Tamás F. Freund

Born:

Zirc, Hungary
June 14, 1959

Education:

Loránd Eötvös University, Budapest, Hungary, Biologist (1983)
Semmelweis University, Budapest and Hungarian Academy of Sciences, PhD (1986)

Appointments:

Postdoctoral Fellow, Anatomy, Semmelweis University–Hungarian Academy of Sciences (1986–1990)
Visiting Research Fellow, MRC Unit, Pharmacology, Oxford University (1986–1988)
Head of Department, Institute of Experimental Medicine, Hungarian Academy of Sciences (IEM-HAS), Budapest (1990–1994)
Professor, IEM-HAS, Deputy Director (1994–2002) and Director (2002–present)
Professor and Head of Department, Péter Pázmány Catholic University, Budapest (2000–present)

Honors And Awards (Selected):

Drs. C. and F. Demuth Swiss Medical Research Foundation Award, Switzerland (1991)
KRIEG Cortical Kudos Cortical Explorer Award of the Cajal Club (1991, USA)
KRIEG Cortical Kudos Cortical Discoverer Award and the Cajal Medal (1998, USA)
Dargut and Milena Kemali Foundation Award, FENS Forum, Berlin (1998)
Fellow, Hungarian Academy of Sciences (1998), Vice President (since 2014)
Bolyai Prize (2000), Széchenyi Prize, (2005), Prima Primissima Award (2013, Hungary)
Fellow, Academia Europaea (2000, London) and Academia Scientiarum et Artium Europaea (2001)
Fellow, German Academy of Sciences Leopoldina (2001)
President, Federation of European Neuroscience Societies (FENS, 2004–2006)
The Brain Prize (2011, Grete Lundbeck Foundation, Denmark)
Fellow, American Academy of Arts and Sciences (2014)
Doctor Honoris Causa, University of Southern Finland (2015)

Tamás Freund's main achievements include the discovery of new molecular pathways in nerve cell communication, identity and principles of neuron connectivity fundamental to cortical circuitry, and the generation of network activity patterns underlying multiple stages of information processing and storage in the brain. He made significant discoveries relating to structure and function of cortical microcircuits, particularly the inhibitory components and their relationship to the oscillations that underlie different stages of memory formation in the hippocampus. He discovered that GABAergic (inhibitory) pacemaker neurons in the septal region selectively innervate GABAergic interneurons in the hippocampus, thereby synchronizing activity rhythmically at theta frequency. His results in the epilepsy field provided evidence that early loss of inhibitory interneurons leads to conditions that allow interictal spiking to generate hyperexcitable circuits and ultimately lead to the chronic phase characterized by spontaneous seizures. He described the pattern of ischemic vulnerability of different cell types and regions in the brain, and its correlation with excitotoxicity. With his research group, he discovered that CB1 cannabinoid receptor activation inhibits neurotransmitter release, described the structure and operational principles of this circuit breaker in several brain regions, and identified its relationship to the pathomechanisms of anxiety and epilepsy.

Tamás F. Freund

My ancestors were German immigrants, who moved to Hungary at the end of the 18th century, and settled as farmers in villages within 10 to 20 km of Veszprém, the capital of the county. Both of my parents grew up in one of these small German-speaking villages, Bánd, within four houses of each other. It did not therefore require much effort from my father to see my mother to her home when they were dating. In the 1950s, it was no longer possible to live from farming alone, due to the agricultural policies of the Communist regime (forced establishment of cooperatives, condemnation of successful, independent farmers, and so on) and my parents moved to Veszprém as soon as they married, in 1957. I grew up in this lovely small city 10 km from Lake Balaton, the largest lake in Central Europe, having plenty of opportunities to swim and to learn sailing and waterskiing from a young age.

Duality has always been present in my life, first my parallel loves of science and music. For a long time, I was unable to decide which field I should chose as a profession and which I should keep as a hobby. Let me share with you how this dual interest developed.

My Early Scientist Self

My parents could not afford higher education. My father's parents' house burned down and he had to leave the high school and go to work. My mother wanted to be a school teacher, but at the age of 15, she became seriously ill for almost a year and had to abandon grammar school. Thus, my father ended up in a chemistry research institute in Veszprém as a car dispatcher, while my mother worked in the adjacent chemistry research institute as a laboratory technician. Their workplace was responsible for my initial interest in research, mainly chemistry.

In elementary school, I went for lunch every day to my "mother's institute," where I could watch her do chemical reactions and measurements of concentrations, pH, and such, and where I could talk to scientists. This is how I gradually fell in love with the world of research in general, exploring what was new, seeing what nobody saw before. From age 12 to 15, I worked one month every summer in these institutes as a schoolboy, serving the scientists when they came to pick up various chemicals from the storehouse. This way I quickly became acquainted with the names of many chemicals, including their formulas, and with many basic reactions. I managed to rescue some leftover chemicals, a discarded Bunsen stand and light, glass test tubes, beakers, retorts, and alembics, and I established a serious-looking

chemistry lab in the woodshed of my father's vineyard at Lake Balaton. Of course, I did all of the reactions in the manner of a schoolkid with an experimental mind. Luckily, my eyes survived all the explosions. Once we even made some use of my hobby. My father bought a derelict wine cellar adjacent to our vineyard and planned to use the stones from its construction to build our own wine cellar. The older cellar had a very sturdy vaulted ceiling, however, which we were unable to break into even with a mallet or a hack. Fortunately, I had all the ingredients to make black gunpowder, with which we exploded the ceiling and caused the cellar to collapse. We soon had all the stones we required for our new wine cellar.

My elementary school teacher found me sufficiently talented in chemistry and sent me to various competitions, which I sometimes managed to win. My father's friend, a chemical engineer, also gave me several practical lessons in analytical chemistry. Thus, I entered grammar school at age 14 with a rather broad knowledge of chemistry and was terribly bored during chemistry classes. However, biology began to interest me even more, especially in the third year of grammar school (age 17), when we learned about the brain for at least half a year. This biology textbook was written by Jozsef Hámori, a pupil of the famous anatomist, János Szentágothai, and contained several beautiful anatomical drawings by Szentágothai as illustrations. I was fascinated by the fact that activity in complex networks of billions of nerve cells can produce behavior, control all bodily functions, store memories, generate thoughts, and give rise to various disorders when malfunctioning. I decided then, at this young age, that I wanted to spend the rest of my life doing brain research.

My Early Musician Self

When I was 7, my parents entered me into music school. They did not ask my opinion, and I just did what I was told, as playing an instrument (mostly accordion) was a long tradition in my father's family. My parents thought I should learn the violin, but luckily for me, the music school had no more violins available, and there were no slots in the violin teacher's schedule either. They asked the music school director what he suggested, and he picked the clarinet for me, because they had one in stock, and there were open slots in the clarinet teacher's schedule. I started rather enthusiastically and made visible—or should that be audible—progress day by day. But after a few years of this, I lost motivation and was annoyed by my parents forcing me to practice every day while my friends were playing soccer in the courtyard.

I was just about to abandon music school at age 12, when I saw a film on television about the life of Benny Goodman. I immediately fell in love with jazz, as well as with the improvisations of Goodman. The next day, I asked my clarinet teacher whether he had any sheet music from Goodman.

Luckily he did. My teacher provided me with scores from "Rachel's Dream" and "Variations on Paganini's Caprice 24," both including the improvisations written down, with piano accompaniment. The jury was rather amazed when we played these tunes at music competitions as elementary school kids at age 13–14, because they thought we really were improvising. Several invitations followed; we played at school celebrations, dancing clubs, and in the Veszprém Theater at public musical events, just to name a few.

Thus, I was already rather busy with music when I entered gymnasium (grammar school) at age 14, which further enriched my musical life. The director of the music school was an excellent conductor, he established the City Choir of Veszprém and the Veszprém Symphony Orchestra, and also conducted the choir of our high school. He knew me well as a clarinetist and convinced me to join the gymnasium choir, which I reluctantly did. I was not terribly enthusiastic about choir music. However, this changed instantly when I first participated in his choir practices, as the director was an amazingly charismatic person. Choir singing became my other lifetime passion, like clarinet, and I have been unable to stop singing in choirs ever since.

The director found my voice rather mature for my years, and I could read music. He therefore invited me to sing bass in the adult City Choir—at age 15. Also in the first year of the gymnasium, I began to play the tenor sax and established a jazz quartet with three schoolmates who played the piano, bass guitar, and drums. I left Benny Goodman's world of swing and bebop and moved toward cool and free jazz, having John Coltrane and Miles Davis as my icons. When the leader and double-bass player of a top jazz quartet in Veszprém—Endre Nyitrai—heard us play, he invited me to join his professional band. I was 16. From then on, I played in two jazz quartets, sang in two choirs, and occasionally played second clarinet in the Symphony Orchestra of Veszprém. But I did not abandon classical music. I continued to play solo clarinet and won a couple of national competitions. My teachers wondered how I managed to study and gain good marks, with all of these musical engagements. I did not even dream, at that point, that my neurobiological studies to come a couple of decades later would shed some light on how music, and indeed, the arts in general, can enhance memory formation and creative thinking.

The Choice of a Professional Career

Up until the third year of gymnasium I was unable to make up my mind whether to become a professional musician or a scientist. My interest in chemistry gradually subsided, while music occupied all my free time. However, at age 17, my passion for neuroscience became dominant, and I made a decision for a scientific career. I thought that as a scientist I could maintain my musical activities as a hobby, but it would not work the other way around. Brain research could never be done as a hobby. There were

other reasons. When touring the country with the Nyitrai Jazz Quartet, we often arrived back in Veszprém well after midnight, or even at dawn, and I hardly had time to get ready for school, sometimes without any sleep at all. I also realized that I would be working in the evenings as a musician and would not be able to participate much in raising children. I grew up in a very close and loving family and wanted to give at least as much love and care to my own kids. This seemed incompatible with a career in music. The only remaining question was how to get my scientist career started (i.e., whether to attend medical school or the Biology Faculty).

My biology teacher advised that if I wanted to do research, the Biology Faculty was the better choice, because there I would learn several subjects relevant for research. The medical school did not offer these subjects. Instead, medicine would require that I study subjects that would not be as important in research. This is how I ended up in József Attila University in Szeged, at the Biology Faculty. I chose Szeged because my girlfriend at the time expected to enter medical school there, whereas I had no special connections to any university. However, after the first year of study, I had a new girlfriend—my future wife—and moved to Budapest, where she was studying in the University of Applied Arts. No such faculty existed in Szeged. I graduated from Loránd Eötvös University in Budapest in 1983 as a biologist. This move to Budapest turned out to be crucial for my research career, because I found the best mentors of all times in Budapest, Péter Somogyi and János Szentágothai. I should thank my first wife for this decision. "Cherchez la Femme"!

The Early Years in Research as an Undergraduate

In September 1979, I enrolled in the Biology Department of Loránd Eötvös University. Serious research in the field of neuroanatomy did not exist there, and I was advised to start as a student research assistant at Semmelweis Medical School, in the Anatomy Department headed by the world-famous neuroanatomist, János Szentágothai. Péter Somogyi was a young scientist in the Szentágothai research group at the time. Because Somogyi was also a biologist by basic training, he welcomed biology students to his lab particularly warmly, including me. I spent all my spare time in his lab after the compulsory university lectures or practicals, and also most of my weekends and holidays. In a few months, I had gained the technical skills that allowed me to work on my own project, investigating the morphology and connectivity of Golgi-impregnated chandelier and double bouquet cells in the cat and monkey visual cortex by correlated light and electron microscopy. This technique had been invented by Péter, and scientists visited him from all over the world to learn it. I was lucky to learn it from him first hand.

Immunocytochemistry was not available to us in 1980, and we began to use high-affinity tritiated gamma-aminobutyric acid (GABA) or nipecotic

acid uptake followed by autoradiography to identify GABAergic inhibitory neurons. Identification of the transmitter, and thereby the presumed inhibitory nature of a cell type, made our morphological studies much more interesting. However, the combination of nipecotic acid uptake, autoradiography, and Golgi impregnation had its own serious limits. We turned finally to immunocytochemistry when this technique became available, and obtained our first antisera against glutamic acid decarboxylase (GAD), the synthetizing enzyme of GABA. This coincided with my very first opportunity to visit Oxford University—for two months in the summer holiday in 1981—via a collaboration between Péter Somogyi and A. David Smith at the Department of Pharmacology. It was during this visit that I managed to solve one of the major technical problems of the lab, namely, to combine immunocytochemistry with Golgi impregnation.

Immunocytochemistry was needed to visualize chemically identified neurons or pathways. In most cases (e.g., in the case of GAD), however, the antibodies available to us did not reveal the dendritic processes or axonal arbors of the immunopositive neurons, and thus the cell type could not be identified. Conversely, Golgi impregnation revealed the processes allowing the identification of cell type, but it provided no information about the transmitter identity of the visualized neurons. Thus, a combination of Golgi impregnation with immunocytochemistry was required. All earlier attempts had failed, however, because immunostaining could be done only on 60- to 80-micron-thick sections because of the limited penetration of antibodies. Golgi impregnation, by contrast, always had been done on much thicker blocks of tissue, which were sectioned only after the impregnation procedure was performed. Immunostaining could not be done after the Golgi impregnation because of the osmium treatment.

The solution to this problem came to me from the physical chemistry practicals held at the university, a subject that I disliked the most. In these classes we used U-shaped glass tubes filled with agar to connect two electrolyte solutions. I realized that if we cut 60- to 80-micron—thick sections from a tissue block and immunostained them as usual, we then could reassemble the sections into the original block of tissue using agar to hold them together. This thin layer of agar allowed the diffusion of potassium dichromate, osmium tetroxide, and silver nitrate into the tissue blocks, as needed for Golgi impregnation. The use of this technique gave rise to several important discoveries, for example, the identification of the postsynaptic elements of dopaminergic fibers in the striatum and the classification of GAD-positive interneuron types in the cerebral cortex. These studies resulted in an invitation from Professor A. David Smith to come back to Oxford for an entire year as an undergraduate in 1982–1983. The dean of Eötvös University gave me permission to spend the fifth year of my undergraduate studies in Oxford, but only if I passed all the fifth-year exams in my fourth year. So I did.

The major project of the Oxford lab led by A. David Smith at that time was the connectivity of the basal ganglia in relation to Parkinson's disease, with a particular emphasis on how the dopaminergic neurons that are known to degenerate in Parkinson's influence striatal neurons. The dogma at that time was that dopaminergic fibers make hardly any synapses. They simply release dopamine nonsynaptically, and the dopamine diffuses to all potential target cells that carry the appropriate receptors. Thus, it was thought, that target selectivity was determined not by synapses terminating on certain cell types, or on their specific compartments, but rather by the cellular and subcellular localization of dopamine receptors. In contrast to this view, I found that dopaminergic fibers did make synapses. This discovery was made possible by my section-Golgi impregnation technique, which allowed for the combination of Golgi impregnation, immunocytochemistry for tyrosine hydroxylase (TH), and retrograde transport of horseradish peroxidase (HRP).

The synapses of dopaminergic fibers most often were established on the necks of dendritic spines of medium spiny neurons that projected to the substantia nigra. The heads of those spines received cortical or thalamic excitatory input. Thus, the dopaminergic fibers were in an ideal position to shunt the excitatory input selectively, at the level of individual spines. This discovery was published in 1984 in the journal *Neuroscience* (Freund et al., 1984) and has been cited extensively each year since publication. When I was just in the middle of these studies in 1982, Francis Crick—who at that time was working on dendritic spines as potential sites of memory storage and had just proposed a model in which spines twitch in response to synaptic stimulation—visited Oxford. David Smith invited him down to the electron microscopy lab, and gave me an opportunity to show Crick my results about dopaminergic fibers selectively innervating the necks of dendritic spines. Crick really liked what he saw and was amazed by this specificity, which led him to think immediately about how to incorporate this phenomenon into his model.

Meeting the Nobel laureate, who with Watson used the data obtained from Rosalind Franklin's unique X-ray crystallography studies to make one of the greatest discoveries in the history of biology, was a really great experience for a young Hungarian, who had just managed to move out from behind the iron curtain. Crick and Watson had seemed unreachable stars, or demigods from my school desk when I made first the decision to become a biologist. But there I was only five or six years later in Oxford, one of the capitals of world science, discussing my discovery with one of these demigods. This was like a real fairytale. Similar experiences at a young age can seriously contribute to a lifetime motivation in science. I was even younger when Sir John Eccles visited János Szentágothai in Budapest, who guided him to our lab in the basement of the Anatomy Department. That was the time when I made my first drawings of double-bouquet and chandelier cells, which he looked at with interest, and gave us encouraging comments.

Another important encounter in 1983 in Oxford was with Anders Björklund, who was on sabbatical in Cambridge, working on transplantation of embryonic dopaminergic neurons into the denervated striatum (a rat model of Parkinson's disease). Björklund popped over to visit David's lab in Oxford. When he saw my data on the termination pattern of dopaminergic fibers in the normal striatum, and I saw his light micrographs of dopaminergic fibers reinnervating the denervated striatum, we raised the obvious question of whether the transplanted embryonic dopaminergic neurons would find the same targets in the host striatum. The results surprised us all. I had already shown that in the normal striatum dopaminergic fibers form synapses mostly with spines and dendritic shafts of medium spiny projection neurons (Freund et al., 1984), but no morphological signs of any interactions with other axon terminals had been observed. We therefore assumed that the pharmacologically demonstrated dopaminergic control of acetylcholine release in striatal slices can be brought about only by dopaminergic receptors located on cholinergic axon terminals, where the receptors bind nonsynaptically released dopamine.

All cholinergic axons in the striatum originate from the giant cholinergic interneurons. Although these account for only about 2 percent of all striatal neurons, they give rise to an extremely dense fiber meshwork. Dopaminergic axons form a similarly dense network. Thus, the conditions are ideal for a presynaptic nonsynaptic interaction between the two transmitter systems. What I found instead in the denervated striatum that had been reinnervated by embryonic dopaminergic neurons was really astonishing. Most of the synaptic contacts on the necks of dendritic spines were reestablished, but in addition, the cell bodies of the giant cholinergic interneurons had been selectively hyperinnervated by dopaminergic fibers! They formed dense basket-like arrays of axon collaterals, some of which even got folded into the cytoplasm of the giant cells. Nothing like that has ever been seen in the normal striatum. Our hypothetical interpretation was that the dopaminergic fiber meshwork originating from the transplanted embryonic nigra was too sparse for an efficient nonsynaptic–presynaptic control of cholinergic transmission. However, the dopaminergic fibers had been able to regain normal control over acetylcholine release by hyperinnervating the parent cell bodies of the vast cholinergic fiber networks (Freund et al., 1985a). This discovery led to the continuation of this exciting collaboration, also involving my good friend and colleague in Oxford, Paul Bolam, and a visit to Lund in 1985.

Motivation and commitment to research is also enhanced by having the opportunity to publish the results in leading journals. I was very lucky from that respect as well, since Péter Somogyi's studies of GABAergic neurons and their roles in the columnar organization of cortex had reached the most exciting stage exactly at the time when I joined his lab. This led to my easily earned, and perhaps not really deserved co-authorship on a *Nature* (Somogyi

et al., 1981) and a *Proceedings of the National Academy of Sciences* (Somogyi et al., 1983) paper as an undergraduate. During my undergraduate years, I also made sufficient contributions to be the first author in a *Neuroscience* and a *Journal of Comparative Neurology* paper, both of which came out in 1983 (Freund & Somogyi, 1983; Freund et al., 1983).

Back in Budapest in 1983, I defended my diploma work at Loránd Eötvös University and received my university doctorate one year later. I carried on with my studies in the Anatomy Department under the official supervision of János Szentágothai, since Péter Somogyi was already working as a visiting professor in Australia. The head of the department in the early eighties was also the head of the university's Communist party section. She was afraid that over the weekends or in the late evenings, we were going to use the photocopying machine of the electron microscopy lab to copy various political *samizdat* literature and confiscated our keys to the lab. However, Péter and I borrowed the keys from the cleaning lady, took them to the workshop of the department, and chiseled copies for ourselves. This illustrates our determination and motivation at that time, and also the presence of a Communist dictatorship, although Hungary was regarded by the west as "the happiest barrack."

Thanks to János Szentágothai's good political connections, Hungarian scientists had a chance to travel to the west for conferences, or even for longer visits coupled to collaborations. Szentágothai has never been associated with the Communist party, but since he was a member of not only western Academies of Sciences, but also of the Soviet Academy, the Communist leadership was unable to push him aside, or prevent him becoming the president of the Hungarian Academy of Sciences. Szentágothai had to provide a written personal guarantee that I would come back from Oxford from my 1982–1983 trip. This was the condition under which the government would allow my wife to come with me. Just a funny illustration of the regime's operations: My first student, Iván Soltesz—now the holder of an endowed chair in Stanford—went to work with Péter Somogyi in Oxford for she Summer holidays in 1984 by train. He took several microscope slides, tissue blocks, and camera lucida drawings of neurons with him to continue analyzing them with Péter. When the train arrived at the Austrian border, Hungarian border guards began checking passports, visas, and luggage. They searched Ivan's plastic cylinder containing the drawings, and when Ivan said these were drawings of nerve cells, they snapped at him pointing to the cell body: "come on . . . , don't be joking! Where is this meeting point? Who is going to meet there? And where are these roads to the meeting point coming from"—they asked pointing to the dendrites. We can hardly stop laughing at this now, but at that time, it was rather scary. Ivan was taken off the train by border police, and into custody, until I was able to reach Szentágothai by phone. He managed to get Ivan out and back on the next train.

In 1985, I went to Oxford again, this time to learn electrophysiology in the lab of Kevan Martin and David Whitteridge in the Experimental Psychology Department. I never learned enough to carry out electrophysiological experiments myself, especially not *in vivo*. Nevertheless, I learned what is possible with these techniques, as well as how to provide a functional interpretation of our anatomical findings. I also learned to always ask functional questions regardless of whether anatomical, electrophysiological, or pharmacological approaches are used. This visit also gave rise to a number of valuable discoveries in the visual cortex and the thalamocortical system in cats, combining *in vivo* electrophysiology with intracellular HRP filling and target analyses using immunocytochemistry at the electron microscopic level (Freund et al., 1985b, 1989b).

Switching to the Hippocampus and the Septohippocampal System

During my visit to Anders Björklund's lab in Lund in 1985, I also met Fred (Rusty) Gage. Interestingly, it was this cooperation with Anders and Rusty that led to my lifelong friendship and collaboration with Gyuri Buzsáki, who also was involved in joint studies with Anders and Rusty at that time. Gyuri has always been an extremely charismatic, enthusiastic, and motivating person. Thus, it is not surprising that even a couple of conversations with him gave rise to my interest in the hippocampus and the septohippocampal system that remained in the focus of my research for the rest of my career. It all started with Anders sending to Budapest about 20 rats that had gone through transplantation of embryonic dopaminergic neurons into the denervated striatum, and one to two months of behavioral testing to demonstrate functional recovery of the nigrostriatal system. Rusty arrived on the same flight with the animals to assist in the anatomical experiments, and we went together to the air cargo depot in the airport to pick them up. However, all 20 rats arrived dead, since—as we learned later—they were left outside overnight in winter when transferred between planes in Copenhagen airport. We were obviously terribly disappointed, but wanted to make a good use of the free time and decided to visit Gyuri Buzsáki in Pécs until Rusty's return flight a couple of days later. On the train toward Pécs, Rusty told me about the discrepancies in the literature concerning the role of septal cholinergic neurons in driving hippocampal theta activity. The slow metabotropic cholinergic actions seemed most unlikely to drive any rhythmic activity, but he mentioned a group of noncholinergic—presumably GABAergic—neurons in the medial septum that might be suited for a pacemaker job. At that time, nobody knew anything about the termination pattern or postsynaptic targets of this cell group in the hippocampus, nor had any direct evidence for its transmitter being produced. These questions

stimulated my first experiments in the septohippocampal system, which I began to investigate when back in Oxford in 1986.

Having defended my candidate of sciences degree (equivalent these days to PhD) in the Hungarian Academy of Sciences, I went back to Oxford in 1986, this time for two and a half years. I was working in the MRC Anatomical Neuropharmacology Unit that had been established around that time under the co-directorship of A. David Smith and Péter Somogyi. My salary came from FIDIA, an Italian pharmaceutical company, to investigate the protective effects of gangliosides in ischemia. However, the ischemia models had to be established first, obviously in the hippocampus, not only because it was my newly found favorite region, but also because it was one of the most vulnerable areas in ischemia. Thus, instead of testing gangliosides, I investigated the basic mechanisms of delayed neuronal death and selective vulnerability of specific cell types in the hippocampus in various models of global forebrain ischemia (Freund et al., 1989a, 1990a; Schmidt-Kastner & Freund, 1991; Freund et al., 1992).

These studies, together with the newly formed friendship with Gyuri Buzsáki, led to my ultimate switch to the hippocampus and related structures. I had a lucky start in this field, given that the study that I had already planned on the train traveling with Rusty to Gyuri Buzsáki in Pécs produced really exciting results. I managed to demonstrate that GABAergic septohippocampal neurons selectively innervate GABAergic interneurons in the hippocampus, which seemed to be an ideal circuit to produce rhythmic population synchrony, and it gave an answer to the age-old question of how septal pacemaker units can induce theta activity in the hippocampus (Freund & Antal, 1988). This conclusion was confirmed later *in vivo* by Gyuri Buzsáki and *in vitro* in a joint study with Richard Miles in Paris, using the septohippocampal slice preparation I developed in his lab together with my former doctoral student, Katalin Tóth (Tóth et al., 1997). This discovery of the innervation pattern of GABAergic septohippocampal neurons stimulated numerous studies that revealed, for example, the neurochemical identity and connectivity of the target interneurons, and the existence of the septocortical pathway with the same target selectivity in the phylogenetically most ancient cortex—the dorsolateral cortex of the lizard (Martínez-Guijarro & Freund, 1992)—as well as in the monkey (Gulyás et al., 1991). We also described the existence of the GABAergic basal forebrain-neocortical projection in rat, cat, and money and demonstrated that these projections were also selective in innervating interneurons only (Freund & Gulyás, 1991; Freund & Meskenaite, 1992).

Most of this work was carried out in Budapest, where I returned in 1989 upon invitation of E. Sylvester Vizi, director of the Institute of Experimental Medicine (IEM) of the Hungarian Academy of Sciences (HAS). He offered me a head of department position in his institute, which was an offer

difficult to refuse, since earlier department heads in this institute were usually senior academicians. Thus, for a scientist of 30 years of age, this was a really great opportunity. I believe that for a young scientist the most important issue, even more important than salary, is being able to test as many of his or her hypotheses as possible, which requires many hands and brains. At the IEM, I had the opportunity to build up a rather large lab in a short time, taking all my students and young postdocs with me from the Anatomy Department of Semmelweis Medical School. The Morphology Department at the IEM changed its focus under my leadership and was renamed the Department of Functional Neuroanatomy. The entire institute was slowly converted into a multidisciplinary brain research center under E. Sylvester Vizi's directorship.

From the mid-1980s, major changes happened also in my private life. With my first wife, who is a talented painter artist, we had our first child, Éva, in 1985. Éva inherited her mother's artistic vein and graduated from the University of Fine Arts in Budapest, as a sculptor. She tried to find some links to her father's profession as well and obtained another diploma as art therapist, working with psychiatrists on the differential diagnosis and treatment of schizophrenic patients. She made excellent portrait sculptures of *Santiago Ramon y Cajal* and *János Szentágothai*, which stand in the Szentágothai square just in front of the main building of Semmelweis Medical School, facing each other as if they were debating serious anatomical questions. A copy of both sculptures also can be found in the entrance hall of our institute. Our son Adam, who was born in 1991, also tried to find a career as far as possible from his father's, and graduated at the University of Theatre and Film Arts in Budapest. He became a successful film director. Adam's diploma film, *Earthly People*, was among the 2017 Student Academy Awards Finalists in Los Angeles. The film is about a crazy scientist father, who works day and night in the barn of their countryside house, trying to make a spaceship from washing machine spare parts and similar bits and pieces. Only his son believed in him. The wife and daughter just scoffed at him until he successfully launched himself into outer space. The film is about how different family members relate to this unexpected event later on. One may wonder where Adam got the mad scientist idea from.

During the 1990s, our circuit and molecular-level anatomy, immunocytochemistry, tracing studies, and light and electron microscopy gave rise to numerous discoveries, mostly in the hippocampus and related structures. However, it was clear from the very beginning that the functional implications of these studies would remain speculative, unless we were to provide direct electrophysiological or pharmacological confirmation. Initially, we handled this shortcoming through collaborations with physiology labs—that is, with Gyuri Buzsáki's *in vivo* lab during the mid-1980s, and later on with the *in vitro* labs of István Módy at the University of California, Los Angeles (UCLA) and Richard Miles of the Pasteur Institute in Paris. In addition to

collaborating with us, each of these three colleagues and close friends took on the responsibility of training young scientists from my lab, so that high-standard electrophysiology could be started in Budapest as well. Most of my students and postdocs—including, for example, Katalin Tóth, Iván Soltész, Attila Gulyás, László Acsády, Norbert Hájos, Attila Sík, and Viktor Varga—had a chance to study for a couple of years in one of these labs, and more than half of them returned to Budapest, which is not a bad rate. In addition to training, we had joint grants with these three labs, which allowed us to build several well-equipped electrophysiology workstations in IEM and to become a truly multidisciplinary department. Young Hungarian talents, like Gábor Nyiri, in addition to some of those just mentioned, were fortunate to have the opportunity to work in Péter Somogyi's lab in Oxford, while István Katona received excellent training in molecular biology while working in Hannah Monyer's and Peter Seeburg's lab in Heidelberg.

With such a research group, having Attila Gulyás as my deputy group leader, we were able to tackle complex questions, such as the electrophysiological characterization of individual, morphologically identified inhibitory and excitatory synapses using intracellular recording and filling of connected cell pairs in hippocampal slices (Gulyás et al., 1993a, 1993b; Miles et al., 1996). We also performed analysis and functional characterization of the hippocampo-septal and the septohippocampal projections, identification of new interneuron types, and localization of transmitter receptors with a molecular level resolution. Perhaps the most interesting finding in the interneuron classification field was the discovery of interneurons that selectively innervated other interneurons in the hippocampus, forming the basis of local circuit disinhibition. Such interneurons had not been mentioned in the literature earlier, not even at the conceptual level. Using immunostaining for vasoactive intestinal polypeptide (VIP) and calretinin (CR), we described three types of interneuron-selective interneurons (Acsády et al., 1996; Gulyás et al., 1996; Hájos et al., 1996). The most interesting type was a bipolar or bitufted cell in stratum radiatum, which sent its axon to the border of stratum oriens and alveus, where it selectively innervated the cell bodies and dendrites of somatostatin-containing GABAergic interneurons: the dendrites visualized using mGluR1a immunostaining. The CR-containing cells could be found in all layers and innervated a less-well-defined, and perhaps heterogenous, group of interneurons in stratum radiatum and oriens. Local disinhibitory interneurons subsequently have been described also in the neocortex.

An interesting finding in 1990 was that the serotonergic projection from the median raphe targeted a select population of interneurons in the hippocampus, a projection similar to the septohippocampal GABAergic projection (Freund et al., 1990b). This suggested that numerically sparse subcortical projections, which carry information about motivation, emotions, and autonomic state, gain powerful global control over cortical activity patterns by

modulating inhibition. This hypothesis gave rise to another series of studies that bear fruit even today. The *in vivo* electrophysiology lab in my group led by Viktor Varga provided evidence that the median raphe input to the hippocampus co-releases glutamate and serotonin, and selectively activates certain interneuron types through both glutamate (AMPA and NMDA) and serotonin (5HT3) receptors (Varga et al., 2009), and thereby controls hippocampal population discharge patterns. Predictions of our hippocampo-septal anatomical studies (Tóth et al., 1993) have been successfully tested by Varga's group. Using optogenetics combined with juxtacellular recordings and Ca^{2+} imaging, they demonstrated that hippocampo-septal GABAergic neurons are activated rapidly during movement onset, and in turn, inhibit septal pacemaker units.

Investigations of the median raphe also continue today. The team of Gábor Nyiri in my lab discovered a group of glutamatergic neurons in the median raphe that did not contain serotonin. These neurons represent the largest known output of the median raphe and serve as a key hub for the acquisition of negative experience. They showed that these cells receive an extensive convergence of inputs from aversion- and memory-related brain centers, and in turn, massively innervate both the lateral habenula/ventral tegmental area axis as well as the septohippocampal system. *In vivo* electrophysiological recordings confirmed that these neurons are activated selectively by aversive but not by rewarding stimuli. Their activation induced acute and conditioned place aversion, pathological aggression, depression-related anhedonia, and a memory acquisition-promoting change in the state of the septohippocampal system. In contrast, precise inhibition of these cells at the moment of the aversive stimulus strongly disrupted the expression of both contextual and cued fear memories and prevented fear generalization. These discoveries may have profound implications for normal and pathological processing of negative experience as well as for the development of new effective therapies for mood disorders (Szőnyi et al., 2019a).

The year 2019 was a really productive and serendipitous year for my lab, because the same group, led by Gábor Nyiri, managed to make another discovery that was found worthy for publication by the editors of *Science* (Szőnyi et al., 2019b). They showed that the nucleus incertus, a small cell group in the brain stem, sends a GABAergic (and relaxin3-containing) projection to the hippocampus, selectively innervating the somatostatin-containing interneurons that in turn inhibit the most distal dendrites of pyramidal cells. Thus, they control inhibition that terminates in conjunction with the entorhinal pathway and regulates its efficacy. Thus, the nucleus incertus, responding selectively to salient stimuli, reduces inhibitory control of entorhinal excitation of hippocampal pyramidal cells, thereby generating ideal conditions for memory formation—that is, they can efficiently influence the selection and sparsity of the group of memory-encoding pyramids.

The knowledge that accumulated in the 1980s and 1990s about inhibition, interneurons, and their subcortical control was assembled into a monograph by Gyuri Buzsáki and myself, titled "Interneurons of the Hippocampus" (Freund & Buzsáki, 1996), which is extensively quoted. It has received, so far, more than 2,000 citations and has been called by our colleagues worldwide the "Interneuron Bible." Writing this review and preparing all the illustrations was a really hard but most enjoyable work, which I did together with Gyuri while I visited him at Rutgers University in Newark, New Jersey, for two months. It was published as an entire regular issue of the journal *Hippocampus*.

A successful new direction of research was opened in the lab in 1996, when Ken Mackie invited me to his poster at the Society for Neuroscience meeting showing his initial immunostainings with his CB1 cannabinoid receptor antibody. For the experienced anatomist eye, it was immediately apparent that a specific type of interneuron expresses the cannabinoid receptor protein at high levels in all cortical areas, most intensely in the hippocampus. We started a collaboration with Ken, and began a series of investigations on this topic, using correlated light and electron microscopy with his antibodies, *in vitro* electrophysiology, pharmacology, and behavior. István Katona, a very talented undergraduate student started research work in my lab around that time. He was ambitious and skillful enough to gain responsibility for the morphological aspects of the project. First, we demonstrated the selective presynaptic expression of CB1 on axon terminals of CCK-containing interneurons in the hippocampus.

Electrophysiological experiments, which paved the way to the discovery of retrograde synaptic signaling, were performed by Norbert Hájos, and pharmacology, demonstrating the CB1 receptor-mediated decrease in GABA release by Beáta Sperlágh and E. Sylvester Vizi (Katona et al., 1999; Hájos et al., 2000). In a review in *Trends in Neuroscience* titled "Rhythm and Mood in Perisomatic Inhibition" (Freund, 2003), I arrived at the conclusion that while parvalbumin-containing basket cells operate as a clockwork for hippocampal oscillations, CCK-containing basket cells fine-tuned the oscillations as a function of motivational and emotional state. If the clockwork malfunctions, cortical operations may collapse, but if CCK basket cells fail, the most likely outcome would be mood disorders.

The CCK basket cells seem to be the common denominators of several types of neurochemical impairments that ultimately lead to anxiety, because they are the only cell type that expresses receptors for all anxiolytics know so far. This gave me the idea that if CB1 is also expressed by CCK basket cells, they should definitely modulate anxiety, which we successfully proved in collaboration with József Haller's group at the IEM, demonstrating that CB1 receptor knockout mice showed significantly high levels of anxiety in all models tested, whereas CB1 agonists were anxiolytic (Haller et al., 2002, 2004). Knowing that the doctor cannot prescribe marijuana for an anxious

patient, at least not in Hungary at the moment, we had to find another way to exploit this discovery. We demonstrated that the endogenous cannabinoid that regulates GABA release from CCK basket terminals is 2-arachidonoylglycerol, or 2-AG, which is synthesized by diacylglycerol lipase and metabolized by monoacylglycerol lipase (Gulyás et al., 2004; Makara et al., 2005) . We hypothesized that by inhibiting the metabolism of 2-AG we can achieve an enhancement of 2-AG levels, which is released specifically in space and time—that is, it continues to be synthesized only when and where it is demanded, but because it stays around longer, it may have effects similar to those of exogenous CB1 agonists, but without the well-known side-effects.

Our cannabinoid studies then changed gear, and—mostly with the direct supervision of István Katona—led to the discovery of CB1 receptors also on glutamatergic boutons, the subsynaptic localization of the synthesizing and degrading enzymes of endocannabinoids, the discovery of the perisynaptic signaling machinery with diacylglycerol lipase coupled to mGluR5 in the perisynaptic membrane to detect glutamate spillover, the retrograde endocannabinoid signaling at glutamatergic excitatory synapses that operate as a circuit breaker, and its role in the generation of epileptic activity (Katona et al., 2006). This series of studies led to the birth of two important reviews in the field, one we wrote together with István Katona and Daniele Piomelli in *Physiological Reviews* (Freund et al., 2003), and the other with István only in *Annual Review of Neuroscience* (Katona & Freund, 2012). In addition, in a paper in *Nature Medicine*, written with István, we postulated the existence of a perisynaptic signaling machinery that, in general, could control the release of several transmitters and could function as a circuit breaker in several neuronal or even non-neuronal systems (Katona & Freund, 2008). István then established his own lab and has since carried on with cannabinoid signaling-related work at very high standards.

During these two decades (1990s and 2000s), my administrative responsibilities also grew considerably. In 1994, Sylvester Vizi appointed me deputy director of the IEM, and when he became president of the Hungarian Academy of Sciences in 2002, I became his successor as director. In the year 2000, the Péter Pázmány Catholic University started the Faculty of Information Technology focusing on bioinformatics, infobionics, robotics, and computational neuroscience. The founding dean, Tamás Roska, and the rector, Cardinal Péter Erdő, invited me to chair the Department of Neuroscience, and organize a one-year curriculum for third-year students in neuroscience. In 1998, I was elected as corresponding member of the Hungarian Academy of Sciences, regular member in 2004, and vice president in 2014. I was also very lucky to gain recognition abroad, becoming a member of the Academia Europaea (London, 2000), the Academia Europaea Scientiarum and Artium (2001), the German Academy of Sciences Leopoldina (2001), and the American Academy of Arts and Sciences (2014). In 2019, I became a member of the Pontifical Academy for Life.

I also took my share of administrative responsibilities in scientific societies and organizations in Hungary as well as abroad. When IBRO reorganized its leadership, I was elected chair of the Central and Eastern European Regional Committee, and I was a member of the Executive Committee of IBRO from 1999 till 2005. I served as president of the Federation of European Neuroscience Societies (FENS) from 2004 to 2006 and of the Hungarian Neuroscience Society from 2009 to 2013. It was a great honor to become a member of President Barroso's Science and Technology Advisory Council in the EU (2013–2014). I also served as a member of the Committee on Committees of the Society for Neuroscience of the USA (2011–2013) and served as chair of its Young Investigator Award Selection Committee.

I was fortunate to be presented with several awards both in Hungary and abroad, although I am fully aware that awards and prizes do not necessarily indicate the value of someone's contribution to scientific knowledge. As a colleague of mine bitterly explained, "those get awards, who are given one." Nevertheless, I would like to mention one, as it indicates the value of the long-term collaboration and friendship with my mentors. The Brain Prize was established in year 2011 by the Lundbeck Foundation in Denmark. This is a €1 million prize given to scientists who have made major contributions to any field of neuroscience. The first awardees were three Hungarian neuroscientists, Gyuri Buzsáki, Péter Somogyi, and myself. This was a great honor, indeed, particularly knowing that the prestige of a new prize is going to be set largely by those who receive it. We were proud of it also because this prize shed new light on Hungarian neuroscience, which had long traditions and had earned worldwide respect in the past. Thus, we considered it as a well-deserved award of our great ancestors: János Szentágothai, who was such an important mentor for Péter and myself, and Endre Grastyán, who was a mentor to Gyuri. The prize also indicated that long-term collaborations between research groups that have overlapping focus, but different approaches, can be very productive and successful. Péter was my first mentor in the first decade of my career, while joint studies with Gyuri began in 1985.

I believe that the greatest benefit Hungarian neuroscience has received from my activities was not my discoveries, but the lobbying for the Hungarian Brain Research Program (HBRP). When I served as president of FENS, I was automatically a member of the board of the European Brain Council (EBC), a lobby alliance that united all European organizations with an interest in neuroscience (i.e., besides FENS, the European societies of neurologists, psychiatrists, neurosurgeons, neuropharmacologists, patient organizations, alliances of drug companies, and medical device companies). One of the major achievements of EBC was that brain research became a priority in two successive EU funding cycles (Framework Programs 6 and 7). This probably also had an influence on the establishment of the Human Brain Project, the largest research funding by the EU in history. This success

was brought about largely by the "Cost of Disorders of the Brain in Europe" study initiated by the EBC, which came up with scary figures that estimated the annual costs to be about €800 billion, which is larger than cardiovascular, cancer, diabetes, and rheumatoid arthritis combined, and still increasing. Using these figures, as well as EBC's lobbying strategy and power, I was able to convince the Hungarian government about the importance of brain research for society, for the healthcare system, and for the economy. I also argued that—in contrast to Hungarian soccer—Hungarian neuroscience not only has great traditions but also remains successful even today (e.g., see winners of the first Brain Prize, and the high number of European Research Council grants won by Hungarian neuroscientists). Thus, numerous arguments suggest that it is worth investing in brain research in Hungary, developing further the successful laboratories, and even establishing new ones. In the first four-year period (2013–2017), the Hungarian Brain Research Program received €40 million in support from the government, and in the second (2018-2022), it received an additional €22 million, which were the largest grants ever given in Hungary in any field of research. About 120 labs have been supported by this funding. More than 30 labs were newly established, several of which are headed by scientists who have returned from abroad as a result of our invitation and the available HBRP funding.

Throughout my scientific career, I've put considerable emphasis on disseminating research achievements to the general public, trying to translate our results to lay language, and making stories relevant and enjoyable to everyone, often by somewhat overinterpreting the data. A favorite topic of mine when delivering such lectures, a topic that also bridged the gap between my neuroscience and music interest, was "brain waves, memory and creativity, the effects of our inner world and information explosion." Our studies on the subcortical control of the hippocampus showed how selective innervation of interneurons by the pathways carrying information about motivation and emotions (i.e., our inner world) explains the great efficiency of these pathways in driving hippocampal oscillations and synchrony, which thereby can control the efficacy of storage and recall of memory traces. A slightly far-fetched conclusion from this discovery is that it also explains the well-known experience of everyone that we perfectly remember events, regardless of how long ago they happened, if they have intense emotional attributes. From this respect, it doesn't matter whether it was a tragic or a joyful experience. Motivation appears to operate in the same way.

If all of this is true, then the most important tasks from the point of efficient storage, recall, and creativity are (1) to let our brain circuits have a chance to incorporate our inner world influences efficiently into processes of memory formation, and (2) to enrich this inner world by artistic impressions, cathartic experiences, positive thinking, a desire to better understand the material world, and our mental environment (i.e., observing moral and ethical values). Inappropriate handling of information explosion, bolting

down nonselected information (e.g., surfing on the internet while gradually loosing initial motivation and emotional attitude) will result in the superficial storage of information from which no creative new ideas will emerge. Such memory traces lack a handle by which we could drag them out from unconscious storage sites during a creative thinking process. According to recent evidence, it is recall rather than storage that represents the bottleneck in making memories useful. In contrast, the involvement of our inner world in the processes of memory formation would not help much, if this inner world is poor and disbelieving. This has led to the conclusion that the inner world—particularly our range of emotions—has to be enriched and be maintained in such a state. Perhaps the easiest and most enjoyable way to achieve this is through artistic experiences, or even more efficiently by actively participating in artistic activities, in the creation of artistic values that could include playing in chamber orchestras, singing in choirs, participating in drama study groups, dancing, folk-art ensembles, and film clubs.

Music Accompanied My Entire Life

When someone regularly practices music in professional choirs or ensembles, or plays an instrument in a jazz band, it is hardly possible to abandon it even for a short period. Thus, wherever life took me, I searched for a possibility to sing or play the clarinet or sax, and if there were no such choirs or bands, we established one. For example, I had to go to the army for 11 months for a basic training at age 18, before starting university. This was the regular practice in Hungary in those years. We established a gospel quartet in our army base, singing Negro spirituals at quite high standards, even winning nationwide competitions that resulted in weeks of extra leave of absence. Thus, even this otherwise-wasted year, could not pass without musical activity. During the first year at the university in Szeged, our gospel quartet joined the Szeged University Choir, but in addition, we formed a chamber choir as well by inviting the best sopranos and altos from the main choir. This gave rise to the establishment of the Canticum Singers, which won the major nationwide cultural competition in Hungary as well as international festivals. At the Anatomy Department of Semmelweis Medical School, where I began my scientific work in 1980, we established a choir called the Budapest Anatomists Choir, which had the nickname "Body-washer's Singers," because, when there were no free rooms for choir practice in the department, we had to use the autopsy room.

Currently, I am singing in Ars Nova Sacra, which is a semiprofessional chamber choir consisting of professional musicians who graduated in the Music Academy of Budapest as instrumental soloists or conductors, as well as medical doctors, lawyers, and teachers. We have recorded three CDs, and we regularly perform in Hungary as well as occasionally abroad. My clarinet and saxophone skills slowly declined, since to keep that in shape,

you must practice almost daily, for which I obviously had no time with all my professional and family obligations. Nevertheless, I had the opportunity to play occasionally with some of the greatest professional jazz musicians of Hungary, most often with George Vukán, a pianist genius and composer, who died in 2013. Two of his film scores have been nominated for Oscar, and he accompanied international jazz icons like Philly Joe Jones, Clark Terry, and Kenny Wheeler. Our joint performances usually began with my lecture on the neuronal mechanisms of learning, memory, and creativity, as well as the involvement of our inner world enriched by arts in these processes. He then would demonstrate the amazing creativity of the human brain by improvising on three or four notes given by the audience. We concluded the evening by playing together his compositions for clarinet and piano. Even today, I occasionally play in clubs or concerts as a guest with world-famous musicians like the Hot Jazz Band.[1] My motivation to practice on the clarinet was considerably increased recently by my fiancée, who is a superb semiprofessional soprano soloist and also is working as an otorhino-laryngologist and phoniatrist physician. We tremendously enjoy playing in a trio with János Balázs, a celebrated classical pianist in Europe, although it is not easy to find music for soprano voice, clarinet, and piano. We have performed in prestigious places, like the Music Academy of Budapest, and have broadcasted live on the major classical music radio channel of Hungary[2] https://www.youtube.com/watch?v=OK9ExM-E9pM and in various cultural centers.

I am convinced that if I ever had any creative and original thoughts in my research career, it was largely due to an intervention of my inner world enriched by music. Whether the mechanism of this involves the entrainment of synchrony through cortical oscillations that depend on subcortical pathways that carry information about motivation and emotions, I am not sure. Nevertheless, I believe every word of the famous Hungarian composer, Zoltán Kodály, who said, "The mission of music is a better understanding, revival and expansion of our *inner world*. And where we reach the barriers of knowledge, music goes beyond these limits, into another world, which cannot be known, only guessed or presumed."

Our mission, as scientists, is to use the most developed, state-of-the-art technologies of our age to push the barriers of knowledge as far out as possible. But unless we can look behind these barriers, we'll never find the next best hypothesis, or the most suitable approach to explore the unknown.

[1]"A Hot Jazz Band vendége Freund Tamás, klarinét," Alexander's Ragtime Band, MOMKult, December 30, 2018, video, 4:24, https://www.youtube.com/watch?v=jni5sGPTe14, https://www.youtube.com/watch?v=UpqGgHQbDr8.

[2]"Schubert: Der Hirt auf dem Felsen, D.965," Bihari, Freund, Balázs, video, 11:49, https://www.youtube.com/watch?v=OK9ExM-E9pM.

Throughout my scientific career, music was the major guide of my instincts that allowed me to flash a glance behind the limits of knowledge and occasionally to come up with hypotheses that turned out to be realistic and useful.

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