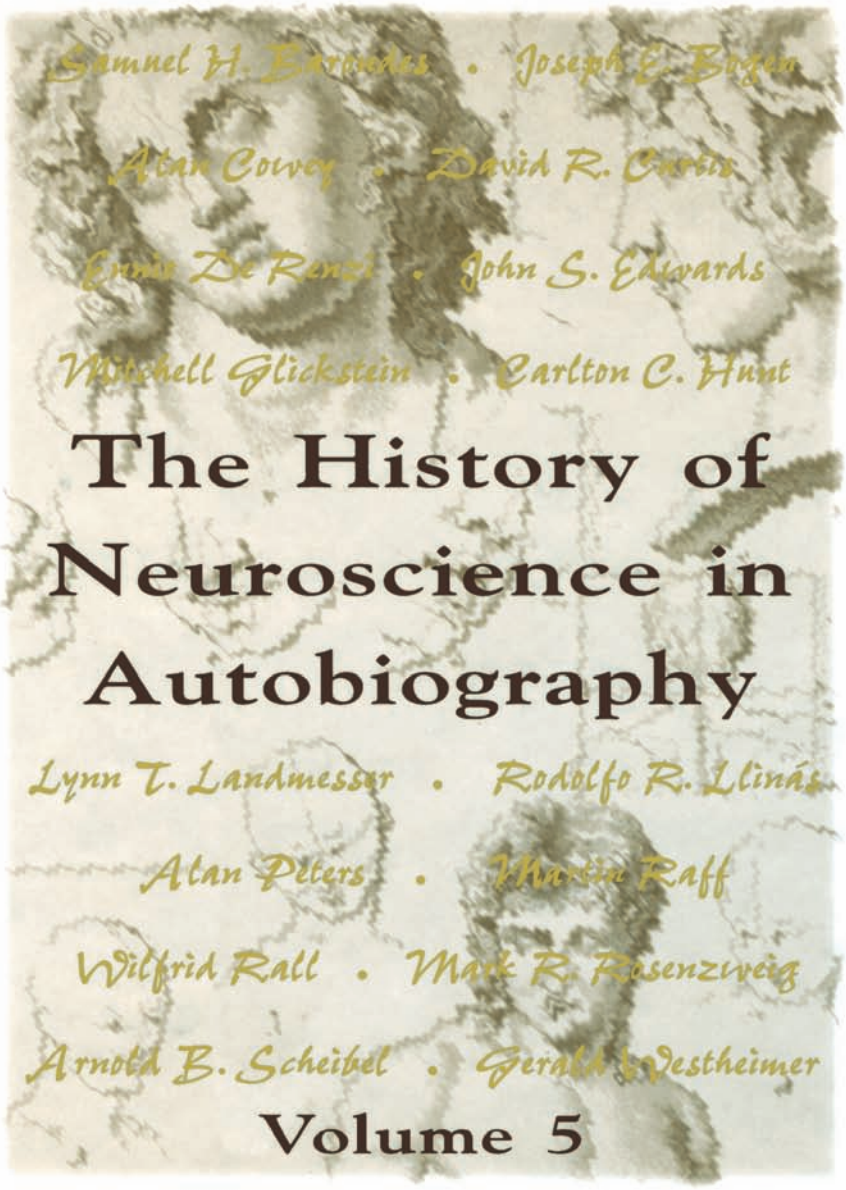

Edited by Larry R. Squire

The History of Neuroscience in Autobiography

VOLUME 5

Edited by Larry R. Squire

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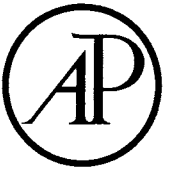

Elsevier Academic Press
30 Corporate Drive, Suite 400, Burlington, Massachusetts 01803, USA
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
84 Theobald's Road, London WC1X 8RR, UK

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Library of Congress Catalog Card Number: 2003 111249

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN 13: 978-0-12-370514-3
ISBN 10: 0-12-370514-2

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Printed in the United States of America
06 07 08 09 10 11 9 8 7 6 5 4 3 2 1

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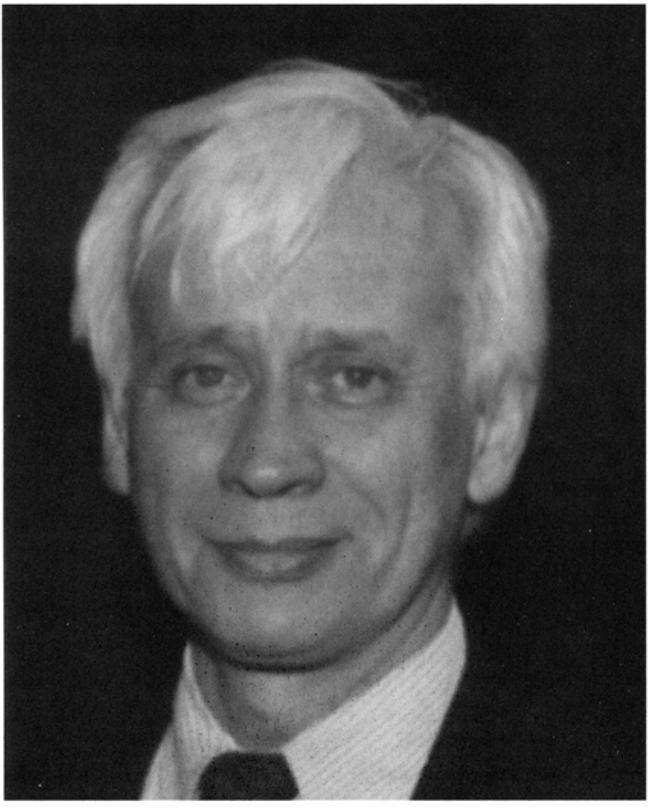

Rodolfo R. Llinás

BORN:

Bogota, Colombia
December 16, 1934

EDUCATION:

Universidad Javeriana (Bogota), Colombia, M.D. (1959)
Australian National University (Canberra), Ph.D. (1965)

APPOINTMENTS:

Head, Department Neurobiology, IBMR, AMA/ERF, Chicago (1968)
Head, Division of Neurobiology, University of Iowa Medical School (1970)
Chairman, Department of Physiology and Neuroscience, New York University School of Medicine, New York (1976–)

HONORS AND AWARDS (SELECTED):

Bowditch Lecturer, American Physiological Society (1973)
Professorial Lecturer, College de France, Paris (1979)
Lang Lecturer, Marine Biological Laboratory, Woods Hole, MA (1982)
McDowall Lecturer in Physiology, King's College, London (1984)
"Doctor Honoris Causa" (Medicine), University of Salamanca (1985)
National Academy of Sciences, USA (1986)
Ulfvon Euler Lecturer, Karolinska Institutet, Stockholm (1987)
F.O. Schmitt Lecture and Award in Neuroscience, Rockefeller University, New York (1989)
UNESCO Albert Einstein Gold Medal Award in Science (1991)
Signoret Award in Cognition, Fondation Ipsen La Salpatriere, Paris, France (1994)
American Academy of Arts and Sciences (1996)
American Philosophical Society (1996)
Real Academia Nacional de Medicina, Spain (1996)
French Academy of Sciences (2002)
Catedra Santiago Grisolia, Valencia (2004)
Neuroscience Prize, University of Zurich (2004)

Rodolfo Llinás has carried out detailed and elegant studies of the electrophysiology of the vertebrate cerebellum. In mammalian neurons, he discovered dendrite calcium spikes, dendritic inhibition, electronic coupling, and subthreshold oscillations. At the squid giant synapse he demonstrated presynaptic calcium current. He studied the electrophysiology of thalamocortical networks in brain slices and related his findings to his pioneering work with humans using magnetoencephalography (MEG). He has also drawn on his physiological findings to propose possible commonalities among certain neurological and psychiatric disorders.

Rodolfo R. Llinás

Bogotá, Colombia

Basic Schooling

The study of nervous system function has been my profession, as well as my passion, in life. I was born in December 1934 in Bogota, Colombia, a city high in the Andes (8,700 feet above sea level), to a family of physicians. My grandfather, father, and one uncle were professors at the National University Medical School. Much of my interest in medicine, and particularly in nervous system function, arose from family discussions and only later was this reinforced by studies at school and at the university.

My first serious "encounter" with the brain occurred very early in my life. When I was four years old, my paternal grandfather, a widower, suggested to my parents that I should spend a year with him. Grandfather, a professor of psychiatry, had his office at home. One day while playing on the second floor, I happened to look down into the glass–covered patio that served as the usually boring waiting room for my grandfather's patients. To my amazement one of the patients started moving in a strange way. He bent over, fell onto the ground, and began making jerking movements and producing very strange sounds. When Grandfather came up to lunch I was waiting to ask what had happened to the patient, and most particularly, I wanted to know why this individual behaved so strangely. He mentioned that the patient did not want to behave that way, but rather, something had happened to his brain so that he could not stop his body from acting like that.

This event completely blew me away. I could not understand how this person would do something he did not want to do. Then this statement came from Grandfather:

> "Not everything we do is under our control."
> And so I asked him, "Who is in control?"
> His answer was "It is controlled by the brain."

Further discussions on the subject convinced me that everything we did was brain-related, from talking, to hoping, to fearing, to every other event in which I was interested. And then I asked the question, "Please can you tell me how the brain works and how is it connected to me?"

> Grandfather answered, "Sorry, I don't know."

I explored such questions with many people for many years and found that it was basically considered a rather nerdy, even gross and unpopular subject for conversation.

The rest of my childhood was spent at home with my parents. Father was a loving, if strict, individual. A professor of thoracic surgery, he was willing to discuss almost anything as long as it was presented cogently. My youth was a bit complex as I was uninspired by religion, and so, while being socially accepted, there was the nagging problem of noncompliance, which bothered my peers and in particular my mother; my father, on the other hand, was willing to hear my arguments. I had the distinct suspicion that God was viewed as "a highly placed executive" who was mostly supposed to judge and do favors for people.

Education actually went ok, although I was far from a star pupil. Most scholastic subjects seemed boring and irrelevant to me. Nevertheless, my schooling was excellent, and I remember my teachers with gratitude. I was taught intellectual rigor at a leading school, the Gimnasio Modemo, where many of the teachers were European intellectuals and university professors who had reached Colombia before and during the Second World War.

At 17 I entered medical school (6 years) and decided to devote myself to the study of nervous system physiology, because that was what I had wanted to do since childhood. Before graduation I had the good fortune to visit R.W. Hess in Zurich. I had a copy of his book titled *Das Zwischenhirn* (difficult to read in a translation done by a German-speaking classmate), which included an interesting description of hypothalamic electrical stimulation and how it modified the behavior of animals. I went to Zurich to visit Hess's lab in 1954 during my second year in medical school and then again in 1956. Hess and his colleagues, Robert Hunsperger and Antonio Fernandez de Molina, the latter a professor of physiology at Madrid University and still a very dear friend and esteemed colleague, kindly offered me the possibility of participating in some of their experiments.

I was totally fascinated by the results: electrical stimulation could repeatedly activate complex stereotyped behavior, day after day. The behavioral pattern was related to the stimulus site. In attempting to reproduce such experiments back in Colombia, I understood that without a proper infrastructure animal research was impossible. Not even the cats cooperated! Cats in South America are not gentle like those in Switzerland; they are a lot more savvy. Interaction with the alley cats of Bogota, which were the ones provided by the university, was rather trying. So I opted for theoretical work for my doctoral thesis under the direction of Drs. Carlo Federici (a professor of physics and math at the National University) and Fernando Rosas (a professor of physiology). After 2 years of physics and math study, in parallel with the clinical years, I produced a thesis on circuit analysis of the visual system, using multivalued symbolic logic.

Boston

The Beginnings

With a medical degree in hand, I came to the United States to do neurosurgery at the prestigious Massachusetts General Hospital (MGH). It was clear to me that Boston would be a wonderful place to do research, especially since the MGH was near to both the Massachusetts Institute of Technology (MIT) and Harvard. MIT was particularly attractive as the external examiner for my thesis had been Warren McCulloch, whose work, in fact, had been the inspiration for my starting theoretical work. He had written important papers at the time concerning the possibility of modeling nerve net activity from a mathematical point of view. At MGH I was greeted by a very kind group of colleagues, from both psychiatry and neurosurgery. Frank Ervin, at the Stanley Cobb laboratory, offered me lab space and was extraordinarily supportive. There I met Teruo Okuma from Tokyo University and Janice Stevens from UCLA, and I published a paper on the effect of brainstem lesions on epilepsy.

After a short stay I moved to neurosurgery, but soon found that it was not what I had expected. Thus, my romantic view of surgeons dealing with human brains every day and having the time to understand better the function of the nervous system was replaced by a different reality. Neurosurgeons were intelligent, hardworking individuals addressing the brain as one would any other organ. They dealt with vascular problems, head trauma, and tumors and attempted to help patients in rather dire conditions. But for the most part, and rightly so, they were more interested in saving lives than in figuring out how the brain works.

Somewhat disillusioned, I decided to spend some time at MIT and I also, on several occasions, visited the newly appointed Professor of Neurobiology, Steven Kuffler, and members of his department at Harvard Medical School. At that time this very distinguished group, which included David Hubel, Torsten Wiesel, Edward Furshpan, David Potter, and Edward Kravitz, were basically interested in the visual system as well as in different aspects of synaptic transmission. At MIT I took some courses with Walter Rosenblith and had the good fortune to meet Jerry Lettvin, Humberto Maturana, and Walter Pitt, in addition to McCulloch.

Minneapolis

Dendritic Inhibition and Disfacilitation

However, my real interest had long been the electrophysiology of central neurons, from an intracellular perspective. I had read J.C. Eccles's book on motoneuronal physiology and thought that such techniques had to

be mastered if one was serious about understanding central nervous system (CNS) circuits. And so after a year and a half in Boston, I went to the University of Minnesota with a National Institute of Health (NIH) grant to work in motoneuronal electrophysiology with Carlo Terzuolo. Working in Carlo's lab was an incredible experience; he was a truly gifted electrophysiologist, having just finished the first voltage clamp study in mammalian motoneurons with Araki at UCLA. He presented me with the possibility of learning intracellular recording at the level that I had hoped.

Carlo was a force of nature. On my arrival, he pointed to a room and said "That is your lab. So, you want to study motoneurons? Fine, let's do some work on the effects of cerebellar stimulation on motoneuron excitability." He spent a day showing me the equipment, gave me the manuals concerning the instrumentation (mostly Tektronics), and proceeded to tell me that I was supposed to do five experiments a week by myself, which I did. I was basically left on my own; I would report to him once a week. I cannot overstate the importance of having had Carlo be as tough as nails concerning science and as hands-off as he was during this time.

The experience was superb; one had to know enough electronics to understand and modify the circuits used because many instruments were home grown or were directly adapted from electrical engineering (oscilloscopes and waveform and pulse generators). I heard comments such as "Rodolfo, don't even think of putting your finger on the glass cover, high-quality resistor in the capacity compensation circuit, OK? It is very expensive and we have only one!" and "Pull good electrodes. Get the animal from the cages left to right. Do laminectomies carefully. Don't damage the brain or spinal cord. Take detailed notes. Deal with the dark room-based photographic development of the intracellular CRT traces. And after the experiment put the cat in the freezer!" One felt a bit like Chaplin in *Modern Times,* but it was a fantastic experience nevertheless.

Woods Hole Interlude

Getting to Know You

During my stay with Carlo, I also had the good fortune to work at the Marine Biological Laboratory (MBL) at Woods Hole, Massachusetts, in the summer of 1962. I am presently writing this manuscript during my 37th summer at the MBL.

That particular summer was quite an experience for me. Harry Grundfest, a major force in electrophysiology at the time, invited Carlo, and I tagged along with him. At the MBL I had the pleasure of meeting many of my life-long colleagues: Mike Bennett, George Pappas, Hal Gainer, Kiyoshi Kusano, Clay Armstrong, John Dowling, Toshio Narahashi, and

John Moore among others, as well as very distinguished senior investigators such as Alan Hodgkin, Andrew Huxley, K.C. Cole, and Ichiji Tasaki. Also present at the MBL at that time was the Harvard group mentioned previously.

Carlo and I did intracellular experiments in lobster giant axon, attempting to create complex repetitive firing properties by manipulating the conductance of the extracellular space with an oil gap. It did not work. I also became acquainted with the squid giant synapse that Ted Bullock, Susumo Hagiwara, and Tasaki had introduced to neurophysiology a decade before. The "high" that followed the first MBL experience has stayed with me ever since.

Return to Minneapolis

Returning to Minneapolis, we continued our electrophysiological investigations of descending inputs to motoneurons, and the results had the immediacy one would wish for in other things in life. Proper recordings allowed incredible questions to be asked and, for once, one would see nature revealing "new findings" everyday. However, when I brought them to Carlo, he would quickly point out that the results had been published *in extenso* by others, and he would tell me, "Please go back to the lab." And so, after about a year and a half of work, we determined that cerebellar and reticular inhibition were different, one being a disfacilitation (Llinás, 1964) and the other a direct inhibition that occurred at somatic and at, contrary to the current dogma, the dendritic level (Llinás and Terzuolo, 1965).

At that time motoneuron electrical activity was considered to be driven by synaptic input to the soma. The dendritic input was basically disregarded because before Wilfred Rall's important modeling work, neurons were assumed to be a single isopotential compartment where all synaptic inputs could be considered equivalent electrically. Moreover, the soma was considered to be the only location for inhibition, being close to the axon hillock, where the action potentials were generated.

Thus, dendritic inhibition represented a rather different view of neuronal integration, and one that we felt was sufficiently important to be presented at the International Physiological Conference in Amsterdam in 1962. Armed with slides, Carlo and I went to the meeting, and I presented our new findings to a very distinguished group of electrophysiologists. Sitting in the audience in a very prominent site was John C. Eccles. After the presentation he immediately got up and mentioned that it was well known that inhibition would only be effective if located near the neuronal axon. After the session he called me aside and mentioned that maybe it would be a good idea if I joined him in Australia to study this in more detail because we were clearly wrong.

Canberra, Australia

The Cerebellar Cortex Electrophysiology

The Canberra years were a very exciting and important time in my scientific career. They began with a seemingly interminable flight from Minneapolis to Canberra. There was plenty of activity on the way, however, because some of my fellow travelers were Australians happy to be going back home, and I left a very lively group of people when we arrived in Sydney. The next leg of the trip was made in a small plane going into Canberra, the somewhat secluded capital of Australia. At that time the Australian National University was located on the outskirts of Canberra and consisted of two main parts: the School of General Studies and the Institute of Advanced Studies. Professor Eccles had his world-renowned laboratory at the John Curtin School of Medical Research, an important part of the Institute of Advanced Studies.

I found the physiology department on the second floor and discovered the professor in his rather modest office working on a manuscript. Also on the same floor were the laboratories headed by Rosamond Eccles, John Hubbard, and David Curtis, all of whom had graduate and postdoctoral students. This was a very vibrant group of people indeed.

Following instructions regarding where to place my luggage, I was directed to University House, a truly magnificent building, then more akin to a four-star hotel than to the student lodgings found everywhere else in the world. Once organized, I returned to the laboratory to discuss the immediate plans with Sir John. Facing each other and becoming rapidly acquainted, he mentioned quite directly his ideas about nervous system function and asked, among other questions, whether I believed in psychokinesis. This was, of course, a bit of a shock because I did not expect that anything like that would come up, and I was not at all prepared. I explained that I knew what the word was supposed to mean, but that I frankly did not understand the mind (a functional property of the brain) being able to move objects at a distance.

Sir John mentioned that psychokinesis was part of the mechanism that he had considered for the relation between mind and brain (Eccles, 1951). I mentioned that I was not religious and considered such views somewhat contrary to my understanding of the world and its workings. That being said, there was no adverse reaction that I could see, and we simply decided that I was going to be working directly with him on the cerebellum. This work would follow a set of electrophysiological studies begun in his lab with two postdoctoral students, Per Andersen and Paul Voorhoeve. Thus, a short time after arriving and shortly after Per Andersen returned to his home in Oslo, I became acquainted with the equipment and began experiments.

At about the same time, Dr. Kazuo Sasaki arrived from Kyoto and was also assigned by Eccles to study the cerebellum. Thus, for 2 or so years, Eccles, K. Sasaki, and I worked on trying to understand the physiology of the cerebellar cortex. Prior to our work, extracellular field potential recordings and some intracellular recordings by Andersen, Eccles, and Voorhoeve had demonstrated that the basket cell terminals onto the soma of Purkinje and hippocampal neurons were inhibitory, which further fueled their view of the somatic nature of inhibition.

A few months after my arrival in Canberra, Eccles was awarded the Nobel Prize for Physiology or Medicine in conjunction with Andrew Huxley and Alan Hodgkin. This was, of course, a wonderful event for the lab and a lovely party was put together, but work otherwise proceeded without much fanfare. A few months later, Eccles went to Stockholm to receive the prize and came back for a rather larger party that included most of the faculty of the John Curtin School of Medical Research.

The relationship between Eccles and his students had been and was at that time extraordinarily cordial. We would work three times a week on experiments that started just after breakfast. We would be ready to record in the early afternoon. We would break at dinner time, then return to the lab and generally, end a recording session somewhere between 11 PM and 1 AM, at which time I would return to the University House while Sasaki and Eccles would drive to their respective homes. Other than work, lab members also visited the Eccles' home occasionally on weekends, where they had garden parties, and played tennis on his private court.

Immediately following his Nobel Prize, Professor Eccles had to spend a rather significant part of his time traveling and fulfilling the many invitations and lectures that are *de rigueur* after such prizes are awarded. So, for most of 1964 Sasaki and I worked in his absence but under his very watchful eye.

By the middle of 1964 we had characterized most of the cerebellar circuit in the sense of having defined the excitatory or inhibitory nature of all synaptic interactions in the cerebellar cortex. The electrophysiological characteristics of the cerebellar cortex turned out to be very beautiful. The climbing fibers, one for every Purkinje cell, elicited an absolutely huge synaptic potential that was, as expected, all-or-none, being in fact a unitary potential (produced by the activation of a single input fiber). This, of course, was unheard of, as central synaptic transmission was viewed as being produced by the summation of many afferent fibers that were activated in unison. In fact, the response was so large that only after Sasaki and I managed to reverse it was Eccles convinced that it was a synaptic potential, rather than an aberrant spike (Eccles, Llinás, and Sasaki, 1964a, 1966a).

The mossy fiber-granule cell pathway, on the other hand, turned out to be an extraordinary subtle synaptic input because the number of parallel

fibers that a Purkinje cell receives is one of the largest in the brain. Thus, in human brains as many as 200,000 parallel fibers impinge on a single Purkinje cell. Cajal beautifully described the anatomy of this circuit in 1888. And indeed, as foretold by the anatomy, synaptic inputs occurred directly on the spines.

At this point the issue of dendritic inhibition came up once again. Most of the synaptic input onto Purkinje cell dendrites, other than parallel and climbing fiber afferents, were represented by synaptic input from stellate cells in the molecular layer of the cortex (Eccles, Llinás, and Sasaki, 1966a, 1966b). We had determined that a direct activation of the surface of the cerebellar cortex (a rather unphysiological stimulus but helpful in determining synaptic organization) evoked an early excitatory input generated by the parallel fiber-Purkinje cell synapse followed by disynaptic inhibitory postsynaptic potentials that were strongly dendritic as well as somatic, as, in fact, stellate cells largely outnumber basket interneurons. Thus, restricting our concept of inhibition to the soma was, scientifically, not a viable option. In addition, the results demonstrated that Golgi cells were also inhibitory and that their input was exclusively dendritic (Eccles, Llinás, and Sasaki, 1964b, 1966d), and so the question of dendritic inhibition had been addressed and confirmed. Over and above these questions the results demonstrated that all connectivities in the cerebellar cortex were inhibitory with the exception of the mossy fiber—the granular cell—parallel fiber system and the climbing fiber input.

This was, of course, a most exciting happening. It was the first region of the brain to be completely mapped from a physiological point of view. And so a set of seven papers to *Journal of Physiology* and *Journal of Experimental Brain Research* and two papers to *Nature* were written. This was easily one of the most productive times in my life. I had produced a thesis that afforded me a second doctorate, this time in physiology, and had become acquainted with a truly wonderful set of colleagues in Canberra.

Among the latter was Gillian Kimber, a student of philosophy who was finishing her Ph.D. on the philosophy of the mind. In fact, in addition to my being very interested in physiology many of the friends I had at that time were philosophers. And so it happened that Gillian and I, having similar views on the nature of mind, mine being physiological and hers being philosophical, became very close and decided to marry. It was a bit surprising for Professor Eccles because others of his students who had married Australian women had always married Catholics, he being himself a devout Catholic, as was his wife. Gillian, on the other hand, was a Protestant and student of J.J.C. Smart, the most outstanding materialist philosopher at that time. And so there was a deep contrast between this particular union and those of his former students and collaborators, such as Bernard Katz, Steven Kuffler, and Paul Fatt, who had all married Catholic women from the Eccles's social circle.

After close to 3 years, I finished my Ph.D. and wrote a thesis on the functioning of the cerebellar cortex. Spirits were, of course, extraordinarily high. The work was presented at the International Physiological Congress in Tokyo, and with the new results of Masao Ito, who had discovered that Purkinje cells were themselves inhibitory, the cerebellum loomed very large in neuroscience.

Return to Minneapolis

A Short Stop

I then returned to Minneapolis in 1965 to take up a position as Associate Professor of Physiology, following several pauses to present our work in Europe. The first was in Budapest, Hungary, at the kind invitation of János Szentagothai, and the second was in Goteburg, Sweden, where Lundberg, who had been studying the spinal cord input to the cerebellum, greeted the new results with genuine joy. Again, everything was coming to fruition with extraordinary speed and a great deal of excitement.

At this time we all had the feeling that having deciphered the neuronal connectivity of the cerebellar cortex, we would be in the position to understand global cerebellar function in short order. In hindsight I find it amazing that all of us, including Eccles, were so naive about the functioning of the nervous system. Indeed, 40 years later we still continue to address cerebellar function without an ultimate resolution. The fact was that shortly after my return to United States, I personally began to feel that our initial enthusiasm had pushed us to be overoptimistic. Clearly, understanding cerebellar function, without a context in which to frame our wonderful circuit, would be an impossible task. So I began to consider embarking on a comparative study of cerebellar function to see if a common denominator could be found that would put into context the electrophysiological details that we had addressed in the mammalian cerebellum. Again, from the work of Cajal it was evident that evolution had maintained, almost unchanged, the morphology of cerebellar neuronal circuits throughout vertebrates. The real issue at hand was whether the maintenance of an almost stereotyped neuronal circuit across vertebrates was actually paralleled by an equal invariance in electrophysiological detail.

At about that time a wonderful letter came from Eccles. He had decided to leave Australia and was planning to come to the United States to work at the American Medical Association in a brand new institute known as the AMA/ERF Biomedical Institute. He further wrote that he would be very happy if I could join him in his American venture as a colleague at the institute. Gillian and I had been married since the end of 1965 and felt that a move to Chicago could be very interesting and although we were moving out of a well-known university to a new institute, I felt that the

move would be more conducive to scientific work. Indeed, there were no teaching requirements, and all the time could be devoted to research. The idea was to have two main labs, one that Eccles would direct and a smaller lab that I would have to do my own research. The downside, of course, was leaving many friends and colleagues, especially Carlo Terzuolo, who had been such a wonderful friend.

Chicago

Comparative Cerebellar Physiology, Dendritic Spikes, and the Vestibular Cerebellum

As we were preparing to go to the AMA at the end of 1966, Gillian and I had our first son, Rafael, who is now a neurologist at Johns Hopkins University School of Medicine. Early life in Chicago was interesting and slightly complicated. Eccles was in Australia getting ready to move to the United States with much of his equipment, while I was helping to set up a new, complex lab, and Gillian was setting up a new household.

Before we left for Chicago, Eccles asked me to train a colleague from Czechoslovakia. Helena Taborikova was a physician who was going to join us in Chicago as a collaborator. Helena had been trained in human electrophysiology and was not acquainted with single cell research. And so I invited her to spend some time with me in Minneapolis, and then she moved to Chicago at about the same time as my family.

The time in Chicago, 1966 to 1970, was scientifically very productive but rather complicated from a human point of view. Although I was quite used to American universities and colleagues, Eccles was not, and so problems began to appear almost immediately after his arrival. Indeed, in Australia he was accustomed to a great deal of deference and respect from all who surrounded him, as befits an extraordinarily distinguished scientist who is recognized throughout the scientific world. The rather rough and ready approach of the scientific community in the United States, as well as the relation to students and technical staff, was less deferential and more confrontational than that with which he was familiar. Setting up the labs was marred by the usual delays that occur with construction efforts in a city, and so it took longer than expected to get the labs working. Meanwhile, and totally unbeknownst to all of us in the department, Eccles had decided to divorce his wife of more than 40 years and leave his family of 9 children in Australia. He had decided to marry Helena, with whom he had become acquainted before coming to the United States. Not knowing about this relationship, I considered Helena a student who was starting physiology and so I did not pay her the attention or show her the deference I would have had I known that she was not coming as a colleague but as the future Lady Eccles.

This early misunderstanding generated innumerable repercussions and complications that resulted in unintended, unfortunate, and sometimes hilarious human relation problems. So, in short, what had begun as an extraordinarily cordial relation with Eccles grew into a very complex and Byzantine interaction in which people from the two labs were not allowed to talk to each other. All this engendered a dysfunctional social environment.

From a research point of view, however, the work on comparative cerebellar circuit function proceeded to generate beautiful results. At that time I had several colleagues who came to work directly with me or had been assigned to my lab by Eccles during our initial departmental structuring effort. The first of the colleagues was Charles Nicholson, a particle physicist and a student of Donald McKay at Keele University who had decided to do electrophysiology of the cerebellum. Dean Hillman, an electron microscopist who had worked with Dr. Fox at Wayne State University in Detroit, followed shortly.

The group was completed by the addition of John Freeman, Wolfgang Precht, and Steve Kitai, and we began to do comparative cerebellar physiology and anatomy. Eccles's lab had a wonderful group of postdoctoral fellows that included Henri Korn, Piergiorgio Strata, Luciano Provini, and Daniel Sax, in addition to Helena.

In our lab the basic idea was to define cerebellar function from an evolutionary perspective. My reasoning was that looking at the "natural history" of cerebellar phylogeny should define the context in which the cerebellar system had adapted to the ever increasing complexity of the CNS, thereby giving us a clue as to the general nature of cerebellar control. Over the next 3 years we studied every type of vertebrate cerebellum we could get our hands on. That included frogs, fish (elasmobranches and teleosts), reptiles (mostly alligators and turtles), birds (pigeons), and of course, several mammalian species.

In a second line of inquiry, I wanted to study the problem of cerebellar control on a well-defined set of movements. Wolfgang Precht, who had studied the vestibular system, joined the lab to do a comparative study of the vestibular system and its relation to postural and eye movement control. The original idea was to develop a horizontal rotating table that would allow quantitative physiological stimulation of the semicircular canals. The steps taken were to record incoming activity by addressing first the vestibular nerve and then the resulting cerebellar activity generated by the vestibular nerve input, which we knew was direct through the mossy fibers as well as indirect through the vestibular nucleus.

The straight electrophysiological study of the frog cerebellar circuits, which I had begun in Minneapolis with my first graduate student, James Bloedel, was combined with the work Precht and I were doing. The organization of the frog cerebellum at both the field potential and intracellular levels served as the basis for the system approach concerning the vestibular

system. This set of studies demonstrated that, in fact, the organization of the frog cerebellum was quite similar to that of mammals, including the physiology of both the climbing and mossy afferent systems. More importantly, it showed that the protracted input from horizontal rotation activated Purkinje cells rather uniquely. Some of these cells responded with gradual tonic discharges, while others, seated next to each other, would respond with a rapid high-frequency short-lasting response (Precht, Llinás, and Clarke, 1971). This was not what one would expect from the results produced by direct parallel fiber stimulation!

Research in the other vertebrate forms basically confirmed that the cerebellar circuit is very much the same throughout evolution and that, although the connectivity of the cerebellum to the rest of the brain becomes more complicated in higher forms, the cerebellum, in principle, represents an almost universal solution from the point of view of circuit properties and dynamics.

It was during this phase of comparative cerebellar research that two very interesting findings were discovered in the lab. First, Purkinje cells turn out to be wonderful neurons in which to study integration. Indeed, a particular study in alligator Purkinje cells illustrated that dendrites were capable of generating action potentials. These massive spikes were conducted in a non-continuous fashion toward the soma. To me, the beauty of the system was that finally dendritic integration was clearly addressable,—but this time not as an appendage to the Purkinje cell soma but rather as the real integration site suggested by the complex connectivity. It was a place where excitation and inhibition could play dynamically on the geometry of neuronal structure.

In fact, it turned out to be the perfect place to understand exactly how it was that inhibition works by shunting excitatory currents. It was easy to show that dendritic inhibition could functionally inhibit dendritic activity without necessarily modifying somatic or axonal excitability. The demonstration of dendritic spikes, published in *Science* (Llinás et al., 1968), produced a real flurry of disagreement in the field with many people voicing objections. These included Calvin and Hellerstein (1969) and Zucker (1969), who wrote papers suggesting that our field potential analysis was defective, and the conclusions about dendritic spiking were wrong. This was actually quite interesting because it demonstrated to me that even good mathematicians can sometimes be misled by lack of direct access to experimental data. I, in fact, had written a book with Quastel and Hubbard (Hubbard, Llinás, and Quastel, 1969) on the electrophysiology of synaptic transmission in which we had treated field potentials in detail. Basically, the presence of dendritic spikes, which were recorded for the first time intradendritically by Charles Nicholson and myself, demonstrated that dendrites really spiked and were robust enough to be impaled, thus allowing the direct study of their electrophysiology (Llinás and Nicholson, 1971).

Moreover, such recording could be combined with good anatomy and with mathematical modeling based on actual data (Nicholson and Llinás, 1971).

At about this time a rather unexpected event occurred. John Eccles left the AMA, and I stayed with my colleagues as director of neuroscience. Also at this time we were taking some steps out of the cerebellum and into brainstem physiology with a new colleague, Robert Baker. With Bob, we studied the electrophysiology and pharmacology of ocular motoneurons and found the first example of electrical coupling in the mammalian CNS. In short, although science was progressing nicely, Eccles' departure on less-than-friendly terms left behind a rather sad wake. He had been a crucial person in my scientific career, and yet, the vicissitudes of life had made it impossible to continue my relationship with him. And so it was that we never spoke again.

About a year and a half after Eccles left, the AMA decided to terminate the Institute for Biomedical Research. This generated great distress because none of us had government research grants. George Beadle had replaced Roy Ritz as head of the Institute and had proposed to move it to the University of Chicago. This was not acceptable to the AMA, and so the decision was made that the Institute was to be closed 2 months after the initial announcement. This abrupt and unprecedented decision was challenged by the many members of the Institute and by the scientific community at large. Indeed, the lab directors decided to engage the AMA legally, and we ended settling out of court after some trying times. And so there it was, a lab of six people plus technical and clerical help looking for a place to go. We had been given all the scientific equipment and sufficient funds to survive for 1 year and to start a lab somewhere else.

In the midst of this turmoil, a most wonderful event that cheered Gillian and myself was the birth of our second son Alexander (presently an M.D./Ph.D. finishing his ophthalmology training at Stony Brook University Medical Center). A second event occurred just before the institute closed. As we were finishing our work on the comparative anatomy and physiology of the cerebellum, we hosted an international symposium on the evolution and development of the cerebellum. Most of the people working in the field came to Chicago. We had a truly spectacular meeting, and basically, most of the things that were known both from a comparative and a development perspective were reviewed and a voluminous book was published: This, in true AMA style, was beautifully produced (Llinás, 1969).

Iowa City

Calcium Spikes and Electrotonic Coupling in Vertebrate CNS

At that point, the issue was to find a place that would accept our group as it stood. We were all very much involved in cooperative research, and the

thought of separation after working productively together for 5 years was very painful. In the midst of looking for a position or a place, the University of Iowa offered a home to our group. So we rented some trucks and moved, in circus style, all our equipment, books, and furniture to Iowa City where we were given proper professorial status. This then concluded our Chicago years and began our years in Iowa City at the Oakdale Campus. Having bid against the university for the construction of a new building (our bid) versus the renovation of an old building (their bid), we prevailed, and the funds we had secured from the AMA were invested in a Butler Building (still up and working 35 years later) twice the size of the renovated proposal.

Iowa City, basically a university town, was quite a contrast to Chicago, and I found it confining. So within a few weeks of arriving, three of us decided to buy a small airplane and we all learned to fly so that we could visit Chicago for theater and music whenever possible. This turned out to be quite a delight. Flying a Piper Cherokee in Middle America is very, very lovely. People are very kind and there are plenty of small airports. The mobility this affords adds immensely to life. It was convenient and easy to run to the plane and then go give a lecture anywhere within a 500-mile radius. This was as far as one could go in 5 hours on one tank of fuel, and it included Chicago, Minneapolis, and the surrounding universities. All of my colleagues thought it was a bit of a riot that I should come in my airplane, give a lecture, and fly out again. Those were wonderful, happy times indeed. Our work on comparative cerebellar physiology continued, and my first graduate student during the Iowa City years received his Ph.D. This was the indefatigable Wise Young of spinal cord fame.

Several interesting scientific events occurred during those years. Having noted that the so-called climbing fiber reflex was very robust, and with almost no latency fluctuation, I began to consider the possibility that inferior olivary neurons were electrically coupled. A set of experiments with Bob Baker had indeed demonstrated a short latency depolarization intracellularly that had no delay with respect to the antidromic field and did not show an obvious equilibrium potential. I got on the phone and called Costantino Sotelo in Paris and suggested to him that he should look for gap junctions in the inferior olive (IO). A few weeks later Costantino called back very excited. The expected gap junctions were there: great deduction works! (Sotelo, Llinás, and Baker, 1974; Llinás, Baker, and Sotelo, 1974).

As part of our drive toward defining cerebellar control, Andras Pellionisz, a young Hungarian mathematician from Szentagothai's lab, and I decided that it would be very important to develop a general model of cerebellar function. The basic issue was to reconstruct the connectivity and electrical properties of the cerebellar cortex and attempt to reconstruct a network circuit that would consider the cerebellar cortex as representing movements expressed in intrinsic coordinate systems. Later on while in New York, Andras carne up with the wonderful idea

that perhaps a coordinate-independent type of vector transformation, as addressed by tensor analysis, could be a good approach to understand cerebellar circuit dynamics. Over the next 10 years we wrote papers on tensor analysis of neuronal networks. This was an original approach that promised the possibility of understanding the functional geometry of CNS activity (Pellionisz and Llinás, 1985). This work was not well received, although it provided the very useful concept of frame-independent vector transformations that is used these days in many labs.

Meanwhile, the work on Purkinje cells was progressing into what we called the "bird phase." Somewhat surprisingly, Purkinje cell dendritic spikes, particularly in alligators and pigeons, were very prominent, and their duration was longer than expected for a rapidly inactivating sodium conductance. This made me wonder whether such dendritic spikes could be supported by anything other than a sodium current—the then accepted dogma concerning the action potential properties of neurons. Well, tetrodotoxin did the trick: Direct activation of Purkinje cell dendrites could not be modified by this sodium channel blocker, but the fast spike in the soma was gone. In short, Rainer Hess, a young student from Germany, and I found that Purkinje cells were capable of generating calcium-dependent spikes (Llinás and Hess, 1976). We published this work in *PNAS,* and once again all kinds of problems arose concerning these novel findings. Indeed, one of the sad side issues concerning this finding was that a colleague and friend of mine, Rafael Lorente de Nó, whom I admired immensely, decided not to speak to me again. During a presentation of this work at UCLA, he got up from the first row and asked in front of a large audience, "Do you really believe that those are calcium spikes? Well, you are wrong." He stomped out of the hall in front of Susumo Hagiwara (Hagi), who had invited me to lecture and had found calcium spikes in invertebrate neurons. We were all stunned and open-mouthed. Later on, Lorente de Nó wrote me a strong letter saying that, "You have either lost your mind or your honesty." By now it was becoming clear that new findings always seem to come with a high price.

Woods Hole from Chicago and Iowa City

Presynaptic Voltage Clamp

In a parallel life, I had been doing research at the MBL in the summer months through both the Chicago and the Iowa City years. There my interests were focused on a totally different area—the physiology and biophysics of synaptic transmission in squid. Synaptic transmission can be studied directly in the squid simply because the very large (giant) size of the synapse allows intracellular recordings from both the pre- and postsynaptic terminals. Using this approach, in 1965 we had discovered that

synaptic transmission can occur in the absence of action potentials (Bloedel et al., 1966). Two other laboratories, that of Kiyoshi Kusano at the National Institute of Health (NIH) in Bethesda and Bernard Katz and Ricardo Miledi at University College London, were doing similar experiments in Woods Hole and Plymouth Labs, respectively. Quite independently we all found that indeed the secretory event resulting in a postsynaptic response does not require action potentials but only presynaptic depolarization. This was, of course, very intriguing because it clearly distinguished the electrophysiological properties of the action potential from those of its ultimate function—the release of transmitter. In 1967 Katz and Miledi demonstrated that in the presence of potassium blockers the calcium current could produce regenerative responses. This and other findings had made it clear that calcium could, in fact, generate action potentials if a sufficient calcium channel density was attained at the plasmalemma.

At about the same time as we were describing calcium spikes in central neurons, my work at the MBL with a graduate student, Kerry Walton, was centered on trying to voltage clamp the presynaptic terminal to determine directly the relation between calcium current and transmitter release. Charles Nicholson, John Blinks, and I had demonstrated calcium entry into the presynaptic terminals some years before using the photon emission protein aquorin, and Charles and I had demonstrated that the suppression potential was really at E_{Ca++} (Llinás and Nicholson, 1975). And so we knew that sufficient calcium flow could be observed if we could successfully clamp the preterminal axon. The voltage clamp experiments turned out satisfactorily. Walton and I observed a calcium current that did not inactivate and that reached equilibrium potential at the expected value given the calcium driving force. Moreover, the equilibrium potential had the same value as the suppression potential that Katz and Miledi had demonstrated some years before. In collaboration with a dear friend, Izchak Steinberg from the Weizmann Institute in Israel, we published the results together with a mathematical treatment of calcium current (Llinás, Steinberg, and Walton, 1976) using a variation of the famous Hodgkin Huxley equations. A more detailed paper followed in the *Biophysical Journal* (Llinás, Steinberg, and Walton, 1981a, 1981b), published after enormous effort and unsavory reviews. However, the findings were original and robust and have weathered well the ravages of time.

As these sets of studies were developing an invitation came from New York asking whether I would accept the chairmanship of the Department of Physiology and Biophysics at the New York University School of Medicine. This was a very attractive offer. Both Gillian and I had been city folk and living in Iowa City, although it was wonderful from certain points of view, could not hold us. And so in 1976, exactly 5½ years after reaching Iowa City, the whole group moved *en masse* to New York.

New York

Still at It

New York University School of Medicine was of course an extraordinary place. Very bright and unexpectedly friendly colleagues in every aspect of medicine populated the Medical Center. And the school had an excellent history in basic science research. A previous department chairman, Homer Smith, had been one of the luminaries of American Physiology. His immediate neighbors in the Department of Pharmacology were Severo Ochoa, a Nobel Prize winner in Physiology or Medicine concerning Molecular Biology, and Otto Levi, who also had a Nobel Prize in Physiology or Medicine for his discovery of acetylcholine as a myocardial modulator. Also present had been Eric Kandel, a colleague and friend from the MGH years who had moved with his group to Columbia University prior to our arrival. This was indeed the beginning of a very special episode in my scientific life. I remember David Sabatini, the chairman of Cell Biology, taking me for a long walk in the city, singing the praises of NYU in his incomparable manner. Very influential in my coming to New York were encouraging words by Dominic Purpura, a dear friend of many years, and at that time already the Dean at the Albert Einstein School of Medicine.

I have now been chairman of that department for close to 30 years (my longest time in a single place) and have seen neuroscience flourish at a level that would have seemed miraculous in 1976. I have also cherished my relationships with the students. I love to teach, as did my ancestors in another time and place.

Coming to New York was, of course, an exciting experience. The medical school was well organized, and we took to the two courses that we had to deliver like fish to water. Fortunately for us, the equipment that we had acquired from the AMA had been bequeathed to our group, and so we came to New York fully equipped. This included not only equipment for our machine shop, all our electronic equipment including an electron microscope, but also benches, etc. We also came with a program project grant from NIH, which we had attained in Iowa and which continues to be with us after 35 years. This grant has been the source of funding for most of the neuroscience research that we have done over the years.

Calcium Conductance in Purkinje cells, the P Type Channel

The move to NYU paralleled a shift in our approach to the electrophysiology of the cerebellum from an emphasis on *in vivo* work to one using *in vitro* preparations. This was begun with Mutsuyuki Sugimori ("Sugi"), one of the brightest persons I have ever met and my closest colleague over the last 30 years. The set of experiments we did from this time on concerned

the ionic mechanisms at the single cell level relating to the so-called intrinsic properties of neurons. Sugi and I published a detailed study on the electrophysiological properties of the soma and dendrites of Purkinje cells *in vitro* (Llinás and Sugimori, 1980a, 1980b). The results clinched the calcium nature of dendritic spikes very nicely, as well as the noncontinuous nature of spike conduction in dendrites. In addition, the results demonstrated a new type of sodium current that we named "non-inactivating" or "persistent," which was independently described by Wayne Crill at University of Washington in Seattle.

Further, studies on Purkinje cells *in vitro* using single channel recordings performed with a postdoctoral fellow, Maria Usowicz (Usowicz et al., 1992) from University College London, demonstrated that the channels were non-inactivating and had a distinct single channel conductance. Because they were discovered in Purkinje cells, we named them P channels. This P channel turned out to be an alpha one subunit channel whose open time is modulated by the delta subunit at a molecular level. The P channels were then described morphologically using an antibody generated by injection of a P channel protein into rabbits and indeed, demonstrated that the channel is present in the dendrites and up to the level of the spines in Purkinje cells.

We had found that funnel web spider poison blocks P channels and used that venom in one of its forms (a polyamine), isolated by Bruce Cherksey, as a synthetic blocker. We found that in its presence Purkinje cells lost their calcium electroresponsiveness (Llinás et al., 1992) (except for the T channel conductance that occurs when Purkinje cells are injured), as did the squid giant synapse under presynaptic voltage clamp. Two more recent steps have allowed us to pinpoint the physiology and distribution of the P channels. With David Tank and John Connors at Bell Labs, Sugi and I defined intradendritic calcium spikes, using calcium dependent dye imaging (Tank et al., 1988), and with Winfried Denk (Denk, Sugimori, and Llinás, 1995), one of the developers of two photon microscopy, we also defined, at the Bell Labs, the electrophysiological properties of single spines. These sets of experiments went on to characterize the repetitive firing properties of Purkinje cells, with our own version of the two photon system developed by Sugi (Sugimori and Llinás, 1990).

The Inferior Olive: T type Calcium Channels and Multiple Electrode Recording

This set of studies was followed in short order by a set of *in vitro* studies in the IO carried out with a then postdoctoral fellow, Yosi Yarom, from the Hebrew University in Jerusalem (today one of the pillars of Israeli neuroscience). Using a brainstem slice preparation, we discovered that

IO neurons were not just strange looking, they also behaved most unconventionally. Thus, they demonstrated a new type of calcium conductance that generated calcium-dependent spikes when the membrane potential was negative to rest! This calcium conductance showed voltage-dependent inactivation at potentials positive to −65 mV (Llinás and Yarom, 1981). We named these events "low threshold spikes." Today we now know that this calcium conductance is supported by at least two different types of T type calcium channels.

We also discovered that near resting level these neurons were capable of self-paced subthreshold oscillations (Llinás and Yarom, 1986). This intrinsic electrical property turned out to have a frequency of 8 to 10 Hz *in vivo* and 1 to 10 Hz *in vitro,* depending on the slice section through the IO, the temperature, and other tissue variables. As always, flack followed. In giving a lecture on subthreshold oscillations at Penn, my friend Brian Salzberg asked in a jocular tone, "Hey, Rodolfo, are you sure that is not 60 cycles?" Indeed, having something as weird as beautifully regular membrane potential oscillations continuously going on and not being either action potentials or synaptic transmission was pretty peculiar and difficult to stomach at the time. Since then, fortunately, many examples of subthreshold oscillations have been found in other neurons.

As this work was in progress, basically defining the electrophysiology of the elements in the IO and Purkinje cells, I began a related set of multiple electrode *in vivo* experiments that were designed to address the functional significance of the olivocerebellar system in a more physiological setting. The fact that IO neurons fired very slowly, were electrically coupled, and demonstrated subthreshold oscillatory activity indicated to me that the system operated in parallel, that is with IO activity probably organized in a time coherent manner, most probably as a pacemaker of sorts. Moreover, if this was the case, the only way to understand its organization would be to record from many Purkinje cells simultaneously and determine if synchronous complex spike activity could be detected.

These experiments used up to 90 individual electrodes that recorded from an equal number of individual Purkinje cells recorded extracellularly. The work in anesthetized rats was initiated with Jim Bower, who at that time was a post doc in my lab, continued with Kasuo Sasaki (Sasaki, Bower, and Llinás, 1989) (not related to the Sasaki of Canberra times), Masaji Fukuda, and Tomoya Yamamoto, and published years after the work was finished (Fukuda, Yamamoto, and Llinás, 2001). As it turned out, the electrophysiological properties of Purkinje cell complex spike activity related directly to the properties of the IO coherent electrical properties. We found that the neurons in the IO, being electrically coupled, generated well organized, and temporally coherent complex spike activity in Purkinje cells. The activation occurred in well-defined Purkinje activity bands oriented in a rostro-caudal direction over the cerebellar cortex. This work

was continued and very much enhanced by Izumi Sugihara, Eric Lang, then a graduate student, and John Welsh. I will get to that work in a more contextual vein momentarily.

Contextualizing Olivo-cerebellar System Function

The ultimate significance of the olivocerebellar system, although murky, does appear a little clearer when put together with the electrophysiology and the neurology of the cerebellar system. Indeed, Holmes working at Queens Square London during the First World War, had described a set of patients with cerebellar lesions that, in addition to ataxia, demonstrated a disappearance of the so-called physiological tremor described by Sherrington as having an approximate 10-Hz frequency. It became evident to me that this 10-Hz physiological tremor was probably generated at the IO and could provide the "time binding" properties (i.e., the ability to engage motoneuronal networks simultaneously along the neuraxis) that characterize active motricity.

In agreement with this conclusion was a pharmacological study using harmaline, an alkaloid known to enhance physiological tremor in animals and man. It is known as "yage" by the Amazonian Indians, who use it as a ritualistic hallucinogen. We knew from early experiments (Llinás and Volkind, 1973) that this drug enhanced climbing fiber activation of Purkinje cell activity that correlated well with the enhanced tremor. When applied *in vitro* to brain slices including the IO, harmaline increased the amplitude of T currents that support oscillations in these cells (Llinás and Yarom, 1986). This increased activity, in theory, would enhance physiological tremor. Harmaline tremor is coherent in time and indicates that under these conditions the IO acts as a single oscillator. A second important finding was that specific pharmacological lesions of the IO prevented harmaline tremor. Having understood something about the property of electrical coupling and the organization of the olivocerebellar system, the question arose: How could a coupled IO be modulated such that it would not behave as a single oscillator? I had proposed in 1974 the possibility that inhibitory terminals that contact the IO glomerulus could work to momentarily decouple neurons of the IO by shunting trans-dendritic currents (Llinás, 1974). A similar inhibitory base decoupling had been described by Spira and Bennett (1972) in Navanax, probably subserved by a different mechanism because invertebrate neurons have no dendrites.

That such a mechanism is in fact present at the olive was demonstrated in collaboration with Eric Lang. He showed that damage to the cerebellar nuclei produced an immediate coherence of olivary neurons, resulting in coactivation of complex spikes throughout the cerebellar cortex (Lang, Sugihara, and Llinás, 1996). Years before, Nelson and Mugnaini (1989) had discovered that a proportion of the cerebellar nuclei cells actually

returned to the IO and that such neurons were GABAergic, and Sotelo et al. (1986) had found that the GABA terminals were part of the IO glomerulus, where we had initially described the location of the gap junctions. Indeed, these three findings had demonstrated that the cerebellum does control the coupling at the IO, and so it can dynamically specify the distribution of coherence at the IO. This was very satisfactory.

This timing view, of course, is in total disagreement with the very popular view that the climbing fiber system had as its ultimate function the modification of parallel fiber Purkinje cell synapses and so serves as the biological basis for motor memory. Once again, as on past occasions, our finding became a source of heated discussions and disagreements. For the timing hypothesis to be correct, the olivocerebellar system had to produce coherent activation of Purkinje cells regardless of their location in the cerebellar cortex. Because the cerebellar cortex is folded, the issue of timing as produced by the conduction time of the climbing fiber action potentials became a critical issue. I remember considering the possibility that the conduction velocity for climbing fibers could be tweaked such that the input to the Purkinje cells would be isochronous regardless of position in the cerebellar cortex. In fact, experiments by Sugihara, Lang, and myself had shown that the conduction time for Purkinje cell activation was roughly 4 milliseconds plus or minus half a millisecond for any region of the cerebellar cortex (Sugihara, Lang, and Llinás, 1993). Indeed, it turned out, as expected, that the conduction velocity of the climbing fibers was indeed related to the length of the pathway between the IO and Purkinje cells. This isochronicity of the olivocerebellar system has been observed in reptile cerebella as well (Ariel, 2005).

Such research was further elaborated in our lab with John Welsh, who came to NYU as a post doc to do behavioral work with the goal of correlating volitional movements with multiple recorded Purkinje cell activity in nonanesthetized rats! The paradigm called for correlating the timing of complex spike activation to tongue movements, which actually worked very nicely (Welsh et al., 1995). Furthermore, our colleague Chris DeZeeuw and his team in the Netherlands had demonstrated that fully half of the cerebellar nuclear cells are GABAergic and that they return to the IO. This indicates the functional importance of this connectivity, given that the half of the total cerebellar output is used to regulate the electrical coupling in the IO. Also with Chris, we found that climbing fiber synchrony actually occurred bilaterally in the cerebellum (DeZeeuw et al., 1996; Leznik, Makarenko, and Llinás, 2002).

An aspect that was missing related to the actual direct demonstration of the existence of clusters of coupled neurons in the IO. This was shown by a graduate student, Elena Leznik, and myself using voltage dependent dye imaging (Leznik, Makarenko, and Llinás, 2002), and their absence was demonstrated with electrical coupling block (Leznik and Llinás, 2005).

Of course, the issue remains whether this olivo-cerebellar arrangement can actually control motor coordination. A couple of significant findings were discovered by Vladimir Makarenko. The first was the demonstration that the oscillations in the 10 neuron are chaotic (Makarenko and Llinás, 1998), with an intriguing phase reset property. That is a robust oscillation that is also nimble, as opposed to the rather dull periodic oscillator. That work proceeded with two colleagues from Russia, Victor Kasantsev and Vladimir Nekorkin, with the development of microchips that simulated IO neurons and the construction of motor controllers (Kazantsev et al., 2003). In short then, one message that arises from this research is that motricity is really a discontinuous function of time (Llinás, 1991). And it makes sense; discontinuous control is simpler and more economical from a computational perspective than continuous control.

At present we have been studying cerebellar long term depression (LTD) and motor learning. We have found that the T-588 neuroprotective drug developed by Toyama Pharmaceuticals can block LTD produced by the temporal coherent activation of climbing and parallel fiber activity on the Purkinje cells. This LTD has been considered to be the mechanism responsible for cerebellar learning. T-588 was found to prevent LTD by acting on the necrotic pathway leading to cell death. And so the possibility arose that LTD was not a learning mechanism, but rather a cell protection mechanism. Indeed, its oral or parenchymal administration to rodents had no effect on either motor or eye blink conditioning but blocked LTD quite completely. So at present there is strong evidence that LTD may, in fact, not be related to learning of motor behavior.

Woods Hole from New York

The Molecular Aspects of Synaptic Release and the Calcium Concentration Microdomain

Concerning research at the MBL after moving to New York, the trip certainly became less laborious than our trips from Iowa City. Over the years the squid giant synapse has continued to afford good experimental food for thought concerning synaptic release. That being the case, Sugi and I have continued to go to the MBL from New York for the last 30 years.

After the voltage clamp experiments, I had decided to study synaptic latency and described with my student, Sandy Simon, and Sugi a method to voltage clamp the preterminal with a spike wave form profile (rather than a step function pulse) and determine the actual characteristics of the calcium current that causes synaptic release following an action potential (Llinás, Sugimori, and Simon, 1982). That worked well and gave us the first direct demonstration of the real calcium current time course and amplitude following a spike potential. Sandy and I also published a biophysics paper

concerning the possible distribution and size of the intracellular calcium concentration profiles at the active zone responsible for transmitter release. The concentration turned out to be rather large (in the hundreds of μM), given the high concentration of this ion in sea water (Simon and Llinás, 1985). Of course, I remember discussing with one of my colleagues in Woods Hole that it was interesting but it would never could be experimentally demonstrated, given the small size of the event. Well, as it happened, we thought of a way using a special low affinity aequorin (a protein that emits light in the presence of calcium) kindly provided to us by Dr. Shimomura at the MBL. The paper demonstrating the existence of such calcium concentration microdomains by using a single photon imaging system was published by Sugi and I in collaboration with Bob Silver (Llinás, Sugimori, and Silver, 1992).

From a molecular perspective our interest in the release problem led Sugi, John Gruner, Jen Wei Lin, and me to work in collaboration with Paul Greengard and his student Teresa McGuinness (Lin et al., 1990; Llinás et al., 1991) on synapsin I, a protein discovered in Paul's lab. This study demonstrated that synapsin I holds synaptic vesicles next to the active zone, and when phosphorylated, it untethers the vesicles and makes them accessible for release.

A second set of studies addressed the calcium-sensing protein synaptotagmin that had been characterized by Thomas Sudhof as the calcium sensor for synaptic transmission. With antibodies against this protein made for us by our colleagues Katsuhiko Mikoshiba and Mitsunori Fukuda, we (Sugi and I) showed that it too blocked synaptic release by preventing vesicular fusion. Indeed, the ultrastructure implemented by our friend and colleague Jorge Moreira demonstrated that the vesicles were kept in the active zone, because in the absence of this calcium sensor release does not take place (Mikoshiba et al., 1995; Sugimori et al., 1998). Other aspects of molecular control of synaptic release were done with such friends as Scott Brady and his colleagues Jordi Marsal, Chi Kun Tong, Herman Moreno, Bernardo Rudy, and Joseph Schlessinger.

This molecular approach has been one of the most rewarding aspects of my work in synaptic transmission and one that I hope to continue for years to come.

Meanwhile in New York

This was a very active time in the lab with many postdoctoral fellows and students from Europe and Japan as well as the United States. In addition to the central setup that Sugi and I used, we had two laboratories with a total of four electrophysiological setups. Work proceeded basically along two lines. One was a study of variations in intrinsic properties in *in vitro* brain slices in the 1980s in which Pepe Lopez-Barneo, Serge Charpak,

Chris Leonard, Tony Grace, Denis Pare, Kerry Delaney, Susan Greenfield, Emilio Geijo-Barrientos, and Atushi Nambu worked over that decade. The second line of research focused on the development of isolated *in vitro* brainstem and whole brain preparations, originally in the early 1980s with Yosi Yarom, and in the middle 1980s with Brian MacVicar, Marco deCurtis, and Michel Muhlethaler. Some of this work was summarized in a review paper I wrote in 1988 concerning intrinsic electrical properties of central neurons (Llinás, 1988).

Neurolab

In a departure from everyday problems on Earth's surface and following a meeting with two friends, Wally Wolfe and Frank Sulzman, the thought came to mind that given that we had all worked on the vestibular system, it would be interesting to propose to NASA a shuttle flight be devoted to the study of all aspects of the nervous system in microgravity. Accordingly, a proposal was sent and NASA responded with the Neurolab, a mission that generated much information about neuroscience in many life forms ranging from fish to humans. Our own involvement was headed by Kerry Walton, with Dean Hillman and myself as co-investigators. We studied the effect of microgravity on spinal cord function. Among the people involved in the overall project was Alain Berthoz, a dear friend with whom I had collaborated over the years and who had a genuine interest in coordinate systems from the time of Pellionisz. The work headed by Walton defined a critical period for motor development for the first time (Walton et al., 1992). We also studied the development of cortical and hypothalamic circuits in collaboration with Javier DeFelipe from the Cajal Institute.

All in all, Neurolab was an interesting experience. We organized meetings at the MBL, and the different groups were assembled. As often happens with research, much was accomplished by some of the projects, whereas others accomplished much less. However, we all learned about team work at a level we had not experienced before. Projects had to be carefully designed at an exasperatingly minute level (e.g., define the animal's cage weight and building material) 1 or 2 years in advance. However, this also provided for a new and truly frustrating experience. Once you had written up your plan you could change nothing, even if newer, better techniques became available. That aspect, for scientists who are accustomed to optimizing techniques "on the fly," was a nightmare.

The Thalamocortical System, Cognition, and Dysrhythmia

As the work on the cerebellum proceeded, I began another line of research in the early 1980s concerning the electrophysiology of thalamic neurons, known to be the major entry into the cerebral cortex. For reasons concerning the rhythmicity, I intuitively expected thalamic neurons to have

electrical properties that were in some way similar to those of inferior olivary neurons. Delightfully enough, low threshold spikes were observed *in vitro* on the first intracellular recording. Indeed, when thalamic neurons were artificially hyperpolarized, a T channel current was deactivated and allowed the cell to oscillate at low frequency near 3 to 4 Hz. The work was published in a set of papers with Hendrik Jansen, a postdoctoral fellow from Denmark (Jahnsen and Llinás, 1984a, 1984b). These have turned out to be a rather significant set of findings. Also to my delight, Mircea Steriade and colleagues from University of Laval in Quebec came out simultaneously with similar findings in the cat *in vivo* (Deschenes, Roy, and Steriade, 1982). Although they could not determine the ionic mechanisms, the electrophysiological characteristics they found were exactly the same we had seen *in vitro*.

Mircea has continued with his research in this area, and we have been friends and at times serious critics of each other's work for over two decades. Although for the most part we agree, he is critical of my intellectual attachment to gamma band activity in relation to consciousness. Another very important colleague in this field is Ted Jones, a superb anatomist/physiologist and a dear friend and colleague, with whom Mircea and I have written books and organized scientific meetings and symposia.

Thoughts About Cognition

A second aspect of interest to me concerning the electrical properties of thalamic neurons was the fact that they all behaved similarly. That was initially unexpected because thalamic neurons provide the main afferent input to all areas of the cerebral cortex, and so one would expect differences pertaining to different cortical properties. The immediate consequence of this homogeneity was the possibility of having a unifying common mechanism that would address cortical activity globally as well as specifically. Thus, modifying membrane potential across the thalamic nuclei would necessarily result in a huge functional event at the cortical level. It became evident then that activation of the T channel conductance in the thalamus, if prevalent, should produce a low frequency activation of the cortex, which had been known to engender sleep. So T channels became a major key into brain function at the thalamic level. Moreover, because T channels in the IO could be considered as engendering the muscular rhythmicity that allows movement to be coordinated, T channels in the thalamus could be viewed as regulating the two huge functional events in the brain—that of being awake and that of being asleep.

Because the thalamus addresses the cortex in a rather powerful way and because the cortex returns to the thalamus an even larger number of synaptic contacts, the thalamocortical pathway is perhaps one of the most important systems operating in the brain. The fact that this neuronal

coherence is capable of being modulated rather rapidly explains, in principle, the ability we have to fall asleep very quickly, as one sees during boring lectures, and our ability to wake up quickly, as when one's neighbor puts his elbow into you and mentions, "You're snoring." So one important issue is that one can fall asleep or wake up very fast. The second is that when one falls asleep all of the systems fall asleep simultaneously, and similarly, when one wakes up, all aspects of one's sensory perception wake up at the same time.

So at this point in my career, the essence of nervous system function appeared to be defined as a set of embedded structures starting with the ionic channels with defined but simple properties that give neurons specific intrinsic electrical properties (Llinás, 1988). The next step would be the harnessing of significant "quorum sensing" states by the weaving of specialized neuronal circuits that would allow neuronal activity to coexist in time in order to produce cognition and motricity. This must occur, as in the case of cerebellar networks, as the product of evolution where complexity is attained by building on circuits sculpted by natural selection. In particular, when considering "higher nervous functions" the thalamus viewed as the hub in brain function appeared a beautiful structure from which to build. It became evident that the anatomical separation of midline thalamic structures—the mediodorsal, centrolateral (intralaminar), and centromedial—from the phylogenetically newer dorsal thalamus was central in global brain function. The beauty of the arrangement becomes clear when one realizes that the phylogenetically old medial group projects directly to the apex of cortical pyramidal cells and that this cortical arrangement characterizes the cortical circuits in reptiles in which the dorsal thalamus is absent. This is in contrast to the six-layered cortex of vertebrates in which the appearance of the dorsal thalamus is accompanied by the appearance of layers two, three, and four, the newcomers to the cerebral mantle.

The interaction between these two systems, known also as the specific system for the dorsal thalamus and the nonspecific system for the medial thalamus, became an important issue in my thinking. Indeed, it has been known from early neurology that damage of the dorsal thalamic nuclei, for instance of the lateral geniculate nucleus, produces visual problems similar to those encountered by damage of the visual cortex itself. Similar damage in the other thalamic relay nuclei, such as the ventral lateral nucleus and the medial geniculate nucleus, produced damage to the sense of touch and hearing, respectively. So, damage of the thalamocortical system was known to remove aspects of cognition without necessarily altering the overall cognitive state.

Contrary to the rather specialized nature of cognitive loss due to dorsal thalamic damage, medial thalamic lesions result in total disappearance of consciousness (akinetic mutism) if a lesion occurs bilaterally or hemineglect

if it occurs in only one side. In the latter case patients would see only half the world and not know about the existence of the other half. For example, clock faces would only go from 12 to 6 o'clock and people's faces would have a right or a left side only. This was known to happen not only with the perceived but also with the imagined world. So for instance, the film director Federico Fellini, a hemineglect patient, when asked to describe his view of a place from memory, would describe the scene differently depending on his imagined location, with one side always missing from his description. Thus, hemineglect encompasses both a perceptual and a cognitive problem as well as anosognosia.

Gamma Band and Cognition

From another perspective, attempting to define what aspect of brain function supports cognition became, to me, a plausible question to ask when considering what other properties thalamic neurons support. It was clear from our work and that of others that high-frequency activity in both the cortex and thalamus seemed to correlate well with cognitive events. The original discovery that gamma band activity (brain rhythms at 30 to 50 Hz) was related to cognition was described at the field potential level in behaving cats by Arlette Rougeul-Buser (Rougeul-Buser et al., 1983). Suddenly, electrophysiology took off with gamma band activity being recorded at the single cell level in the visual cortex by Wolf Singer and Charles Gray (Gray and Singer, 1989) and in our lab at the intracellular level as a subthreshold oscillation both at a cortical (Llinás and Grace, 1989; Llinás, Grace, and Yarom, 1991) and thalamic neuronal level. Thus, in thalamic neurons, Christine Pedroarena and I showed that dendritic P channels are most probably responsible for this subthreshold oscillation at gamma band frequency (Pedroarena and Llinás, 1997). This has recently been modeled mathematically by Paul Rhodes and myself (Rhodes and Llinás, 2005).

The significant issue here was that the thalamocortical system, given its loop connectivity, could easily go into recurrent gamma band oscillation. This was aided by the intrinsic properties of both thalamic and cortical neurons as well as the tendency for synaptic facilitation, as demonstrated in a second set of experiments with Pedroarena (Pedroarena and Llinás, 2001).

The question, then, was whether gamma band oscillation had a functional significance and if so, how such significance could be determined. I felt that if we could measure from a substantially large number of neurons, then perhaps the significance of this oscillatory property would become apparent. In a set of *in vitro* experiments using voltage dependent dye imaging, implemented initially in collaboration with Diego Contreras, the activation of the cortex with different rhythmic electrical white matter stimuli gave us a clear result. Indeed, the results indicated that the

geometry of thalamocortical activity is determined by the frequency of the thalamocortical oscillations (Contreras and Llinás, 2001). Thus, low frequency stimulation produced coherent activation of a large portion of the cortex, whereas high frequencies produced punctate activity. These experiments were repeated in a thalamocortical slice with Francisco Urbano and Leznik. Those results demonstrated that, in addition to functional geometry, coactivation of specific and nonspecific thalamic systems produced supralinear summation as expected if the two systems were temporally coincident (Llinás, Leznik, and Urbano, 2002).

This geometry of activation, I believe, is fundamental in defining sleep/wake cycles as well as the dream state and the moment-to-moment internal representation of our perceived world. I also feel that in order to support the ongoing perceptual state that characterizes wakefulness, the system would have to be sufficiently agile to the point of constructing such images at better than 30 different "frames" a second (the picture presentation rate of movies and TV).

The next step in this analysis was that of defining and trying to understand cognitive binding. That is, the ability we have to bind cognitive sensory bits that arrive separately into our brain and are anatomically distant from each other in brain space. Thus, for instance, the perception of a small bird in one's hand requires the brain to assemble the bird's color, form, weight, sound (if it is making one), and smell into a single percept, a "mental object," as my friend Jean Pierre Changeux has so eloquently named it (Changeux, 1983).

So now the question is: How can we experience this "object" as one single perceptual entity when different parts of the percept are represented in different parts of the brain? This is a fundamental issue that is still generating great debate. Singer and Gray (1995) and Francis Crick and Chris Koch (1995) represent the cortical view of binding, which assumes that coherence in time, at cortical level exclusively, is the probable mechanism. A second view, which we have pushed, holds that cognitive binding must indeed operate as a time binding functional entity but as a recurrent thalamocortical loop, as indicated by the experiments described later. A similar view on theoretical grounds, is held by Gerald Edelman and Giulio Tononi (Edelman, 1989; Edelman and Tononi, 2000) and Francisco Varela (Varela et al., 2001). In our case however, we define the issue of binding as related to temporal coincidence in involving the specific and nonspecific thalamocortical loops (Llinás, 1987; Llinás and Paré, 1991) based on direct experimental evidence to be reviewed later. Accordingly, through synchronous activation, the thalamocortical system binds in time not the activation of muscles as in the case of movement but rather binds the components of the percept into a single event. A corollary to this view impacts not only normal physiology of the brain but the variations of physiology conventionally known as pathology.

Enter Magnetoencephalography

Although research into the thalamocortical system was proceeding at the single cell and at the multicellular level *in vitro* using voltage dependent dye imaging, I was desperately wanting to determine whether events similar to those recorded in our animal experiments could be confirmed or excluded in human brain function. We needed a noninvasive technique with enough temporospatial resolution to test the hypothesis that arose from the *in vitro* and *in vivo* electrophysiological research.

And so in the mid 1980s, we began research using magnetoencephalography (MEG). An NYU School of Medicine lab was given to us in Bellevue Hospital, where we installed the first multichannel commercially generated system in the United States. It was produced by a company originally called SHE, then Biomagnetic Inc. (BTI), and now 4D. MEG was initially developed at MIT by David Cohen and then at NYU by Sam Williamson and Lloyd Kaufman. I started recording human MEG signals with a few superconductive coils in one Dewar, then graduated to a set of two Dewars, one on top of the head and the other one at its bottom, in a yaws type arrangement we jokingly referred to as "the nut cracker." We finally graduated to the present system with 148 recording coils that cover the whole cranial surface. The virtue of MEG consists in recording the magnetic rather than the electrical signature of neuronal activity. The technology is more expensive and complex than electroencephalography (EEG), but it has the important advantage that, as beautifully demonstrated by Yoshio Okada, the fields recorded are not modified in any way by the intervening brain tissue, the cranium, or the scalp, making the recordings very close to what can be attained using electrocorticography. Having been convinced that the device was capable of generating meaningful information about brain activity, we began to analyze the coherent activity of the brain generated by spontaneous events. Fourier transform analysis of spontaneous brain rhythms described the normal rhythms seen by EEG recordings and, to our delight, much of the electrophysiology that we had seen *in vitro* and *in vivo* using field potential recordings.

It seemed, therefore, that this MEG approach was a possible link between research at the circuit level in laboratory animals and at the macroscopic level in humans. Several bright graduate students and post docs began to rotate through the lab. And so after a few years of perfecting techniques and computer programs to localize and image the recorded data, Urs Ribary, Andy Ioannides, myself, and a set of students began experimenting with this new recording methodology. Together we produced a paper that addressed the issue of the recurrent nature of gamma hand activity (Ribary et al., 1991). It related directly to the recording of the thalamic loop activity at gamma hand frequency. This paper was followed by MEG recording of gamma band activity during dreaming, which I believe

demonstrates that dreaming and wakefulness are related states (Llinás and Ribary, 1993), and a correlation study indicating that temporal binding is congruent with coherent gamma band activity (Joliot, Ribary, and Llinás, 1994) and more generally, to global cognition (Llinás et al., 1998). At the experimental level more directly, Mircea Steriade and colleagues had demonstrated with simultaneous thalamic and cortical recordings in cat that a real recurrent loop at gamma band is operant (Steriade et al., 1996).

Beyond cognition, MEG has served as a good approach to brain neuroscience and has generated a group of Ph.D.s in the field. Among the students during the early 1990s Fred Lado, studying somatosensory responses, and Josh Cappell, studying fictive language, were the first to obtain their combined M.D./Ph.D. degrees in MEG. Josh Schulman and Rey Ramirez followed with Ph.D.s in this field. The latter did much more development at the mathematical level and implemented good localization techniques. Rey also studied vision, while Schulman studied patients with psychiatric disorders and central pain, and Kevin Sauve, a Ph.D. in philosophy, is presently finishing his second doctorate in temporal binding of somatosensory aspects of cognition.

To make a very long story short, MEG results continued to provide a good correlation between cognition and the electrical activity of the brain, suggesting, indeed, that different cognitive events can be related to specific electrical events at brain level. We felt that a good way to test such a hypothesis further was to consider pathological conditions in which brain electrical activity could be related to the neurological characteristics of several conditions. So we began a study in patients with Parkinson's disease, having theorized in an early paper that the frequency of Parkinson's tremor could be similar to the frequency obtained in thalamic cells *in vitro* when the cells were hyperpolarized. The connection was the well-known fact that damage to the substantia nigra nucleus is the basic etiology of Parkinson's disease. This is produced by a depletion of dopamine input to the basal ganglia resulting in pallidal disinhibition that releases inhibitory background synaptic inhibition on the motor thalamus. This being the case, it was proposed that this membrane hyperpolarization would activate the thalamic T channel system and trigger low-frequency oscillation, i.e., a "disconnection" syndrome produced by excess inhibition. This low-frequency oscillation could then have two effects: make the motor system behave as though it was asleep—also known as paralysis agitans—and also, generate tremor, these being the two known symptoms of Parkinson's. The results demonstrated very clear thalamocortical oscillatory activity and a paper was published with Jens Volkmann (Volkmann et al., 1996), a postdoctoral student who was finishing his neurological training. This work is continuing in more detail and utilizing more sophisticated analysis routines with graduate student, Kimberly Moran.

I had the good fortune at that time to meet Daniel Jeanmonod, a professor of neurosurgery and neurology at Zurich State University who, based very much on our original papers on thalamic electrophysiology, had been thinking of the so-called low threshold oscillation of the thalamus as generating Parkinson's disease. Daniel, from a neurosurgical neurological point of view, and I, from a physiological point of view, had arrived at very similar conclusions. Namely, that both the negative symptoms, e.g., the inability to generate movement, and the positive symptoms, e.g., presence of tremor, of Parkinson's disease were the products of low frequency oscillation of the thalamus.

Based on commonality of thought and instant friendship, we decided to collaborate and proceeded to put our heads together concerning the origin of Parkinson's disease. An obvious question that had been troubling us both was what would happen if similar disconnection syndromes were to occur in other nonmotor thalamic nuclei. So putting together MEG and human neuron recordings Daniel had made during surgery, we produced a hypothesis that suggested that many neurological and psychiatric diseases had a similar neuronal origin. The difference between the diseases was the localization of these abnormalities in different groups in the thalamus or in different parts of the cortex that could also result in a low frequency thalamocortical activity. This general concept was named thalamocortical dysrhythmia (TCD). We thought TCD included petit mal epilepsy, which in fact had been previously shown to be due to T channel activation of thalamic neurons, Parkinson's disease, irreversible depression, phantom limb pain, tinnitus, obsessive-compulsive disorders, and some aspects of schizophrenia. All of these, according to our hypothesis, were produced by disconnection of the thalamus allowing a low frequency thalamocortical attractor to be generated that would have sufficient resiliency to be maintained for long periods. A paper was written on this subject (Llinás et al., 1999) that was coauthored by two physics math colleagues, Eugene Kroneberg, a real rocket physicist from St. Petersburg, Russia, and Partha Mitra, a well-known and respected theoretical neuroscientist.

This hypothesis is still very much under discussion. The success of deep brain stimulation and stereotaxic micro lesions in many of these diseases strongly suggests that, indeed, they are all disconnection properties that generate abnormal brain rhythms and well-defined pathologies. A very constructive aspect of all this is a unification of psychiatry and neurology under neuroscience with the possibility of studying common mechanisms capable of producing the abnormal conditions relating to these two fields of knowledge.

The advantage of a unified view such as this (despite its obvious simplification) is the possibility of addressing many of these ailments pharmacologically because they ultimately relate to disconnection syndromes or channelopathies. The possibilities for the future are bright indeed.

New pharmacological compounds may be developed using rational drug development, which take into account the small differences that channels may have at different levels in the CNS. The other avenues that become extraordinarily important are the attempts to generate particular disease models by knocking out specific types of channels in given nuclei. So, at present we find ourselves beginning collaborations with colleagues such as Soonwoo Shin in Korea, who has developed a thalamic knock-out mouse where either T or P channels are impacted. These, to our delight, demonstrate similar types of neurological conditions, as expected in thalamocortical dysrhythmia both from a behavioral and an electrophysiological phenotype.

All in all, my scientific life has been a set of experiments done with different animals with a variety of techniques, but all based on the assumption that brain function is ultimately understandable. Indeed, the scientific method is sufficiently powerful to provide the answers in our struggle to understand the properties of the brain. From afar this set of studies may seem a kaleidoscope of unrelated experiments and ideas. In reality the many aspects of my work have been orchestrated by a deep sense that we *can* understand the nature of brain activity, or more fundamentally, the nature of what we are (Llinás, 2001). The brain is, basically, more capable than it is complex. Deep in the recesses of my being, I have always felt that our nature is no more mysterious than the rest of the physical world. The biggest impediment to our true understanding seems to lie with us; by wanting to be angels we deny our nature. I remember writing, some years back, that we may ultimately prove to be simpler than our intellect feared or our vanity hoped.

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