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Terje Lømo discovered long-term potentiation (LTP) and with Tim Bliss did the first systematic study of LTP. Lømo discovered that evoked muscle impulse activity per se controlled the properties of muscle fibers outside the neuromuscular junction and provided evidence against the idea of such control by "trophic factors." He described the normal firing patterns of motor neurons by 24 h recordings of single motor unit activity from unrestrained rats and demonstrated the importance of such patterns in controlling the phenotype of muscle fibers with respect to both membrane and contractile properties. He introduced the concept of adaptive ranges, within which muscle fibers adjust their contractile properties to the impulse patterns and hence to the work imposed on them.

Terje Lømo

To be invited to write about yourself in a book like this must mean that you have had some success in life. In my case, this success, such as it is, has always amazed me. With no particular intention or ambition to do research, how did it come about that I have spent most of my life doing it? I read of others in these collections of autobiographies, about hardships, courage, narrow escapes under persecution in some cases, dedication to learning, and hard work followed by success. And I think of countless others, surely equally able and strong, who perish in periods of wars and civil breakdowns when their luck runs out and who leave no trace outside their nearest circle. There is nothing similar in my story. Growing up in occupied Norway during World War II, I was too young to be put to any test of hardship. I enjoyed an almost free ride at school and university, meeting a post-war world where jobs were abundant and Western societies were becoming ever more affluent.

It was thought in the family that I would study medicine and so I did. There was nothing else I particularly wanted to do. Medicine meant being able to postpone any real decision about my future since it opened doors to many types of workplaces, not only the clinic. I cannot remember that research entered my mind and, if it did, surely I must have concluded that I had neither the interest nor the ability. In fact, I barely got into medical school. In 1935, when I was born at the time of the Great Depression, there were exceptionally few births in Norway and the number of applicants to medical school 18 or so years later was correspondingly low. Consequently, I could enter with lower grades than ever before or since. In those days, it was mainly the sons and daughters of the bourgeoisie who entered University (my father was a dentist). In all, there were about 40 of us who finished *gymnas* (high school) in 1954. At that time, there was only one high school serving Ålesund, the town where I grew up, and the population on surrounding islands and along the fiords totaling perhaps 30,000–40,000 inhabitants. Today, the schools are many, the number of pupils many-fold higher with not all that many more inhabitants. Norwegian, mathematics, and physics were the main subjects for those wanting to study medicine, dentistry, or engineering. The grades in these subjects were weighted heavily and had to be good to obtain one of the 100 places at the medical school in Oslo, then the only one in the country. Many went abroad for their studies, mainly to Universities in Europe. For the United States, an affidavit, documenting local financial support in case of need, was required, making it difficult for many to go there. Entrance to law and liberal arts studies was not restricted.

Biology and chemistry were minor subjects at high school. I cannot remember being much engaged by these subjects and certainly put little effort into them.

I remember being more interested in political subjects than anything that had to do with medicine or biology. At medical school I happened to come across the monthly periodical *Encounter* at the public library and found it of immediate interest. I soon became a loyal subscriber and remained so until it folded when the Soviet Union collapsed in 1990. To me, it represented an appealing corrective to much left-wing writing and politics of the day. I found Hans Eysenck's *Sense and Nonsense in Psychology*, *Use and Abuse of Psychology*, and *You and Your Neurosis* very interesting. Neurosis, inferiority complexes, anxiety, feelings of insecurity, and the like preoccupied me. I felt I had more than my fair share of such attributes and was prone to contemplate my own navel to nobody's pleasure. Throughout my life I have experienced at times long periods of very low spirits. I considered leaving science but held back knowing that would hardly solve the problem. In such periods I would withdraw. Exposure at meetings was often an ordeal. There were challenges and possibilities that I could not take on. Making a career did not seem important. I preferred the more lonely existence of being in the lab with my experiments. This explains, I think, my lack of ambition, the modesty that has been attributed to me in a Norwegian biographical lexicon. Perhaps this also explains why I have never looked for heroes or role models, but rather have maintained a skeptical awareness of human imperfections.

More rarely, I felt on top of the world. But most of the time, I guess, it was a matter of going about an ordinary daily life with trying not to make a fool of myself as my main driving force. Unexpectedly, however, the intermittent feelings of doom that often gripped me appear to have lifted in recent years, particularly after retirement, and to have been replaced by unaccustomed feelings of contentment.

Childhood

I grew up in Ålesund, a small town on three islands on the west coast of Norway, between fiords and steep mountains on the inside and a string of larger islands on the outside, protecting Ålesund from the Atlantic Ocean and its winter storms. The people living there are industrious and entrepreneurial, a gift from ancestors surviving under harsh conditions as small farmers and fishermen along the fiords and on the islands. Home industries took off early—boat building, the making of furniture and textiles. Today, Ålesund is home to a fleet of modern fishing boats and trawlers plowing the waters all over the world. Smaller and larger industries are scattered along the coastline, supplying offshore installations, building ships and advanced machineries, farming salmon, producing furniture for the often distant

international markets, and yet highly competitive. For some time after the war the region also had a large textile industry until most of it went under as this industry went to places with cheaper labor, first in Southern Europe, then Asia.

During World War I, when Norway was neutral, my father moved to Ålesund from eastern Norway. His oldest brother had inherited the family farm, and the other children had to seek their fortunes elsewhere. Some in similar situations went to the United States A few went to St. Petersburg before the Russian Revolution made that option unattractive. For my father it happened to be Ålesund. My father always liked Ålesund. He found it less class ridden than where he came from, the wealth spread across many families, opportunities aplenty for the hard working, so he admired the people there. Growing up in Ålesund, the town seemed to me to be split into two types of people: on the one hand the religious, the puritans, and the teetotalers for whom dancing, drinking, and playing cards was considered sinful and, on the other, those who liked to do just those things. My family and my friends belonged to the second category. While many of my friends, and my oldest brother as well, knew from early on that they would return to Ålesund after studies and live there, I never felt quite at ease there. I felt constrained by the jargon, the customs, and the social pressures that came from belonging to a particular group. I wanted to start from scratch, on my own, freer, and without social bonds. So I left, never to return, except for brief visits, and without regrets then or later. Such sentiments, I think, have followed me since. Prone to feelings of awe toward people in command, I would tend to shy away from situations that I felt might require commitments and expression of loyalties that would undermine my sense of freedom.

Medical School

At medical school one of my teachers in anatomy was Alf Brodal, the author of *Neurological Anatomy*, a widely known book for many years and described by Todd Sacktor as his "bible" during preparations to become a neurologist. In one of his early lectures, Brodal invited some students to form a study group that would come to his office once a week for discussions about his research project at the time, the reticular formation of the brain stem. I joined that group and found it very much to my taste. We did not do any research ourselves but were introduced to relevant literature and offered his experimental material to look at. Brodal's approach was to cut axons by making small lesions in the spinal cord (and elsewhere in the central nervous system) and then determine their cells of origin by identifying swollen cell bodies undergoing retrograde chromatolysis (tigrolysis) in sections of the brain stem. Using young kittens (his modified Van Guddens technique), such cells would stand out clearly from intact neurons in the region. To be able to trace pathways in the brain in this rather precise way appealed to me

as did reading about and discussing their possible functional implications. At the end of this exercise, I presented the group's experiences to the rest of the class to Brodal's satisfaction, according to his concluding remarks. And that apparently was the end of it. As it turned out, however, it probably had a decisive influence on my later choice of what to do in life. It was also, I think, a fine and relatively rare example for its time of a professor making a strong effort to introduce and recruit young students to research.

Early Days in Pisa

In 1958, in the midst of clinical studies, I felt I needed a break. I contacted Brodal and told him that I would like to go abroad perhaps to do research. Did he have any suggestions? He suggested Pisa, wrote to Professor Guiseppe Moruzzi, the head of the "Istituto di Fisiologia" at the University of Pisa, who immediately agreed. So with a fellowship from the Italian State, I left Oslo by train one Wednesday evening in August 1958 and arrived in Pisa on Friday afternoon nearly 2 days later. My father enthusiastically supported this study abroad as he had earlier, in 1951–1952, supported my spending a year in a high school in Fort Wayne, Indiana, with a scholarship from the American Field Service, and even before that when I bicycled with a friend over much of England at the age of 14.

Arriving in Pisa I was met at the station by Arnaldo Arduini. He took me to a nice hotel along the river Arno, where the institute had booked a room for me, and asked me to come to the institute on Monday morning, which suited me fine. The room turned out to be on the top floor, where I quickly stepped into the bathtub to take a shower, looking more like a chimney sweeper after 2 days on the train and hours of leaning out of the train to take in the Italian scenery. Then with black foams of soap all over my body, the water disappeared and did not come back that day. Although trivial, it left an impression on me. I can still see the layout of the room, the bathtub, and myself scraping off the foam before I went out to have my first Italian meal in a nearby trattoria with wine and fresh fruit for dessert, feeling free and great, but also wondering what the next year would bring.

On Monday, I was shown a little corner room on the second floor, which was to be my home for the next year. To reach it I had to pass through a student laboratory, where I later assisted Amilcare Mollica in demonstrating the vagus effect on the heart of anesthetized rabbits for the medical students. Moruzzi was in Moscow, I seem to remember, at an international conference on brain research, particularly sleep, that attracted many high-profile scientists in the field from Europe and the United States. When he returned, he arranged for me to work with Mollica, who had a large laboratory with high windows facing Via S. Zeno and a large Faraday cage in the middle. In a similar, neighboring room worked, among others, Ronald Melzack, who with Patrick Wall, would later publish the classic Gate Control

Theory of pain. At the time, the Institute in Pisa was an international centre for brain research, attracting researchers from across the world, and over it all presided Moruzzi, *Il Professore*, in somewhat splendid isolation it seemed to me. The language in the institute was English, and partly for that reason I was very slow in learning Italian, even though friends among the local students helped me along. But I have always regretted that I never learned to speak Italian well.

Pisa and Oslo collaborated at the time. Gian Franco Rossi and Ottavio Pompeiano, who would later replace Moruzzi as head of the Institute, both spent a year or more in Brodal's lab in Oslo, which explains, I think, why I was so readily and so well accepted in Pisa. But I remember Mollica's surprise when he realized that I was only a medical student with no experience doing research.

Research in Pisa

Moruzzi wanted Mollica to study sleep. I remember Moruzzi telling us about a presentation at the meeting in Moscow, claiming that it was possible to induce sleep in humans by letting them rest in a bed that was rocked back and forth at a certain frequency. So he suggested we do something similar with rabbits. We had a cage made that swung to and fro while suspended from the ceiling of the Faraday room and placed a rabbit inside with screws in the skull for electroencephalogram (EEG) recordings. The problem was that we could never make these rabbits sleep. The EEG was more or less continuously desynchronized and if hopeful signs of synchronization should appear, sudden loud noises from Via S. Zeno would put an end to that, as when the local rag-and-bone man made his rounds shouting out his goods.

We gave up the project, which Mollica from the beginning did not believe in anyway, and went on to a more conventional approach to sleep research based on the encephale isolé preparation. Mollica did not believe in this approach either and was happy to leave the surgery to me, first on rabbits and, when that turned out difficult, on cats, the more common preparation. I don't remember the purpose of the effort and before long this project also was abandoned. Occasionally, Moruzzi would come into our lab to ask how we were doing. As progress was zero, also eventually he gave up making suggestions and left Mollica to his own devices. We then began recording from single units in the cortex of awake rabbits, using electrolytically sharpened, insulated tungsten electrodes, the technique for which David Hubel had published a little earlier in *Science*. I don't remember Moruzzi taking any interest in this project but, when we told him about it, he suggested that I go to Rome at the Institute's expense to see G. M. Ricci, who had recently returned from Herbert Jasper's lab in Montreal and was setting up his own lab to do similar single-unit recordings. I was well received by Ricci, saw Rome for the first time, and enjoyed a marvelous couple of days there.

But well before this, when Mollica and I were at our most frustated, Ron Melzack, perhaps concerned that I was having a difficult time, suggested that I could join his group. In this group there were also C. J. Smith, F. Magni, and sometimes Moruzzi would come down from his office on the third floor to participate in the experiments. However, Mollica and I got along very well together and I was happy to work with him. It is true, that at times of frustation, we would distract ourselves by shooting down flies from the high ceiling by squirting ether on them from syringes. With every hit, the fly would drop to the floor and then quickly recover from the anesthesia, which allowed the game to continue. It is also true that Mollica was extremely upset after every visit by Moruzzi, not because Moruzzi, as far as I could see or now remember, was unkind or unreasonable, but rather because hierarchic traditions and Mollica's character were such that, when facing Moruzzi and even strongly disagreeing with Moruzzi's proposals, he was unable to express his own. Perhaps Mollica took as orders what Moruzzi meant as suggestions and generally answered Moruzzi with "Si Professore" in his presence, while resorting to fits of temper as soon as Moruzzi left the room.

By New Year 1959 Mollica and I were on our own. We designed a microdrive and asked a machine shop in Piacenza to make it. The shop, which specialized in high-precision tools, belonged to the father of an engineering student in Pisa who had become a close friend of mine. We attached the drive to the skulls of rabbits under anesthesia and later recorded from single units in the visual cortex with the rabbit loosely attached with a strap to a wooden cage open at both ends, head and chest sticking out at one end, tail at the other. I made the electrodes according to Hubel's description, realizing soon that this was not always straightforward. Despite efforts to standardize the procedure, particularly the coating of the electrodes with insulating material, the electrodes varied enormously in quality and most of them had to be discarded. Nevertheless, over the ensuing month we were able to record many units with high signal-to-noise ratios over periods of up to several hours. We exposed rabbits to light, sound, odors, pinpricks, and squeezes of the tail, described the firing patterns of responding neurons and the modulatory effects of different sensory inpurts, as wells as effects of barbiturates and local applications of strychnine to the cortical surface.

I was probably the last person to collaborate with Mollica, and our paper together was probably his last. Later, I heard that Mollica had had a serious mental breakdown and that he never returned to science. I found this very sad. Our relation and collaboration had been very good. Mollica was interested in many things, which we frequently discussed. He had top grades from medical school. I found him very clever, even brilliant, in many ways. But, as far as I could tell, he had little or no social life outside work. He lived alone with his mother. After work he would take his bicycle and apparently go straight home. Outside work, we never met and I never saw him with

others. When difficulties arose and he stopped working, I believe that Moruzzi tried to help him. It is one of my many regrets in life that after I returned to Norway and the correspondence relating to the paper came to an end, I failed to keep in touch with him.

In June or July, I returned to Norway. Mollica had started writing the paper, which appeared in 1962 in *Archives Italiennes De Biologie*. There, we acknowledge that the research was carried out with funds from the U.S. Air Force and the Rockefeller Foundation. This was a time when the United States was still very active in rebuilding and supporting Europe after the war with direct donations in the best traditions of American philanthropy.

I now see what I had forgotten, that the paper was translated into English by T. D. M. Roberts and J. D. Christie. Roberts visited Pisa when I was there. We went out together with my Italian friend who spoke no English. Roberts was walking with notebook and pencil in hand asking for the Italian names of just about everything that caught his eye. I was impressed and I remember well wanting to take up that behavior myself, but it never came to that then or later. I may well have seen the paper before it was submitted for publication, but I cannot remember that I took any direct part in writing it. Perhaps it wasn't even considered that I might be of use, being a student with no previous research experience and without English as my native language. As it was, it suited me fine. I was busy taking up my medical studies again and would have found it very difficult to enter into the mindset and vocabulary needed to describe and discuss such complex issues.

I don't know that our paper has ever been referred to. It is not listed under PubMed. Although these were early days for recording from single cortical neurons in awake animals, the paper was anecdotal rather than systematic, descriptive rather than mechanistic, and rather deficient in revealing new and interesting phenomena or principles. But then it becomes another example of the ease with which one can spend much time and effort on research and obtain results that then disappear without leaving a trace except in CVs for job applications. And yet, the year in Pisa was not only personally unusually enjoyable, satisfying, and educational but also decisive for my later choice in life (more later).

Memories from Pisa

I had a good year in Pisa. I was on my own and was never homesick. I liked living in the institute and having my own private room there. Seeing it again last summer for the first time in more than 50 years filled with outdated instruments and computer ware, I was amazed at how small it was, room for a bed, a wash basin, and a writing desk in front of the window and little more. But I don't remember having any problems with that. Coming from the north to the sunny Mediterranean of my imagination I had not expected some of the winter days to be so cold but with an electric stove installed, my

room was fine. In the mornings I went straight to lab without breakfast of any kind, which was contrary to everything that had been drilled into me for as long as I could remember, namely the absolute necessity of a regular, substantial, and healthy breakfast. But again, no adverse effects came to pass, only a healthy appetite building up before lunch. My stipend and supplements from my father allowed me to buy a wonderful new Vespa, travel, eat and drink well, and see Toscana, the nearby mountains, and the sea. I enjoyed the library in the Institute but found Eccles' *The Physiology of the Nerve Cell* rather difficult. Every midday I would walk across Piazza dei Cavalieri and Piazza dei Miracoli to a little bar just outside the old city wall where, in a little room behind the darkness of the bar, the wife of the bar owner served a delicious, simple, low-cost lunch for a few regular customers, including myself and one or two friends among the students. This became such a routine that at one point I told my surprised friend that I needed a break and wanted to walk around on my own. Before long, however, I fell back into the routine. After Norway, where I was used to partying, drinking, dancing, and the rest from the time of my confirmation, Pisa at the time appeared very provincial. Everybody, it seemed would dress up and walk up and down the streets before disappearing behind gates and shutters for dinner, leaving a good number of male students to wander aimlessly around. From what I understood, the church controlled the choice of films shown by most of the cinemas, so that was not much fun either.

In my experience there was little social interaction between the people working in the institute, no common room where one could go for a cup of coffee or tea, or to talk about science, ongoing work, or whatever, no seminars that I can remember. Had I known better, I think I would have missed it. But I may also have missed much of what was going on in the institute since outside working hours I was away most of the time. By some happy coincidence I had met an Italian engineering student as I stepped out of the train that brought me to Pisa. He became an almost daily companion during my year in Pisa and a life-long friend. He introduced me to many of his friends and made my days in Pisa anything but lonely.

Of many memorable experiences in Pisa, one in particular made an impression on me. One day I heard loud noises from the lab next door, went in, and saw a cat on the loose knocking down bottles and making a great confusion. I tried to grab it, but it turned around in its loose skin, biting and scratching my hand. This caused some concern and I was sent to the hospital with a prescription for immunization against rabies. There the doctors filled a large syringe with an opaque, withish fluid that they injected under the skin of my belly. I did not like the sight of the fluid and when they told me to come back for an astonishing number of additional injections, I started thinking about risks, about the likelihoods of rabies in the cat, and immune reactions in my brain in particular. No more injections for me, I quickly concluded, and went back to observe the cat, which in the meantime had

been caught and brought back to the *stabulario* in the garden. When it saw me, it snarled at me in great anger but when it continued to do so on subsequent inspections without any signs of disease, I dropped the matter without at any time having felt really worried. This may have been the first time that I became concious of an enduring scepticism of doctors for their readiness to overtreat and, in the process, underappreciate risks, side effects, and complexities. Sometimes it is better not to treat and give the body time to heal itself.

Return to Oslo and Medical Studies

I returned to Norway in July 1959. Soon afterward, I was invited to present the work from Pisa at a meeting of the Neurological Society in Oslo. There I met Per Andersen for the first time and on his suggestion presented the work again at the Scandinavian Physiological Congress in Oslo in 1960. The session was chaired by Ragnar Granit, who made some comments I did not understand. I don't remember my answer, but I remember talking to others afterward who also had not understood what Granit had been talking about. By then I had moved to Bergen to finish the final 2 clinical years of medical school.

Life as a Doctor in Northern Norway

After graduation, I was doctor in a hospital for 1 year and assistant community doctor for another half year. Such practice was required in Norway, and it still is to obtain a medical licence. I went to the hospital in Tromsø and then to the community of Skjærvøy, a small island north of Tromsø with about 1400 inhabitants. There was only one car on the island, no bridge to the mainland, but northbound and southbound coastal steamers that stopped over once a day. I often visited patients at home, by foot on the island or by boat to nearby islands or the mainland, trips that could take many hours. The boat *Medicus* owned by the community, was slow, had a crew of two who made delightful company, and whose job it was to take the doctor around and transport patients. Contact could be made by radio, but it was seldom used. Thus, weather permitting, the boat calls often became restful outings, sometimes in the company of my wife, Anne, in spectacular landscapes as the season changed from no sun in the winter for 2–3 months to sun all day and night in the summer.

For many, life was hard in a harsh climate, the men away as fishermen or sailors, the women at home often in isolated places looking after children, a cow or two, or some sheep. Pain from muscles and joints, usually in the back or neck was a common complaint, and I soon adopted my own procedure for dealing with it. There were four main components. Application of an ointment over sore muscles, resulting in a local flush and feeling

of warmth; injections of dilute solutions of a local anaesthetic into sore spots; aspirin; and a belladonna derivative, a relaxant before the time of valium and similar drugs. I would use them in this order depending on the condition and its resistance to treatment, only for restricted periods of time with counterindications in mind, aiming to break a vicious circle, always with instructions to the patients to make sure they had a good bed or mattress and, if possible, to take time off during the day in which to concentrate on relaxing, massage the ointment into sore regions, or better have a partner to do that, then inform me of any progress after a week and, if disappointing, to come back for further treatment. I believed in the approach, was enthusiastic, and as a result the patient probably benefited from a strong measure of placebo effect together with any specific effects there might have been from breaking a vicious circle and adopting a lifestyle that stressed the muscles less. Anyway, I had the impression that sometimes the treatment was helpful. As an indication of that, at the end of my time at Skjærvøy, a woman travelled the considerable distance from Tromsø to see me because she had heard that I was good at treating backaches.

I remember one patient in particular, a young woman complaining of chronic headache. The muscles at the back of her head were sore on palpation for which I found no reason and therefore started treating as described. She came back after a few days not seeing well. The belladonna, a muscarinic antagonist, had blocked accomodation in her eyes, revealing a hyperopia that eyeglasses immediately corrected. She did not come back, in all likelihood because her problem had been solved. This event had some lessons for me. Had I continued being a doctor, I would never again have treated headaches without checking eyesight. It demonstrated how chronic tension in some muscles, in this case in the eye, could somehow cause chronic tension and pain in other muscles, in this case the neck. Assuming that the eyeglasses resolved the headache, it also demonstrated how effective breaking a vicious circle can be.

I liked being a doctor. Initially, it was hard due to inexperience, unnessarily long hours because I was slow, responsibilities beyond my competence, and, I think, an inborn feeling of insecurity. But before the end of my time at Skjærvøy, and faster than I had feared, I had acquired a certain assurance based on a conviction, drilled into us at medical school, that it was essential to be systematic in taking the history of the patient and in the subsequent physical examination, so as not to overlook anything. On many occasions, surprise findings made it clear to me that being systematic was important even when it might seem to be a waste of time. Moreover, it was essential when things did not turn out well or as expected. One had done one's best and mistakes, when they occurred, at least were not due to negligence. With hindsight, I think this was the only way I could deal with the anxieties and feelings of guilt that could otherwise so easily come with being a doctor. I see my background in medicine and medical practice, however short, as important

for my subsequent career in science. The search for a diagnosis in medicine and a resolution to a problem in science have similarties, among them the lack of a priori answers. Perhaps it has helped me to better see the organism as a whole with many interacting parts at the level not only of cells and molecules but of organs and to see functional significance in new observations made in the laboratory.

Apprenticeship with Per Andersen

After Skjærvøy, I returned to Bergen to complete my military service in the Norwegian Navy. I had already done 5 months of service during summer holidays as a student. There remained 13 months as a Navy doctor, partly on land at the NATO naval headquarter outside Bergen and partly on board one of the Norwegian frigates. A few years earlier, I had served as an attendant to the officers on board another frigate on a training expedition for Norwegian naval cadets that took us to O'Porto in Portugal and Jan Mayen in the Arctic Ocean with many calls at ports in between. Some impressions remain: the extreme tiredness from late nights in ports, crowdedness and irregular hours of duty on board, rough weather, and sea sickness at sea. Around Jan Mayen there were several large Soviet factory and storage ships serving numerous trawlers inside and outside Norwegian territorial waters, at that time positioned 3 or 4 nautical miles from the shore. As our frigate moved very slowly along this border, the fishing boats pulled up their gear and moved out of Norwegian waters only to move in again as soon as we had passed. But Norway had shown its flag and Norwegian jurisdiction over Jan Mayen and its surrounding waters had been respected, and that was, surely, the purpose of the visit. But this was in a time of peace. I can only imagine the conditions on board such ships in times of war.

Before discharging from the Navy in July 1964, I asked for a leave of absence for a few days, went to Oslo, and looked for a job that combined research and clinical medicine. Neurology had no appeal, too little to offer in terms of treatment. Neurophysiology, as I knew it, was too far removed from the clinic. I had visited one hospital and was on my way to another when I accidentally met Per Andersen in the street. He had done highly successful postdoctoral work with John Eccles in Canberra, Australia, was back to set up his own lab, and was looking for people to join him. He remembered me from 4 years earlier and promptly said we needed to talk, the result of which was that I joined Per in August 1964 with a fellowship from the Norwegian Research Council that Per and Alf Brodal helped me to obtain. I had a letter of recommendation from Moruzzi saying that he was "quite pleased" with my stay in Pisa, but I did not I use it, thinking that it meant fairly or not so pleased, according to English usage. Now I learn that "quite" according to American usage might be an intensifier, an interesting

ambiguity in the meaning of words. I still think Morzzi used it in the English sense, and rightly so, since what I did in Pisa was quite ordinary.

I assisted Per with his experiments until Christmas 1965, usually twice a week and, as was common among serious neurophysiologists at the time, often long into the night. We focused on CA1 in the hippocampus of rabbits and cats, the identity and location of excitatory synapses on dendrites in collaboration with Theodor Blackstad and dendritic conduction. Soon I was setting up the preparation and moving the electrodes while Per controlled the instruments. Upon penetrating a cell, the loud noise from the loudspeaker made Per jump to life and in the fleeting moments before we lost the cell, he would play the instruments like a virtuoso and obtain incredible amounts of information. With Sven Andersson, visting from Gothenburg, Sweden, I also participated in the experiments on thalamo-cortical interactions that led to the book by Per and Sven: *The Physiological Basis of the Alpha Rhythm*. Early on I learned the Golgi technique from Blackstad and, in one of the sections, I could see one neuron, and only one, a CA1 pyramidal cell, standing out pitch-black apparently in its entirety. This cell and influential ideas by Wilfred Rall on the electrophysiology of dendrites inspired Per to suggest the following project: model the stained neuron in copper, run hot water over the cell body, and measure temperature gradients along the cell's branches as simulation of passive current flow. Bill Letbetter, a postdoctoral students in Per's lab, made the model in our workshop with help from specialized tool shops in downtown Oslo. The cell body and dendrites were in solid copper, extending more than 1 m with relative dimensions similar to the stained cell and with strips of copper clamped to the dendrites to simulate spines in a completely unrealistic manner. Letbetter, another student in Per's lab, and I did the experiments, placed the model horizontally on a bench, attached homemade thermocouples at different positions along the cell, opened a valve from a large container with 60 liters of boiling water on a high shelf above to let the water flow rapidly over the cell body and into a reservoir on the floor through custom-made tubes and a tightly sealed plastic housing around the cell body. With a new multichannel HP A-D converter we obtained beautiful curves of temperature changes along the dendrites. As expected, the temperature rose progressively more slowly with distance from the cell body. But that was the end of it, presumably because we did not quite know what to do with the data we had collected. Unfortunately, all records from this rather heroic or reckless experiment from an earlier era, considering all the boiling water above our heads, have disappeared.

On other occasions, I went into the garden around the University buildings in Oslo's center to collect hedgehogs, which were then placed in the refrigiator for hibernation. The idea was to remove the hippocampus in toto under cold conditions, hoping that it would survive and allow in vitro experiments,

but it did not work. The few hedgehogs we found did not properly hibernate and it was not easy, I remember, to remove the spines and anesthetize them.

At the end of 1965 it was time to start my own Ph.D. project. In agreement with Per I began studying frequency potentiation in the dentate area. Per had earlier reported this phenomenon, which refers to the dramatic increase in firing of pyramidal or dentate granule cells caused by repetitive stimulation of their afferent fibers, usually at frequencies around 10–20 Hz. It was expected that work for the Ph.D. should be independent. Therefore, I used the lab on days when Per had other things to do, and did all the experiments, subsequent analysis, and writing myself. Under Per's guidance I had participated in all aspects of this type of research, from the idea to the finished paper, except the writing. This was essential background. But now I wanted to be on my own, choose my own directions, and prove, above all to myself, that I could be independent. I am grateful to Per, and the tradition of that time, for the opportunity to do so.

The Discovery of Long-Term Potentiation

Initially, the focus was on what happened during repetitive stimulation. I cannot remember that Per and I ever discussed that looking for the aftereffects of such stimulation should be a specific part of my project. That just happened as I did the experiments. I then saw the dramatic build-up in granule cell population responses with repeated trains of stimuli, a build-up which then could last for hours. It must have been a natural thing to look for. I was then reading papers by Eccles and coworkers on the cerebellum and was impressed by how they used the paired-pulse paradigm to demonstrate excitability changes and the spread of such changes at different times after the first pulse. All I needed to do was to replace the single pulse with a train of pulses. Furthermore, others had already looked for such aftereffects and not found them, and I wanted to have my own look. I reported the new phenomenon at a meeting of the Scandinavian Physiological Society in Åbo (now Turku), Finland in August 1966, and in an abstract from that meeting in *Acta Physiologica Scandinavica.*

I remember showing the result to Per and how impressed we both were by the magnitude and the duration of the effect. But I am sure we had no idea then that this would be the beginning of the LTP story as it is known today. Rather, I think it is fair to say, we saw it as one of many other exciting phenomena brought to light by the brain studies of the day. Nevertheles, in seminars that I gave at the time, my records show that I used expressions such as "The effect is long-lasting, shows no tendency to fade after periods of rest up to 22 min in this experiment. In other experiments increased efficiency is seen for hours," "If it is correct that the hippocampus is involved in memory function, this is a region where one should expect long lasting

changes to occur," and "The phenomenon may represent a kind of *bahnung* [opening] of individual synapses and may have relevance to theories of learning."

Trying to Understand the Dentate Gyrus

I had many records and plots related to frequency potentiation and its after-effects lying around at the time but, except for the abstract in *Acta Physiologica Scandinavica*, I never published any of them, nor did I on my own follow up or publish anything on what was later to be known as LTP. I remember sitting there by myself in the lab, often late at night, being overwhelmed by the complexity of the field potential changes unfolding before my eyes on the ocilloscope screen. I obviously needed to know much more about the basic properties of the structure I was studying. I therefore changed direction. Along the lines pioneered by Eccles and coworkers in the cerebellum, I began using field potential analysis to explore the distributions of responses to orthodromic perforant path stimulation and antidromic mossy fiber stimulation and obtained evidence for a lamellar organization of both the input to and the output from dentate granule cells. Using the paired pulse paradigm I demonstrated marked potentiation of intra- and extracellularly recorded excitatory postsynaptic potential (EPSPs) at the perforant path-granule cell synapse. The potentiation occurred independently of any detectable granule cell firing, which required substantial EPSP summation. When the intensity of the conditioning stimulus was increased to discharge granule cells, profound postfiring inhibition occurred, lasting up to 100 ms and spreading several mm along the longitudinal, septal-temporal axis of the dentate gyrus to granule cells that had not been activated by the conditioning stimulus. When granule cells were discharged antidromically by stimulating mossy fibers in CA3, granule cells were profoundly inhibited along a similar time course and with a similar longitudinal spread, which I took as evidence that the inhibition was mainly recurrent. This work then made up my thesis, which did not include frequency potentiation or LTP. I defended my thesis in Ocober 1969. The first opponent was Anders Lundberg, professor of Neurophysiology in Gothenburgh, Sweden. He made some critical remarks with which I agreed and, as I remember it, I then went on to mention other aspects of the work with which I was unhappy. To the amusement of the audience, we then entered into a discussion where he would defend and I would criticize the thesis.

The work consisted of four single-author papers, which I submitted to *Experimental Brain Research* at the end of 1969. The papers were returned with both favorable comments and critical remarks calling for changes. I made the necessary changes for two of the papers, which were published in 1971. The other two remained in my files for nearly 40 years until I merged them into one paper using the same original figures but an updated text and

had it published in *Hippocampus* in 2009. But before then I received a letter dated March 31, 1970, from Eccles, the editor of *Experimental Brain Research* and one of the reviewers of the four papers saying: "I have been wondering for some time about your hippocampal papers. I am sorry that you have not made some of the suggested alterations and sent them in to me because they would certainly have been accepted for publication. Perhaps though it is better that you think some more about them. Please do appreciate that the referee and I both think very highly of the papers so you should not be depressed about them."

Enter Tim Bliss

While I was busy in Oslo, Tim Bliss was doing a Ph.D. on synaptic plasticity in the neocortex at McGill University, Montreal, with Ben Delisle Burns as his supervisor. When Burns became Head of the Division at the National Institute for Medical Research, Mill Hill, United Kingdom, in 1966, Tim followed him a year later and has been there ever since. From the beginning of his research career, his interest had been in possible neural mechanisms underlying learning and memory. But work on the neocortex appeared difficult and unrewarding. In Tim's own words: "However, the main conclusion I reached after devoting nearly 3 years to this approach [undercut slabs of neocortical tissue developed by Burns] was that it was misguided. The preparation was too complex. It was essential to simplify." Tim then happened to read a paper by Per on the hippocampus describing its relatively simple structure, with the cell bodies condensed in a single layer and afferent fibers from different sources terminating on different discrete regions along the dendrites. This structure appeared much more suitable, particularly for field potential studies, and when Per made a visit to London in 1968, Tim contacted him and asked him about the possibility of spending a year in his lab to learn the hippocampus and field potential analysis. On hearing of Tim's interest in memory mechanisms, Per, according to Tim, then said: "You must come and talk to Terje Lømo who has found something that will interest you."

Tim visited Oslo briefly and, as he remembers it, we agreed to carry on with the LTP experiments but, since I had to finish my Ph.D., I would not be able to spare more than 1 day a week. Then, in August 1968, Tim came to Oslo for a year. With Per and Knut Skrede he did experiments that led to papers on the "Lamellar Organization of Hippocampal Excitatory Pathways" and "Unit Analysis of Hippocampal Population Spikes," both published in 1971 in *Experimental Brain Research*. They confirmed the lamellar organization that I had shown in my thesis work for the perforant path input and the mossy fiber output of the dentate gyrus (published in 1971 and 2009, respectively), expanded it, and went on to demonstrate a similar organization for the CA3 input by Schaffer collaterals to CA1. Thus, they

showed that it should be possible to preserve the entire trisynaptic circuit from the entorhinal cortex (perforant path fibers) to CA3 (mossy fibers), and thence to CA1 (Schaffer collaterals) in a narrow, nearly transverse segment of the hippocampus, an essential requirement for the success of the transverse hippocampal slice maintained in vitro that was to come later.

Bliss and Lømo (1968–1969): The Start of Long-Term Potentiation Research

When Tim came to Oslo in 1968, a new lab had been set up across the corridor from Per's lab. About once a week, Tim joined me there to follow up my preliminary results on LTP 2 years earlier. Usually, the experiments went on until late at night or into the morning, either because we were busy with other projects and therefore started late or because some of the experiments lasted that long. We both remember our tremendous excitement when, in the very first experiment, the granule cell population spike increased dramatically and progressively after each stimulus train in the experimental pathway, while the control pathway on the other side was unaffected. And, into the next morning, our excitement was no less when we found similar long-lasting effects after briefly tetanizing what had been the control pathway. Our minds were prepared and, when further experiments showed that the results were reproducible, we were well aware of their significance.

With the setup just described, we used the same stimulation electrode to tetanize (condition) the pathway and to monitor the aftereffects with single test stimuli. We therefore worried, and Tim may have been the first to express this concern, that changes at the site of stimulation might lead to activation of more perforant fibers after the tetanization. If so, the posttetanic increase in the population spike might be an artifact. In my own studies, I had observed that the perforant path fibers run as through a bottleneck in the angular bundle before they fan out to excite the dentate gyrus along narrow, nearly transverse strips of tissue along its entire dorsal extent. We therefore changed our approach and for all the reminaing experiments placed the conditioning, tetanizing electrode up front to stimulate only a narrow strip of granule cells in what we called the experimental pathway and the test electrode some distance away in the bottleneck of the angular bundle to stimulate together perforant path fibers both in the experimental pathway and in a more lateral control pathway not receiving tetanization. Obtaining comparable LTP with this approach, we became convinced that the phenomenon was genuine. There was now no longer any reason to think that the test stimulus had activated more axons in the experimental pathway than in the control pathway.

We also routinely measured not only the size of the population spike but also its latency, and most important, the population EPSP in the molecular

layer. We knew that this was monosynaptic and by measuring its rate of rise and amplitude no more than 1 ms after its onset we could be reasonably sure that it reflected the synaptic current evoked by the perforant path volley uncontaminated by other activity. In this way we convinced ourselves that the observed potentiation was real and occurred at the synapses that perforant path fibers make with the dendrites of the granule cells. Nevertheless, there was much variability in the result, as described in Bliss and Lømo (1973), from one stimulus to the next and from one animal to the next in the size and duration of the potentiation. Interestingly, the varibility from one stimulus to the next was much reduced after induction of LTP. In some animals, the spike latency was shorter than accounted for by EPSP potentiation, and in some cases spike potentiation could occur without EPSP potentiation (this uncoupling of spike and EPSP potentiaton was later termed EPSP-to-spike potentiation or E-S potentiation). Why we had such variability, we did not know (but see the next section).

The Paper (Bliss and Lømo, 1973)

We did our last experiments in the summer of 1969 and submitted the paper based on them early in 1973. There were several reasons for this delay. I was finishing my thesis and preparing to defend it in October 1969. Tim was busy finishing other work in Per's lab. Soon after my thesis we both left Oslo for London. Tim returned to Mill Hill. I went with a fellowship from the Wellcome Trust to work for 1½ years on the nerve-muscle preparation at the Department of Biophysics, University College London after a letter from Per to Ricardo Miledi had elicited an invitation to work with him. There were still experimental records in Oslo waiting to be analyzed. Things moved less fast in those days, there was no immediate interest in our results (see later), and neither Tim nor I could have felt any urgency for a rapid communication. Moreover, both Tim and I tended never to be fully satisfied with our drafts and would go on and on, intermittently, trying to make improvements. Until one day, according to Tim, Tony Gardner-Medwin took matters in his own hands. Tim and Tony had earlier completed a study in Tony's lab in the Department of Physiology, University College London, similar to ours but on unanesthetized rabbits. In exasperation at our procrastinations, Tony finally and in a short time came up with a nearly submission-ready paper describing their work. This must have pushed Tim and me to a final spurt of energy, and both papers were submitted together to the *Journal of Physiology*. Two or three weeks later, and I cite Tim: "Bliss was startled to be informed by the *Journal of Physiology* referee, Bernard Ginsborg, who had taken the unusual step of telephoning him from Edinburgh to discuss 'this interesting work,' that while the second manuscript was fine as it stood, the first, Bliss and Lømo, showed signs of having been hurriedly written."

Bliss and Lømo (1973) was reprinted in the *Journal of NIH Research* in 1995 as a landmark paper. There, Roger Nicoll writes: "So, the question is, Why did this paper start this dramatic field? First of all, it describes all of the basic phenomena of the process of long-term potentiation. These include pathway specificity, saturation, and an increase in the coupling of the synaptic potential to the discharge of the granule cells. Second, there's not a single controversial result in that paper—a very remarkable thing in this field." Pathway specificity was not directly demonstrated in our work in the sense that synapses belonging to inactive inputs to the same target cells were shown to be unaffected. That demonstration would come later in slices. But we had indirect evidence, which Tim, Tony, and I published in a book article in 1973. We plotted stimulus response curves before and after tetanization and showed that there was an increased slope after the induction of LTP, but only for stimuli with an intensity equal to or less than the intensity used for the tetanization. After that, the curves were parallel. This, we argued, implied input specificity because at higher intensities nontetanized axons were being recruited, and these, as indicated by the parallel curves, had not been potentiated.

The Reception

In 1969, at a NATO-sponsored summer school in Varenna, Italy, we presented our work as students to many of the top names in neuroscience at that time without causing any obvious excitement. As late as 1981, LTP was not mentioned in the textbook *Principles of Neuroscience* by Kandel and Schwartz or in Alf Brodal's *Neurological Anatomy* despite Brodal's extensive treatment of the hippocampus in his book. Interest in LTP began in earnest in the first half of the 1980s when G. L. Collinridge, P. Ascher, G. Lynch, and others discovered that LTP in CA1 requires both activation of postsynaptic NMDA receptors by glutamate and sufficient postsynaptic depolarization to remove the Mg^{2+} block of NMDA receptors, and that postsynaptic injection of calcium chelators blocks the induction of LTP (see Bliss and Collinridge, *Nature*, 1993, review).

A few scientists did show an early interest in our work. John Eccles, visiting Per's lab at the end of 1968, was very excited. He had looked unsuccessfully for such plasticity in the spinal cord and in his book *The Physiology of Synapses* (1964), he writes: "Perhaps the most unsatisfactory feature of the attempt to explain the phenomenon of learning and conditioning by the demonstrated changes in synaptic efficacy is that long periods of excess use or disuse are required in order to produce detectable synaptic changes." Our results were just what he had been looking for but in the wrong system. He incorporated the results into his own thinking about the brain and presented some of our results and figures as unpublished results in books that he wrote at the time, one of them with Karl Popper, *The Self and Its Brain* (Springer

International, 1977). Other persons showing an early interest were Gary Lynch, Graham Goddard, and Bruce McNaugthon. I first met Bruce, then a Ph.D. student with Graham Goddard, at a summer school in 1973 in Erice, Sicily, where Bernard Katz had set up the scientific program. I remember Bruce at lunch, after a lecture I had given, asking many questions and wanting to discuss the implications of our findings.

Thoughts on the Function of the Hippocampus as Perceived in the 1960s

At times during my Ph.D. work people would ask, Why do you work on the hippocampus? What does it do? and I was embarrassingly short of answers. I knew that its main output, the fimbria, projected to the hypothalamus, so I said it probably participated in the control of the endocrine and autonomic systems. The older idea that it served olfaction, reflected in the term *rhinencephalon*, was out, as convincingly argued by Brodal in his book *Neurological Anatomy*. Citations from the latest edition of that book in 1981, pages 683–685, illustrate the dearth of knowledge: "The anatomical complexity of the hippocampal formation makes it clear that it will be difficult—and perhaps impossible—ever to define satisfactorily the 'function' of the hippocampus or any other part of the hippocampal formation. One type of theory suggests that a major function of the hippocampus is to modulate motor control systems directly. The second type of theory suggests that the major function of the hippocampus concerns some non-motor behavioral or psychological process (Black, 1975). Some authors have concluded that a main function of the hippocampus is to exert a nonspecific inhibition of emotional reactivity in general."

In fact, I did not really care much about "the function" of the hippocampus. The structure had its own intrinsic beauty, first displayed by the drawings of Ramon Y Cajal and Lorente de No, later by the anatomical studies of Blackstad and his students; and a simplified cortex well suited for electrophysiological approaches, as exploited early on by Per, and appreciated by Tim from afar, a structure ready to reveal its secrets. On joining a lab, one does what that lab is good at, and so, in Per's lab, I soon became fascinated by the internal workings of the hippocampus, in particular the dentate gyrus, as reflected in the title of my thesis: "Synaptic mechanisms and organization of the dentate area of the hippocampal formation." Answers to what the hippocampus does, I felt, would come in due time and were of little concern to me then.

Why did Bliss and Lømo not refer to Hebb? many have asked, and McNaughton was "amazed that neither Hebb nor Marr was referred to in our paper" when he first saw it on his return from Erice in 1973 (see earlier). In the book chapter that Tim, Tony, and I published in 1973, and where we review and discuss our findings from both anesthetized and

unanesthetized rabbits, we do refer to both Hebb and Marr together with several others as follows: "Synapses with this sort of properties, in one guise or another, feature in many theoretical discussions of learning."

I knew about H. M., the patient with intractable epilepsy treated in 1954 by bilateral resection of the temporal lobes, including the hippocampal formation, who lost the ability to remember events that had occurred minutes earlier. This knowledge and related knowledge from other reports of memory dysfunctions after damage to the hippocampus probably expain that I did refer to theories of learning in describing my findings (see earlier). But again, things appeared less clear then than now. Citing Brodal again:

> The belief that the hippocampus is involved in memory functions is based chiefly on experience with patients . . . Autopsy was carried out on only a few of the patients, and convincing evidence has never been produced that damage to the hippocampus is responsible for the loss of recent memory that occurs following bilateral removal of the medial part of the temporal lobe (see Horel, 1978, for references). Are the memory disturbances due to affections [*sic*] of non-hippocampal parts of the temporal lobe? Horel (1978), who discusses these relations in an extensive study, produces good arguments for an affirmative answer to this question. No final conclusion is as yet possible about the structures in the temporal lobe whose damage results in loss of recent memory. However, at present evidence for a close relation of the hippocampus to memory function is rather weak. There appears to be a growing skepticism about theories that consider certain brain regions specifically related to memory. Perhaps the most important common factor is a general disturbance of brain function. There are interesting features in common between the search for "the site of memory" and the search for "the site of consciousness," discussed in Chapter 6.

Brodal then cites Weiskrantz (1978): "The striking aspect of the hippocampus is the anatomical elegance of its structure, revealed in detail in the past few years. In contrast there is really appalling ignorance about what this elegance means" (pages 686–689). In our 1973 paper Tim and I refer to the hippocampal formation as "a region of the brain which has been much discussed in connection with learning and memory," and give some references, although we do not specifically refer to Hebb or the patient H. M.

Hebb's *neurophysiological hypothesis* (1949) famously starts with the words, "When an axon of cell A is near enough to excite a cell B. . . ." It has always surprised me that this formulation is treated with such reverence among neuroscientists. I did not know about the hypothesis in 1966 and I don't see now that it would have made any difference to what I did then or

Tim and I would do later, based as it was on speculation without evidence. Many scientists at the time and before, Konorski, Eccles, Burns, Bliss, and others, believed that memory and learning had to involve long-lasting changes in synaptic efficacy and some looked for such changes in structures outside the hippocampus without finding them. Some studied the effects of high-frequency stimulation in the hippocampus without looking for aftereffects (Cragg and Hamlyn, *J. Physiol.*, 1955). Others did look for aftereffects but found that they lasted only up to some minutes, too short to be of relevance to learning and memory (Green and Adey, *EEG Clin. Neurophysiol.*, 1956; P. Gloor et al., *EEG. Clin. Neurophysiol.*, 1964; Andersen, *Acta Physiol. Scand.*, 1960; Andersen et al., *Acta Physiol. Scand.*, 1966).

What Hebb did, in my view, was to formalize some current ideas with an apparent rigor that presumably helped his theory to become widely accepted. It did not have any obvious relevance to the homosynaptic potentiation that Tim and I described. It emphasized a requirement for coincident pre- and postsynaptic firing, although later work showed that such firing is not necessary. For example, in our own experiments, brief conditioning 100 Hz trains induced LTP despite failure of granule cell firing after the first stimulus due to the high frequency-induced depolarization and spike inactivation. It did not describe the more interesting model where two cells converge on a third so as to explain associative conditioning between weak and strong inputs, later to be amazingly well explained by the discovery of NMDA receptors requiring strong depolarization (the strong input) to allow glutamate from the weak input to open NMDA receptors and to allow influx of calcium for strengthening of the weak input. Hebb himself expressed surprise at so much excitement about this particular part of his theory. The idea was, he said according to McNaughton, "an old one, dating back at least to Lorente de No, and the principle was obvious to anyone who had considered the principles of associative learning. Something like cooperativity had to be present in the nervous system." Would the enormous progress of recent years in uncovering mechanisms of learning and memory not have occurred if Hebb had not formulated his hypothesis? I doubt it.

Failing to Reproduce Bliss and Lømo (1973)

When Bliss and Lømo (1973) appeared, I had already left the field. This had not at all been my intention. Arriving In London in 1969, I joined Tim at Mill Hill about once a week to continue our work. To our dismay, LTP was now far less impressive and often absent. We could not bring the experiment under sufficient control for further systematic analysis and had to abandon the project. We considered importing rabbits from Norway. Perhaps they were smarter. But this idea, only partly a joke, had to be discarded when similar difficulties arose in Oslo when I returned there in 1971. Back in Oslo, I was joined by Tony Gardner Medwin to continue work on LTP. Tony had recently published a theoretical paper in *Nature* entitled

"Modifiable Synapses Necessary for Learning" and, with Tim Bliss, had just finished experiments in his own lab that demonstrated LTP in the unanesthetized rabbit lasting up to 3 days after a single conditioning train of stimuli and, in one animal, lasting several weeks after a series of such trains. Again, what little LTP Tony and I saw was modest and highly variable. We tried all we could think of to improve the situation, but to no avail. I never worried that what Tim and I had found was not true. One cannot begin to doubt something one has seen over and over again. But how to explain it?

Until recently, I had no idea how to explain it. But now, papers have appeared showing that stress markedly suppresses LTP, as observed in vivo and in slices from stressed animals, effects mimicked by administration of corticosteroids. The rabbits that Tim and I studied were bought from a local farmer for the equivalent of about £3 and brought to the institute by the farmer. Perhaps his rabbits were less stressed than the rabbits I studied with Tim in London or with Tony later in Oslo. Differences in animal strain, age, handling, and previous history are all factors that affect an animal's response to stress and might be responsible for the variability in LTP that Tim and I also observed (see earlier). Perhaps such factors also explain why Per and others studying frequency potentiation in the hippocampus before the LTP era failed to see long-lasting aftereffects. I cannot know, of course, but it seems plausible. It strengthens the impression that LTP, like learning and memory, is a rich and subtle phenomenon sensitive to a variety of modulators.

Failing to study LTP in vivo, Tony and I then tried to do so in slices of the hippocampus. Across the corridor in Per's lab, Knut Skrede, who had worked with Tim and Per on the lamellar and population spike experiments, and was taking a year off from his medical studies, had set up such a technique. Skrede had learned the technique from Chris Richards during a visit to Tim at Mill Hill. Richards, in turn, had come from the lab of McIlwain who, with Chosaburo Yamamoto, had pioneered the technique. Skrede's setup consisted of a metal cooking pot containing the perfusion fluid, an aquarium heater bought from a local hardware shop, a silk mesh floating on the surface to support the slices, and a humid gas mixture of O_2+CO_2 blowing over the slices. He was later joined by Rolf Westgaard and together they did the experiments that showed for the first time that the entire trisynaptic circuit, perforant path to dentate gyrus to CA3 to CA1, survived in the transverse slice and could be activated in sequence by stimulating the perforant path. For most of this time Per was away on sabbatical in Charles Philllip's lab in Oxford and was not, as I understand it, directly engaged in the project. Yamamoto (1970) introduced the transverse slice, but not in the context of the trisynaptic circuit. How the idea to study the trisynaptic circuit in slices arose is not clear but from the lamellar organization of this circuit already demonstrated, it was a natural thing to do.

Tony and I set out to improve on Skrede's setup, or so we thought, by submerging the slices in a small perfusion chamber that we placed on the

stage of a high-power compound light microscope for transmitted light. Layers, cell bodies, and dendrites now stood out in much greater detail than with a low-power dissection microscope and incident light, beautiful to see and useful for more precise placements of electrodes. But again, we failed. The excitability of the submerged slices remained poor. Perhaps we did not master the technique of cutting the slices by hand, as we had to in those days, or the slices did not like being submerged, not getting enough or too much oxygen, which we tried to vary as best we could. I did not want copy the setup across the corridor because I liked ours and because I sometimes heard sudden sharp noises from their loudspeaker. It turned out that drops of water condensed on the outside of the recording electrode and every time it dropped or flowed down onto the slice, bursts of spike discharges, amplified by the loudspeaker, resounded in the air. Impressed by this observation, Tony and I started to dilute our perfusion fluid with distilled water, and for every dilution the excitability in the slice improved. But when we reached near half-normal tonicity, we felt we were studying pathology rather than physiology and gave up. Skrede and Westgaard (1971) even refer to this finding as follows: "Preliminary experiments indicate that the excitability of the preparation in vitro is highly dependent on the ionic composition of the bathing medium (Gardner-Medwin and Lømo, personal communication)." After all this travail, Jan Jansen came back from a sabbatical at Harvard and suggested collaboration on a problem related to my work in London on the nerve–muscle preparation. That work left a mine of interesting problems that appeared much easier to address than LTP. So I switched to explore that mine for the next nearly 40 years, while Tony, who wanted to stick with brain, went on to study on his own, and for the rest of his time in Oslo, impulse conduction in parallel fibers in the cerebellum, work that was published in the *Journal of Physiology*.

While Tony and I struggled in Oslo, Tim did likewise in London. He tells me that he and Chris Richard were unable to demonstrate LTP in the dentate gyrus in vitro, because, as became clear later, they did not add bicuculline, which was needed to block the tonic inhibition that prevented LTP from developing (though not, luckily for the transverse slice, in areas CA3 and CA1). Tim then turned to other problems until Graham Goddard came to visit Tim in 1974. He was then on sabbatical with John O'Keefe but came to Tim at Mill Hill for a few days to look for LTP in rats. In the one rat they tried, they did not see any LTP but when Goddard returned to Halifax he did, and in collaboration with Rob Douglas, the study of LTP in vivo, now in rats, was born again.

Concluding Remarks on the Discovery of Long-Term Potentiation

Concluding these recollections about LTP, I note that the events just described took place 40 years ago. Few notes remain, no diaries, a few letters,

no records from the experiments that Tim and I, or Tony and I, did. To my regret they must have disappeared, unintentionally, in 1990, when our institute moved from the old University buildings downtown to new buildings in the suburbs of Oslo. Papers and records related to the work for my thesis I did keep because it was always my intention some day to come back to them and publish the papers so far unpublished, as I finally did in 2009. As I write, questions arise: What did I or people central to the story do then? What did they say? Whose idea was it? And repeatedly I am unable to answer because I have forgotten. Again, as I was writing, my wife came with some letters I wrote to her from London in 1971 when I stayed on alone for a few months to finish the work there. Reading them now brings back memories that would have been gone forever without these letters. What better illustration of the value of letters and diaries and of the regrets one feels at not having been more disciplined and forward looking when interesting things happen in one's life? And how difficult it is, without such records, to be sure that one's recollections are reasonably close to what actually happened.
With such thoughts in my mind I will try to answer a question Tim has asked: Would Oslo have followed up my observations in 1966 if he (Tim) had not come to the lab in 1968? Probably not. I might well have wanted to return to my early observations of long-term changes but would hardly have had the time considering the direction my Ph.D. work was taking (see earlier). I would have finished my thesis and gone to London for further training. There, as it turned out, the answers I obtained to different problems on a different preparation were received with much greater interest than any results relating to LTP (see later discussion). On my return to Oslo, would I then have chosen the hippocampus over muscle, with the success in London behind me and without the work that Tim and I did together? Hardly.

Without Tim's year in Oslo, Knut Skrede would almost certainly not have gone to Mill Hill in the summer of 1970, where he learned the slice technique and was inspired to set it up in Oslo. The purpose of his visit was to write a paper with Per and Tim as coauthors, and it was Tim's suggestion that, when there, he might be interested in learning the slice technique. At that time Tim and Richards used "longitudinal" slices. As Tim writes: "the hippocampus was laid out flat and a slice was cut that contained the whole septal-temporal extent of the lower blade of the dentate gyrus." How it came about that Skrede chose the transverse slice, containing the pyramidal cell fields in addition to granule cells, is not clear but as already noted it was a very natural thing to do at that stage. Without this background, Schwartzkroin and Wester would probably not have demonstrated LTP for the first time in slices in 1975 in Per's lab.

As far as I can remember and understand from talking recently with those involved, Per did not directly engage himself in these early projects on LTP or on the slice. That would come later. We were doing our projects. Per was busy with his. He provided essential support but did not interfere with

the way we did the experiments and we were very grateful for this. I felt that he considered our results on a par with other results obtained in the lab at the time. So I think the answer to Tim's question must be that neither Per nor I would have followed up my findings from 1966 had Tim not come to the lab in 1968, at least not for some years.

One of the more rewarding experiences of my involvement with LTP has been the lifelong friendship that Tim and I developed from our first days together, the fun of working together, the satisfaction of discovering how easily we agreed on how to do things and view the developments in the field, and, above all, especially now that LTP has become so important to neuroscience, that our memories and interpretations of what happened then match so well. Wonderful also that now 40 years after our last experiments together we are collaborating again, setting up experiments to try to solve some unsolved problems from back then. What will come of it remains to be seen. Time is running short.

London (1969–1971): The Trophic Hypothesis

I arrived in London in October 1969 with Anne, three children between 1½ and 7 years of age, and an au pair, a young girl from our neighborhood in Norway. We stayed in a hotel in Gower Street with money running out and the au pair desperate with homesickness. After 3 or 4 days, the girl already on her way home, Tim came to our rescue. Together we looked at apartments for rent and after about a week we were nicely settled in a small but charming "cottage" in Hampstead Garden Suburbs.

Ricardo Miledi suggested that Jean Rosenthal and I should team up in a small lab down the corridor on the fifth floor of the Department of Biophysics. Jean had just arrived from Yale after a Ph.D. with Bob Martin. Other labs down the corridor housed Nick Spitzer, Wolfgang Grampp, John Heuser, Dale Purves, Bill Betz, Bert Sakmann, Lincoln Potter, Mike Dennis, among others. Miledi suggested that Jean and I should try to block impulse conduction in the sciatic nerve of rats without damaging the nerve and causing denervation. In normal muscle fibers the sensitivity to transmitter acetylcholine is restricted to the neuromuscular junction. But after denervation the sensitivity increases dramatically along the entire length of all the muscle fibers (acetylcholine supersensitivity), as was well known at the time. By blocking nerve impulse conduction and paralyzing the muscle without causing denervation, we might find out whether the supersensitivity was caused by lack of impulse activity in the muscle or a putative motor neuron-derived trophic factor believed at the time to be released from the nerve terminals at neuromuscular junctions. Miledi had already done experiments, mainly in the frog, which he interpreted to mean that the supersensitivity occurred because denervation (axotomy) interrupted the flow of the trophic factor and not because it interrupted nerve-evoked muscle impulse activity.

In their classic work on the effects of cross-reinnervating fast and slow muscles, Buller, Eccles, and Eccles (*J. Physiol.*, 1960) discussed whether alterations in impulse activity or trophic factors were responsible for the observed changes in muscle contractile speed. They favored the "trophic hypothesis" and presented a figure showing schematically how the putative trophic factor travelled down the axon of a motor neuron, crossed the synapse, and continued travelling intracellularly to either end of the muscle fiber. Regarding the acetycholine supersensitivity caused by denervation, Eccles wrote as follows in his book *The Physiology of Synapses* (1964, p. 246): "In summary of the preceding section it can be stated that the evidence for a trophic influence from nerve on to muscle membrane is conclusive, but it is still uncertain whether it is entirely effected by unique trophic influences or whether in part by the spontaneous emission of quanta of acetylcholine. The trophic influence apparently travels along the motor axon with a velocity ranging from 1–2 mm/hour." This being the accepted idea of the day, one might expect that muscle fibers paralyzed by blocking nerve impulse conduction would retain normal sensitivity to acetylcholine, as predicted by the trophic hypothesis. Some distinction, however, is in order. Since the heyday of the trophic hypothesis, neural agrin has been established as a substance that induces and maintains the neuromuscular junction after axonal transport and release from motor nerve terminals. However, agrin is not considered trophic, as the hypothesis was understood then because its actions are restricted to the neuromuscular junction. Target-derived substances acting retrogradely on motor neurons also fall outside the trophic hypothesis.

At the Institute of Neurology, Queen Square in London, research on demyelination was ongoing, and Jean and I heard that diphtheria toxin was used to induce it. As a first approach, we therefore injected diphtheria toxin under the perineurium of the sciatic nerve in rats. A week or so later, the leg on the injected side was paralyzed and the muscle fibers were supersensitive to acetylcholine, like the supersensitivity observed after denervation. And yet spontaneous acetylcholine release (MEPPs) and muscle responses to stimulation of the sciatic nerve below the injected site were essentially normal. Apparently, apart from the conduction block, the axons were intact. Then one day, Steve Roper in Jack Diamond's lab in the next building came over and told us about a paper by Robert and Oester (*J. Pharmacol. Exp. Ther.*, 1970). This paper reported that in rabbits no acetylcholine supersensitivity was observed in muscles distal to a silicone cuff mixed with lidocaine, a local anesthetic, around the sciatic nerve despite complete impulse conduction block and muscle paralysis. This seemed a more direct and controlled way of blocking impulse conduction than using diphtheria toxin and we immediately adopted it. And again, with this technique too, full acetylcholine supersensitivity appeared in muscle fibers that appeared normally innervated. Later, Jean tried to replicate the findings of Robert and Oester in the rabbit without getting a clear answer. The experiment was more

difficult on the larger nerve and muscles of the rabbit. In Lømo and Rosenthal, 1972, we therefore wrote: "So far, we have no explanation for this contradictory finding [Robert's and Oester's], but only note the species differences."

The Importance of Denervating Muscles and Stimulating Them Electrically

Denervation-like acetylcholine supersensitivity in muscles innervated by "silent" but otherwise apparently intact nerve fibers suggested that spontaneously released acetylcholine or some unknown factor might not be responsible for the acetylcholine supersensitivity as postulated by the trophic hypothesis. But we could not be certain because blocking impulse conduction might also affect axonal transport. The next step was obvious. We would have to remove all neural influences by cutting the nerve and substitute nerve impulse-evoked muscle activity by direct stimulation of the muscle through implanted electrodes. If such muscles became insensitive to acetylcholine outside the denervated endplates like normally innervated fibers, evoked muscle impulse activity would have to be the essential factor. This was the critical experiment, and I cannot remember that we discussed it with Ricardo at that time. When things started to go well, we were left to ourselves and liked it that way.

We were into January, 1971, before we had our first successful experiments with direct stimulation of denervated muscles. There had been several difficulties. Ordinary electric wires under the skin invariably broke, usually within 1 day of implantation due to the rat's movements. I remember going to a shop in nearby Tottenham Court Road selling hearing aids to get flexible multistranded wires, which solved the problem. Polarization of electrodes was another problem, which was solved when we began using stimulus pulses that were only 0.1 ms in duration, unusual at that time for stimulating denervated muscle, and switching polarity between each train of stimuli. More of a nuisance than a problem was the observation that the rats under stimulation in the lab tended to move around more in one direction than the other. We therefore spent much time "unwinding" the wires that connected the rat to the stimulator on the shelf above it, done also outside "normal" working hours since intermittent stimulation went on day and night for up to 2 weeks. Today, the use of commercially available rotating contacts easily solves this problem. For stimulation of the soleus muscle we ended up using platinum wires inside a silicone cuff that we molded to fit around the Achilles tendon, while the most distal portion of the muscle was activated with the short platinum wires connected to the hearing aid wires that ran under the skin to a plug attached to the skull with screws and dental cement. I remember contacting Patrick Wall in the next building to hear about how they attached wires to record from rats that moved about. I also

visited John O'Keefe who was developing his technique for recording from single cells in the hippocampus of freely exploring rats, cells that he later called place cells.

Then, finally, one day in February we had a group of four or five rats, each in its own wide plastic bucket with its sciatic nerves cut in both legs and the soleus muscle in one leg apparently well stimulated for at least 5 days. I remember well the first experiment. I removed both soleus muscles, placed them side by side in a perfusion chamber, inserted a recording electrode into a surface fiber of the denervated unstimulated muscle and, on the outside of that fiber, positioned a micropipette containing 3M acetylcholine chloride. Moving the electrode to different positions along the length of the fiber and at each position passing a short electric pulse through the electrode to eject acetylcholine, I evoked large depolarizing endplate-like potentials along the entire length of that fiber and others tested in the same way, as expected from the supersensitivity known to occur after denervation. In the other denervated muscle, however, which had been chronically stimulated, I got no response. Could there be something wrong with the electrode? A broken pipette tip, leakage of acetylcholine, and desensitization of acetylcholine receptors in the muscle fiber membrane were common problems. How reassuring then that subsequent tests on the unstimulated muscle, using the same pipette, evoked the same large responses as before. I then returned to the stimulated muscle fibers, moved the acetylcholine-containing pipette along the fibers, and saw no response except at one restricted site on each fiber that corresponded to the denervated endplate. The distribution of acetylcholine sensitivity at such sites was in exact agreement with that observed at normally innervated endplates but MEPPs were absent because these endplates were denervated. The result was enormously exciting. The difference between stimulated and unstimulated fibers stood out like the difference between night and day. In one stroke, existing understanding seemed turned upside down. Absence of a nerve-derived "trophic factor" could not be responsible for the acetylcholine supersensitivity of denervated muscle. The controlling factor had to be evoked muscle impulse activity.

Immediately, however, the question arose: Is the result reproducible? The following day, Jean examined the next rat in the series. I remember walking up and down in the corridor outside the lab nervously awaiting the result. To my immense relief, Jean got the same result. Thereafter, it was relatively smooth sailing. Chronic stimulation starting at the time of denervation not only prevented acetylcholine supersensitivity from appearing but made the supersensitivity disappear when the stimulation started after it had fully developed. The denervated extensor digitorum longus responded similarly to direct stimulation. While many denervated stimulated fibers were indistinguishable from normally innervated fibers (except for the absence of MEPPs), others displayed varying degrees of supersensitivity in some stimulated muscles. In some of these muscles it was obvious that the

implanted electrodes had not stimulated effectively all the fibers. But, as would become clear later (see later discussion), by causing peripheral nerve branches to degenerate, the axotomy also elicited an inflammatory response in the muscle that in itself could induce some degree of acetylcholine supersensitivity, thus counteracting the effect of stimulation. Finally, we showed that stimulating the sciatic nerve through electrodes implanted on the nerve below a paralyzing block also prevented supersensitivity from appearing. But this result could not answer what to us was the fundamental question: Was there or was there not a factor carried by axonal transport as postulated by the trophic hypothesis? Only by removing the nerve altogether and replacing nerve-evoked impulse activity with stimulation-evoked impulse activity could we get an answer and the answer seemed unequivocal. It had to be muscle impulse activity.

Life in the Department of Biophysics

Jean and I started out doing experiments together but found it difficult. Together in the small lab with both of us having our hands on the experiment, we got into arguments about who was to do what next. Since one person could easily do the experiments, we therefore early on decided to split the project between us. Jean would address some aspects of the project. I would address others. She would have the lab to herself on some days, and I would have it on other days. Jean took care of all the electron microscopy showing that neuromuscular junctions belonging to axons in cuffed sciatic nerves appeared structurally normal. She worked on replicating the results of Robbin and Oester and began studying effects of our interventions on muscle contractile properties. I developed the approach for stimulating denervated muscles and did most of the tests for acetylcholine supersensitivity until I returned to Oslo at Easter in 1971. We both worked at writing up the bulk of the paper and, when I left, Jean finished it after consultations with Miledi. There were also further stimulation experiments to be done. I was very happy with our arrangement. It allowed us to stay on friendly terms, enjoy each other's company outside the lab, and to collaborate effectively to finish the work.

I very much enjoyed my time at the Department of Biophysics. Considering the stimulating research environment and the good outcome of our work, it was also unusually rewarding. I remember the "time off" periods in the department's seminar and lunchroom around 11 o'clock when people from the labs along the corridor would come in for coffee or tea and chats. Miledi was often there, a source of stimulating and interesting conversations. Katz came in rarely, and I remember being disappointed when he let us understand that he was not interested in "talking shop" there. He would rarely, if at all, discuss his own or other people's work at coffee time. At that time Katz and Miledi were studying acetylcholine-induced membrane

"noise," but I did not know about this important work before it was published in *Nature*. Katz received the Nobel Prize in 1969 and some time after the announcement I remember him coming into our lab asking us how we were doing and what we thought about some more general aspects of science. Otherwise, I saw little of Katz during my time there. I am sure he would have been very helpful had we needed help, but for me our problems appeared too trivial and were mainly of a practical nature that we had to sort out ourselves.

We hesitated to tell Miledi about our results. They stood in such contrast to his own results and interpretations that I felt awkward at breaking the news. However, when a moment came at coffee time, his response was short, and as far as I can remember, something like: Oh, I had expected it to be different. Later, when an abstract was being prepared for a talk that Jean would give in Paris and later still, when the full paper was being completed, relations with Miledi became somewhat difficult. We could not agree on how references to earlier literature should be phrased or on the wording of some other aspects of the paper. In the end, Miledi did not want to have anything more to do with the paper because, as I remember Jean telling me, "We did not listen to him anyway."

The day before I left London Jean and I had an appointment with Katz to discuss our work. However, when Katz learned that we had not yet written up the Discussion, he seemed irritated, commented that the Discussion is an important part of any paper, and left me at least with the impression that we were wasting his time. Hence the meeting became quite short as I said we would be back when the paper was closer to completion. On the last day of my 1½ years at the institute as I stood wating for the lift with a bag containing some of my belongings from the lab, Katz emerged from the lift together with John Eccles and Stephen Kuffler. Seeing my bag and addressing Eccles and Kuffler, he then said something to the effect that "there go our fine scissors". Perhaps some people did take with them expensive instruments when they left the department but I hardly liked the suggestion that I would be one of them. Perhaps I was being considered "difficult" by Katz and Miledi since we did not agree with Miledi about how to write certain parts of the paper (see earlier discussion), and perhaps this accounts for what I felt was a rather odd farewell.

The Reception of Our Results on the Trophic Hypothesis

Our results were immediately well received by neurophysiologists not studying the trophic hypothesis, and less well by others who did (see later discussion). The issues involved were well known and the significance of the results promptly appreciated, in contrast to the reception of the results on LTP (see earlier discussion). I remember being invited to give a lecture on neural control of muscle properties at a high-profile meeting in Oxford in

1972 or early 1973. I suggested to the organizers that perhaps I could talk about some interesting unpublished results from the hippocampus instead, but it was the muscle they wanted to hear about. I find it interesting that while Lømo & Rosenthal (1972) was received with immediate interest but now seems largely forgotten, Bliss & Lømo (1973) took many years to be widely recognized in a way that now seems to endure.

In the following I describe some examples of the criticisms that were leveled against our experiments in London and later in Oslo because I think they contain a lesson. Today, it seems clear that the trophic factor postulated by so many does not exist (see later discussion). It may therefore be instructive to go over the evidence that led to the trophic hypothesis and see how they now can be given alternative more plausible explanations.

R. J. Blunt and G. Vrbova (*Pflügers Arch.*, 1975) reported that the local anesthetic used in our cuffs was too toxic, that such cuffs produced extensive denervation, and that the effects were irreversible. However, we had noted the problem of partial nerve degeneration in our paper. In our experiments, the effect was reversible in cases where there was little or no degeneration. The presence of some degeneration was not important for the conclusions we made, and subsequent work by others and us fully confirmed them. As it turned out, the addition of a local anesthetic to the cuff was unimportant. The long-term block was caused by nerve compression and occurred (with a delay) also with plain cuffs, as used later. The critical part was the diameter of the cuff's central canal that accommodated the nerve; slightly too wide and no block occurred, slightly too narrow and much nerve degeneration ensued.

S. S. Deshpande, E. D. Albuquerque, and L. Guth (*Exp. Neurol.*, 1976), concluding that "both the prejunctional nerve membrane and the postjunctional muscle membrane are regulated by a neurohumoral factor," made the following comment: "Furthermore the experiments using prolonged electrical stimulation [Lømo and Westgaard, 1975; Westgaard, 1975] can be criticized because they were performed on chronically denervated muscles. The denervated muscle fiber is a cell that has been released from many of its physiological regulatory controls; given our present state of knowledge one cannot use such pathological tissue to make inferences about the role of muscle activity on physiologically normal muscle fibers; this experimental approach appears to us to be inappropriate for the study of trophic nerve function." This seemed an odd criticism. To me it was perfectly in order, following common research practice, first, to remove some unidentified factor and, then, add a specific, identified factor to see to what extent it could replace the unidentified one and restore normal function. In our case, direct muscle stimulation did restore normal nonjunctional properties, not only with regard to acetylcholine sensitivity but also, as later work showed, with regard to a host of other nonjunctional properties.

V. Witzeman, H. -R. Brenner, and B. Sakmann (*J. Cell Biol.*, 1991), comparing the effects of denervation, direct muscle stimulation, and nerve blockage by botulinum toxin, α-bungarotoxin, or tetrodotoxin on acetylcholine receptor subunit expression in muscle, concluded that "levels of the γ-subunit in the entire fiber are reduced by a negative neural factor and possibly also by nerve-induced electrical muscle activity." This conclusion surprised me because Brenner had earlier shown that stimulation of denervated muscles blocked the expression of γ-subunits, a necessary component of the extrajunctional acetylcholine receptors induced by denervation and underlying the acetylcholine supersensitivity that Jean and I had studied. This result and many others (see later discussion) have been cited as evidence for the trophic hypothesis in textbooks and elsewhere. For example, in his Chapter 10 in the handbook *Myology* (1994) Alan Grinnell writes: "Because the direct-stimulation experiments are so convincing, it is tempting to conclude that muscle activity is the only factor responsible for many of the muscle properties changed by denervation or that it is essential to their regulation. However, there is compelling evidence that activity-based effects are superimposed on at least two other mechanism of trophic regulation." He then discusses this evidence under the headings: "Stump length effects," "Nerve breakdown products," "Nerve conduction block," "Block of axonal transport," and "Pharmacological block of synaptic transmission."

Attempts to Prove the Fallacy of the Trophic Hypothesis

The evidence that Grinnell found compelling, I did not. The demonstration over and over again that direct stimulation of muscle could restore normal muscle properties outside the neuromuscular junction made me ask: What can then be the role of a hypothetical trophic factor? How then to explain the "compelling" evidence listed by Grinnell? After London, and my failure to pursue LTP, trying to answer such questions was what much of my work was about in the years that followed. And here are my answers.

But first some background is necessary. Jones and Vrbova (*J. Physiol.*, 1974) showed that a foreign body, such as a small piece of thread, placed on the surface of a muscle induced a local supersensitivity to acetylcholine underneath the inflammation at the site of the thread. Later Clarke, Slater, and I confirmed this and spent much time trying to find out what was going on without succeeding. In Pisa, Italy, Alberto Cangiano and his colleagues showed that in partially denervated muscles, fibers with intact innervation developed a moderate degree of supersensitivity to acetylcholine (and other denervation-like properties) in proportion to the degree of denervation. Moreover, when they reversibly blocked impulse conduction in the intact axons, the innervated fibers became as supersensitive as the denervated fibers. By comparing the development of denervation-like changes in denervated and in

innervated but chronically blocked muscles, they also showed that the earlier and greater effects of denervation, already well known, were transient and that after 2 weeks there was no longer any difference between denervated muscles on the one hand and innervated but blocked muscles on the other. Finally, in denervated muscles, re-innervation by chronically blocked axons did not suppress their supersensitivity to acetylcholine or other signs of denervation. Such results have two important implications. First, denervation (axotomy) results in transient changes in the muscle, probably of an inflammatory nature related to the breakdown of intramuscular nerve branches that by themselves induce acetylcholine supersensitivity and other denervation-like changes. Second, no evidence emerged that blocked but otherwise intact nerves deliver any trophic signal capable of counteracting the signs of denervation. Others disputed some of these findings or obtained contradictory results. However, in my view, Cangiano's experiments, some of which were published in *Nature* and *Science*, were by far the most careful and convincing.

Our own results were consistent with these findings. In addition, they revealed the importance of impulse activity and impulse patterns in controlling muscle fiber properties, perhaps the first demonstration of the importance of impulse patterns in controlling postsynaptic phenotypic expressions. Here is a brief summary of our findings.

1. "Fast" pattern stimulation (100 Hz for 1 s every 100 s) starting at the time of denervation prevented the development of acetylcholine supersensitivity altogether. In contrast, "slow" pattern stimulation (10 Hz for 10 s every 100 s) restored normal sensitivity only after an initial marked but transient increase in acetylcholine supersensitivity, maximal on day 3 and over by day 10.
2. Doubly innervated soleus muscle fibers were obtained by letting the denervated soleus become re-innervated first ectopically by a "fast" nerve (the superficial fibular nerve) and then at the original endplates by the original "slow" soleus nerve. In such muscles cutting the slow soleus nerve caused no supersensitivity to appear around the denervated soleus endplates, whereas cutting the fast nerve resulted in marked local transient acetylcholine supersensitivity around the denervated fibular endplates. In the first case, only the fibular nerve delivering fast activity remained intact. In the second case, only the soleus nerve delivering slow activity remained intact.

Points (1) and (2) show that fast impulse activity (high frequency) prevented the development of transient acetylcholine supersensitivity around denervated endplates, whereas slow

activity (low frequency) did not. Moreover, the result was the same whether the activity had been imposed naturally by a nerve or artificially by electrical stimulation. Evidently, fast activity was more efficient than slow activity in counteracting the expression of acetylcholine supersensitivity induced by degeneration of peripheral nerve branches in the muscle, an effect of what Cangiano and I have called "products of nerve degeneration."

3. The rate at which fully developed acetylcholine supersensitivity declines after onset of direct stimulation depends strongly on the stimulus patterns used, the decline being faster with higher frequencies than lower frequencies, and with larger amounts than smaller amounts.
4. The rate of decline also becomes slower with longer intervals between stimulus trains, being weak but still noticeable with as much as 5.5 h separating each short single stimulus train.
5. Delivering a certain number of short stimulus trains over a 6 h period every 24 h is much less effective than when the same number of trains is spread out evenly over the same 24 h.
6. Fast pattern stimulation is only effective if it starts earlier than 1 day after denervation. If it starts after 1.5 days, marked and transient acetylcholine supersensitivity follows.

Points (3)–(6) lead to the following conclusions. Acetylcholine supersensitivity and other denervation-like changes develop when a period of inactivity exceeds a certain critical duration. Even very small amounts of activity are effective provided this period is not exceeded. When exceeded, processes causing acetylcholine supersensitivity (and other denervation-like changes) are set in motion and will run their course lasting some days even as activity is reinstated. Fibrillatory activity has little effect on acetylcholine supersensitivity because the periods of inactivity are too long.

With this background it is now possible to provide more plausible explanations of the results listed by Grinnell and others as evidence for a neurotrophic factor. The acetylcholine supersensitivity observed after cutting one of two nerves to doubly innervated muscle fibers in the frog is more likely the result of "products of nerve degeneration" than interruption of any hypothetical trophic factor by the axotomy. The delay in onset of denervation changes caused by cutting the nerve far from the muscle rather than close is more likely due to the later onset of nerve terminal degeneration in muscles with the "long nerve stump" rather than a longer release time for the postulated trophic factor. Given that very small amounts of muscle impulse activity is needed to suppress acetylcholine supersensitivity in

denervated muscles (and probably even less in innervated muscles), it must be very difficult to rule out the presence of such activity in experiments based on spinal cord isolation, immobilization, or cuffs around nerves. Cangiano has convincingly demonstrated how difficult it is to completely block all axons over long times by cuffs around the nerve and how much care is needed in interpreting the results.

The use of toxins presents its own problems. For example, when colchicine is applied to the sciatic nerve, acetylcholine supersensitivity (and other denervation-like effects) appears in distal leg muscles with preserved neuromuscular transmission. Since colchicine blocks axonal transport, Albuquerque, Thesleff, and their colleagues concluded that failure of the trophic factor to reach the muscles was responsible. However, knowing the effects of direct muscle stimulation, I thought there might be another explanation. I therefore applied colchicine to the sciatic nerve in one leg, as the others had done, and like them observed the supersensitivity downstream. But I also looked at other muscles in the body and found, as Cangiano did independently, that these muscles were similarly supersensitive. Could there be a systemic effect of colchicine on muscle independent of any effects on the nerve (Lømo, Nature, 1974)? I then cut the sciatic nerve in one leg, allowed supersensitivity and other changes to fully develop, injected colchicine in the thigh (away from the nerve that had been cut anyway), and started stimulation. The result was exciting. In the presence of colchicine in the thigh, stimulated denervated muscles developed the same supersensitivity as innervated muscles, whereas in the absence of colchicine, stimulated muscles became as insensitive as normal muscles. Evidently, the effect of colchicine on the muscle had nothing to do with its effect on the nerve. Again, contradictory reports and criticisms followed but soon interest in colchicine as a way to identify activity-independent trophic effects petered out. In the case of Witzeman et al. (see earlier) they based their conclusion on comparing the effects of denervation with the differential effects of several toxins on acetylcholine receptor subunit expression. The expression of γ-subunits after botulinum toxin was unexpectedly high. Could injection of the toxin, a foreign protein, into the muscle and accompanying inflammation have boosted the effect? The expression of γ-subunits after 9–10 days of a tetrodotoxin block of the nerve was unexpectedly low. Could the block have been incomplete for some of the axons for some of the time? One does not know, but the possibility is there. So how much trust should one place on such results knowing that evoked muscle impulse activity can account for all the effects of the nerve on these properties?

Concluding this part of my story, the controversy that marked the field for many years now seems over. The essential role of nerve-evoked muscle impulse activity is generally accepted. No convincing evidence for the postulated trophic factor appears to exist. That Chapter 10 in the 1994 edition of *Myology* entitled "Trophic Interaction between Nerve and Muscle" has been

removed from the latest edition strengthens this impression. Despite many attempts to isolate a candidate trophic factor from motor nerves, no one has yet succeeded. In fact, what has been isolated usually has the opposite effect from that expected, for example, increasing the expression of acetylcholine receptors. Of course, it is difficult to prove that something does not exist. Convincing evidence may still arise in species not yet examined. Nevertheless, with hindsight I find it surprising that so many have invested so much energy in defending a concept for which there was so little direct evidence, so many plausible alternative explanations, and such strong evidence for the essential role of evoked impulse activity.

Control of Muscle Contractile Properties

The cross-reinnervation experiments by Buller et al. (1960) referred to earlier showed that muscles are remarkably plastic with respect to their speed of contraction. It was a surprising discovery, noted inadvertently when muscles were seen to contract either slower or faster than normal after an operation performed to study plasticity of synapses in the spinal cord. To explain it, Buller et al. opted for the trophic hypothesis in keeping with the thinking of the day, although the possibility that impulse activity might play a role was discussed at length. In 1972–1973, knowing the importance of impulse activity for the control of membrane properties, we began to study whether it is similarly important for contractile properties. And sure enough, during chronic "fast" (high-frequency) direct stimulation, the denervated rat slow soleus muscle became much faster than normal. The average twitch time to peak dropped from ~40 ms to ~16 ms, or close to that of the normal extensor digitorum longus. Moreover, fiber typing based on myosin ATP-ase activity revealed that considerable slow to fast transformation had occurred also with respect to the type of contractile protein expressed (Lømo, Westgaard, and Dahl, 1974). Similar effects of chronic stimulation had already been demonstrated by G. Vrbova, S. Salmons, F. A. Sreter, and D. Pette, but only for indirect stimulation through the nerve. An influence by a putative trophic factor affected by the stimulation was therefore still a possibility. To circumvent this possibility, we started to stimulate denervated muscles directly and look for effects on their contractile properties.

A detailed study (Westgaard and Lømo, 1988), using different frequencies and number of stimuli for about 2 months, showed that both frequency and number were important factors controlling contractile properties in the rat soleus. Increasingly higher frequencies for a given number of stimuli caused increasingly faster twitch speed, and greater numbers for a given frequency caused slower twitch speed. In addition, greater number of stimuli caused higher fatigue resistance. Thus, the denervated soleus maintained slow fatigue-resistant properties or turned into a fast fatigue-resistant, or fast fatigue-sensitive muscle depending on the stimulus pattern used. As a

consequence of briefer twitch durations, the tension-frequency curve moved toward higher frequencies, that is, toward the imposed frequency, suggesting that muscles adapt their speed to the firing frequency of motor neurons for optimal force output control. In fast extensor digitorum longus, studied in less detail, high-frequency stimulation maintained normal fast speed, whereas high amounts at low frequencies caused a change in twitch speed from normal ~11 ms to ~24 ms but not to ~40 ms, which is normal for the soleus. Interestingly, however, ~24 ms is the normal speed for slow type 1 motor units in extensor digitorum longus. Therefore, the transformation from fast to slow twitch speed appeared complete with respect to normal slow type 1 fibers in extensor digitorum longus but incomplete with respect to normal slow type 1 fibers in soleus.

Such findings led us to introduce the concept of an "adaptive range" subject to the following rules:

1. Muscle fibers adapt their contractile properties within certain limits.
2. These limits are different for different types of muscle fibers owing to intrinsic differences between the fibers.
3. Adaptation within these limits is controlled in a graded way by patterns of impulse activity.

Later work confirmed this concept. In collaboration with Stefano Schiaffino and his colleagues in Padova, Italy, we showed that slow pattern stimulation for 2 months maintained the type 1 phenotype in soleus but failed to induce it in extensor digitorum longus. Conversely, fast pattern stimulation maintained type 2B phenotype in extensor digitorum longus but failed to induce it in soleus (although fast types 2A and 2X were induced). In addition, intrinsic shortening velocity in soleus became only about half as fast as that in normal extensor digitorum longus, confirming the absence of the fastest type 2B fibers. Since twitch time to peak undergoes essentially complete slow to fast transformation in soleus, these results also demonstrate that individual properties within a muscle display different degrees of plasticity, or adaptive ranges. In a more recent work, we showed that intrinsic differences exist not only for different mature muscle fiber types but also for satellite cells from different muscles. Thus, muscle fibers regenerating from satellite cells in denervated soleus and extensor digitorum longus muscles acquired distinctly different phenotypes during identical fast or slow pattern chronic direct stimulation (Kalhovde et al., 2005).

We continued collaborating with Schiaffino's group in Padova until quite recently. The focus in Padova was on intracellular signaling pathways that transform muscle impulse activity patterns into gene expression patterns for the control of type and size of muscle fibers. We contributed by providing muscles subjected to different stimulation regimes.

The Importance of Patterns of Impulse Activity

Of course, we were aware that the stimulus patterns we used to begin with were quite artificial. To learn more about the natural firing patterns of motor neurons, Rune Hennig in our lab therefore embarked on a very challenging project, to record single motor unit activity continuously over 24 h from slow soleus and fast extensor digitorum longus in rats moving or resting unrestrained in their cages. The differences in firing patterns for different types of motor units were remarkable (Hennig and Lømo, Nature, 1985). In soleus, slow units with type 1 muscle fibers fired from 300,000–500,000 impulses per day often as trains lasting minutes at nearly constant frequency of ~20Hz. In extensor digitorum longus, two types of firing patterns stood out. In the first type, brief, high frequency bursts of impulses dominated. Total number of impulses per 24h was only 2600–11,200, number of impulses per burst usually only 1–6, median frequency within bursts 70–90 Hz, with the first interval particularly short, up to 250 Hz (initial doublet). Almost certainly, these units were fast fatigue-sensitive units consisting of type 2B (and probably some 2X) fibers. The second type was similar to the first type except for considerably longer trains of impulses at somewhat lower frequencies making up a much larger total number of impulses per 24 h. These units were probably fast fatigue-resistant units consisting of type 2A (and probably some 2X) fibers. With this knowledge we could let our chronic stimulation protocols mimic more precisely the natural firing patterns of motor neurons.

Torsten Eken in our lab took over studying single motor unit activity when Rune Hennig left and later became professor of neurosurgery at the University of Tromsø. Early on in this work, Eken established a close collaboration with Ole Kiehn in Copenhagen. One major focus of their work was on plateau potential generation in motor neurons, and convincing evidence was obtained that such plateau potentials generated the long duration "tonic" impulse trains in the soleus just referred to. I was intrigued by seeing such trains go on and on while the rat was resting or asleep and making no movements. Obviously, they had nothing to do with motor control. Could they be engaged in temperature control? We have explored this possibility and found that the number and duration of tonic episodes increase progressively with decreasing ambient temperature from 26°C to 17°C (T. Eken, E. Bekkestad Rein, and T. Lømo, in preparation). Accordingly, we propose that this type of tonic activity in deep-lying, well-circulated muscles such as the soleus is generated by plateau potentials in motor neurons. The tonic activity serves to fine tune body temperature around its set point by producing heat that is transferred to the blood and distributed to the rest of the body. The control goes on at normal ambient temperatures well above those that cause shivering and is a novel mechanism for body temperature control not mentioned in textbooks.

The "size principle" for recruitment of motor neurons is well known. It describes the finding that motor units are recruited in a certain order during voluntary contractions of increasing intensity, small units being recruited before large motor units. Eken's findings show that during the involuntary tonic contractions just described, the activity may switch from one unit to another in no apparent order. According to textbooks, two mechanisms participate in increasing the force output of muscle: motor unit recruitment and increased motor unit firing rates with force output increasing according to the tension-frequency relation. Our work (Hennig and Lømo, 1985, 1987) shows that there is a third mechanism based on the finding that the fastest units in extensor digitorum longus rarely fire more 1–6 pulses in brief bursts of activity. For such units in rats moving unrestrained in cages, the interval between successive impulses is constant and independent of the number of impulses in the burst. Most often there is only 1 impulse, less often 2, and so on. Consequently, there is a marked increase in force output for each additional impulse in the burst without any rate modulation. But, like many other findings, this one has disappeared from view and is never referred to, as far as I can tell.

Ectopic Neuromuscular Junction Formation: An Instructive Model

Formation of ectopic neuromuscular junctions has been a major experimental model of our lab. Fex and Thesleff (*Life Sciences*, 1967) introduced the model. They cut the deep fibular nerve and moved the proximal cut end onto an endplate-free region of the gastrocnemius. Two weeks later, they denervated the gastrocnemius by cutting the tibial nerve. After a further 2 weeks they stimulated the foreign transplanted nerve and observed strong contractions resulting from transmission at new ectopic neuromuscular junctions. The important stratagem here was to allow time, 2 weeks in this case, for the transplanted nerve to grow to the "starting line" for synapse formation, then to denervate the muscle by cutting the original nerve to induce receptivity to ectopic innervation with the result that new neuromuscular junctions began to form underneath the terminals of the regenerating transplanted nerve. Without denervation, no ectopic junctions appeared. With simultaneous transplantation and denervation, only a few ectopic junctions appeared in some animals.

We adopted this approach with the difference that we transplanted the foreign superficial fibular nerve onto the soleus muscle. Often, the number of ectopic junctions was impressive, covering all fibers in large parts of the muscle. At other times, ectopic innervation might fail altogether in batches of rats in certain periods and for unknown reasons. Our earlier failure to consistently induce LTP comes to mind (see earlier). Nevertheless, the model has proved very useful for studying basic features of neuromuscular

junction formation. On superficial muscle fibers, ectopic synapse formation starts nearly synchronously 1–2 days after the denervation, proceeds over the next 1–2 weeks to fully formed and functional neuromuscular junctions, recapitulating all the essential steps in neuromuscular junction formation in the right sequence, including aggregation and stabilization of acetylcholine receptors underneath nerve terminals, appearance of acetylcholine esterase after a couple of days' delay, folding of the postsynaptic membrane, and synapse elimination.

In 1975 Clarke Slater and his wife Helen and two young children came to Oslo for a year. Together, Clarke and I set out to study inflammation-induced supersensitivity to acetylcholine in muscle fibers with much effort but little success. We therefore switched to studying ectopic synapse formation, which turned into a productive (three papers in *Journal of Physiology*) and exciting enterprise full of lively discussions, much enthusiasm, and good companionship. A few years later, Johannes Skorpen took a year off from his medical studies to make a substantial contribution to our studies of ectopic synapse formation.

Here are some main results of our combined efforts using this model.

1. Blocking impulse conduction in the original nerve (rather than cutting it) allows ectopic synapse formation to occur, whereas direct muscle stimulation after cutting the original nerve prevents it. Therefore, receptivity to ectopic innervation is controlled by evoked muscle impulse activity.
2. Extensive reorganization of ectopic inputs on individual muscle fibers occurs as the junctions mature. Some sites develop into mature adult-type neuromuscular junctions (the winners), while multiple others disappear (the losers). The winners become spaced out, separated by ~1.5 mm on average. Thus, a refractory zone appears around ectopic winners, like that observed on either side of the original endplate band where ectopic junctions never form closer than ~0.75 mm, although foreign axons may "jump" this zone and innervate original endplates.
3. No such reorganization occurs if impulse conduction in the transplanted nerve is blocked or the transplanted nerve is cut early, but it does occur if the transplanted nerve is cut early and the muscle, in addition, is stimulated directly. Therefore, evoked muscle impulse activity causes the appearance of winners and refractory zones, and the elimination of losers.
4. If the foreign nerve is cut at the time of onset of synapse formation, subsequent discrete sites of acetylcholine esterase activity, representing mature endplates, will not appear. But if the muscle is stimulated directly after the cut, such sites

appear just as they do with an intact nerve. Evidently, the cut nerve leaves a trace, or an imprint, in the muscle fiber, which will then persist and provide instructions for subsequent impulse activity-dependent processes setting up the postsynaptic apparatus, including the appearance of acetylcholine esterase.

5. In the presence of such a nerve-derived transient trace, stimulation-evoked muscle impulse activity causes the now denervated winners to attain a limited size, comparable to the size of normally innervated junctions. This limitation and the accompanying refractoriness suggest that impulse activity-dependent processes within the muscle fibers determine both the size and spacing of future neuromuscular junctions. Furthermore, such restriction in available "synaptic space" seems likely to affect competitive interactions between axons during periods of synapse elimination.
6. Individual soleus muscle fibers support from 1 (most common) to 5 ectopic neuromuscular junctions depending on the length of the region covered by transplanted axons. The "synaptic domain" of one winner, that is, the length of fiber required to "support" each one, would be about 1.5 mm (the winner + a refractory zone about 0.75 mm long on either side). These results may explain why normal muscle fibers (unless they are very long) end up with only one neuromuscular junction roughly in the middle of the fiber. In the embryo, axons form neuromuscular junctions when the muscle fibers are very short. Nerve evoked impulse activity occurs early, setting up refractory zones on either side of the initial site. Conducted impulse activity then makes the rest of the fiber refractory to innervation as the fibers grow in length by additions to either end, and the initial site ends up roughly in the middle.

Agrin at the Neuromuscular Junction

In 1993, with the experience of ectopic synapse formation behind me, I went to Jack McMahan at the Department of Neurobiology, Stanford University, for a sabbatical. In a series of important experiments Jack and his coworkers had discovered agrin and shown its essential role in the formation of neuromuscular junctions. Could agrin be responsible for the persistent "trace" that axons leave on the surface of muscle fibers when removed at an early stage of synapse formation (see earlier discussion)? To answer this question, Mendell Rimer, Ilana Cohen, and I started to inject neural agrin cDNA into denervated rat soleus muscles. The effect was impressive.

In some muscle fibers short segments began to express neural agrin, while clusters of mature acetylcholine receptors containing ε-subunits appeared on fibers immediatley surrounding these segments. Moreover, at the sites of such clusters, other components and structural features characteristic of the postsynaptic apparatus of normal neuromuscular junctions also appeared. Evidently, the injected cDNA had entered some muscle fibers where it caused local expression and release of neural agrin, which in turn induced the appearance of a mature postsynaptic-like apparatus on neighboring fibers. Similar results were obtained independently and at about the same time by Hans Brenner and his colleagues.

Back in Oslo, Iacob Mathiesen, a Ph.D. student in my lab joined the project and injected, as before, the cDNA into denervated soleus muscles. In addition, he stimulated the injected muscles directly. The results were strikingly similar to those observed during ectopic innervation. In the absence of stimulation, multiple clusters of acetylcholine receptors appeared along the length of fibers surrounding the agrin-expressing segments. In the presence of stimulation, all but one of these clusters disappeared (the losers), while the one that survived (the winner) acquired a size comparable to that of the original endplates in the same muscle. In addition, the winner displayed all the normal components of the postsynaptic apparatus that we looked for. Thus, the formation of ectopic neuromuscular junctions can be accounted for by the combined effects of neural agrin and evoked muscle impulse activity, normally secreted or evoked by motor nerve terminals. There is one exception. Since no nerves are present at agrin-induced acetylcholine receptor clusters, the distinct structural features that normally reflect overlying nerve terminal branches and swellings are missing.

I think these results answer the question raised earlier: Yes, agrin probably is responsible for the postsynaptic trace that nerve terminals leave after early removal, a trace that then provides instructions for further postsynaptic development. Other evidence, based on estimates of the number and stability of postjunctional acetylcholine receptors obtained in collaboration with Jacopo Andreose and Guido Fumagalli, suggests that the trace persists at least 2 months in the presence of stimulation-evoked muscle activity but disappears within 2 weeks in the absence of such stimulation.

Recently, there has been much interest in the possibility that during development muscle fibers are preprogrammed to display acetylcholine receptor clusters that determine where the first neuromuscular junctions will form. Certainly, for ectopic neuromuscular junction formation no such programming is needed. The new junctions form at random sites without the presence of any clusters before the nerve terminals "touch down" on the surface of muscle fibers. For this reason, I have earlier suggested that the appearance of acetylcholine receptor clusters in the middle of some muscles around the time of nerve arrival may be an epiphenomenon that is unnecessary for normal neuromuscular junction formation (Lømo, 2003).

Ectopic synapse formation is rarely used as an experimental model today. It does not normally occur and may therefore be seen as artificial. Nevertheless, ectopic synapse formation recapitulates all essential aspects of normal neuromuscular synapse formation and allows studies that are difficult or impossible to do in the embryo. I think the processes of ectopic synapses, as outlined earlier, throw light on important general principles of synapse formation.

Life at Stanford (1993–1994)

Anne and I enjoyed our stay at Stanford very much—the good life, the Sundays with coffee, newspapers, and books at Printers Ink in Palo Alto, the climate, the walks in nearby hills and from our apartment across the road to the department, and the extraordinary generous hospitality of Jack and Sandra McMahan in whose home I lived for more than 2 months in Anne's absence. There I was introduced to molecular biology, working at the bench with Mendell Rimer next to me explaining and showing me how to do things. Thus, after nearly a year, I felt that I was on speaking terms with molecular biologists, if only barely. Before Stanford we had been without a car for 16 years. We thought we could do likewise at Stanford but quickly experienced its absolute inconvenience. So from a neighbor we bought a sleek bottle green 1979 two-door Cadillac Eldorado with brown leather seats and a rather cozy backseat, which very soon required very expensive repairs. After that, it served us wonderfully. In fact, we liked it so much that I contacted authorities in Norway to learn that it would cost us ~$24,000 in customs duty alone to bring it to Norway. In the end, the day before leaving the United States, we drove down El Camino Road to a park-and-sell place where we paid the guy $100 to sell it for us at some unspecified later date. And after about half a year we received $1800 for the car.

Setting up a Biotech Company

Injection of cDNA into muscles for transformation of muscle fibers was very inefficient. To improve the efficiency, Iacob Mathiesen began to stimulate the muscle with brief, high-frequency, and high-voltage electrical pulses immediately after the injection. This procedure, called electroporation, increased the efficiency substantially. He then did a systematic study of stimulus parameters to optimize the procedure and initiated a collaborative project with a Merck subsidiary in Rome, Italy. This resulted in the demonstration of dramatic and long-lasting expression of erythropoetin in muscle after intramuscular injection of its cDNA, an effect that could be attributed mainly to the electroporation. The two of us then applied for and obtained a U.S. patent for the method of electroporation that Iacob had used. With money from venture investors and licenses sold, Iacob set up a company,

Inovio, with me as a partner, which was later sold to a company in the United States taking the name of Inovio Biomedical Corporation. The entire enterprise was the result of Iacob's ideas and efforts. For me, watching from the sideline how it developed, it was a totally unexpected and exhilarating experience. I even gained some money so that Anne and I now buy more books, subscribe to more newspapers and periodicals than we will ever have time to read, and travel more often and more comfortably than we would otherwise have done. I have invested in powerful machinery in my carpentry shop in our basement and I have already had plastic surgery on one badly injured finger to show for it. Our needs have always been quite modest, and I am sure we would have been just as fine without the extra money and some shares in the new company by now much reduced in value.

Back to Oslo

Returning from Stanford in the summer of 1994 with ideas for new projects that combined my experience with chronic experiments on live animals with my newly acquired experience of techniques in molecular biology, I joined others in applying for larger and more ambitious grants from the European Union and the Norwegian Research Council. Some of the projects built on already established collaborations with labs in Europe, others on new ones, most of them fruitful. Instead of having a Ph.D. student or two in the lab, I now had several such students and two or three postdocs. In general, I found these projects productive and enjoyable but also stressful with meetings, deadlines, and reports coming up all the time. Among many results from these efforts, here is one set of results that I find particularly interesting and that owes much to Gabriela Bezakova, a postdoc in the lab who did most of the work.

Examining muscle fibers with agrin-induced acetylcholine receptor clusters outside the endplate, Gabriela noticed that the clusters consisted of smaller aggregates that lined up along transverse stripes in electrically active fibers and along longitudinal stripes in denervated or electrically inactive fibers. Further work revealed that the transverse stripes overlapped with costameres, a chain of proteins made up of α-dystroglycan on the outside, β-dystroglycan in the sarcolemma, and underneath the sarcolemma, dystrophin, F-actin, and α-actinin connecting to the Z-disks of each sarcomere. After denervation, all these proteins changed organization and appeared instead along longitudinal stripes. During direct muscle stimulation, they then returned to their normal transverse orientation. Similar changes were seen at endplates where acetylcholine receptor clusters underwent comparable reorientations, as expected if they were firmly attached to underlying cytoskeletal proteins. Importantly, in denervated muscles, muscle agrin induced a similar restoration of the normal transverse orientation when applied to the muscle surface in very low concentrations. Unlike neural

agrin, muscle agrin does not cluster acetylcholine receptor on the surface of muscle fibers because it lacks eight amino acids required for such clustering, and its function was a mystery. Muscle agrin was known to be secreted by muscle fibers and to bind to α-dystroglycan on the outside. Moreover, the chains of costameric proteins, positioned at Z-lines along the entire length of muscle fibers, transmit forces generated by each sarcomere to the extracellular matrix. With this background we proposed a function for muscle agrin. Outside the endplate, muscle activity (and/or accompanying mechanical stresses) controls the expression (and/or processing) of muscle agrin. After secretion, muscle agrin binds to α-dystroglycan and acts back in an autocrine way to adjust the cytoskeleton in accordance with the mechanical demands posed by the activity. At the endplate, muscle agrin similarly assists in stabilizing the postsynaptic apparatus. These and other results from that work were published in *Journal of Cell Biology* (2001) and *Proceedings of the National Academy of Sciences* (2001). I felt that our results demonstrating such dramatic effects of muscle impulse activity and muscle agrin on the organization of the cytoskeleton of muscle fibers were novel and important. I have therefore been surprised to see that at least so far they have not made much of an impact. Perhaps their time will come.

Resurrecting the Lamellar Hypothesis

Finding the unpublished manuscripts that I had submitted to *Experimental Brain Research* 40 years ago, I immersed myself in the literature of the intervening years, wrote a new manuscript based on the old experiments and figures, and saw it published in *Hippocampus* in 2009. Two conclusions stood out.

1. The perforant path input to and the mossy fiber output from the dentate gyrus were both organized according to the lamellar hypothesis.
2. Marked inhibition spread for several millimeters along the septo-temporal axis of the dentate gyrus following the discharge of granule cells along a narrow transverse strip of the dentate gyrus. Orthodromic activation by perforant path fibers or antidromic activation by mossy fibers produced comparable inhibition, indicating that the inhibition was primarily recurrent.

The lamellar hypothesis fell into disfavor after an influential review of anatomical data by Amaral and Witter (*Neuroscience*, 1989), and it is not discussed in the recent *The Hippocampus Book* (2006). In their review, Amaral and Witter write: “The overwhelming consensus in all these studies is that aside from the mossy fibers, none of the intrinsic connections of the

hippocampal formation is organized in a lamellar fashion. Quite the opposite organization seems to be true.” They pointed to findings showing that extracellular injections of tracers into small regions of the entorhinal cortex labeled perforant path fibers along “substantial portion of the long axis of the dentate gyrus.” Conversely, small injections into the dentate gyrus labeled “cells in a long, rostrocaudally oriented zone of the EC.” Therefore, they write: “it is clear that the fibers that cross the hippocampal fissure to enter the dentate area are collaterals of axons that contribute a far more widespread pattern of termination within the dentate gyrus.” And they conclude, surely correctly, that a point source in the entorhinal cortex cannot activate a lamella of hippocampal tissue, as argued by Andersen, Bliss, and Skrede (1971). But the idea of widespread collaterals of a single axon required an explanation of my conclusion (Lømo, 1971), which they cite: “Provided the stimulating electrode was located close to the hippocampal fissure where the perforant path enters the dentate area, it was consistently found that the incoming perforant fibers divide the dentate area into a series of parallel segments.” The explanation they suggest is that “these collaterals terminate with lower density at progressively greater distances from the stimulation point, the synaptic effect on distant granule cells may be insufficient to generated sufficiently synchronous activation to produce detectable populations spikes.”

There are two arguments against this view.

1 The perforant path-evoked field EPSP drops rapidly just outside the strip of activated granule cells, as my contour plots showed. And even if some collaterals spread further and affect distant points only weakly, the much greater efficiency centrally may well turn the dentate gyrus into a series of functional lamellae. I see such lamellae not as well-demarcated anatomical lamellae but as narrow strips of discharging cells, one strip flowing into others, shifting positions as events unfold, and interacting by longitudinal connections and accompanying excitability changes. Such functional lamellae would be much narrower than any anatomical lamella encompassing the entire terminal field of a perforant path axon.
2 The entorhinal cortex may be organized in a modular manner, and there is supporting anatomical data, much like the situation in primary sensory cortical areas where information from large visual, somatosensory, or auditory fields is split for similar processing in a mosaic of columns. This suggests a model where one cell with a particular set of properties in one module projects to one transverse lamella, and other cells with the same set of properties in other modules project to that same lamella. Similarly, any other lamella

> receives inputs from cells in many modules having another set of similar properties. Such a model is consistent with the anatomical data reviewed by Amaral and Witter (1989) and with other more recent anatomical and physiological data.

I presented this model at the Spring Hippocampal Research Conference in Verona, Italy, in 2009. In the discussion after the session, one of the participants made the following comment: "There is no evidence supporting the lamellar hypothesis, and trying to resurrect it is like trying to beat a dead horse back to life." This to me appears close minded. The lamellar hypothesis as presented earlier may be wrong, of course, but is not impossible, and to me seems the more plausible. Anyway, we have now started experiments to test it. Tim Bliss will come to Oslo to participate in some of the experiments, an unexpected and wonderful opportunity to take up again a collaboration that was so enjoyable and rewarding 40 years ago. It seems important to try to find out which of the two models referred to earlier is the more correct since it must strongly influence how one will understand and model dentate gyrus function in the future. Today, so-called pattern separation appears to be an important function of the dentate gyrus, a process of splitting the information from the entorhinal cortex to the dentate gyrus before it is sent forward for pattern completion in CA3. While my understanding of pattern separation may well be incomplete, it seems to me that the concept is better served by perforant path fibers targeting a series of lamellae in the dentate gyrus than by extensive longitudinally running axon collaterals spreading the same information along the entire dorsal dentate gyrus.

Concluding Remarks

In summing up I am struck by how lucky I have been, having lived and nearly completed a life in a peaceful corner of the world in good health, in a happy marriage of almost 50 years, with three sons with successful careers who have given their parents nothing but pleasure, and with grandchildren, all of whom appear set for normal and fulfilling lives. One goes through different epochs in life, each epoch requiring different approaches and efforts for a good outcome. Marriage, family, and work are especially important. To my continual amazement, somehow and largely unplanned, I have managed to land on my feet. Perhaps surprisingly, life in retirement has turned out particularly rewarding with access to a part of what was my own lab, now under the control of the younger professor and good collaborator Arild Njå, ready for interesting experiments with my own hands, as in the old days when I did my own experiments in the lab.

The move away from the lab that came with advancement in age and position I found problematic. It was in the lab that I made my discoveries, doing experiments sometimes over and over again to convince myself that

they were reproducible, to allow time for separating the significant from the noise, and for seeing new and interesting patterns emerge. One of the reviewers of my paper in *Hippocampus* commented: "I think it might be worth adding something here [to my claim that the figures shown were representative], for there may have been a change in etiquette over the years. Does 'representative' mean just that, or are these the experiments that 'worked'? This was my answer:

> An interesting question. The more correct answer is probably "representative of the experiments that worked." But this phrasing may leave an impression of undue selection of results, which I hope was not the case. Over those 3–4 years many experiments were ignored for different reasons. Some early ones were ignored because I could not make sense of them. It took time to reasonably master the technical procedures, to obtain reproducible results under particular experimental conditions, and to get a grasp of what was happening on the oscilloscope screen in front of me. Others were ignored for merely technical reasons. Still others were ignored because they were part of an exercise in trial and error, looking for phenomena that did not materialize, make sense, or were not reproducible (or they did catch my eye and made grounds for a different paper, for example Bliss & Lømo, 1973). The present paper is not the outcome of experiments to test well-conceived, preformed ideas but rather a synthesis of an understanding based on many different experiments, slowly arrived at by asking many different questions over a relatively long period of time, with certain parts selected to make a coherent whole for this particular paper. With this approach it is critical, I think, to do the experiments often enough to become convinced that they are reproducible and thus 'true' under the conditions described. I believe and hope that that was so in this case.

My work has pleased me the most when the results spoke for themselves and the use of statistics was unnecessary. I have never felt comfortable with statistics and the conventions behind them. At a 5% significance level the result might be spurious anyway and often of marginal importance.

So in doing research, what has been rewarding for me? I think the process of finding out more than the result itself. Is LTP an essential process for learning and memory? Probably. But if it is, other processes, most of them perhaps still unknown, are likely also to be essential. Once we know, the fun is over. The excitement is in the uncertainty and in trying to find the right answers. Finding out about learning and memory involves problems of mechanism and are therefore probably soluble. Harder questions relate

to consciousness. Will we ever know? To me it is no less and no more than shifting patterns of activity in the networks of my brain. When that activity stops, it is all over. Why such activity gives me self-awareness and subjective feelings I don't know. And I will never know. The problem seems insoluble and therefore I am not particularly interested. For others the question obviously is not as simple as that. There will always be people who believe in religious or other forces affecting us from outside in mysterious ways. That may or may not be real. If it is unreal, as I believe it is, then the fact that the unreal is so real to many people is a paradoxical aspect of reality. Similarly, the fact that there is so much irrationality in the world, people believing in the unreal, is also part of our reality. Consequently, the prospects for a rational world appear bleak, even as we find out ever more about the workings of the brain and reach for an understanding of the nature of consciousness.

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Selected Bibliography

Lømo T, Mollica A. Activity of single units in primary optic cortex of the unanaesthetized rabbit during visual, auditory, olfactory and painful stimulation. Arch Ital Biol 1962;100:86–120.

Andersen P, Andersson SA, Lømo T. Patterns of spontaneous rythmic activity within various thalamic nuclei. Nature 1966;211:888–889.

Andersen P, Blackstad TW, Lømo T. Location and identification of excitatory synapses on hippocampal pyramidal cells. Exp Brain Res 1966;1:236–248.

Lømo T. Frequency potentiation of excitatory synaptic activity in the dentate area of the hippocampal formation. Acta Physiol Scand 1966;68(Suppl 277):128.

Andersen P, Andersson SA, Lømo T. Some factors involved in the thalamic control of spontaneous barbiturate spindles. J Physiol 1967;192:257–281.

Andersen P, Andersson SA, Lømo T. Nature of thalamo-cortical relations during spontaneous barbiturate spindle activity. J Physiol 1967;192:283–307.

Lømo T. Patterns of activation in a monosynaptic cortical pathway: the perforant path input to the dentate area of the hippocampal formation. Exp Brain Res 1971;2:18–45.

Lømo T. Potentiation of monosynaptic EPSPs in the perforant path - dentate granule cell synapse. Exp Brain Res 1971;12:46–63.

Lømo T, Rosenthal J. Control of ACh sensitivity by muscle activity in the rat. J Physiol 1972;221:493–513.

Bliss T, Gardner MA, Lømo T. Synaptic plasticity in the hippocampal formation. London: MacMillan, 1973; 93–203.

Bliss T, Lømo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 1973;232:331–356.

Frank E, Jansen J, Lømo T, Westgaard RH. Maintained function of foreign synapses on hyperinnervated skeletal muscle fibres of the rat. Nature 1974;247: 375–376.

Lømo T. Neurotrophic control of colchicine effects on muscle? Nature 1974;249: 473–474.

Lømo T, Westgaard RH, Dahl HA. Contractile properties of muscle: control by pattern of muscle activity in the rat. Proc Roy Soc Lond, Ser B 1974;187:99–103.

Frank E, Jansen JK, Lømo T, Westgaard RH. The interaction between foreign and original motor nerves innervating the soleus muscle of rats. J Physiol 1975; 247:725–743.

Lømo T, Westgaard RH. Further studies on the control of ACh sensitivity by muscle activity in the rat. J Physiol 1975;252:603–626.

Lømo T, Westgaard RH. Control of ACh sensitivity in rat muscle fibers. Cold Spring Harbor Symposia on Quantitative Biolology 1976;XL:263–274.

Lømo T, Slater CR. Control of acetylcholine sensitivity and synapse formation by muscle activity. J Physiol 1978;275:391–402.

Cangiano A, Lømo T, Lutzemberger L, Sveen O. Effects of chronic nerve conduction block on formation of neuromuscular junctions and junctional AChE in rat. Acta Physiol Scand 1980;109:253–296.

Lømo T. What controls the development of neuromuscular junctions? Trends Neurosci 1980;3:126–129.

Lømo T, Slater CR. Control of junctional acetylcholinesterase by neural and muscular influences in the rat. J Physiol 1980;303:191–202.

Lømo T, Slater CR. Acetylcholine sensitivity of developing ectopic nerve-muscle junctions in adult rat soleus muscles. J Physiol 1980;303:173–189.

Hennig R, Lømo T. Firing patterns of motor units in normal rats. Nature 1985;14: 164–166.

Lømo T, Massoulié J, Vigny M. Stimulation of denervated rat soleus muscle with fast and slow activity patterns induces expression of acetylcholinesterase molecular forms. J Neurosci 1985;5:1180–1187.

Lømo T, Wærhaug O. Motor endplates in fast and slow muscles of the rat: what determines their differences? J Physiol (Paris) 1985;80:290–297.

Brenner HR, Lømo T, Williamson R. Control of end-plate channel properties by neurotrophic effects and by muscle activity in rat. J Physiol 1987;388:367–381.

Hennig R, Lømo T. Effects of chronic stimulation on the size and speed of long-term denervated and innervated rat fast and slow muscles. Acta Physiol Scand 1987;30:115–131.

Hennig R, Lømo T. Gradation of force output in normal fast and slow muscles of the rat. Acta Physiol Scand 1987;130:133–142.

Lømo T. Formation of ectopic neuromuscular junctions: role of activity and other factors. In: The Neural and Molecular Bases of Learning (Changeux J-P, Konishi M, eds). New York: John Wiley & Sons Ltd. 1987; 359–374.

Gorza L, Gundersen K, Lømo T, Schiaffino S, Westgaard RH. Slow-to-fast transformation of denervated soleus muscles by chronic high-frequency stimulation in the rat. J Physiol 1988;402:627–649.

Lømo T, Gundersen K. Trophic control of skeletal muscle membrane properties. In: Nerve-Muscle Cell Trophic Communication (Fernandez HL, ed). Boca Raton: CRC Press, Inc. 1988; 61–79.

Lømo T, Pockett S, Sommerschild H. Control of number and distribution of synapses during ectopic synapse formation in adult rat soleus muscles. Neurosci 1988;24:673–686.

Schiaffino S, Ausoni S, Gorza L, Saggin L, Gundersen K, Lømo T. Myosin heavy chain isoforms and velocity of shortening of type 2 skeletal muscle fibres. Acta Physiol Scand 1988;134:575–576.

Westgaard RH, Lømo T. Control of contractile properties within adaptive ranges by patterns of impulse activity in the rat. J Neurosci 1988;8:4415–4426.

Schiaffino S, Gorza L, Sartore S, Saggin L, Ausoni S, Vianello M, Gundersen K, Lømo T. Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. J Muscle Res Cell Mot 1989;10:197–205.

Ausoni S, Gorza L, Schiaffino S, Gundersen K, Lømo T. Expression of myosin heavy chain isoforms in stimulated fast and slow rat muscles. J Neurosci 1990; 10:153–160.

Fumagalli G, Balbi S, Cangiano A, Lømo T. Regulation of turnover and number of acetylcholine receptors at neuromuscular junctions. Neuron 1990;4:563–569.

Andreose J, Xu R, Lømo T, Salpeter MM, Fumagalli G. Degradation of two AChR populations at rat neuromuscular junctions: regulation by electrical stimulation. J Neurosci 1993;13:3433–3438.

Wærhaug O, Lømo T. Factors causing different properties at neuromuscular junctions in fast and slow rat skeletal muscles. Anat Embryol 1994;190:113–125.

Andreose JS, Fumagalli G, Lømo T. Number of junctional acetylcholine receptors: control by neural and muscular influences in the rat. J Physiol 1995;483: 397–406.

Cohen I, Rimer M, Lømo T, McMahan UJ. Agrin-induced postsynaptic-like apparatus in skeletal muscle fibers in vivo. Mol Cell Neurosci 1997;9:237–253.

Mathiesen I, Rimer M, Ashtari O, Cohen I, McMahan UJ, Lømo T. Regulation of the size and distribution of agrin-induced postsynaptic- like apparatus in adult skeletal muscle by electrical muscle activity. Mol Cell Neurosci 1999;13: 207–217.

Rimer M, Mathiesen I, Lømo T, McMahan UJ. γ-AChR/ε-AChR Switch at agrin-induced postsynaptic-like apparatus in skeletal muscle. Mol Cell Neurosci 1997;9:254–263.

Rimer M, Cohen I, Lømo T, Burden SJ, McMahan UJ. Neuregulins and erbB receptors at neuromuscular junctions and at agrin- induced postsynaptic-like apparatus in skeletal muscle. Mol Cell Neurosci 1998;12:1–15.

Sala C, Andreose JS, Fumagalli G, Lømo T. Calcitonin gene-related peptide: possible role in formation and maintenance of neuromuscular junctions. J Neurosci 1995;15:520–528.

Windisch A, Gundersen K, Szabolcs MJ, Gruber H, Lømo T. Fast to slow transformation of denervated and electrically stimulated rat muscle. J Physiol 1998;510:623–632.

Skorpen J, Lafond-Benestad S, Lømo T. Regulation of the size and distribution of ectopic neuromuscular junctions in adult skeletal muscle by nerve-derived trophic factor and electrical muscle activity. Mol Cell Neurosci 1999;13: 192–206.

Vult von Steyern F, Martinov V, Rabben I, Njå A, deLapeyrière O, Lømo T. The homeodomain transcription factors Islet 1 and HB9 are expressed in adult alpha and gamma motoneurons identified by selective retrograde tracing. Eur J Neurosci 1999;11:2093–2102.

Murgia M, Serrano AL, Calabria E, Pallafacchina G, Lømo T, Schiaffino S. Ras is involved in nerve-activity-dependent regulation of muscle genes. Nat Cell Biol 2000;2:142–147.

Awad SS, Lightowlers RN, Young C, Chrzanowska-Lightowlers ZMA, Lømo T, Slater CR. Sodium channel mRNAs at the neuromuscular junction: distinct patterns of accumulation and effects of muscle activity. J Neurosci 2001;21: 8456–8463.

Bezakova G, Helm JP, Francolini M, Lømo T. Effects of purified recombinant neural and muscle agrin on skeletal muscle fibers in vivo. J Cell Biol 2001;153: 1441–1452.

Bezakova G, Lømo T. Muscle activity and muscle agrin regulate the organization of cytoskeletal proteins and attached AChRs aggregates in skeletal muscle fibers. J Cell Biol 2001;153:1453–1463.

Bezakova G, Rabben I, Sefland I, Fumagalli G, Lømo. The neural agrin controls acetylcholine receptor stability in skeletal muscle fibers. Proc Nat Acad Sci USA 2001;98:9924–9929.

Martinov VN, Sefland I, Walaas SI, Lømo T, Nja A, Hoover F. Targeting functional subtypes of spinal motoneurons and skeletal muscle fibers in vivo by intramuscular injection of adenoviral and adeno-associated viral vectors. Anat Embryol 2002;205:215–221.

Ralston E, Ploug T, Kalhovde J, Lømo T. Golgi complex, endoplasmic reticulum exit sites, and microtubules in skeletal muscle fibers are organized by patterned activity. J Neurosci 2001;21:875–883.

Serrano AL, Murgia M, Pallafacchina G, Calabria E, Coniglio P, Lømo T, Schiaffino S. Calcineurin controls nerve activity-dependent specification of slow skeletal muscle fibers but not muscle growth. Proc Nat Acad Sci USA 2001;98: 13108–13113.

Lømo T. Nerve-muscle interactions. In: Handbook of Clinical Neurophysiology: Clinical Neurophysiology of Disorders of Muscle and Neuromuscular Junction, Including Fatigue (Stålberg E, ed). Elsevier Science B. V. 2003; 47–66.

Lømo T. What controls the position, number, size, and distribution of neuromuscular junctions on rat muscle fibers? *J Neurocytol* 2003;32:835–848.

Lømo T. The discovery of long-term potentiation. Philos Trans R Soc Lond B Biol Sci 2003;358:617–620.

Reid B, Martinov VN, Njå A, Lømo T & Bewick GS. Activity-dependent plasticity of transmitter release from nerve terminals in rat fast and slow muscles. *J Neurosci* 2003;23:9340–9348.

McCullagh KJ, Calabria E, Pallafacchina G, Ciciliot S, Serrano AL, Argentini C, Kalhovde JM, Lømo T, Schiaffino S. NFAT is a nerve activity sensor in skeletal muscle and controls activity-dependent myosin switching. *Proc Natl Acad Sci USA* 2004;101:10590–10595.

Grønevik E, Mathiesen I, Lømo T. Early events of electroporation-mediated intramuscular DNA vaccination potentiate Th1-directed immune responses. *J Gene Med* 2005;7:1246–1254.

Kalhovde JM, Jerkovic R, Sefland I, Cordonnier C, Calabria E, Schiaffino S, Lømo T. "Fast" and "slow" muscle fibres in hindlimb muscles of adult rats regenerate from intrinsically different satellite cells. *J Physiol* 2005;562:847–857.

Vult von Steyern F, Lømo T. Postnatal appearance of 5-HT2A receptors on fast flexor and slow extensor rat motor neurons. *Neuroscience* 2005;136:87–93.

Eken T, Elder GC, Lømo T. Development of tonic firing behavior in rat soleus muscle. *J Neurophysiol* 2008;99:1899–1905.

Lømo T. Excitability changes within transverse lamellae of dentate granule cells and their longitudinal spread following orthodromic or antidromic activation. *Hippocampus* 2008;19:633–648.

Lømo, T. Modulation of the Muscle Fiber and the Neuromuscular Junction by Activity. In: New Encyclopedia of Neuroscience (Squire LR, ed). New York: Elsevier 2009; xx–xx.