuello, 1990), but I believe that much of the glycosphyngolipid–ganglioside pharmacology has been left undone.

Our work with NGF revealed that this trophic factor could not only rescue atrophic cholinergic cell bodies but also provoke an upregulation of ChAT enzymatic activity in the remaining cortex after stroke-type lesions (Cuello *et al.*, 1989; Garofalo and Cuello, 1995). The importance of this upregulation in ChAT activity was that it provided the first evidence for a trophic factor-induced cholinergic presynaptic effect in the CNS. This was accompanied by a robust increase in high-affinity choline sites in cortical synaptosomes obtained from cortically lesioned, NGF-treated animals (Garofalo and Cuello, 1995). The changes could be attributed to changes in the turnover of acetylcholine, but we suspected that a synaptic reorganization could also occur. To investigate this we launched a very extensive and painstaking study counting ChAT varicosities in the various experimental situations, including cortical lesions and NGF treatment. During her thesis work, Lorella Garofalo found that the actual number of ChAT IR varicosities at the light microscopy level in lesioned cortices with NGF treatment was larger than that of control unlesioned cortical tissue (Garofalo *et al.*, 1992). Furthermore, at the electron microscopy level, she discovered that the actual number of cholinergic synaptic contacts increased twice over controls and that the size of the cholinergic presynaptic boutons increased as well (Garofalo *et al.*, 1992, 1993). These observations amounted to drug-induced *de novo* synaptogenesis in the mature and fully differentiated CNS of adult animals, in this case by a growth factor (NGF). How much of the actual number and pattern of synaptic contacts in the CNS of adult animals is regulated day to day by the endogenous release of neural growth factors? Thoenen and collaborators convincingly showed that growth factors are produced and released in an activity-dependent manner (Thoenen, 1995). The pattern of cortical synapses probably changes with experience since cortical maps do change with modifications to sensory input (Merzenich and Kaas, 1982), a phenomenon that in the auditory cortex is enabled by the basalis cholinergic projection (Kilgard and Merzenich, 1998). We have shown that the application of exogenous NGF can alter cortical patterns and that diverse

neurotrophins impact differentially the cortical termination of geniculate projections in the visual cortex (Cabelli *et al.*, 1995). Would the suppression of the normal, baseline supply of NGF have an impact on NGF-sensitive cholinergic synapses in the cerebral cortex? The investigation of this problem was made possible by the development by Saragovi and collaborators at McGill of small cyclic peptides mimicking loops of NGF and acting as competitive antagonists of NGF on TrkA receptors (LeSauteur *et al.*, 1995). By infusing small amounts of this peptide in the cerebral cortex for 2 weeks and examining the density of total presynaptic boutons with anti-synaptophysin antibodies and the density of cholinergic presynaptic boutons with anti-VAChT (vesicular acetylcholine transporter) antibodies, we observed a selective loss of preexisting cholinergic boutons in the animals treated with the TrkA receptor antagonist (Debeir *et al.*, 1998). This would indicate a trophic factor dependency in the maintenance of synapses in the mature CNS well after the developmental period. From these experiments we could also extrapolate that endogenous growth factors would selectively modulate synaptic numbers within diverse neurotransmitter systems according to their intrinsic trophic dependency. The trophic modulation of the steady-state number of CNS synapses in the adult could offer the microanatomical framework to the Hebbian concept that the strength of synaptic connections is conditional on use and that a 'growth process' takes place with improved synaptic efficacy (Hebb, 1949).

## My Current Work

Having completed my term as chair of the McGill Department of Pharmacology and Therapeutics, and taking on a research chair, I look forward to uninterrupted research for many years to come. I would now like to follow in the footsteps of some of my more distinguished teachers, such as Bernardo Houssay and Eduardo De Robertis, who continued at the bench until very late in life. I enjoy science today with the same enthusiasm and candor as when I was given my first opportunity at the Institute of Cell Biology (chair of histology) at the University of Buenos Aires. I have seen science being done with different emphases and styles in Argentina, the United States, Great Britain, and Canada. Funding programs come and go, sometimes in a very disruptive manner. The sudden changes in granting modalities remind me of the complaint Petronius made in Rome approximately 2000 years ago: 'We trained hard but it seemed that every time we were beginning to form teams we would be reorganized. I was to learn later in life that we tend to meet every situation in life by reorganizing, and a wonderful method it can be for creating the illusion of progress while producing confusion, inefficiency and demoralization.'

The stars of scientists also come and go. Some are rushing furiously for credit or power, at any cost; others do science simply because they like it.

I believe I belong to the second category. The drive lasts longer. I enjoy people and the friendships that come along with entertaining common projects. I have very long-lasting collaborations with Cesar Milstein and with Alfredo Ribeiro da Silva. In recent years I have been dreaming about generating a rat transgenic model to reproduce features of the Alzheimer's pathology, an adventure that is proving very costly and difficult to fund through regular granting channels. This effort, however, has brought the first product, a rat animal model with mutated APP and PS1 transgenes expressing intracellularly human A-beta fragments in cortical pyramidal neurons and also in the CA3 region of the hippocampus. To date, the rats do not display abnormal behavior but offer a unique opportunity to study the impact of intracellular amyloidogenic A-beta fragments in neuronal cellular biology. Other transgenic rat lines are in the making and we hope to contribute significantly to the quest of viable and valuable transgenic rat models to investigate aspects of, and potential therapies for, this most disabling disease. This enterprise is possible due to close collaboration with many friends, new and old: at the Virtanen Institute in Kuopio, Leena Alhonen and Juhani Janni; at the UN International Center for Genetic Engineering and Biotechnology in Trieste, Alberto ('Tito') Baralle and Andrés Muro; at the 'Severo Ochoa' Center for Molecular Biology in Madrid, Jesús Avila and Filip Lim; and at the Nathan Kline Institute in New York, Karen Duff. With Karen, we have also started collaborating on a phenotypic characterization of diverse transgenic mice models. The first product of this collaboration has been the demonstration that cortical amyloid burden results in synaptic remodeling of the cerebral cortex with selective vulnerability of the cholinergic system. This work indicates that a single genetic factor of Alzheimer's disease pathology is sufficient to affect a CNS transmitter system (Wong *et al.*, 1999).

## In Closing

I wish to say a few words about the countries in which I have lived. I miss the Argentina of my dreams, the Argentina of great writers and poets such as Borges, Sábato, or Cortazar; the fine humor of Landrú or Quino (who the Italians believe is theirs); the great music and theater of the 1960s; and the long discussions over the meaning of life in suburban coffee shops. I miss the many friends of my youth and overall I miss the loss of an Argentina in which there were real opportunities to create and contribute to a society without fear. I have much of the 'old' Argentina left in me. I miss Great Britain because of the incredible cultural wealth and richness of its traditions. I dare to think that I understand, at least partially, the British psyche. Every corner of Cambridge and Oxford has something to tell me and my family. We became British as a declaration of our commitment to Britain, and I am eternally grateful for the opportunity I was

given to start a scientific career in earnest and to fully participate in its institutions. I still feel a part of Lincoln College. Finally, there is Canada, my accidental country. I have in Canada a level of recognition and acceptance that I could not have had in my own country of birth. This made us convinced Canadians. Canada is the epitome of fair play. I have even made modest inroads in Quebec institutions. I truly enjoy my life in the charming district of Westmount and the rich scientific environment of McGill. Other countries are becoming significant for us, for different reasons, in particular Spain and Italy. We will continue dreaming and living in this wonderful cultural variety.

In closing, I acknowledge and thank my wife Martha for her uncompromising love and remarkable resilience to overcome difficulties. Her loyalty and dedication to the family have made possible whatever I could have accomplished in life. My daughters, Paula and Karina, and their husbands, Richard and Marcus, have also to be thanked for being such good friends and superb people who have reassured us, and many others, that the next generation will be better than ours. Whatever experimental success I have had I owe to excellent teachers, collaborators, and friends whose names appeared in various parts of this testimonial. They have made my life richer. I am eager to meet my future friends and colleagues with whom I will share further adventures in research.

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