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Kjell Fuxe

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Kjell Fuxe

Born:

Stockholm, Sweden
April 25, 1938

Education:

Karolinska Institutet, Stockholm, BMedSci (1959)
Karolinska Institutet, Stockholm, PhD (1965)
Karolinska Institutet, Stockholm, MD (1971)

Appointments:

Assistant Professor of Histology, Karolinska Institutet (1966–1968)
Associate Professor of Histology, Karolinska Institutet (1968–1979)
Professor of Histology, Karolinska Institutet, (1979–2005)
Professor Emeritus, Karolinska Institutet (2005–present)

Honors and Awards (Selected):

Member of the Nobel Assembly 1986, of Royal Swedish Academy of Sciences (1980)
Honorary Doctorates, Lyon (1992), Barcelona (1998), Ferrara (2002), Malaga (2007)
Kaiser Award, Medical Faculty, University of Honolulu, Hawaii, (1975)
Gold Medal, Research on Prolactin, First International Prolactin Symposium (1977)
Erik H. Fernströms Reward at Karolinska Institutet (1979)
Hilda and Alfred Erikssons Prize, Swedish Royal Academy (1979)
International Madonina Prize of the Biological Sciences, Milan, Italy (1980)
New York Academy of Sciences Award, Biological and Medical Sciences (1985)
IPSEN Prize, Paris, France (1992)
Albert Einstein International Academy Foundation, Cross of Merit (1993)
Humboldt Prize Award, Germany (1998)
ECNP Neuropsychopharmacology Award, Prague (2003)
The Palay Award in Structural Neuroscience, Cajal Club (2006)
Camillo Golgi Medal Award, Brescia (2012)
Sigillo d'Ateneo, University of Urbino (2012)

Kjell Fuxe has been a pioneer in brain and neuroendocrine communication and integration. He provided evidence for the existence of central monoamine neurons, volume transmission and its different forms, and receptor-receptor interactions in heteroreceptor complexes in the central nervous system. He applied this knowledge to develop novel strategies for treatment of schizophrenia, depression, Parkinson's disease, and cocaine addiction. He introduced, for example, several DA receptor agonists for treatment of Parkinson's disease and became a pioneer also in molecular neuropychopharmacology. Integration of synaptic and volume transmission signals can take place in synaptic and extrasynaptic heteroreceptor complexes, becoming a new major mechanism for neuromodulation of synapses. The reorganization of the homo- and heteroreceptor complexes in the postsynaptic membrane with novel adapter proteins may form the molecular basis of learning and memory.

Kjell Fuxe

Stockholm in the Early Years

Sweden is my country, and I was born in its capitol in 1938 just before the beginning of World War II. My father and mother came from the farming country of Västergötland at the heart of Sweden. They both grew up in the same village, which had the name Kållands-Mellby, located outside a small town named Lidköping. I do not remember having met any of my grandfathers and grandmothers on my mother's or father's side. My parents had to leave and start a new life because the oldest brother inherited the small farm. I still dream of the waving golden cornfields in the sun and the green meadows that I never saw.

My father, Nils Johansson, worked hard as a firewood dealer to take care of our family in Norrmalm in the city of Stockholm, but I never got to know him. My mother, Linnea, called Nea, was a housewife, but she wished she had become a nurse. She gave me the love I badly needed and I grew up as a happy boy. My mother was at the basis of my existence, although I did not understand it at the time. She remained close to my heart all my life. My older brother was kind, but he was six years older and we did not interact much.

I did not realize how lucky I was to live in Sweden, a neutral country during World War II. The rest of Europe suffered in its fight against Nazi Germany, while I was playing on the safe and quiet streets of Norrmalm in the city of Stockholm and in its parks. I had no idea what war meant but remember the strong voice of Winston Churchill on the radio and how happy everyone was the day the war was over. The Americans became my heroes, especially after seeing all the Hollywood war movies.

Entering a New World: Norra Latin

I began school at seven years of age and enjoyed to study and learn already from the beginning. The teachers were kind and the discipline was fair.

After passing four years in elementary school of Adolf Fredrik in Norrmalm, my marks allowed me to enter in Norra Latin, one of the best schools in Stockholm and also located in Norrmalm. There I started in a secondary school for four years. With good marks, you could pass into its high school for another four years and receive a high school diploma.

This eight-year period was full of hard work and was fundamental for my life because I learned foreign languages and began to understand biology, chemistry, physics, and mathematics. I remember that almost every morning I had to run over the alleys of the cemetery of Adolf Fredriks church,

where Prime Minister Olof Palme now is buried, because the gates of the school closed at 8 a.m.; being late had consequences. The teachers did a good job, and it did not matter that my parents were poor and lacked academic background. The only drawback was that it was a school only for boys. It made it difficult for me to understand girls. I finished my last year at the top of my class, got a stipend for this achievement, and could enter any university education of my choice. I chose medical studies because it seemed to be the beginning of something important, namely, understanding life.

Karolinska Institutet, Medical Studies

In the autumn of 1957, my studies began at the Karolinska Institutet. The first year was devoted to anatomy and histology, the second year to chemistry and physiology. I became a bachelor of medical science in 1959 and a teaching assistant at the department of histology. I loved to study the beauty of stained cells in the light microscope. In 1960, while learning pharmacology and microbiology, I managed to set up a histochemical method for demonstration of cholinesterases at the histology department. It became my first paper and I enjoyed my collaboration with histologist Bengt Fredricsson and pharmacologists Bo Holmstedt and Folke Sjöqvist at the Karolinska Institutet. The latter two became famous in pharmacology and clinical pharmacology, respectively. My next teacher was Dr. Ove Nilsson at the histology department with whom I published a number of papers in 1962 on the histochemistry of the uterine epithelium in different hormonal states.

In 1962, the important event was that Professor Nils-Åke Hillarp became the new chairman at our department of histology. He was brilliant and full of enthusiasm and energy. He wanted me to set up the histochemical fluorescence method for the cellular localization of monoamines, which he and Bengt Falck had just introduced the same year. With this method the catecholamines (CA) and 5-hydroxytryptamine (5-HT) were transformed into fluorescent compounds by means of a condensation with formaldehyde followed by a secondary dehydrogenation yielding a green and yellow fluorescence, respectively. I was more than happy to accept and chose the subject for my thesis to be focused on the possible existence of monamine neurons in the central nervous system (CNS). Professor Hillarp also collaborated with Professor Arvid Carlsson at the Department of Pharmacology, University of Göteborg, and in 1962, they published a fine article on the existence of a high density of noradrenaline (NA) nerve terminals in the hypothalamus.

Karolinska Institutet, Thesis Period, 1962–1965: Evidence for the Existence of Monoamine Neurons in the CNS

During this period, I stopped my medical education to complete my thesis. A two-years younger medical student, Annica Dahlström, helped me when

she was not occupied by her medical studies. My first publication on the brain was based on the indications of the existence of tubero-infundibular dopamine (DA) neurons, which may have a significant role in neuroendocrine regulation. The work on my thesis turned out to be a tremendous struggle because it was impossible to standardize the Falck-Hillarp method, at least with regard to the analysis of the CNS. Working day and night, never giving up, I could obtain a sufficient number of excellent sections from many rats, which made it possible for me to obtain evidence for the existence of DA, NA, and 5-HT cell bodies and nerve terminals and their architecture (Dahlstrom and Fuxe 1964; Fuxe 1965a, 1965b). The axon bundles could be visualized by means of monoamine oxidase inhibitors increasing the monoamine levels and ablations and lesions, which led to the high accumulation of monoamines in axons, especially on the cell-body side of the injury.

This work led to the discovery of the nigro-striatal DA pathway (Anden et al. 1964), degenerated in Parkinson's disease (PD), and to the mesolimbic DA pathway innervating mainly the nucleus accumbens and the olfactory tubercle (Fuxe 1965a, 1965b). The papers with Dr. Anden were not part of my thesis but rather were published in parallel with the thesis work. They represented the results of a fine collaboration with Professor Arvid Carlsson's team, where Dr. Nils-Erik Anden gave us the biochemical correlates to the results with the Falck-Hillarp histofluorescence method. Also in parallel, we found the impressive NA and 5-HT nerve terminal networks in the ventral, lateral, and dorsal horns of the entire spinal cord from the rostral to the caudal levels after a number of failures.

What stands out in my memories of the thesis work is the magnificent DA nerve cell groups of the ventral tegmental area, the unique beauty of the NA cell groups of the locus coeruleus (LC), and the subcoeruleus and the 5-HT nerve cell groups of the dorsal and median raphe. I remember these moments when the images created by the green CA fluorescence or the yellow 5-HT fluorescence reached my eyes in the darkness of the microscopy room and changed my life. Happiness came around and said hello.

The opponent (external examiner) at my thesis on April 25, 1965, was Professor Theodore Blackstad from the University of Oslo. He was an outstanding neuroanatomist. Much to my relief he liked my thesis. We had a nice discussion and became friends.

It seemed to me that the identification of the central DA, NA, and 5-HT brainstem neurons in my thesis (1965), innervating more or less globally the brain and the spinal cord, represented the beginning of chemical neuroanatomy. It was made possible through the visualization of these neurotransmitters at the cellular level with the Falck-Hillarp histofluorescence method. The innervation of the brain and spinal cord from the brainstem NA and 5-HT neurons appeared to take place through extensive collateralization of their ascending (Fuxe 1965a, 1965b) and descending pathways. The nigro-striatal and mesolimbic DA neurons were more

restricted in their collateralization and mainly involved an innervation of the dorsal and ventral striatum, respectively. Later on in 1973, however, the Glowinsky group discovered the cortical projections of the mesolimbic DA neurons that had a major impact in the DA field, which we rapidly validated.

There were shadows over my thesis celebration because my mentor and supervisor Professor Nils-Åke Hillarp died from a malignant melanoma just one month before my thesis defense. I had a close relationship with him, as I was his first graduate student at the Karolinska Institutet. I respected and admired him very much, and he became my friend. In spite of increasing bad health, he gave me invaluable support and help. His enthusiasm and encouragement made the tough work possible. After having been fortunate to meet, get to know, and work together with this great scientist, it was bitter, sad, and depressing to face the fact that he was gone and no longer with us.

Karolinska Institutet after My Thesis, 1965: Reorganization and Meetings in Stockholm, Milan, and New York

It was far from clear how the Hillarp students would survive at the histology department after the death of Hillarp. However, the prosektor (associate professor) Lars Gyllensten helped us a lot. He became a good friend.

Gyllensten was also a brilliant author and wrote several outstanding novels. He was invited to join the Swedish Academy, which gives out the Nobel Prize in literature, and soon led the Academy, being selected to become its secretary.

Two other Hillarp students defended their thesis after me in the spring of 1965: Torbörn Malmfors and Karl-Axel Norberg. It was decided that the Hillarp students should form an Amine Group at the department built up of three subgroups, each led by one of the three that had obtained their doctor degree in the spring. Medical student Annica Dahlström decided to leave and have her thesis made at the department of pharmacology at the University of Göteborg where Professor Arvid Carlsson was the chair. Instead, medical student Urban Ungerstedt decided to join me. He was a young man with many unique talents. We became good friends, and he made an excellent thesis on the central CA neurons, which he defended in 1971. He became professor of neuropsychopharmacology at the Karolinska Institutet because of his outstanding scientific contributions. At the end of 1965, the Swedish Research Council gave equal support to all the three subgroups, and the Amine Group was able to survive for at least a three-year period.

As a result of my thesis work, I was invited to speak on the topic of central monoamine neurons at several conferences in 1965. One was organized on mechanisms of release of biogenic amines at the Wenner-Gren foundation by U. S. von Euler, S. Rosell, and Börje Uvnäs, professors at the Karolinska Institutet. Another one was the second international CA symposium in Milan in July, where I for the first time presented our illustrations of

the central DA, NA, and 5-HT neurons and their pathways. It was an exciting meeting, and I got to know great young scientists in the CA field like Floyd Bloom and Jacques Glowinsky. After the meeting, I went to Venice for a day or two to enjoy the Mediterranean Sea together with Nils-Erik Anden, Arvid Carlsson, and Annica Dahlström. On the way back, I had a nice lunch with the outstanding chemist Hans Corrodi and his family on the green hills of Zurich. I still remember the scent of cut grass and the spectacular view, which made me think of Earth as being close to paradise.

The most exciting meeting for me was held on November 29, 1965, in New York. It dealt with the biochemistry and pharmacology of the basal ganglia. It was organized by Drs. Costa, Cote, and Yahr at Columbia University. I spoke on the nigro-striatal DA neurons and how its degeneration can cause the motor symptoms of PD and can explain the disappearance of DA levels as found by Hornykiewicz. The audience was excited, but one classical neuroanatomist claimed that the ascending DA pathways did not exist. I explained to him that transmitter histochemistry can discover pathways not previously detected by classical neuroanatomy. Arvid Carlsson and Nils-Erik Anden were also there, and we had a good time in Manhattan together, especially at Gallagher's steak house on the west side, close to Times Square. We felt that our work was becoming recognized in the neurology field.

Even so, there were groups, especially from Chicago, that still claimed that the monosynaptic NA and 5-HT pathways from the lower brain stem to the cerebral cortex did not exist. It took more than 10 years and lots of work before our long ascending and descending monoamine pathways to the cerebral cortex and the spinal cord were accepted in the entire neuroanatomy field.

After New York, I traveled to Los Angeles. I had been invited by Dr. Arnold Scheibel at UCLA, School of Medicine to give a lecture on the central monoamine neurons. Arnold Scheibel is known from his work on the architecture of the brain and spinal cord using inter alia Golgi techniques. He was kind to me and genuinely interested in our work. He appeared to believe in our evidence that the NA and 5-HT brainstem neurons from the reticular formation, including the raphe regions, could directly innervate the cerebral cortex. It was the first time such neuron systems were shown to directly reach and form vast networks of varicose terminals in the limbic and neocortex.

I also met Professor Horace Magoun at UCLA. I was honored to meet him because he was already famous from his work together with Dr. Giuseppe Moruzzi on the ability of electrical stimulation of the brainstem reticular formation to produce arousal and awakening in animal experiments. He was the director of the Brain Research Institute on the campus, and I remember him as full of life and devoted to his institute. He was a charming host at the lunch in Bel Air Country Club on the hills facing the university buildings in the sun. He indicated that perhaps our ascending monoamine neurons may be involved in the arousal mechanism they had discovered. I was happy to

enjoy the world of California and its science and felt its attraction. I realized, however, that it would be difficult to work effectively in such a lovely climate. It was sad saying goodbye to California.

Karolinska Institutet, 1966–1968: Application for a Prosektor (Associate Professor) Position at the Department of Histology

This was another important period filled with hard and exciting work. It became clear that to stay on at the Karolinska Institutet it was crucial to obtain a permanent position. It was also necessary for the survival of the Amine Group. I was lucky that such a position became free at the department in this period. To increase my chances, I felt it was important to study also the function and pharmacology of the central monoamine neurons. This became possible through the development of methods to study changes in monoamine turnover through inhibition of tyrosine hydroxylase (TH) and trytophane hydroxylase and measuring the disappearance rate of DA, NA, and 5-HT neuronal levels with biochemistry and histochemistry (the Falck-Hillarp technique). I was happy to collaborate in this project especially with the biochemist Hans Corrodi and the pharmacologist Nils-Erik Anden at Arvid Carlsson's department in Göteborg. The work was initiated by Hans Corrodi, and I was already friends with both of them. I am sorry to say that they are no longer with us.

We showed that the disappearance of the monoamines after synthesis inhibition was dependent on impulse flow. Anden, Corrodi, and I wrote a summary of this work in the proceedings of a meeting of the British and Scandinavian Societies in Edinburgh in July 1968. I still remember the excellent lecture of Professor John Vane, which was performed in great style.

The degree of disappearance of monoamines was highly correlated with the activity of the discrete neuron systems analyzed, and we frequently used this method to characterize the dynamic changes in discrete DA, NA, and 5-HT neurons in various physiological and pathological states and after treatment with neuropsychopharmacological compounds like the hallucinogen lysergic acid diethylamide (LSD). The reductions of striatal DA turnover we observed in 1967 with apomorphine also strongly indicated that apomorphine possessed DA agonist properties, as previously proposed by A. M. Ernst the same year based on behavioral work.

Later on in 1969–1970, Dr. Gordon Arbuthknott was a guest scientist at the department working, especially, with Urban Ungerstedt in my group and also Lars Olson in another group. They were three friendly, young, and fine scientists. Together, we obtained indications that the mesolimbic DA neurons represented a reward pathway. Thus, electrical stimulation of the ventral tegmental area DA cells, using the same protocol as for electrical self-stimulation, appeared to enhance the neuronal activity of the mesolimbic DA neurons. This was indicated from the enhanced disappearance of

the accumbens DA fluorescence after TH inhibition giving evidence for an increase of DA turnover in the mesolimbic DA neurons. Dr. T. J. Crow working with us, now a psychiatrist and researcher at University of Oxford, also played a significant role in this work.

In a series of papers, we also studied DA turnover changes in the median eminence terminals of the tubero-infundbular DA neurons in different hormonal states. This work made it possible to propose that they participated in the inhibitory regulation of gonadotrophin and prolactin secretion at the median eminence and anterior pituitary level.

The most interesting finding in this period was the demonstration in 1967 that after intraventricular injections of 5-HT, Ungerstedt and I, using the Falck-Hillarp technique, observed an accumulation of 5-HT in the central 5-HT neurons. The overall analysis indicated the existence of an uptake-concentration mechanism in the 5-HT neurons (Fuxe and Ungerstedt 1967). It also indicated the possibility that not only the NA uptake-concentration mechanism discovered by Nobel Laureate Julius Axelrod but also a 5-HT uptake-concentration mechanism could be a target for antidepressant drugs. I approached Arvid Carlsson for a collaboration on the effects of antidepressant drugs on this 5-HT uptake mechanism. He agreed to be part of the project. In 1968, we could in fact show that imipramine blocked the 5-HT uptake mechanism (Carlsson et al. 1968). This was further documented the same year by our group, and imipramine was also found to reduce 5-HT turnover in the brain as part of a negative feedback mechanism. The same was true also for other classical antidepressants. This was the beginning of the development of new antidepressant drugs based on their selective ability to block the 5-HT uptake mechanism. It led to a new era in antidepressant therapy with the introduction of serotonin selective reuptake inhibitors (SSRI), the first being fluoxetine.

I was also happy to collaborate in this period with Dr. Donald Reis who later became famous in the cardiovascular field. We found under his leadership that sham rage following brainstem transection was linked to NA release. We became friends, and he always remembered his time in Sweden with joy.

Happy Ending in 1968

I could add manuscripts from the work in this period to the application for the *prosektor* position and believe it helped me in the competition with the other applicants. Two of the three reviewers voted for me, but there were complaints from another applicant and many in the medical faculty turned against me, especially those from the physiology department. At the end, I was finally approved by the faculty at the Karolinska Institutet. I was lucky.

The year 1968 turned out to be my best year ever, and not only because I got my permanent position. I fell in love with a beautiful girl working as first lab assistant at one of the departments at Karolinska Institutet.

We married in April 1968, and I got one son for free from her previous marriage, Jonas, who now is associate professor at Karolinska Institutet. My other son Jörgen arrived in August 1968 and looks a little bit like me. My daughter entered the world in June 1971. Our children were all healthy and wonderful. I am a proud father and have a great wife. I believe it is rare to be so lucky, especially in the world of science, who is a demanding lady.

Meetings in 1968–1969 in Edinburgh, Mexico City, Puerto Rico, and Milan

I gave a lecture at the Third Symposium on PD in Edinburg in 1968 on the nigro-striatal DA neurons. I had the pleasure to meet Professor George Cotzias, a tall and friendly scientist with a strong voice, who was the first to provide the evidence that levodopa in fact possessed anti-Parkinson actions.

The same year I was invited to the Third International Symposium on Endocrinology in Mexico City. I introduced our concept that the tuberoinfundibular DA neurons played an inhibitory role in the regulation of luteinizing hormone-releasing hormone (LHRH) release from the external layer of the median eminence based on our results on DA turnover changes in the external layer of the median eminence in various endocrine states. There was no agreement on the neuroendocrine role of the tuberoinfundibular DA neurons because inter alia microinjections of CA into the third ventricle gave opposing results, namely, an enhancement of LH secretion. This controversy stayed around for a long time.

In 1969, a meeting was held in Puerto Rico on contemporary research methods in neuroanatomy. I was invited to speak on fluorescence microscopy in neuroanatomy with a focus on the Falck-Hillarp technique for the cellular demonstration of DA, NA, and 5-HT in the CNS. I met the organizers Professor Walle Nauta and Professor Sven Ebbesson and liked both of them. I was proud to meet and play golf with Walle Nauta, who was one of the world leaders in neuroanatomy.

The most important meeting for me was the Second International Meeting of the International Society for Neurochemistry in Milan in the summer of 1969. I was part of a symposium on "Neurochemical Aspects of Hypothalamic Function" organized by Professors L. Martini and J. Meites. I gave a presentation on central monoaminergic neurons and their participation in the regulation of anterior pituitary function. It was a friendly meeting with fine discussions, and I met Professor Menek Goldstein working at the New York University Center. He was one of the world leaders on the neurochemistry of CAs. We immediately liked each other, and Menek was interested in understanding central CA pathways. We decided to take a train ride to the famous lake country of Northern Italy. As we watched the beautiful countryside with the green hills of Italy passing by, we decided to start a collaboration and map out the central CA pathways with immunohistochemistry based on the use of his antibodies against TH, dopa decarboxylase, and

DA-beta-hydroxylase. We became truly happy because we had great expectations, and this led to a great collaboration and a large number of papers that further established the architecture of the central monoamine neurons. A warm friendship developed from the very beginning. I still remember the joy I felt at seeing the DA-beta-hydroxylase immunoreactivity (IR) in the LC for the first time. At the end of 1969, I invited Dr. Hökfelt to join my collaboration with Menek Goldstein.

1970s Quantitation of 5-HT and CA Fluorescence

It was important to move away from subjective evaluations to quantitative measurements of monoamine fluorescence. Microfluorimetric measurements were introduced to quantitate the monoamine fluorescence in cell bodies and nerve terminals in combination with a microspectrofluorimetric analysis. In 1974 quantitative comparisons were made on amine fluorescence in NA terminals based on counts in photographs using different transmittance gratings. In this way, quantitative comparisons could be made not only on fluorescence intensity but also on the number of NA varicosities. Luigi Agnati was an Italian postdoc in my laboratory who became a professor of physiology of the Department of Physiology at the University of Modena. We became great friends, and we have worked together in a large number of projects for more than 40 years together with our groups in a splendid way.

Together with Kurt Andersson, an excellent doctoral student; Dr. F. Wiesel, an outstanding neurochemist and psychiatrist; and Professor Luigi Agnati, we also developed a method in 1979 to determine the DA levels and turnover rate in DA terminal networks of the brain by quantitative use of the DA fluorescence derived from the Falck-Hillarp formaldehyde histofluorescence technique.

1970s Novel Developments in the Histochemical Analysis of the Central Monoamine Neurons

6-Hydroxytryptamine became a new tool in monoamine fluorescence histochemistry, because it gave a high fluorescence yield with the Falck-Hillarp technique. It could be taken up into both CA and 5-HT terminal networks, and by use of selective 5-HT reuptake inhibitors, it was possible to have an improved visualization of the brain 5-HT nerve terminal networks. The high success of immunohistochemistry with excellent morphology through formalin fixation was also clear to us. This was based on our own work on the localization of CA synthesizing enzymes in the 1970s. We reported our findings at a meeting organized by Professor O. Eränkö in Helsinki in 1971 and published in *Progress in Brain Research* the same year. Therefore, 5-HT immunohistochemistry appeared to be the best approach. In 1981, the brain 5-HT nerve terminal networks were mapped out in all their details in an

excellent way by Dr. Harry Steinbusch. We published a paper in 1974 on the theory, practice, and application of the Falck-Hillarp technique in the *Journal of Histochemistry and Cytochemistry*. The immunohistochemistry was taking over, however, especially in view of the varying quality of the results obtained with the Falck-Hillarp technique, which could not be well controlled.

1970s Novel Observations on the Striatal DA Terminal Networks: Striatal Islands of DA Nerve Terminals

In 1970, I was invited to a symposium by Professor de Ajuriaguerra and Professor Gauthier on the "The Central Grey Nuclei of the Monoamines and the Parkinson Syndrome" held in Bel Air, Geneva. I hoped to find novel types of striatal DA nerve terminal networks in the rat by also analyzing the striatum in postnatal development. The reward came quickly with the discovery of highly fluorescent striatal DA islands with densely packed DA nerve terminals against a dark background, because DA terminals in the surrounding striatal tissue had not yet been developed or lacked DA storage. These primary observations were published in a symposium book by Georg and Cie (Ajuriaguerra and Gauthier 1970). This work was done in collaboration with Drs. Lars Olson and Åke Seiger. Both became professors at the Karolinska Institutet. Professor Lars Olson played a significant role in the Nobel assembly and became an international leader of high recognition in neuroscience thanks to his outstanding work.

These observations gave the first indications that the striatum may be organized as islands and as a matrix to integrate information from, for example, glutamate terminals from the cortex cerebri and dopamine terminals from the substantia nigra of the midbrain. In 1972, we published the original article, including evidence that these DA islands exist also in the adult rat (Olson et al. 1972). In parallel and independently, the striatal DA islands also were demonstrated in the rabbit by the Tennyson group at Columbia University.

It is of substantial interest that a new type of ergolene derivative was indicated to be a preferential agonist at ventral striatal DA receptors and at DA receptors belonging to the DA islands but not to the diffuse matrix DA terminal system of the dorsal striatum. This observation led to a selective reduction of DA turnover in the ventral striatum and in the dorsal striatal islands as determined by the TH inhibition method. Using this ergolene derivative in combination with the TH inhibition, the islandic DA system could be excellently demonstrated in the adult rat as well, because of the higher rate of disappearance of DA in the diffuse matrix system as a result of its higher DA turnover.

Professor Ann Graybiel, at McGovern Institute for Brain Research at the Massachusetts Institute of Technology, called the striatal islands striosomes and made pioneering work on their connections and function over many years. The gamma-aminobutyric acid (GABA) output from the

striosomes were found inter alia to participate in the integrative afferent compartments of nigral DA dendrites. It has been a great pleasure to meet and discuss this finding with Ann Graybiel at several meetings over the years. She is one of the world leaders in the basal ganglia field.

1970s Novel Observations on the Locus Coeruleus NA Projections

The Histology Department made major contributions to the LC field in the 1970s. The results gave evidence that the NA LC nerve cells formed the entire NA nerve terminal networks in the ventral and dorsal horn through descending projections, the global cerebellar terminal networks, and the global terminal networks of the neocortex and limbic cortex. The hypothesis was advanced that many LC nerve cells may each produce both ascending and descending NA projections as well as cerebellar projections from which massive NA collaterals can arise. This view is now being challenged by a few groups. Nevertheless, I believe our concept is correct, but it is possible that certain NA LC cells may preferentially produce ascending projections targeting, for example, limbic or neocortex. Others may preferentially produce descending projections targeting the ventral or dorsal horn.

To me, early on, it was highly attractive to look upon a single NA LC neuron as a reticular formation brainstem neuron modulating globally the entire CNS from the spinal cord to the cerebellar cortex and the cerebral cortex with collaterals innervating the reticular formation and the thalamus.

We had similar ideas on the subcoeruleus NA system, which like the other remaining NA cell groups, mainly send projections to the autonomic and neuroendocrine regions of the CNS. We proposed that the subcoeruleus NA neurons may have ascending axons to the hypothalamus and preoptic regions and descending axons to the nucleus tractus solitarius (NTS) and the sympathetic lateral column of the spinal cord. Their nerve terminal varicosities were larger with a more intense NA fluorescence and a higher density of these NA terminals was formed compared with the LC NA terminal networks. These features of the autonomic NA nerve terminal plexus may be related to the need for a safe and tonic activation of multiple adrenergic receptor types at different micrometer (μm) distances from these NA varicosities dependent on NA diffusion.

1970s Understanding Communication in the Central Monoamine Neurons

From the beginning of this work, in my thesis it was unclear whether the CA terminal networks in the brain only operated via synaptic transmission (Fuxe 1965a, 1965b). The hypothalamic NA nerve terminal plexa looked the same as the NA nerve terminal plexa in the autonomic nervous system with large varicosities and an intense NA fluorescence as shown by Torbjörn Malmfors, another student in the Hillarp lab. These varicosities did not

belong to synapses. We proposed that the varicosities represent parts of the central monoamine terminals specialized for synthesis, storage, and release of CAs and 5-HT (Fuxe 1965a, 1965b). Synaptic transmission was the dogma for communication in the CNS. It seemed possible, however, that diffusion of monoamines could take place from the varicosities into the extracellular space.

It was therefore of high interest that amphetamine, a DA-releasing drug, produced an extraneuronal DA fluorescence around the DA cell bodies of the zona compacta and the ventral tegmental area in the presence of monoamine oxidase inhibition. Urban Ungerstedt and I presented these findings at a meeting on Amphetamines and Related Compounds in Milan in 1969, organized by E. Costa and S. Garattini. The book chapter was published in 1970 (Fuxe and Ungerstedt 1970). Dr. Costa was always a dominant force, but no one seemed to be interested in our results. He was a first-class pharmacologist but is unfortunately no longer with us.

At the 1969 meeting in Puerto Rico on Contemporary Research Methods in Neuroanatomy, I also showed that after a 5-HT uptake blockade with chlorimipramine, an extraneuronal 5-HT fluorescence was demonstrated outside the 5-HT cell bodies of the dorsal raphe in the presence of monoamine oxidase inhibition. These two findings gave me the first indications that monoamines may be able to reach the extracellular space after release from cell bodies and dendrites and, through diffusion in the um range, may reach and activate extrasynaptic monoamine receptors.

In support of this view that monoamines also can communicate via the extracellular fluid, we found that application of DA crystals into the neostriatum led to the appearance of a DA diffusion gradient (Ungerstedt et al. 1969). It was the beginning of the discovery of a novel mode of brain communication that we, along with Luigi Agnati, called *volume transmission* (VT).

1970s 5,6-Dihydroxytryptamine and 5,7-Dihydroxytryptamine as New Tools in the Mapping of Central 5-HT Neurons and in Producing Their Selective Degeneration

This work was possible through a close collaboration with the outstanding biochemist John Daly at the National Institutes of Health (NIH) who synthesized the compounds and played a great role in our discussions. Dr. Gösta Jonsson, also in our department, played an important role. It was always great to meet John, a complete person both in science and life who traveled in the jungle to find biological material from which new compounds could be obtained. He always faced the risk of getting a tropical disease. I miss him deeply and feel truly sad that he is no longer with us.

5,7-Dihydroxytryptamine turned out be the most selective in lesioning the ascending 5-HT neurons. Together with especially Drs. B. Everitt, K. Hole, and K. Kiianmaa, we published a number of papers on the impact of their degeneration on sexual and aggressive behaviors and on the sleep-wakefulness cycle.

In the work on the sleep-wakefulness cycle, we followed in the footsteps of Professor Michel Jouvet and his group in Lyon who pioneered this field. We could validate his discovery that the ascending 5-HT neurons are required for the maintenance of SWS2. Peter Lidbrink, my graduate student, also was able to validate Jouvet's work showing that the cortical NA terminals have a role in maintaining cortical arousal. I remember the genuine appreciation Jouvet showed us in response to our neurohistochemical findings on the existence of the ascending NA and 5-HT neuron systems with monosynaptic projections to the entire cerebral cortex. He was a great scientist full of charms and French culture at its best.

A summary of this work was presented at a meeting on serotonin neurotoxins organized by the New York Academy of Sciences. The neurotoxic effects on the 5-HT axons made possible an outstanding demonstration of the ascending 5-HT axons through the accumulation of the yellow 5-HT fluorescence in the axons on the cell body side of the lesions because of the axoplasma flow of the 5-HT storage vesicles from the 5-HT nerve cell bodies of the midbrain 5-HT cell groups.

We wrote many papers with Dr. Barry Everitt in this period on the role of DA and 5-HT in sexual behavior using mainly a behavioral pharmacology approach. I remember our fine interactions. Barry Everitt is currently one of the leaders in the world on drug addiction and works as a professor at the University of Cambridge.

1970s Development of DA Receptor Agonists as a Novel Treatment of PD

We had been interested in developing DA receptor agonists for the treatment of PD since our discovery of the nigro-striatal DA pathways. The idea was that in contrast to levodopa, DA receptor agonists could directly activate the DA receptors and were not dependent on the presence of DA nerve terminals in which DOPA decarboxylase converted levodopa to DA for subsequent release. We assumed that with progressive degeneration of the striatal DA terminals in PD, levodopa treatment could still exert its anti-Parkinson actions through diffusion of DA in the extracellular fluid from remaining DA terminals into regions with DA receptors lacking DA terminals. Thus, in line with our early work (see previous section), we assumed the existence of a DA transmission operating via extracellular fluid diffusion in the extracellular space enhanced by the relative absence of DA terminals with DA uptake mechanisms.

This novel DA transmission was also enhanced by the development of DA receptor supersensitivity in the striatal regions with few or no DA nerve terminals. This was first demonstrated by Urban Ungerstedt in 1971 in his hemi-Parkinson rat model produced through 6-hydroxydopamine neurotoxin (6-OHDA) microinjections into the rostromedial substantia nigra. We were aware in 1970 through collaboration with Drs. Butcher and Engel in Arvid Carlsson's lab that levodopa could be taken up and decarboxylated in

the central 5-HT neurons. Therefore, after levodopa treatment, DA also can be formed in striatal 5-HT terminals and can be released as a false transmitter from these terminals and diffuse via extracellular fluid transmission to activate supersensitive DA receptors in PD.

We therefore proposed that DA receptor agonists should be an interesting therapeutic strategy for the treatment of PD. Apomorphine (see the previous section) was potent but short lasting. We found an interesting compound called ET495 (a piperonyl-piperazine derivative) that produced hypotension, as also found after levodopa treatment, likely because of activation of peripheral dopaminergic receptor systems. In behavioral studies, it showed anti-Parkinson-like actions in hemi-Parkinson rat, as found after levodopa and apomorphine, and reduced striatal DA turnover (Fuxe 1979). In view of my collaboration with Menek Goldstein, I contacted him about also testing ET495 in a monkey model of PD. I was happy to visit his laboratory at the New York University Medical Center and met his colleagues, Dr. Battista, a neurosurgeon, and Drs. Ohmoto and Anagnoste, who were working in the laboratory. It was of high interest for me to watch the antitremor actions of ET495 and the involuntary movements induced. In 1973, a report was published in *Science* in which the effects of levodopa and the DA receptor agonist ET495 were compared with regard to effects on tremor and involuntary movements. We demonstrated that DA receptors mediated the antitremor actions and the induction of involuntary movements (Goldstein et al. 1973). It was tested in clinical trials for anti-Parkinson effects under the generic name of Piribedil and was shown to have therapeutic actions by Chase and colleagues at NIH in 1974. We were happy that this translational research had been successful. ET495 was later characterized as a D2/D3 agonist.

The work on Piribedil took me to Tunisia in 1975 for the first time. Here I had the pleasure of meeting another outstanding neurologist, Professor Charles Marsden who at the age of 34 already had been appointed to first chair of Neurology at King's College in London. He turned out to be a great person, brilliant and highly critical in a friendly and humorous way. We were of the same age and in love with neuroscience. In the 1990s, Charles Masden died. He is missed by all of us.

Our intense research on the tubero-infundibular DA neurons indicated that these neurons were involved in the inhibitory regulation of prolactin and LH secretion, later on it was demonstrated that DA receptors existed not only in the brain but also on the prolactin-producing cells of the anterior pituitary. There were indications that 2-br-ergokryptine-methanesulfonate (CB154) could inhibit prolactin and LH-dependent functions, such as ovulation. Therefore, I became interested in testing this compound on the amine turnover in the tubero-infundibular DA neurons. The tubero-infundibular DA neurons, however, did not change their DA turnover in the median eminence in response to CB154. Instead, I found that the striatal DA turnover was reduced by CB154, which indicated a DA agonist action.

The reduction of striatal DA turnover was validated biochemically by Dr. Hans Corrodi in Arvid Carlsson's laboratory. Then, the DA agonist action of CB 154 was demonstrated in the hemi-Parkinson rat model by Ungerstedt, as shown by contralateral turning behavior of long duration (Corrodi et al. 1973). Menek Goldstein and his group became interested in CB154 and demonstrated its long lasting antitremor activity in their monkey model of PD.

We then started clinical trials with CB154 under the leadership of Dr. A. Lieberman under its generic name bromocriptine, and its long-lasting anti-Parkinson efficacy was demonstrated. These results validated the positive results published by Dr. Donald Calne on bromocriptine in PD in 1974.

It was a true pleasure to meet and get to know Drs. Lieberman and Calne, two outstanding neurologists. They contributed in a significant way to the clinical development of bromocriptine as an anti-Parkinson drug. For many years, bromocriptine became a highly used drug for treatment of PD, second only to levodopa. In 1978, I organized with Dr. Calne an international Wenner-Gren Center symposium on "Dopaminergic Ergot Derivatives and Motor Function" in Stockholm to understand their future potential in the treatment of motor disorders in view of their potent anti-Parkinson actions.

Our research and others indicated that the DA receptors activated by bromocriptine were not linked to activation of adenylate cyclase (AC) but involved a DA receptor now known as D2 receptors with an inhibitory coupling to AC mediated via Gi/o activation. Thus, multiple DA receptors appeared to exist. These results indicated that activation of D2 receptors played a significant role as a target for treatment of PD. Many ergot drugs appear to act as partial DA agonists with the ratio of DA agonist–DA antagonist activity varying from one DA receptor subtype to another one. We realized that ergot drugs in general appear to target multiple DA, NA, and 5-HT receptor subtypes, each of them having their own profile with regard to potency (Fuxe et al. 1978).

The most potent ergot DA agonists in our hands were agroclavine and elymoclavine, but there was no support to go ahead with these interesting ergots of the clavine type. I believed that an in-depth analysis of these ergot compounds would be rewarding in spite of side effects reported on many ergot drugs leading to their withdrawal from clinical use.

Our work on DA receptor agonists as tools in neuroscience and as anti-Parkinson drugs was summarized in a *Trends in Neuroscience* article in 1979 (Fuxe 1979).

1970s On the Mechanism of Action of d-LSD and the Hallucinogenic Indolamines

On the basis of findings in 1968 that the hallucinogen d-LSD could potently reduce brain 5-HT turnover and activate postjunctional 5-HT receptors, we made the suggestion that d-LSD can produce hallucinations via activation of postjunctional 5-HT receptors in the brain. In 1970–1974

the hallucinogenic indolamines, psilocybine, dimetyltryptamine, and 5-methoxy-N,N-dimetyltryptamine were shown to produce similar actions, which was true also for the hallucinogenic phenylethylamines. These results strengthened our hypothesis.

In parallel, George Aghananian, a pioneer in neuropsychopharmacology and working at Yale School of Medicine, had found d-LSD and hallucinogenic indolamines to have their highest potency at presynaptic 5-HT receptors, leading to reductions in the firing of the dorsal raphe cells. Aghajanian and his group therefore proposed that the hallucinations produced by these compounds were caused by their highly potent actions at presynaptic 5-HT receptors. However, they obtained similar results also with antidepressant drugs, and therefore, this hypothesis appeared to be less likely to them also. I have had a good relationship with George over the years, and we always said a friendly hello to each other at the meetings we attended.

In 1977, we found that the antidepressant drugs amitriptyline and nortrypyline had affinity for some of the d-LSD binding sites in the cerebral cortex, but they had no or very weak affinity for the high-affinity 5-HT binding sites. Both these drugs blocked d-LSD-induced head twitches. We therefore believed that these results indicated the existence of two types of 5-HT receptors, but we were not allowed by the reviewers to put this suggestion into the text. The evidence came a couple of years later in the pioneering papers from Professor Solomon Snyder and his group at Johns Hopkins School of Medicine.

In view of these results, we were of the opinion that the hallucinogens reported earlier targeted a distinct type of 5-HT receptor at which a special type of signaling could be induced by the hallucinogens versus 5-HT, the natural transmitter.

At a meeting on neuroleptics in Belgium in 1970 organized by Janssen and Bobon, I underlined the possible impact of dysfunctional mesolimbic DA neurons on schizophrenia development. It seemed reasonable that there should be a link between the mesolimbic DA neurons and the 5-HT receptor subtype targeted by the previously noted hallucinogens.

In 1975, I was invited to give a lecture at a conference in Capri on "Schizophrenia Today" organized by Kemali, Bartholini, and Richter. This was a golden opportunity to give a summary of our work on the biochemistry and pharmacology of hallucinogens. We received no negative or positive response to the lecture, but it felt good to bring it all together, including the use of 5-HT receptor antagonists. Above all, I loved the island of Capri. The view over the mountains and the Mediterranean Sea was spectacular. At the terrace, there was a delightful lemon tree close by and an icy cold limoncello within reach at sunset. The feeling of being in an Italian heaven entered my mind.

1970s GABA-DA Interactions

With the arrival of the biochemist Dr. Miguel Perez de la Mora in the laboratory from the National Autonomic University of Mexico in Mexico

City with high expertise in the GABA field, it became possible to study GABA-DA interactions in detail. We found in 1975 that the DA receptor agonist apomorphine can increase GABA turnover in the ventral tegmental area and the substantia nigra rich in DA nerve cells. The results suggested that activation of DA receptors can induce a GABAergic feedback control of the ascending DA neurons. We also found an inhibitory GABAergic control via GABA-B receptors of the amine turnover in the mesolimbic DA neurons. This finding opened up the possibility of improving treatment of schizophrenia through a combined treatment with GABA-B drugs and DA receptor antagonists. We were invited in 1976 to present our results at a meeting on "Psychopathology and Brain Dysfunction" organized by Shagass, Gershon, and Friedhoff. We also were invited to a meeting on GABA-neurotransmitters in Copenhagen in 1978 organized by Krogsgaard-Larsen, Scheel-Kruger, and Kofod.

This pharmacological approach involving GABA-B receptors made sense to me because we had postulated that the mesolimbic DA neurons may be overactive in schizophrenia. Arvid Carlsson in 1962 had found indications that antipsychotic drugs block DA receptors. Our treatment strategy, however, still remains unexploited.

In this period, we also obtained results indicating the involvement of GABA mechanisms in the actions of benzodiazepines on central CA neurons. These results were reported at a meeting on "Mechanism of Action of Benzodiazepines" organized by Costa and Greengard in 1974. Treatment with chlordiazepoxide and diazepam, drugs with preferential actions in the limbic regions in low doses, reduced DA turnover in the dorsal striatum and especially in the nucleus accumbens and olfactory tubercle. These effects of the benzodiazepines were similar to the effects of the GABAergic drugs. Diazepam also reduced GABA turnover, which we explained on the basis that the drug elicited an increased GABA receptor signaling, leading to an inhibitory feedback on GABA neuronal activity. These actions of benzodiazepines may contribute to their antianxiety effects because, inter alia, the ascending DA neurons also innervate the amygdala. A reduced DA drive to the D1R-enriched intercalated cell masses of the amygdala may bring down the exaggerated fear output from the medial subnucleus of the central amygdala.

Our chapter was published in a book edited by Costa and Greengard for Raven Press in 1975 (Fuxe et al. 1975). It was one of the first reports on the involvement of GABA mechanisms in the actions of benzodiazepines.

This work on GABA in the 1970s with Dr. Miguel Perez de la Mora was the fine beginning of a long-term collaboration, and we became great friends. Miguel for many years now has been a professor at his university in Mexico City. Our work together over many years made it possible for me to become a foreign member of the Mexican Academy of Sciences.

1970s Indications of Adenosine-DA Interactions Using Caffeine and Theophyllamine

In 1972, we observed evidence that caffeine can produce a reduction of 5-HT turnover and especially of DA turnover in the brain but an increase of NA turnover. We therefore tested whether caffeine and theophyllamine could modulate the actions of levodopa and DA receptor agonists in the hemi-Parkinson model. We obtained a marked enhancement of the actions of levodopa and the DA receptor agonists by pretreatment with caffeine and theophyllamine (Fuxe and Ungerstedt 1974) as seen from the marked enhancement of contralateral turning behavior.

In a subsequent paper with Bertil Fredholm, we obtained indications that phosphodiesterase inhibition was not involved in the actions caffeine and theophyllamine but rather adenosine receptor mechanisms. This was the beginning of the work on adenosine-dopamine interactions and opened up a novel field in integrative neuroscience. Bertil Fredholm became a professor of pharmacology at the Karolinska Institutet, a leading member of the Nobel assembly, and an international leader in the adenosine field second to no one through his outstanding research.

1970s Continuation of the Work on the Neuroendocrine Role of the Tubero-Infundibular DA Neurons and the Hypothalamic-Preoptic NA Terminal Networks

We published in this period a large number of papers in the neuroendocrinological field and were invited to give lectures at various neuroendocrine congresses on topics like neuroendocrine regulation of fertility, aspects of neuroendocrinology, neurochemical aspects of hypothalamic function, hormones and brain function, endocrinology, basic applications and clinical uses of hypothalamic hormones, clinical reproductive neuroendocrinology, progress in prolactin physiology and pathology, or brain-endocrine interactions.

We obtained further support for the view that the tubero-infundibular DA neurons were involved in the inhibitory regulation of the LHRH release from the lateral palisade zone (LPZ) of the external layer of the median eminence, including the inhibitory feedback actions of 17-beta-estradiolbenzoate on LH secretion. Furthermore, TH and LHRH IR nerve terminals codistributed in the LPZ but not in the medial palisade zone (MPZ), which received very few LHRH IR terminals.

In the rat MPZ, DA appeared instead to be released as a prolactin inhibitory factor into the hypophyseal portal vessels (Andersson et al. 1981) to reach the DA receptors on the prolactin secretory cells of the anterior pituitary to inhibit its secretion into the circulation. Thus, DA could operate as a prolactin inhibitory factor through release into the capillaries of the median eminence connected to the portal vessels.

Anders Löfström, an excellent graduate student, did his thesis on this topic in the 1970s. Through microfluorimetric quantitation of CA fluorescence in the rat median eminence, he obtained evidence for the distribution of DA and NA nerve terminal networks in the median eminence. He found DA and NA turnover changes in the median eminence in several hormonal states and could validate our previous work in an excellent way. Of special value was his work on the dopamine and NA turnover changes in the critical period for ovulation and his pharmacological analysis of pregnant mare serum induced ovulation in the immature rat. His thesis gave further strong indications for an inhibitory role of the tubero-infundibular DA neurons in LHRH release by targeting the LPZ.

In line with the original findings of Professor Tom Sawyer at UCLA, evidence was obtained for the existence of a stimulatory influence of the hypothalamic NA networks on LHRH release into the portal vessels. I had the pleasure and honor to meet Tom Sawyer, a pioneer in neuroendocrinology, several times, and once I gave a lecture in his laboratory. He was a man I looked up to and respected and remember his gentle and kind way that made me feel at ease.

We found that gut hormones also influenced the hypothalamus and the median eminence. Glucagon, for example, reduced dopamine turnover in the external layer of the median eminence. Instead secretin increased dopamine turnover in the external layer and vasoactive intestinal polypeptide (VIP) produced no action. NA turnover in these regions also was differentially affected by the gut hormones. Dysfunction of the gastrointestinal tract in terms of hormonal release therefore may lead to disturbances in neuroendocrine function and in food intake.

1970s Moving into Nicotine Effects on the Tubero-Infundibular DA Neurons to Understand the Mechanism for Its Neuroendocrine Actions

We started in this decade a highly fruitful collaboration with Dr. Peter Eneroth, an excellent biochemist with a focus on determining hormone levels in blood. He was based at the Karolinska Hospital. I liked him, and we became friends. Together, we felt it was time to evaluate whether certain neuroendocrine actions of nicotine in man could be related to targeting the tubero-infundibular DA neurons.

In three papers from 1977, we demonstrated that acute intermittent treatment with nicotine produced an inhibition of serum prolactin, LH, and FSH levels, mainly seen in the castrated female rat, and associated with an increase of the DA turnover in the external layer of the median eminence. We proposed that nicotinic cholinergic receptors may be located on the tubero-infundibular DA neurons or on other neurons connected to the tubero-infundibular DA neurons. This opened up the possibility that these neurons were targeted by nicotine also in man. Thus, at least part of

the nicotine-induced changes in the neuroendocrine panorama, like effects on prolactin and LH secretion may be mediated over the tubero-infundibular DA neurons.

1970s Setting Up a Small Cardiovascular Laboratory

We were interested in understanding whether the NA nerve terminal networks in the brain stem, especially in the NTS, were involved in cardiovascular regulation. They were formed from the non-LC cells in the medulla oblongata and pons (Fuxe 1965a, 1965b). We had certain indications in 1970 that central NA receptors may participate in mediating the hypotensive actions of clonidine.

Per Bolme and I had been medical students together, and he had made his thesis on cardiovascular mechanisms related to cholinergic transmission at the department of pharmacology. We discussed a possible collaboration, and he agreed to be the leader of this project in my laboratory and teach one of my laboratory assistants, Beth Hagman, to measure blood pressure and heart rate as well as respiration rate and depth in rats. Beth was outstanding in performing animal experiments.

We were able to validate the hypotensive effects of clonidine and the involvement of alpha-adrenergic receptors in this action. In addition, we found that clonidine also reduced respiration rate and increased respiration depth. This was the first indication that alpha-adrenergic receptors participated in modulating respiratory centers in the brain stem.

With the findings also of phenylethanolamine N-methyltransferase (PNMT) positive nerve cells and nerve terminals in the brain stem, including cardiovascular areas, we started experiments to see whether adrenaline also was involved besides NA in activating the alpha-adrenergic receptors in the brain stem. We could not obtain a clear-cut answer, especially in view of the low amounts of adrenaline versus the high amounts of NA found in the cardiovascular regions, including the nuc tractus solitarius. It seemed to us that both NA and adrenaline could reach and act as a transmitter at the alpha-adrenergic receptors. In regions of overlap of NA and PNMT-positive nerve terminals, it appeared likely that NA dominated in view of its higher concentrations. The possible role of adrenaline in the brain still remains to be determined. I give Per Bolme my sincere thanks for his contribution in the 1970s after which he devoted his life in medicine to pediatrics.

End of 1970s Moving into Ibotenic Acid with Robert Schwarcz as a Leader

Robert Schwarcz arrived in the laboratory with new ideas. I liked him right away. He had published a great paper with Joe Coyle at Harvard Medical School in Boston on lesions of striatal nerve cells with kainic acid (KA), a conformationally restricted analogue of glutamic acid. It had given a model

for Huntington's disease. He soon found among my chemical compounds ibotenic acid, an isoxazole isolated from the mushroom *Amanita muscaria*, which also was a conformationally restricted analogue of glutamic acid. I received ibotenic acid from a Swiss biochemist, Dr. C. H. Eugster. I am truly grateful to him for this great gift.

Like KA, ibotenic acid induced neuronal degeneration after microinjection into brain regions like striatum and substantia nigra without affecting axons of passage and nerve terminals of extrinsic origin. It, however, was less toxic and produced more discrete lesions. This made it an improved tool versus KA for the analysis of the architecture of distinct brain regions. Ibo in contrast to KA does not depend on the integrity of a glutamatergic innervation. Unlike KA, it also had a low affinity for ^{3}H-KA binding sites.

We observed compensatory bilateral changes in dopamine turnover after a striatal kainate lesion on one side of the brain with a reduction of striatal DA turnover on the contralateral side to the lesion. Such mechanisms may remove striatal network asymmetry and help maintain symmetrical motor behavior in animals with unilateral lesions.

We wrote 14 papers together in this period from 1978 to 1980. I was happy and honored to be invited to his wedding with Damian in Stockholm. Afterward, we had a wonderful time at the best café in Stockholm. Since then, I have had great interactions with them over three decades. We also organized an international symposium held in Stockholm at the Wenner-Gren Center in 1982 on excitotoxins together with Peter Roberts.

This was the year the secretary of the Wenner-Gren Foundation died. His name was Yngve Zotterman, and he was an outstanding sensory physiologist in love with neuroscience. He was also a great person. I organized my first international Wenner-Gren Center symposium with him in 1973 together with Lars Olson on the dynamics of degeneration and growth in neurons. I respected him and felt close to him, although he was several decades older.

Robert Schwarcz is now a professor of psychiatry, pharmacology, and pediatrics at the University of Maryland School of Medicine, and deputy director of the Maryland Neuroscience Research Center, Baltimore. He pioneered the field of kynurenines in the mammalian brain and is an outstanding neuroscientist.

1980s Introduction of the Concept of Receptor-Receptor Interactions in the Plasma Membrane

At the end of the 1970s, we were convinced that the plasma membrane had been neglected as a place for integration of signals. The integration process was believed to mainly take place in the intracellular pathways toward the nucleus and in the promoter regions of genes regulating gene expression. We therefore tested in membrane preparations to see whether

activation of one G protein-coupled receptor (GPCR) could modulate the affinity and density of another GPCR, assumed to be colocated in the CNS, as studied in biochemical binding experiments. We started out by testing whether substance P and CCK8 could modulate the binding characteristics of ^{3}H-5-HT binding sites and dopamine D2R antagonist binding sites using ^{3}H-spiroperidol. Significant changes in the binding characteristics were observed both in the 5-HT and D2 receptors (Fuxe et al. 1981; Fuxe et al. 1983). In the 1981 paper, we were not allowed to use receptor-receptor interactions in the text, but in the 1983 paper, we could even use it in the title. We also found indications of significant neurotensin receptor-D2R, neuropeptide Y receptor–alpha2 receptor interactions, and glutamate receptor-D2R interactions in 1983 and 1984, again using biochemical binding techniques.

We wrote the first review on intramembrane receptor-receptor interactions in 1985 where we also correlated the biochemical findings to results in functional models (Fuxe et al. 1983). We published the first results in journals like *Acta Physiologica Scandinavica*, *Journal of Neural Transmission*, *Neuroscience* letters, *European Journal of Pharmacology*, and *Medical Biology*. We were invited to present our work on CCK peptides and the CCKR-D2R interactions at a meeting on neuronal cholecystokinin in Brussels in 1984 organized by Professor Jean-Jacques Vanderhaeghen and Dr. J. N. Crawley. It was a great meeting, and I got to know the Brussels aperitifs.

Jean-Jacques and I were good friends after skiing together at the alpine neuroscience meetings organized by the French neuroscientists at various ski resorts. I especially remember our time together in Megeve not far from Geneva. We had great discussions and a lot of fun and the magnificent Mont Blanc was not far away. I was happy to be invited to his 80th birthday celebration in the house of his son Pierre in Brussels, also a famous neuroscientist like his father. Jean-Jacques and I planned for future meetings but sudden death intervened one year later. Sadness is there, but I know he had a fine life and was proud of his son.

To us, the intramembrane receptor-receptor interactions represented a novel integrative mechanism for biological signals, a mechanism that could be of fundamental importance. It gave a new understanding to the existence of isoreceptors (Agnati et al. 1980; Fuxe et al. 1981; Fuxe et al. 1983), because each one of them could have a differential capability to interact with other types of receptors. It opened up a dramatic increase in molecular integration in the plasma membrane and thus in information handling in the synaptic and extrasynaptic membranes because of the receptor diversity obtained. They should have a major place in learning and memory as we proposed in a meeting on neural transmission, learning and memory in Argentina in 1982 organized by Professors Caputto and Marsan. The meeting book was published in 1983.

This molecular integrative mechanism also offered a novel therapeutic approach. Thus, instead of targeting the orthosteric receptor binding

sites directly with agonist–antagonist drugs, its receptor partner could be targeted, upon which agonist activation could modulate its recognition and signaling process through receptor-receptor interactions. In fact, at a meeting in 1983 in Corfu on frontiers in neuropsychiatric research organized by Goldstein, Friedhoff, and Georgotas, we discussed receptor-receptor interactions as a new mechanism for antidepressant drugs.

Later on in 1988, we found an antagonistic galanin receptor-5-HT1A receptor-receptor interaction in the limbic cortex of likely relevance for depression. We presented these findings at a meeting organized by Professor Pauletti in Florence in 1989 on "Serotonin from Cell Biology to Pharmacology and Therapeutics." In view of the therapeutic potential of 5-HT1A agonists in depression, galanin receptor antagonists seemed to be one possible new strategy for development of novel antidepressant drugs.

We gave a comprehensive report on the relevance of intramembrane receptor-receptor interactions for drug development in the CNS in 1988 in Washington, D.C., at a meeting on "Neurochemical Pharmacology: A Tribute to B. B. Brodie" organized by Erminio Costa. It was an honor and pleasure to meet Brodie and give him my genuine congratulations for his lifetime achievements in pharmacology.

Agnati and I organized our own international symposium on "Receptor-Receptor Interactions: A New Intramembrane Integrative Mechanism" at the Wenner-Gren Center in Stockholm in 1986. However, clear-cut evidence for our concept was missing and most of the participants did not believe in our story to put the plasma membrane in the center for integration of biological signals. Only Dr. W. Rostène from Paris was positive. We were disappointed and a little depressed. Already as a medical student, I had believed that the plasma membrane should be an important center for integration of biological signals. The receptors, ion channels, and G proteins were there in the synaptic and extrasynaptic membranes and could be reached by protein kinases and phosphatases. For information handling to work, their signaling and recognition should become integrated already in the plasma membrane followed by molecular integration in the cytoplasmatic pathways and at the transcription level in the nucleus.

Gabriel von Euler arrived in my laboratory as a graduate student in the second half of the 1980s. Together, we agreed that he should focus his thesis on the antagonistic neurotensin receptor (NTSR)-D2R interactions we had previously found. In his thesis, he obtained evidence that made the relevance of these receptor-receptor interactions more convincing. The NTSR interacted with D2Rs and was biochemically characterized. The interaction was G-protein independent and could be demonstrated also after intraventricular NT injections and was not related to the coexistence of DA and NT in nerve terminals. NT also reduced the affinity of the D2R in human caudate-putamen.

Of great interest was that my postdoc Sergio Tanganelli demonstrated that NT in the striatum counteracted apomorphine-induced inhibition of

dopamine release, which could be elegantly explained by the antagonistic NTSR-D2R interaction in the DA nerve terminals (Tanganelli et al. 2012). We regarded this receptor-receptor interaction as a major mechanism for the neuroleptic-like action of neurotensin and its antipsychotic potential. These results taken together strengthened the functional relevance of the antagonistic intramembrane NTSR-D2R interactions.

1980s Central CA-Neuropeptide Y Interactions in Cardiovascular Centers. Focus on the Neuropeptide Y Receptor (NPYR)–Alpha2 AdrenergicR Interactions

Dr. Anders Härfstrand entered my laboratory after completing his medical study in the beginning of the 1980s. He was a highly competent graduate student. He brought new life into our lab, and we agreed his thesis should be on brain NPY mechanisms and involve cardiovascular and neurochemical studies, including interactions with the alpha2-adrenergic receptors in the NTS. The NPY-positive neurons recently had been demonstrated in the brain.

Harfstrand demonstrated that intraventricular injections of NPY produced hypotension, bradycardia, and bradypnoea in the awake unrestrained rat, which is similar to the actions of clonidine. The target region was probably the NTS where the baroreceptor afferents terminate. It is rich in both NPYR and alpha2-adrenergic receptors. It was highly interesting that simultaneous central administration of adrenaline and NPY led to antagonistic interactions in vasodepressor responses in awake male rats (Harfstrand and Fuxe 1987). We then had the idea based on our concept of receptor-receptor interactions that NPYR and alpha2-adrenergic receptors formed a complex in which antagonistic reciprocal receptor-receptor interactions developed, reducing the affinity and signaling of each other. Evidence for this view was obtained by Harfstrand and colleagues in 1989 (Harfstrand et al. 1989). In vitro incubation and central administration to NPY or clonidine reduced the binding of an alpha2 adrenergic receptor agonist and of an NPY radioligand, respectively.

Taken together, the results indicated that antagonistic receptor-receptor interactions can play a major role in cardiovascular homeostasis. I am proud of Anders Harfstrand for his great thesis that was defended in 1987 (Harfstrand 1987). He played a leading role also in other projects in the laboratory. Härfstrand was the first to obtain evidence for a cardiovascular role of central galanin neurons, published in 1987. He was, for example, the first author of a paper on glucocorticoid receptor (GR) IR in monoaminergic neurons, which had a major impact on understanding the glucocorticoid regulation of the central monoamine neurons, especially in relation to stress. It was part of a collaboration with Professor Jan-Åke Gustafsson at the Karolinska Institutet, whose GR antibody made this paper possible. He is one of the world leaders on steroid receptors.

I remember giving a lecture on steroid hormone regulation of brain function in Paris in 1984 at a conference on steroids and the brain. I was a coorganizer together with Bruce McEwen and Etienne-Emile Baulieu, pioneers in the field, at this Table Ronde Roussel Uclaf meeting. I knew Bruce already from the beginning of my time at the Karolinska Institutet. We always had a good time together, and sometimes with our families.

Harfstrand also played a leading role together with Dr. M. Kalia at Thomas Jefferson University in Philadelphia in the discovery that somatostatin produces apnea, which through our work together indicated a possible role in apneic syndromes. We became good friends, and I remember we had a great dinner together in Texas in relation to a meeting on CNS mechanisms in hypertension organized by Drs. Buckley and Ferrario in 1981.

Harfstrand made an outstanding career as a leader for drug development in several of the leading drug companies in the world and presently is a consultant to Karolinska Institutet Innovations AB.

1980s Research on the Role of CA Mechanisms in Neuroendocrine Regulation (continued)

Dr. Kurt Andersson came to my laboratory at the end of the 1970s. He was interested in neuroendocrinology and became the scientist in our group who would do his thesis on the role of DA and NA nerve terminal networks in regulating the endocrine function of the anterior pituitary. Dr. Andersson became an outstanding expert in quantitative microfluorimetry. Together with his fine knowledge of brain microanatomy, his experiments were highly successful. Our expert in animal experiments, Beth Hagman, was also available to help him with the animal experiments in a fine way. Dr. Andersson was the leading author on the method paper on determinations of CA half-lives and turnover rates in discrete CA nerve terminal systems of the hypothalamus, the preoptic region, and the forebrain by quantitative histofluorimetry. Dr. Kurt Andersson is now chief physician in medicine and diabetes at the Karolinska Hospital in Huddinge.

In this period with Andersson as the leading author, we mainly focused our studies on the effects of rapid feedback by pituitary hormones prolactin and growth hormone on the DA and NA terminal networks of the median eminence and the hypothalamus-preoptic area. We also studied the effects of ultrashort feedback via the hypothalamic hormones like LHRH and corticotropin-releasing factor (CRF) on the terminal networks. These experiments were performed with Dr. Eneroth, and in the case of rapid feedback also with Drs. Nyberg and Roos. As an example, I will mention that rat prolactin given intravenously produced a rapid and highly significant enhancement of dopamine turnover exclusively in the MPZ of the median eminence of the hypophysectomized rat. These results strongly indicated that DA as a prolactin inhibitory factor was released from the MPZ in the rat, rapidly

responding to increases in prolactin levels in the blood. Thus, DA can operate via long-distance signaling in the portal blood vessels to the anterior pituitary gland. NA turnover was also increased in discrete regions of the hypothalamus, indicating that rat prolactin modulated other functions of the hypothalamus.

The next example I will highlight is the selective LHRH-induced activation of DA turnover in the LPZ of the median eminence using quantitative microfluorimetry, where the DA and LHRH nerve terminals codistribute in high densities (Andersson et al. 1984). Thus, the ultrashort feedback action by LHRH may involve release of LHRH from the nerve terminals in the LPZ, which may enhance DA release from adjacent DA terminals via their postulated LHRH receptors or via adjacent LHRH-induced tanycyte signaling, with the tanycytes possessing postulated LHRH receptors. LHRH was given intravenously. It was of high interest that LHRH in the same experiment selectively reduced NA turnover in the medial preoptic nucleus where many of the LHRH immunoreactive nerve cell bodies are found. Such events should lead to a reduced activity in the LHRH neurons, in view of the excitatory influence of noradrenergic neurons on their activity. The mechanism may involve diffusion and flow of intravenously injected LHRH from the median eminence within paraaxonal channels along the LHRH axons. In this way, LHRH can reach the LHRH nerve cell bodies and dendrites in the preoptic region and can modulate NA release through the activation of postulated LHRH receptors located on the local NA nerve terminals or the local astroglia. The change in local astroglia signaling may then modulate the local NA release. These results again indicated the existence of a major transmission in the extracellular fluid.

Through the excellent work of Dr. Andersson and Professor Eneroth, we determined in a large series of papers that many of neuroendocrine actions of nicotine and exposure to cigarette smoke (e.g., inhibition of prolactin, LH, and thyroid-stimulating hormone secretion) were linked to changes in the tubero-infundibular DA neurons and to changes in NA turnover in the NA terminal networks in discrete parts of the hypothalamus. Nicotinic cholinergic receptor activation was the receptor targeted by nicotine and cigarette smoke and induced the changes observed. One review of this work was presented at a Ciba Foundation Symposium in 1989 on "The Biology of Nicotine Dependence." Here we also reported on our findings from 1981 that nicotine induced increases of dopamine turnover in the mesostriatal and mesolimbic dopamine neurons. We proposed that the nicotine-induced increase in DA turnover in nucleus accumbens was involved in mediating the rewarding actions of nicotine. In a 1989 review in *Psychoneuroendocrinology*, we discussed the medical implications of the neuroendocrine actions of nicotine.

In 1988, we were the first group to show the neuroprotective effects of nicotine treatment on lesioned nigrostriatal dopamine neurons in the rat.

This was part of Ann-Marie Janson's thesis in my group. She is now one of the experts at the Swedish Medical Products Agency in Uppsala because of her excellent analytical capability.

Together with Drs. Belluardo, Mudo, and Blum we found in 1998 that acute intermittent nicotine treatment produced regional increases of basic fibroblast growth factor (bFGF) messenger RNA and protein in the tel- and diencephalon of the rat. Such effects may be involved in the mechanism for the neuroprotective actions found with nicotine treatment. We have a highly rewarding long-term collaboration on nicotinic receptor agonists as neuroprotective and neurotrophic drugs with the Belluardo-Mudo team at the Department of Physiology, University of Palermo, Italy. Together, we wrote one review on central nicotinic receptors, neurotrophic factors, and neuroprotection that appeared in *Behavioral Brain Research* in 2000 and another one on neurotrophic effects of central nicotinic receptor activation published in the *Journal of Neural Transmission* the same year.

Note that in the early 1990s, an excellent postdoc Gershon Chadi from Sao Paulo, Brazil, came to our laboratory. He demonstrated the neuroprotective effects of bFGF upon lesion of nigro-striatal DA neurons with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and studied the astroglia bFGF dynamics after lesions. He performed great work in his studies on trophic response in the lesioned brain.

1980s Toluene Vapor Exposure Alters CA Turnover and the Binding Properties of CA Receptor Subtypes in the Brain: Possible Mechanism

These experiments were a joint effort by our team, especially Dr. Andersson, the Toftgård toxicology group, Dr. Celani of the Agnati group, Dr. Martire, and Dr. Eneroth. It was of high interest that decreases in DA turnover in the anterior dorsal striatum were induced by toluene at concentrations lower than the current Occupational Safety and Health Administration threshold limit value (100 ppm). It was published in *Toxicology Letters* in 1982. Such changes may lead to disturbances in motor functions and may involve changes in membrane fluidity altering the properties of pre- and postjunctional DA receptors. At high concentrations of toluene, DA turnover in the subcortical limbic regions, including the nucleus accumbens, was instead increased.

This last reported result is of high interest because these regions are innervated by the mesolimbic DA reward neurons. We therefore proposed that these actions may represent the neurochemical basis for its abuse in humans. It was therefore interesting that subacute treatment with toluene produced a dose-related decrease of affinity in the [3H]-spiperone binding sites labeling striatal D2Rs. It was also published in *Toxicology Letters*. The D2Rs are in part located as autoreceptors on the striatal DA terminals. If a decreased affinity of the D2 autoreceptors also takes place in the mesolimbic DA neurons, the increased DA turnover in these neurons found with toluene

may be the result of a reduced affinity of the D2 autoreceptors. Such effects on receptor binding characteristics may be related to the physico-chemical properties of toluene, which can produce alterations in membrane fluidity. As a consequence, the receptor structures may be modulated as well as their receptor-receptor interactions.

Notably, subacute treatment with toluene in a dose-dependent way increased the density and reduced the affinity of beta-adrenergic receptors in the cerebral cortex without affecting the binding characteristics of alpha1-adrenergic and alpha2-adrenergic binding sites in this region. These findings were published in *Acta Physiologica Scandinavica* in 1987 and indicated that certain receptors may be more sensitive to changes in membrane fluidity than others.

1980s Studies on the Neuroprotective and Trophic Actions of the GM1 Ganglioside

Professor Luigi Agnati wrote a number of papers on the impact of the GM1 ganglioside on neuronal repair and survival of neurons, including studies on the nigro-striatal DA neurons. I was happy to help him in this endeavor, and we were able to observe neuroprotective actions. In this project, Agnati used computer-assisted morphometry and microdensitometry to study the effects of the GM1 ganglioside on the degenerative and regenerative features of the nigro-striatal DA neurons.

1980s Quantitative Neuroanatomy in Transmitter Research

Thanks to Luigi Agnati's excellence in developing new methods in neuroanatomy, we were able to introduce the principles for the morphological characterization of transmitter-identified cell groups in 1982. This work was followed by the development of a method for rostrocaudal integration of morphometric information in 1984. This year we also organized an international Wenner-Gren Center symposium in Stockholm on "Quantitative Neuroanatomy in Transmitter Research," with Professor Agnati having a leading role. The book I coedited with Agnati was published in 1985 (Agnati and Fuxe 1985). I remember that quantitative autoradiography was excellently discussed at the meeting by Dr. Fabio Benfenati in Agnati's group.

1980s Effects of Treatment with the Antidepressant Drug Imipramine on 5-HT IR in Nerve Cells of Four Different 5-HT Cell Groups

The psychiatrist Dr. Isao Kitayama came to my laboratory from Japan in the second half of the 1980s. He was an excellent scientist and performed the morphometric and microdensitometric analysis of, for example, 5-HT IR in a fine way together with members of my group. We appreciated each other,

and our families have met in Stockholm and Japan, sometimes with Dr. Yuji Odagaki and his wife in recent years. This has been a fruitful collaboration. Today, Dr. Kitayama has also become a known artist, and he presents his work at international exhibitions, for example, in Paris.

We were both interested in depression and in understanding the actions of antidepressant drugs. We decided to study the effects of acute and chronic treatment with imipramine in normal rats on 5-HT nerve cell groups, namely, raphe pallidus, raphe obscurus, and the raphe magnus nuclei with the ventral part of the reticular gigantocellular nucleus and the large dorsal raphe nucleus.

Only acute imipramine treatment significantly enlarged the field area of 5-HT IR in the nerve cells in the dorsal raphe. The mechanism was unknown. In view of recent work by Dr. Borroto-Escuela and colleagues in our group it seems possible that 5-HT reuptake blockade can contribute by activation of 5-HT1A autoreceptors through increased extracellular 5-HT levels. The 5-HT1A autoreceptors may activate FGFR1 signaling via a receptor-receptor interaction. The FGFR1 signaling may then increase 5-HT synthesis in the dorsal raphe nucleus, projecting into the tel- and diencephalon.

Instead, however, only chronic imipramine treatment selectively enhanced 5-HT IR in the nerve cells of the ventral part of the reticular gigantocellular nucleus, which is continuous with the raphe magnus nucleus, evaluated from the median grey values. Thus, the antidepressant drug imipramine can differentially affect the 5-HT cell groups, which is quite interesting. The mechanism for these differential effects is unknown, but it may be that the FGFR1-5-HT1A autoreceptor complexes are different between 5-HT cell groups, leading to different dynamics in response to imipramine treatment. In addition, the degree of NA innervation also can differ and can play a role because imipramine also inhibits the NA transporter.

It is of substantial interest that Kitayama and colleagues also found that chronic imipramine treatment altered GR IR in distinct regions of the lower brain stem. We found a selective increase of nuclear GR IR in the 5-HT nerve cell group from the ventral part of the reticular gigantocellular nucleus of the rostral ventromedial medulla in continuity with raphe magnus nucleus. These results were obtained with microdensitometrical analysis. In the morphometric analysis, there were instead changes in the mean profile area of the nuclear GR IR, secondary to the significant increase in GR IR in the raphe magnus nucleus. Serum levels of corticosterone and aldosterone turned out to be unaffected.

The increased GR IR may be the result of an altered number of GR in these two 5-HT cell groups or an altered translocation of GR to their nuclei. With current knowledge, we proposed a modulation induced by imipramine, through inhibitory effects on the 5-HT transporter, on the organization of the 5-HT1A autoreceptor containing heterocomplexes of which the 5-HT transporter may be part. The resulting alterations in the signaling from

these heteroreceptor complexes can alter the gene expression of GR or the recruitment of the GR to the nucleus. The selectivity observed among the 5-HT cell groups may be that each 5-HT cell group has its own panorama of 5-HT1A heteroreceptor complexes that determine the sensitivity to imipramine treatment. The 5-HT1A-FGFR1 heteroreceptor complex may play a role also in this case.

The most impressive change with chronic imipramine treatment was the increase of GR IR in the NA nerve cells of the LC. In this case, the NA reuptake inhibition may play the major role. The NA transporter may be part of a complex with alpha2-adrenergic autoreceptor and the increase in extracellular NA levels leading to activation of the alpha2 autoreceptor. This receptor can have other functions than being an autoreceptor by being part of heteroreceptor complex containing, for example, FGFR1.

The net outcome with increases in GR IR after chronic imipramine treatment in distinct monoamine cell groups seems to be of value for its therapeutic actions, because responsivity to glucocorticoids remains or is increased in at least one relevant system. The locus coruleus NA system and antistress actions therefore may develop.

Effects of a 14-day immobilization stress on 5-HT and GR IR in 5-HT nerve cell groups was evaluated on day 7 after the end of the immobilization to understand the relationship between behavioral recovery and changes in GR and 5-HT IR in raphe pallidus, raphe obscurus, and the raphe magnus nuclei with the ventral part of the reticular gigantocellular nucleus and the large dorsal raphe nucleus. The first three raphe nuclei send descending 5-HT pathways to the spinal cord, and the last one gives rise to ascending 5-HT pathways to the tel- and diencephalon. At day 7 after the 14-day stress, food intake and locomotion were fully recovered. Nevertheless the 5-HT IR in the 5-HT cell groups studied was substantially and significantly reduced. This was observed in the dorsal raphe, in spite of an increase in nuclear GR IR in this 5-HT cell group known to play a relevant role in mood regulation. Thus, in this case, the increase in GR IR cannot produce a recovery of 5-HT IR, which probably indicates a lack of recovery of 5-HT synthesis. The explanation may be that GRs, even if increased, cannot function properly and therefore fail to increase 5-HT synthesis. A brake may have been developed in the promotor regions where GRs may act to increase 5-HT synthesis. Future experiments are needed to test this proposal.

1980s Introduction of the Concept of Two Main Types of Communication in the CNS: Volume Transmission and Wiring Transmission

The major result of this study was the finding of a lack of an overall correlation of the regional distribution of central enkephalin and B-endorphin immunoreactive terminals and of mu and delta opioid receptors in adult rat brain (Agnati et al. 1986). These results indicated that the opioid peptides needed

to diffuse in the extracellular fluid before reaching their receptors to induce opioid transmission. Thus, a transmitter-receptor mismatch existed. These findings together with our work in 1969–1970 indicating that monoamines can diffuse in the brain, as well as the overall architecture of the central monoamine neurons, led us to introduce the concept of VT in this paper. It was described as a widespread mode of intercellular communication that occurs in the extracellular fluid and in the cerebrospinal fluid (CSF) of the brain. VT signals move from source to target cells via concentration, temperature, and pressure gradients leading to diffusion and flow (convection) in the extracellular space. Thus, there are in VT no private channels as in wiring transmission (WT), the prototype being synaptic transmission. The latter operates via axons, terminals, and synapses. In 1988, at a meeting on the regulatory role of opioid peptides organized by Illes and Farsang, we reported that the opioid peptide systems operated via VT to produce their actions in the brain. This represented a new major transmission in the CNS that ranged from very short (um range) to long distances (mm range) with global effects introduced via CSF.

1990s to Present: Evidence for VT and Further Development of the Concept

Dr. Börje Bjelke came to the laboratory around 1987, and he was interested in having his thesis on VT and did a great job. He started out in an excellent way with a *Neuroscience Letter* paper in 1988 on the survival of adenohypophyseal homologous transplants in the rat striatum associated with diffusion of prolactin-like IR into the surrounding neuropil of the striatum. In 1989, he found an increased diffusion of prolactin-like material into the brain neuropil from homologous adenohypophyseal transplants in the rat neostriatum after a 6-OH-dopamine-induced degeneration of the nigrostriatal dopamine neurons. Thus, the D2 receptors on the prolactin cells inhibiting prolactin release were inhibited by DA originating from striatal DA nerve terminal networks communicating via VT.

We organized an international Wenner-Gren Center Congress in 1990 on "Volume Transmission in the Brain. Novel Mechanisms for Neural Transmission" and the book, *Advances in Neuroscience,* Volume 1, which I edited with Agnati, was published in 1991 by Raven Press (Fuxe and Agnati 1991). There was a mixed response to the meeting. We provided further evidence for VT based on findings of transmitter-receptor mismatches in the NPY neuronal systems and of biological effects of NPY fragments. Miles Herkenham instead interpreted receptor-transmitter mismatches as implying endocrine functions in brain.

Bunnemann in my group, in collaboration with Professor Detlev Ganten, reported on a major role of VT in communication within the brain renin-Angiotensin system (RAS) and the angiotensin AT1 receptors. Bunnemann presented his thesis on RAS and VT in 1992. The angiotensinogene mRNA expression was restricted to the astroglia.

The most important support for VT in the brain was obtained from Professor Laurent Descarries in Canada. In his outstanding presentation, he demonstrated nonjunctional relationships of monoamine axon terminals in the cerebral cortex. In many cortical monoamine varicosities, there was a lack of synaptic junctions, which supported a role for VT. I am sad that Laurent is no longer with us, and I am happy that I could meet him at a neuroscience meeting a couple of years ago at Christmas time in Uppsala. We had a nice discussion on brain communication, especially VT. I did not know that he was ill.

In 1991, Professor Paul Trouillas and I organized a meeting at the Claude Bernard University in Lyon on "Serotonin, the Cerebellum and Ataxia." The proceedings were published by Raven Press in 1993. Here we presented indications that the 5-HT, DA, and NA nerve terminal networks in the cerebellum can modulate synaptic transmission in the cerebellar cortex via VT. The Purkinje neurons were hypothesized to be major integrators of synaptic and VT.

We were also happy for the great work of Charles Nicholson along with Margaret Rice and of Eva Sykova on diffusion of ions and transmitters in the brain cell microenvironment presented at the meeting in 1990. This represented fundamental work. Börje Bjelke presented his experimental evidence on VT (see the previous section) along with a three-dimensional reconstruction of the prolactin-positive extracellular pathways. Our meeting on VT ended with the outstanding concluding remarks of Professor Jean-Perre Changeux.

In his 1994 thesis work, Börje Bjelke presented indications for VT in the dopamine terminal denervated striatum in the hemi-Parkinson model after amphetamine treatment. Dopamine transmission may be partly restored via systemic amphetamine treatment through the release of dopamine. The origin is unknown, but it may involve the release of DA from remaining DA nerve terminals on the lesioned side as well as from DA terminals on the unlesioned side. The results obtained were mainly from eletrophysiological (Dr. Strömberg) and microdialysis (Dr. O'Connor) studies. This paper illustrated the impact of having a team of experts to get the work done.

In 1995, Börje Bjelke, as the first author, obtained evidence for long-distance pathways of diffusion for dextran along myelinated fiber bundles in brain as well as along perivascular pathways. It indicated the existence as well of long-distance pathways for VT and was performed in collaboration with Dr. Englund and the groups of Professor Agnati and Professor Nicholson (Bjelke et al. 1995). In 1996, he produced another great paper on evidence for VT in dopaminergic communication in the rat retina, along with other experts like Drs. Goldstein, Tengroth, Seesack, Steinbusch, and Watson. He defended his great thesis on VT in an excellent way.

Dr. Anders Jansson started his thesis with me on VT in 1998. The title of his thesis was "Experimental Studies on the Sources, Pathways, and Targets for Volume Transmission in the Rat Brain." He was a rock in the

lab, and we established a great relationship. His first paper on VT was on the relationship of 5-HT neurons to 5-HT 2A receptor-immunoreactive neuronal processes in the brain stem of rats. The results indicated the existence of extrasynaptic VT (in the micrometer range) in 5-HT2A mediated 5-HT transmission in certain brain stem nuclei.

In 2000–2001, Jansson and colleagues including Barbro Tinner, a great laboratory assistant, and Steinbusch again explored the relationships of 5-HT immunoreactive terminal-like varicosities to 5-HT2A receptor-immunoreactive neuronal processes, this time in the forebrain (Jansson et al. 2001). The observations again suggested that 5-HT2A receptor-mediated 5-HT transmission in the forebrain mainly involves a VT process mediated through extrasynaptic diffusion in the um range in the extracellular fluid, which seems to be true for all monoamine transmitters (see the review by Fuxe et al. 2012).

Jansson and colleagues, including Barbro Tinner, also focused on the distribution patterns of D1 and D2 receptors in relation to TH and dopamine transporter immunoreactivities in the ventral striatum. The most important finding was the existence of nerve cell patches of strong D1 receptor IR associated with low D2 receptor, dopamine transporter, and TH immunoreactivities (Jansson et al. 1999). They may represent a compartment in which dopamine VT mainly acts on D1 receptors via a short diffusion distance in the range of 30–50 um, producing a delay in their activation by DA. This may represent part of a temporal-difference learning based on VT. Taken together, the findings give a new understanding of the chemical microarchitecture of the nucleus accumbens. These patches formed a continuous rostro-caudal tubular nerve cell system in the shell part of the nucleus accumbens.

That same year Jansson and colleagues, which included the Sykova group, studied the effects of nitric oxide inhibition on the spread of biotinylated dextran and on extracellular space parameters in the neostriatum. The observed changes after nonspecific nitric oxide synthase inhibition appeared to affect the extracellular space concentration of neurotransmitters. The actions on VT pathways were mainly linked to increased capillary and cellular clearance, although changes in extracellular space diffusion were lacking.

In 2000, Jansson and colleagues discussed clearance mechanisms for long-distance VT signals in the brain. This involved clearance over the brain-blood barrier and over the leaky brain-CSF interface. It can also occur through receptor-mediated uptake into other discrete nerve cell systems through internalization.

We were happy that Professor Laurent Descarries agreed to be the opponent of the Anders Jansson thesis on VT. I remember the fine interactions between them and the ambiance we shared at the celebration in a castle after the approval of his thesis, which was enhanced by excellent food and wine. It was a great day.

In 2002, we published a review together in the book *Brain Homeostasis in Health and Disease*, edited by Dr. Walz (Jansson et al. 2002). It dealt with transmitter-receptor mismatches in central DA, 5-HT, and neuropeptide systems. It gave further evidence for VT. Dr. Anders Jansson was the first author.

The next graduate student interested in doing a thesis on VT was Malin Höistad. She was highly motivated, showed excellence in the performance of the experiments, and came to my laboratory around 2000. Her coadvisor was Dr. Jan Kehr, an excellent biochemist and pharmacologist.

Her first paper dealt with dual-probe microdialysis to study long-distance diffusion. It involved intracerebral infusion of ^{3}H-dopamine and the extracellular marker ^{3}H-mannitol in the striatum. Unmetabolized ^{3}H-dopamine could not be detected at the 1 mm distance. It was concluded that the spread of transmitters and compounds in the brain was highly dependent on the nature of the molecules infused. Classical transmitters like monoamines appear to be used for VT mainly at an extrasynaptic range of um as observed in the majority of our studies on the relationships of monoamine terminals and monoamine receptors. Finally, there were indications that in the DA nerve terminal denervated rat striatum versus striatum of controls, ^{3}H-DA may cause an immediate increase of local CBF and capillary permeability. This enhanced the clearance processes of extracellular ^{3}H-DA and its metabolites, which was demonstrated by a decline in their extracellular levels.

In the coming year, we continued our collaboration with Dr. Charles Nicholson and his team (Dr. Chen) in New York. This made it possible for Höistad to evaluate [^{3}H]mannitol diffusion in agar and rat striatum involving the use of quantitative dual-probe microdialysis. This approach will be valuable in determining interstitial diffusion and the clearance processes supporting the dynamics of brain VT. A mathematical model and analysis was also introduced.

The last paper of the Höistad thesis (Hoistad et al. 2005) involved the detection of intact beta-endorphin in the CSF after intrastriatal microinjection of beta-endorphin. We needed experts from the Karolinska Institutet (Jenny Samskog, Ernst Brodin, Annica Olsson) and the University of Göteborg (Hans-Arne Hanson) to get the manuscript into a final state. Höistad found a significant intracerebral spread of the injected beta-endorphin, for example, into the globus pallidus via diffusion in the extracellular space. It was of high interest that beta-endorphin-IR became colocalized with the mu-opioid receptor-IR at the cell surface of the globus pallidus cell bodies. It was of high relevance to have indications that beta-endorphin may operate via long-distance VT to reach mu-opioid receptors and also via CSF VT globally activate the mu and delta opioid receptors in the CNS.

We have been interested in understanding the role of thermal microgradients for VT besides thermal macrogradients between active and inactive brain regions. The uncoupling proteins (UCPs) may help in the generation

of thermal microgradients. They are found in the inner mitochondrial membrane in which they in part uncouple oxidative phosphorylation from ATP synthesis by decreasing the proton gradient over the membrane. This leads to the generation of heat and to a reduction of oxygen production. Therefore, UCPs can cause local temperature gradients and can increase the flow of neurotransmitters in the extracellular fluid. It was of special interest to study the presence of UCPs in transmitter-receptor mismatch areas. UCP2 like IR was found to be increased in the DA terminal rich regions in the nucleus accumbens shell surrounding the D1 receptor rich mismatch area. UCP2 like IR therefore may increase the diffusion or flow of DA into the mismatch regions by producing local temperature gradients, which will increase DA VT mediated by D1 receptors.

These results indicated the dynamics of VT and suggested a relevant role of UCP2 in VT. In this work, Dr. Alicia Rivera at the University of Malaga and Dr. Tamas Horvath at the Yale School of Medicine played an especially important role. Malin Hoistad was also a coauthor. Rivera established and gave evidence that UCP-like IR surrounded the transmitter-receptor mismatch region. She also observed UCP2-like IR in discrete dopamine terminal networks in the nucleus accumbens and in the cerebral cortex, whereas the IR was found in scattered noradrenergic terminals in the caudate putamen and Islands of Calleja Magna. I am especially fond of this paper.

We were happy that the expert Dr. Margaret Rice from the New York University School of Medicine accepted to be the opponent of Malin Hoistad's thesis. Already at this time, Rice was one of the international leaders in the field of brain communication. I enjoyed the friendly battle between them on the day of the thesis defense. Both of them did an excellent job.

Malin Hoistad was my last graduate student, and I retired from my professor position in 2005. However, I was able to continue my research because I could keep my grant at the Swedish Research Council. I dramatically reduced my laboratory and office space to bring down the high costs of renting space at the neuroscience department.

In this period, I also had an excellent postdoc Alberto del Arco in my laboratory. He came from the Department of Physiology at the University of Complutense in Madrid. I also got to know his professor. His name was Francisco Mora. We were all interested in VT, so we wrote a review together in 2003. We introduced the concept that changes in dialysate levels of GABA and glutamate can be regarded as a result of GABA and glutamate VT in the CNS targeting receptors on neuron-astroglia networks. Professor Mora kindly invited Luigi Agnati and me to be coorganizers of an international conference in Madrid hosted by the Ramon Areces Foundation in 2008. It dealt with "Brain Plasticity, Aging and Neuropsychiatric Disorders." At the lunch, I discovered Pacharan, a very old drink from the Middle Ages. It was originally made from sloe berries in the Navarra country and had a reddish color and a novel attractive taste, unknown to me. The president of the

foundation was a kind man who spoke of science with high respect. I gave him my genuine thanks for accepting our conference. I was happy to help Professor Mora with a few additional conferences. We had great interactions and became friends.

In the coming years, we wrote several reviews on our concepts on VT together with Luigi Agnati and many other coauthors with titles like "From the Golgi-Cajal Mapping to the Transmitter-Based Characterization of the Neuronal Networks Leading to Two Modes of Brain Communication: Wiring and Volume Transmission" and "The Discovery of Central Monoamine Neurons Gave Volume Transmission to the Wired Brain" (Fuxe et al. 2007; Fuxe et al. 2010). Along with Luigi Agnati and Dasiel Borroto-Escuela, we also included the extracellular vesicles into VT and compared the role of transmitter diffusion and flow versus the role of extracellular vesicles in VT to understand their differential role (Borroto-Escuela et al. 2015). We also discussed the same year VT in central dopamine and NA neurons as they target the astroglia. It seems to me that today most neuroscientists accept the existence of VT in the CNS as a major mode of communication inter alia mediating glial transmission and modulating synaptic transmission.

VT also has a special value in neuropsychopharmacology because the VT concept is relevant for pharmacokinetics and thus for the actions of neuropsychoative drugs. Drugs may be considered as exogenous VT signals because they flow or diffuse in the extracellular space (volume). Their migration is limited by the same factors that limit the endogenous VT signals like transmitters, modulators, and ions. Neuropsychoactive drugs may interact more effectively with VT, which is less constrained than synaptic transmission.

There is also an impact of VT on Chinese medicine. Together with Professor Zhang at China Academy of Chinese Medical Sciences, Beijing, we had previously proposed that VT pathways of diffusion and flow in the interstitial fluid can underlie the acupuncture actions and the meridian phenomena. I had given lectures on VT at his department earlier, and we were in full agreement on this concept. To obtain indications whether or not this was true, alcian blue was microinjected under anesthesia into the fish *Gephyrocharax melanocheir*. It had the advantage for us of having a translucent body.

We discovered that the alcian blue could migrate and be recorded with a digital camera. Eight longitudinal threadlike blue tracks were found with positions similar to the meridians found on the body of humans (Zhang et al. 2017). The migration tracks were located in intermuscular septa and in different layers of loose connective tissue. Sometimes the blue tracks had an association with lymphatic vessels. These extracellular pathways may operate via VT through diffusion and flow of biological signals, which need to be identified. They give a new understanding of the acupuncture meridians and why they play a major role in Chinese medicine.

In my mind, based on the work with Professor Weibo Zhang, who is an excellent scientist and fully understands Chinese medicine, I believe that

Eastern and Western medicine must be integrated. In doing so, we will begin to understand the biological mechanisms involved in Eastern medicine as exemplified in our work on VT. Improved treatment of human disease will then develop.

1990s Central GR Immunoreactive Neurons as Targets for Glucocorticoid Action

Graduate student Antonio Cintra came to my laboratory in the mid-1980s. He was a fine and quiet scientist of Brazilian origin. Antonio performed his experiments in an excellent way and gave support to our group under stressful conditions. I had great trust in him and I liked him. Dr. Antonio Cintra is now a medical doctor working in the field of gerontology in Sweden.

As part of his thesis, Cintra found GR IR in different classes of peptidergic neurons. Enkephalin nerve cells, especially in the basal ganglia, and all arcuate beta-endorphin nerve cells were GR positive. Conversely, the majority of the dynorphin nerve cells lacked GR IR. For most peptide systems, there were substantial differences among regions of the brain. In cerebral cortex, including hippocampus, the majority of the various peptide nerve cells did not show GR IR. Instead, the same types of peptide nerve cells in the arcuate and paraventricular nucleus like neuropeptide Y, somatostatin, and the cholecystokinin nerve cells exhibited intense GR IR. It seems likely that such differences depend on their participation in stress circuits. The paraventricular nerve cells show GR IR in their corticotropin-releasing factor, growth hormone-releasing factor, thyrotropin-releasing hormone, and somatostatin IR neurons, which indicates a neuroendocrine regulation by glucocorticoids.

Cintra also found prenatal development of GR gene expression and IR in the rat brain and pituitary gland. Thus, it seems possible that neurochemical and behavioral impairments can be caused by prenatal stress.

Cintra's major papers describe the mapping of the GR IR neuronal and glial cell populations in the rat CNS. This also involved computer-assisted morphometric and microdensitometric evaluations. Nuclear GR IR profiles of neuronal and glial cells were found all over the CNS but in a heterogeneous way. Thus, glucocorticoids can modulate a large number of nerve and glial cells by changing their gene expression. The GR IR neurons, however, seem to be major target for glucocorticoids. The results emphasize the actions of glucocorticoids both in regions known to be involved in stress responses and in regions not linked to such responses, indicating that glucocorticoids have more widespread effects in their modulation of brain function. Our interpretation is that glucocorticoids can modulate the neural-glial networks of many brain regions to produce an optimal integration of these networks to assist in the survival of the individual under stress conditions. Taken together, the Cintra papers indicate that GR is a modulator of neuronal and glial plasticity throughout life from the beginning in the fetal and postnatal

period until the adult and aging period, when the intensity of GR IR in the neuronal cells becomes reduced.

1990s Functional Interactions between Different Types of Receptors and G-Proteins in Native Brain Membranes: Activation of High-Affinity GTPase Activity by Receptor Agonists in Hippocampal and Striatal Membranes

Dr. Yuji Odagaki came to my laboratory in the mid-1990s on a fellowship from Japan. He was interested in developing a technique to determine agonist-induced high-affinity GTPase activity, which I thought was a fine idea. He established the method in a perfect way, and we got beautiful results. Our families met, and we had good interactions with Yuji and his wife Tomoko. We have had great discussions in Kyoto, Seoul, Berlin, and Copenhagen in relation to ECNP and CINP congresses and congresses in biological psychiatry.

The method was established by determining DA-stimulated high-affinity GTPase activity in striatal membranes. D2 receptors mediated the actions via activation of Gi proteins. High-affinity GTPase activity was also stimulated by carbachol and acetylcholine and was mediated by pirenzepine-insensitive muscarinic receptors. Both receptors were coupled to Gi proteins, which inhibited AC activity. A detailed method paper was published in 1997 focused on "Agonist-Induced High-Affinity GTP Hydrolysis as an Index of Receptor-Mediated G Protein Activation in Mammalian Brain Membranes." GTP hydrolyzing activity was determined from the amount of 32P released from [gamma-32P] GTP in the membranes.

Dr. Odagaki and I were both interested in depression and the role of 5-HT receptor subtypes in this disease. We found that 5-HT-stimulated high-affinity GTPase activity of the G protein involved the 5-HT1A receptor in the hippocampal membranes and its coupling to Gi. A pharmacological analysis of high-affinity GTP hydrolyzing activity was also performed involving the use of 5-HT receptor agonists and antagonists. The functional interaction between the 5-HT1A receptors and G proteins, in particular the Gi subfamily, can be studied using this method.

The method could be modified to determine high-affinity GTPase activity mediated via adenosine receptors in hippocampal membranes. It was possible to determine via a pharmacological analysis that the adenosine A1R mediated this interaction, producing Gi/o activation and inhibition of AC. It is of substantial interest that additivity between high-affinity GTPase activities mediated via A1Rs and 5-HTlA receptors was observed. These results suggest that A1R and 5-HT1A receptors are linked independently to distinct pools of Gi/o-proteins. How these events at two pools of Gi/o proteins become integrated at the level of, for example, AC, mitogen-activated protein kinases (MAPK), and G protein-gated inwardly rectifying potassium (GIRK) channels is unknown.

1990s to Present: Evidence for the Existence of Allosteric Receptor-Receptor Interactions in Homo- And Heteroreceptor Complexes in the CNS, a Major Molecular Integrative Mechanism

In 1993, we introduced along with the Agnati group the concept that the intramembrane receptor-receptor interactions took place in receptor heterodimers, which could be formed from the corresponding homodimers or monomers (Zoli et al. 1993). The heterodimer formed the molecular basis for the receptor-receptor interaction. We underlined that the receptor heterodimer represented a novel integrative mechanism in the plasma membrane.

The A2A-D2 Receptor Heteromer and Its Function

In 1974, we had found that the methylxanthines could enhance the levodopa and DA receptor agonist–induced contralateral turning behavior in the hemi-Parkinson rat model (Fuxe and Ungerstedt 1974), which was indicated to be related to an adenosine receptor action. Based on this paper and on the ability of methylxanthines to enhance DA receptor action, we decided to mainly focus our work on the possible existence of adenosine receptor-dopamine receptor interactions. In my group, mainly Dr. Ferre and graduate student Maria Torvinen were involved. In a collaboration with Professor Fredholm, we were able to demonstrate antagonistic A2A-D2 receptor-receptor interactions, leading to a reduction of D2R affinity in the high-affinity state (Ferre et al. 1991). These results obtained in the striatal membranes supported our 1974 findings that adenosine receptor antagonists enhanced DA receptor actions.

On the basis of this paper, we proposed the use A2A receptor antagonists in the treatment of PD. Thanks to Dr. Dasgupta in our lab, in 1996, we were able to validate the striatal results in A2A-D2 stably cotransfected fibroblast cells with the additional help of Dr. Björn Kull and Dr. Ernest Arenas.

In 1995, we had observed in studies on intracellular calcium levels a reduction of dopamine D2 receptor transduction by activation of adenosine A2a receptors in stably A2a/D2 (long-form) receptor cotransfected mouse fibroblast cell lines. We were happy for a collaboration with Professors Lledo and Vincent and their team, especially Dr. Salim at Institute Alfred Fessard at Gif-sur-Yvette. Through this fine collaboration, it was possible to study A2A-D2 receptor-receptor interactions in D2R stably transfected human neuroblastoma cells constitutively expressing A2AR. They found that the A2 receptor agonist CGS-21680 acutely counteracted the D2 receptor-induced [Ca2+] responses. We appreciated very much the important results from these outstanding neuroscientists who provided evidence that A2A receptors can reduce D2R signaling. They showed warm hospitality at our visit to the Albert Fessard Institute, and I regarded them as my friends.

Note that in 1991 Ferre found that stimulation of A2A receptors produced catalepsy, and in 1997, Roberto Rimondini and colleagues in our laboratory

established the atypical antipsychotic features of A2A receptor agonists, which included help from Dr. Sven-Ove Ögren at the department. He completed his thesis with me in 1999 with the title "Behavioral and Biochemical Pharmacology of Adenosine-Dopamine Receptor-Receptor Interactions." He did an excellent job and is now back at the University of Bologna, Italy. Together with Professor Urban Ungerstedt and Dr. O'Connor working in his group, in 1993, we demonstrated with microdialysis that the main locus for A2A-D2 receptor-receptor was in the striato-pallidal GABA neurons.

An important step in demonstrating the relevance of A2A receptor antagonists for the treatment of PD came in the beautiful work led by Dr. Ingrid Strömberg at our department in 2000. She demonstrated in the hemi-Parkinson model that subthreshold doses of the A2A receptor antagonist MSX-3 substantially increased the action of the D2R agonist quinpirole on striatal firing rate; instead, the A2A receptor agonist produced the opposite actions.

Through a collaboration with Dr. Yasmin Hurd and Professor Vanderhaegen in Brussels, my outstanding postdoc Zaida Diaz-Cabiale was able to demonstrate that adenosine A2A agonist CGS 21680 decreased the affinity of dopamine D2 receptors for dopamine in human striatum. Thus, the A2A-D2 heteroreceptor complexes also may exist in the human brain.

Around 2000, we started our collaboration with Professor Rafael Franco and his group at the University of Barcelona. The Fuxe and Franco teams, which also included the Agnati team, made a major effort to obtain indications for the existence of A2A-D2 heteromers, which we believed to exist based on our concept from 1993. The first paper came out in 2002, and Joel Hillion in my group was the leading author.

A2AR-D2R heteroreceptor complexes were found in coimmunoprecipitation experiments in membrane preparations from cell models expressing A2A and D2 receptors. Combined treatment with A2AR and D2R agonists produced coggregation, cointernalization, and codesensitization of the A2A and D2 receptors. In 2003, came further evidence for the existence of A2AR-D2R heteromerization using fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET) performed in the Franco laboratory in collaboration with the expert Professor M. Bouvier. The A2A homodimerization was found the following year by the same techniques in the Franco laboratory.

In 2001, there were indications from the work of Dr. Patrizia Popoli together with our group that an mGlu5R agonist could reduce the D2R agonist-induced contralateral turning behavior in the hemi-Parkinson model and also could reduce the affinity of the D2R agonist binding sites in line with our early work in the 1970s on the glutamate modulation of D2 agonist binding sites. Synergistic interactions between mGluR5 and A2A receptors were indicated. In a follow-up paper in 2002, Ferre and colleagues found the existence of A2A-mGluR5 complexes based on immunoprecipitates from inter alia striatal membrane preparations, which may be the basis for synergistic

A2AR-mGluR5 interactions. In 2009, the Francisco Ciruela group at the University of Barcelona found evidence that mGluR5, dopamine D2, and adenosine A2a receptors form higher-order oligomers in living cells.

In 2004, Dr. Amina Woods pioneered the understanding of the A2A-D2 receptor interface. She demonstrated direct epitope-epitope electrostatic interactions between adenosine A2A and dopamine D2 receptors involving cytoplasmic intracellular loop3 of the D2R and the C-terminal of the A2AR. Mass spectrometry was used, and Dr. Ciruela validated her results with pull-down techniques. It is a beautiful paper. In 2008, I was happy to be part of another paper by Woods and colleagues. Calmodulin was indicated to disrupt the electrostatic interactions between the D2 receptor epitope and the more distal A2A receptor epitope.

In 2005, we wrote a review on the A2A-D2 heteroreceptor complexes and their function in a special issue of the *Journal of Molecular Neuroscience* (Fuxe et al. 2005). Writing this review, I realized how little you can do on your own with the limited budget you have. You need national and international teams to work together to determine whether or not novel concepts on molecular mechanisms are correct. Out of this effort also novel or revised hypotheses may develop.

This special issue contained the proceedings of a Wenner-Gren Foundation symposium we organized in Stockholm in 2004 on receptor-receptor interactions among GPCRs, "From Structure to Function." I enjoyed my discussions with Fiona Marshall who gave a fine lecture on the GABAB heterodimer. I was happy and honored that Nobel Laureate Robert Lefkowitz gave the concluding remarks of the meeting. They made clear his outstanding leadership in the GPCR field. I had great interactions with Robert.

A2A-D2 Heteroreceptor Complex Studies after the Arrival of Postdoc Dasiel Borroto-Escuela in the Laboratory in 2009

Dr. Dasiel Borroto-Escuela was an outstanding biochemist from the University of Havana and a PhD from the Universitat Politècnica de Catalunya, of Cuban origin. He mastered molecular biology and markedly increased the strength of our laboratory by setting up BRET and FRET as well as luciferase reporter gene assays and proximity ligation assays. Through his great contribution, we could together obtain evidence for the existence of a large number of receptor-receptor interactions in heteroreceptor complexes and their function. This work is still ongoing and intense and involves crucial collaboration with outstanding teams in Europe.

With regard to A2A-D2 receptor heteromers, we found that a point mutation of serine 374 to alanine reduced the A2A-D2 receptor heteromerization. This point mutation also abolished the A2A receptor agonist–induced reduction of the high-affinity D2 receptor agonist binding and signaling (Borroto-Escuela et al. 2010b). In a subsequent paper, we found

that mutations of two negatively charged aspartates in the C-terminal tail (D401A/D402A) of the A2A receptor together with the S374A mutation markedly diminished the heteromerization with the loss of the antagonistic allosteric A2A-D2 receptor-receptor interaction. These results gave further evidence for an important role of the electrostatic receptor-receptor interactions in the receptor interface of the A2A-D2 receptor heteromer. Incubation with peptides corresponding to the transmembrane (TM) domains IV and V significantly reduced the A2A and D2 receptor heteromerization. Thus, these TM domains of the D2 receptor play a major role in the A2A-D2 receptor interface. It presented a novel development.

In this paper, postdoc Wilber Romero-Fernandez also participated. He had just arrived from the same University in Barcelona as Dasiel and became part of our team on receptor-receptor interactions in heteroreceptor complexes. Mutation of the arginine residues (217-222 and 267-269) on the intracellular loop 3 of the D2 receptor performed by the Ciruela group finally established a major role of these positively charged residues in producing the allosteric A2A-D2 receptor-receptor interaction.

In 2010–2011, we pointed out the importance of adenosine-dopamine interactions in A2A-D2 heteroreceptor complexes in the pathophysiology and treatment of CNS disorders also involving a regulation of glutamate transmission, especially mGluR5 signaling.

Of substantial interest were the findings of Borroto-Escuela and colleagues (Borroto-Escuela et al. 2011) that the antagonistic A2A-D2 receptor-receptor interactions enhanced beta-arrestin2 recruitment to the D2 receptor protomer leading to subsequent cointernalization and an earlier time onset of Akt phosphorylation, rapidly followed by a dephosphorylation. The results opened up the existence of a possible A2A-D2-beta-arrestin2 complex.

Jeffrey, in 1999, introduced the term "moonlighting protein" for a multifunctional protein in which different functions are found in single strands of amino acids not linked to splicing. GPCR protomers can moonlight through the allosteric receptor-receptor interactions. As an example, the third intracellular loop of D2 can moonlight through the allosteric mechanism by switching between interactions with different types of G proteins, between G proteins and beta-arrestin2, or between G proteins and calmodulin.

A2A-D2 Receptor Heteromers and Drug Addiction, Especially Cocaine Addiction

This work became possible through a highly rewarding collaboration with Professor Malgorzata Filip and her group, notably with Malgorzata Frankowska and Karolina Wydra at the Laboratory of Drug Addiction Pharmacology, Institute of Pharmacology, Polish Academy of Sciences in Krakow. Our collaboration started around 2005 and is still intensely ongoing, in which Dr. Borroto-Escuela has a crucial role. The first paper was published in 2006 and suggested

an antagonistic modulation of D2 receptor-induced actions by cocaine involving A2A/D2 heteroreceptor complexes. Through a collaboration with Professor Luca Ferraro and Professor Sergio Tanganelli and their groups, we were able to obtain indications that a novel mechanism independent of DA transporter blockade was involved in the cocaine actions. That same year Filip and her group wrote a review, which I was happy to be part of. The review proposed that A2A-D2 receptor-receptor interactions may play an overall important role in drug addiction in view of the fundamental role of the midbrain mesolimbic DA neurons in reward. It was interesting to observe that in the ventral and dorsal striatum differential changes were observed in the A2A and D2 receptors during cocaine self-administration maintenance and its extinction.

Another finding of interest was that changes in DA and GABA extracellular levels in nucleus accumbens using microdialysis during cocaine self-administration maintenance appeared to follow the motivational aspects of cocaine intake as demonstrated especially by Drs. Wydra and Krystyna Golembiowska. Both pharmacological and transgenic approaches were employed in findings that A2A receptor signaling counteracts locomotor sensitization known to be linked to nicotine reward-learning.

Of high interest was the finding published in *Psychopharmacology* that an antagonistic A2AR-D2R interaction can help mediate the inhibitory actions of an A2AR agonist, injected into the nucleus accumbens shell, on cocaine seeking and cocaine reward using cocaine self-administration. The A2AR-D2R interaction led to an increase in GABA release in the nucleus accumbens, likely reflecting an increased activity in the ventral striato-pallidal GABA anti-reward pathway (Fuxe et al. 2008). In a subsequent psychopharmacology paper, it was shown that A2AR agonists counteracted cocaine seeking as well as its reinstatement by cues. Instead, A2AR antagonists could reinstate cocaine seeking, blocked by D2 receptor antagonists. Food seeking was modulated in a similar way. Brain-penetrant heterobivalent drugs with, for example, A2AR agonist and D2R antagonist pharmacophors, may be one novel strategy to treat cocaine abuse. They should preferentially target the A2AR-D2R heteromer to increase activity in the GABA antireward neurons.

In 2016, we studied the allosteric A2AR-D2R interactions after cocaine self-administration versus yoked saline controls. A significant reduction in the affinity of the D2R high-affinity state was found after A2AR activation following cocaine self-administration in the ventral striatum but not in the dorsal striatum versus respective control groups. We proposed that differential recruitment of sigma 1 receptor to the ventral striatum can help understand the mechanism involved. In this way, the A2A-D2-Sigma1 receptor may be formed only in the ventral striatum after maintenance of cocaine self-administration. This event can lead to the observed increase in the antagonistic A2A-D2 receptor-receptor interactions. The target for the anticocaine actions of A2A receptor agonists therefore may be the A2A-D2 heteroreceptor complexes in the ventral striatum.

The details of our hypothesis focused on A2A-D2 heteroreceptor complexes, as a target for cocaine actions was found in the *Journal of Neural Plasticity* in 2016. In 2017, using the proximity ligation assay, we followed up the Pintsuk paper (Pintsuk et al. 2016) with findings that cocaine self-administration can reorganize the panorama of A2A and D2 homoreceptor complexes and receptors monomers into an increased formation of A2A-D2 and D2R-sigma1R heteroreceptor complexes in the nucleus accumbens shell (Borroto-Escuela et al. 2017). Such a reorganization can reflect the development of an increased density of A2AR-D2R-sigma1R heterocomplexes with improved antagonistic allosteric A2A-D2 receptor-receptor interactions as indicated by Pintsuk and colleagues. This is the first evidence that A2A-D2 heteroreceptor complexes can be a novel target for the treatment of cocaine abuse and cocaine addiction.

The A1-D1 Receptor Heteromer

We provided biochemical and behavioral results indicating the existence of antagonistic A1R-D1R interactions in collaboration with Dr. Popoli in Rome and Dr. Lydia Gimenez-Llort at our department at Karolinska Institutet. In 1998, we validated the antagonistic adenosine A1 receptor-mediated modulation of dopamine D1 receptors in stably cotransfected fibroblast cells. Graduate student Torvinen played an important role with help from Ferre, Arenas, Civelli, and the Fredholm team. The antagonism exerted by A1 receptors led to a reduction in both D1 receptor recognition and signaling. In 2000, Torvinen was a coauthor in our papers with Professor Franco, demonstrating by means of coimmunoprecipitation the existence of agonist regulated A1R-D1R heteroreceptor complexes in fibroblast cells cotransfected with A1R and D1R receptors (Franco et al. 2000). The article by Franco and colleagues was published in a special issue based on presentations at a special symposium on receptor heteromerzation being linked to the Society of Neuroscience meeting in 1999. We appreciated the good work performed by Dr. Gines on the Franco team.

In 2002, Torvinen as first author published the last paper of her thesis. It dealt with interactions among adenosine deaminase, adenosine A1 receptors, and dopamine D1 receptors in stably cotransfected fibroblast cells and neurons. It was part of a collaboration mainly with the Franco group. She demonstrated the importance of having adenosine deaminase bound to the A1 receptor for the development of the antagonistic A1-D1 receptor-receptor interaction. I was proud of her thesis and liked her fine spirits. She did a great job, and in 2005, she was the first author of a paper demonstrating the existence of A2A-D3 heteroreceptor complexes with FRET in cell lines with antagonistic A2A-D3 receptor-receptor interactions. Antagonistic interactions were demonstrated leading to reduced affinity and signaling of the D3 receptor. The results opened up a new target for treatment of schizophrenia,

should the A2A-D3 complex exist in the brain. This was the result of a fine collaboration with the Franco team.

In the brain, we found in 1996 the antagonistic A1R-D1R receptor-receptor interactions to be located in the strio-entopenduncular/nigral GABA pathway, known as the direct pathway. The paper involved the participation of the Fuxe, Fredholm, and Ungerstedt teams and Dr. Strömberg at the Karolinska Institutet. The role of the A1R-D1R heteroreceptor complexes in the reward neurons of the nucleus accumbens remain to be studied. Rafael Franco and I wrote reviews on this work in 2007.

The Neurotensin R(NTSR)-D2 Receptor Heteromer and Its Function

In 1992, together with the Ungerstedt and Tanganelli groups using microdialysis, evidence was obtained that antagonistic NTSR-D2R interactions at the pre- and postsynaptic level resulted in a DA transmission dominated by D1Rs. Thus, the heteroreceptor complexes could alter the plasticity of striatal DA transmission. In a lecture in New York, we also proposed that intramembrane interactions between neurotensin receptors and D2 receptors represented a major mechanism for the neuroleptic-like action of neurotensin.

In 1992, graduate student XM Li arrived in the laboratory. He did a great job and found in 1993 that the NT peptide neuromedin N (NN) also was a potent modulator of striatal D2 receptor agonist binding sites in dorsal striatum by reducing their affinity. That same year he also found that the C-terminal neurotensin fragment (8-13) could reduce the affinity of the neostriatal D2 receptors and with higher potency and efficacy than NT.

In 1994, Li made the important observation that NT/NN peptides demonstrate a stronger reduction in the affinity of the striatal D2R agonist binding sites in brain sections compared with membrane preparations. This indicated the participation of cytoplasmatic factors like enzymes, such as protein kinases, to optimize the receptor-receptor interactions in the plasma membrane, and a likely demand for a relatively intact plasma membrane structure. In two lectures in New York in this period, we pointed out that the mechanisms for the high modulatory potency of neuropeptides can involve their ability to act as VT signals and also often via active fragments to modulate WT through receptor-receptor interactions at nanomolar concentrations. This also gave diversity to the receptor protomers involved.

Tanganelli and colleagues in 1994 through microdialysis work made the discovery that NT in nucleus accumbens, in contrast to dorsal striatum, reduced DA release. This involved increased GABA release induced by NT through the antagonistic NTSR–D2R interaction on the ventral striato-pallidal GABA pathway or their glutamate afferents (Tanganelli et al. 2012). The findings indicated that through GABA VT mediated via GABAA receptors on the DA terminals, DA release was reduced. The NT receptors were not present on the accumbens DA terminals. These results offered

one mechanism for the proposed antipsychotic role of NT in schizophrenia proposed by Nemeroff. I met Nemeroff a couple of times, but he did not seem to like our NTSR-D2R story very much.

Through a combined D2R binding and microdialysis study in 1995 performed by Drs. XM Li, Dr. Ferraro, and Professor Tanganelli, further evidence was obtained that NT peptides via NTSR-D2R interactions antagonistically regulated postsynaptic D2Rs in the nucleus accumbens. This evidence strengthened our hypothesis that the antagonistic NTSR-D2R receptor-receptor interactions in the nucleus accumbens was part of the mechanism for the atypical antipsychotic profile of NT peptides.

Dr. Luca Ferraro was an outstanding associate professor of pharmacology in the University of Ferrara and Sergio Tanganelli and Luca Ferraro formed a great team. They found in 1997 that the NT fragment 8-13 lacked effects on the striatal DA release in contrast to NT itself. This finding opens up the possibility that the NTSR-D2R autoreceptor complex is different from the corresponding postsynaptic complex, leading to a differential change in the NTR recognition through altered allosteric receptor-receptor interactions.

In 2002, through a collaboration with Dr. Diaz Cabiale at the University of Malaga, we could block the NTSR-D2R receptor-receptor interactions with an NTSR1-like receptor antagonist. Ferraro and colleagues published a review in *Brain Research Reviews* in 2007 on mesolimbic DA neurons and cortico-accumbens glutamate afferents as major targets for the regulation of the ventral striato-pallidal GABA pathways by neurotensin peptides.

In 1995, the Ferraro-Tanganelli team found that NT can release endogenous glutamate in the dorsal striatum in the awake rat. In 2002, Dr. Tiziana Antonelli at the University of Ferrara found that NT enhances glutamate excitotoxicity in midbrain neurons in collaboration with Ferraro and Tanganelli. Antonelli also found that NT receptors can be involved in N-methyl-D-aspartate (NMDA)-induced excitotoxicity in primary cultures of cortical neurons and published a review on these significant results in *Progress in Neurobiology* in 2007, including their relevance for neurodegenerative diseases and their treatment. Of high interest was the review in *Mini-Reviews in Medicinal Chemistry* by Ferraro, Antonelli, and colleagues in 2009 on "Emerging Evidence for NT Receptor 1 Antagonists as Novel Pharmaceutics in Neurodegenerative Disorders." Also, the idea of NMDA-NTSR1 receptor-receptor interactions was introduced in addition to the NTSR1-D2R receptor-receptor interactions (Ferraro et al. 2012; Tanganelli et al. 2012). It was proposed that NTSR1 antagonists represent a novel strategy for treatment of PD.

The paper in 2013 by Borroto-Escuela and colleagues was of high importance. For the first time evidence could be given for the existence of D2LR-NTS1R and D2SR-NTS1R heteromers in cell models using the BRET^2 technique. The cAMP responsive element binding (CREB) reporter gene assay demonstrated that NTSR1 activation inhibited the CREB signaling. Instead, using the NTSR1 agonist, there was an enhanced activation of the

MAPK intracellular pathway, which likely was mediated by enhanced activation of PKC in the MAPK cascade. Instead, the antagonistic allosteric receptor-receptor interactions in the plasma membrane was responsible for the reduction of the D2R mediated inhibition of the Gi/o-AC-protein kinase A (PKA)-CREB pathway by agonist activation of NTSR1. The Borroto-Escuela paper in 2013 gave evidence that the antagonistic NTSR1-D2R interaction (Borroto-Escuela et al. 2013), first observed in 1983 by Agnati and colleagues, likely took place in an NTSR1-D2R heteromer as observed 20 years later.

Putative CholecystokininR-D2R Heteromers and Their Receptor-Receptor Interactions

In 1990, Tanganelli and colleagues followed up our early work from the 1980s (Fuxe et al. 1983) by giving a functional correlate to the CCK-8 induced reduction of D2 receptor affinity. He found that CCK-8 in low concentrations could enhance striatal DA extracellular levels likely through an antagonistic CCKR-D2R autoreceptor interaction on the striatal DA nerve terminals. In 1993, Tanganelli also found that nanomolar concentrations of NT and CCK-8 synergistically counteracted the apmorphine-induced inhibition of DA release in the dorsal striatum. Li, in the same paper, found that these effects were associated with a synergistic reduction of D2R affinity in biochemical binding experiments. The mechanism involved may be linked to the observations that CCK-8 reduced the association rate constant of the D2Rs, while NT increased their dissociation rate constant.

The CCK-8 Induced Reduction of the Affinity of D2R Agonist Binding Sites Was Blocked by a CCKB Receptor Antagonist

It was unexpected that a nanomolar concentration of CCK-8 instead increased the affinity of the D2R agonist binding sites in DA competition experiments for D2R antagonist binding sites performed in dorsal striatal membranes. This action was blocked not only by CCKB but also by CCKA receptor antagonists. The fact that the D1R antagonist also blocked the CCK-8-induced increase in D2R affinity helped us understand the mechanism of action. We proposed that DA, through its activation of D1Rs, can recruit D1Rs to D2R-CCKB heteroreceptor complexes. The formation of putative D1R-D2R-CCKB heteroreceptor complexes with the possible presence of CCKA receptors will then alter the D2R-CCKB allosteric receptor-receptor interaction and an increase in the D2R protomer affinity develops. Susan George has demonstrated that D1R-D2R heteromers do exist. Jonathan Javitch, however, has pointed out that these heteromers exist only in a low percentage of the neurons. In our minds, we should consider the possibility that agonist activation of the D1Rs in neurons that express both D1 and D2R may increase the formation of D1R-D2R heteroreceptor complexes, which

can help explain our findings. We plan to start by testing this hypothesis in cellular models.

In 1995, XM Li and colleagues made one highly interesting observation, namely, that CCK-8 can much more strongly modulate the D2R affinity in the dorsal striatum and in the nucleus accumbens when using receptor autoradiography, which involves sections compared with biochemical binding experiments using membrane preparations. As discussed previously, this may be related to the need for a more intact membrane structure and participation of intracellular mechanisms like protein phosphorylation and dephosphorylation to optimally perform the receptor-receptor interactions in the plasma membrane, at least for certain types of receptor complexes in the plasma membrane. Another interesting finding was that strong modulation was also found *ex vivo* after intraventricular injection of CCK-8.

In 1996, Dasgupta and colleagues found that the reduction of D2R affinity by CCK8 also was found in cells cotransfected with CCKB and D2L receptor cDNAs. Notably, the enhancement by CCK-8 of GABA and dopamine release in the accumbens likely reflects an inhibitory effect CCK-8 on both pre- and postsynaptic D2Rs through CCKB receptors. Instead CCKA receptors inhibit GABA release via cholinergic mechanisms.

In brief, the findings indicate the existence of antagonistic CCKB-D2 receptor-receptor interactions on the ventral striato-pallidal GABA antireward neurons, which can reduce the D2R brake on GABA transmission in this system. CCK-B agonists therefore may have antipsychotic actions, which can enhance the therapeutic effects of D2R antagonists, representing known antischizophrenic drugs. Likewise CCKB agonists should synergize with NTSR1 agonists to increase the brake on the D2Rs of these antireward neurons, which should enhance their activity and produce antipsychotic actions. There is also a need to increase the activity of these antireward neurons in drug use disorders. Therefore, in this case, CCKB-D2R heteroreceptor complexes also should be a new target for treatment of drug abuse. This story on the putative CCKB-D2R heteroreceptor complexes is far from complete and demands the demonstration that these receptor complexes in fact exist in the brain.

Neuropeptide Y (NPY) Y1-Alpha2 Adrenoceptor, NPYY2-Alpha2 Adrenoceptor, and Angiotensin AT1-Alpha2 Adrenoceptor Receptor-Receptor Interactions in Central Cardiovascular Regulation: Focus on the NTS

In the early 1990s, we had an excellent graduate student Shao-Nian Yang from China interested in central cardiovascular regulation. We were lucky to have Professor Jose Narvaez from University of Malaga and his postdoc Jose Aguirre in the laboratory who were experts in cardiovascular regulation. Jose Narvaez is now rector of the University of Malaga. Dr. Debora R. Fior from San Paulo, Brazil, also were with us and had interest in cardiovascular transmitters and modulators. In addition, we had also a long-standing

collaboration with Professor Detlev Ganten in Germany on the brain renin-angiotensin system, which operated via VT. One of his students Bernd Bunnemann had become a graduate student with me in this period. The stage was set for understanding how the NPY receptors and angiotensin II receptors modulated the alpha2 adrenoceptors as well as their reciprocal interactions in the NTS. They formed a great group.

In 1993, we found that microinjections of the NPYY2 agonist NPY (13-36) into the NTS counteracted the vasodepressor actions of NPY (1-36) and of a NPYY1 receptor agonist. Thus, the NPY fragment may exert an inhibitory feedback on the NPYY1 receptor through a possible direct feedback via antagonistic NPYY2-NPYY1 receptor-receptor interactions. We demonstrated that the NPYY1 receptor IR was located on CA nerve cells in the medulla oblongata, including the NA cell bodies of the NTS, known to have alpha2 adrenoceptors. Yang and colleagues in 1995 could validate the findings of Harfstrand (Harfstrand and Fuxe 1987) by demonstrating that coinjections of NPY (1-36) or a NPYY1 agonist with adrenaline could block the vasodepressor responses to adrenaline. These findings taken together opened up the possibility that at least part of the action may take place at NPYY2-NPYY1-alpha2 adreno-heteroreceptor complexes located on the NTS NA neurons at the presynaptic or postsynaptic levels.

It is of substantial interest that upon alpha2-adrenoceptor activation in the NTS, there is the appearance of an antagonistic alpha2-adrenoceptor-NPYY2 interaction associated with an enhanced signaling over the NPYY1 receptor, as demonstrated by Yang and colleagues in 1994. This may be related to the removal of the inhibitory NPYY2-NPYY1 receptor-receptor interaction.

In the spontaneously hypertensive rat, the potency of NPY to antagonize the alpha2-adrenoceptor signaling was enhanced relative to the normotensive strain, which may contribute to the hypertension development. Errors in receptor-receptor interactions thus may contribute to disease processes.

An antagonistic angiotensin IIR-alpha2-adrenoceptor interaction was also demonstrated in the NTS, which was disturbed in spontaneously hypertensive rats. In 1995, Fior and I also found a bradykinin receptor modulation of the alpha2 adrenoceptors in NTS upon its activation by bradykinin.

The possible existence of NPYR-AT1R-alpha2-adrenoceptor heterocomplexes with multiple receptor-receptor interactions is still unknown. However, NPY-angiotensin II interactions were found in central cardiovascular regulation by Aguirre and colleagues in 1991 and published in *Brain Research* (Aguirre et al. 1991).

GalaninR-5-HT1A, 5-HT1A-5-HT2A, and GalR1-GalR2 Receptor-Receptor Interactions

Around 1990, Peter Hedlund came to our laboratory as a graduate student. His thesis project was to develop the concept on the existence of the

galaninR-5-HT1A receptor-receptor interactions. He started by studying the reciprocal interaction and found that 5-HT1A activation increased the affinity of the high-affinity galanin receptors in the tel- and diencephalon using receptor autoradiography. We also found along with Jose Aguirre and Jose Narvaez in 1991 that galanin and a 5-HT1A agonist synergize to produce vasodepressor responses.

In view of the failure to observe high-affinity galanin receptor binding sites in the dorsal hippocampus, the neocortex, and the dorsal striatum, we started to also use [^{125}I]galanin-(1-15) as a radioligand. We discovered [^{125}I] galanin-(1-15) fragment binding sites in the previously noted areas of high specificity. The results suggested that a special type of galanin receptor may exist in brain with a high and selective affinity for N-terminal galanin fragment binding sites. In line with these results, we found that galanin (1-15) but not galanin (1-29) reduced the affinity of the 5-HT1A agonist binding sites in membrane preparations of the dorsal hippocampus.

In support of these results, José A. Narvaez and his collaborator Zaida Diaz-Cabiale found that galanin-(1-15) has a different role than galanin-(1-29) in central cardiovascular regulation. The action of the fragment was blocked by a galanin receptor antagonist. Diaz-Cabiale demonstrated in 1996 that galanin-(1-15) but not Gal-(1-29) decreased the baroreceptor reflex.

We wrote three reviews on this work for the *Annals of the New York Academy of Sciences*. One was prepared in 1996 by Hedlund and I on galanin receptor-5-HT1A receptor-receptor interactions as an integrative mechanism in 5-HT neurotransmission. I prepared the second review with colleagues in 1998 on how galanin and galanin fragments modulates 5-HT receptors and their function. The third review was prepared by Diaz-Cabiale and colleagues in 1998 on the role of galanin and N-terminal galanin fragments in central cardiovascular regulation.

GalR-5-HT1A autoreceptor interactions also exist in the 5-HT cell bodies and dendrites of the dorsal raphe shown by Haleh Razani and colleagues in 2000 as part of her thesis.

Dr. Inmaculada Bellido and colleagues in our group found in 2002 a significant increase of galanin receptors in dorsal raphe in a genetic rat model of depression, which correlated with an increase in immobility in the forced swim test. Thus, Gi/o-coupled galanin receptors in the dorsal raphe may contribute to depression.

In an exciting paper from 2010, evidence for the existence GalR1-5-HT1A receptor heteromers was finally obtained in cellular models by Borroto-Escuela and Manuel Narváez using FRET (Borroto-Escuela et al. 2010c). Through CRE-luciferase and SRE-luciferase reporter assays, the signaling over MAPK or AC pathways in this heteromer was demonstrated. Results revealed an allosteric cross-inhibition between both signaling pathways, which avoids excessive inhibition of the AC pathway or an increase in the MAPK pathway.

We hypothesized in 2008 and 2012 that the Gal fragment-preferring sites developed through the existence of a GalR1/GalR2 heteroreceptor complex (Fuxe et al. 2008). The evidence was obtained in a great paper from 2014 by Borroto-Escuela and colleagues using BRET^2 assays in cellular models. Through proximity ligation assays, evidence was obtained that GalR1-GalR2 heteroreceptor complexes were also present in the raphe-hippocampal system. We found a preferential activation by Gal (1-15) of the GalR1 protomer versus galanin (1-29). In contrast, galanin (1-29) preferentially activated GalR2 with increased Gq/11-mediated signaling determined in NFAT reporter gene assays. This may be the mechanism for the ability of GalR1 agonists to produce depressive actions.

In parallel, the Diaz-Cabiale group in Malaga, using an in vivo model of siRNA GalR2 knockdown or siRNA GalR1 knockdown rats, provided evidence that Gal (1-15) produced depression and anxiety-like behaviors by targeting GalR1-GalR2 heteroreceptor complexes in brain.

On the basis of these results, it seems likely that GalR1–GalR2–5-HT1A heterotrimeric complexes also may exist. Zaida Diaz-Cabiale and colleagues in 2000 also observed that Gal-(1-15) exerted a stronger decrease of 5-HT1A receptor recognition in the ventral limbic cortex than gal-(1-29).

It is of high interest that Millon and colleagues found in 2016 that Galanin (1-15) enhanced the antidepressant effects of a 5-HT1A receptor agonist. The results suggested that upon activation of all receptor protomers of putative GalR1-GalR2-5-HT1A heteroreceptor complexes linked to the raphe-hippocampal 5-HT neurons, antagonistic allosteric GalR1-5-HT1A receptor-receptor interactions no longer dominate but instead are replaced by an enhancing GalR2-5-HT1A interaction. This indicates the possible impact of agonist regulation on the dynamics of the receptor-receptor interactions in this putative heteroreceptor complex. This possibility seems to open up new strategies for the treatment of depression. In the Diaz-Cabiale group, Flores-Burgess and colleagues also demonstrated an enhancement of the antidepressant-like actions of fluoxetine by Gal-(1-15), which leads to a novel strategy for treatment of depression. The mechanisms involved can be an increase in the facilitatory GalR2-5-HT1A receptor-receptor interaction caused by the increased 5-HT1A protomer activation in the putative GalR1-GalR2-5-HT1A complex through increased extracellular levels of 5-HT. An increased expression of 5-HT1A receptors also can be involved.

It is of substantial interest that we, under the leadership of Professor Garriga in Barcelona, could find that the zinc binding receptor GPR39 interacts with 5-HT1A and GalR1 to form dynamic heteroreceptor complexes with signaling diversity. In view of the antidepressant effects of zinc, it is of interest that the GalR1-5-HT1A complex with inhibitory receptor-receptor interactions on 5-HTA signaling is the dominant complex at low zinc concentrations.

Galanin receptor-NPY receptor interactions were found in the brain by Diaz-Cabiale and Jose Narvaez and their colleagues. In a fine paper from

2016, Manuel Narvaez, Borroto-Escuela, and colleagues observed GalR2-NPYY1 receptor-receptor interactions in the dentate gyrus that are associated with antidepressant actions. These receptor-receptor interactions in the amygdala led to increased anxiolytic actions, as also found by Manuel Narvaez and colleagues. These are important findings because they suggest a new basis for integration of Galanin and NPY peptides at the molecular level in the brain.

In October 2017, our paper on 5-HT1A-5-HT2A isoreceptor complexes was published with Dr. Dasiel Borroto-Escuela as first author and corresponding author. Thus, two subtypes of 5-HT receptors from two major families of 5-HT receptors can form a heteroreceptor complex and produce integration and diversity of 5-HT receptor signaling, which likely is relevant for depression. These experiments were done thanks to the financial support of the Hjärnfonden, a neuroscience-focused foundation from Sweden.

FGFR1-5-HT1A Heteroreceptor Complexes and Their Receptor-Receptor Interactions: Relevance for Depression

Postdoc Dasiel Borroto-Escuela was leading this project and did so in an outstanding way. Postdoc Wilber Romero-Escuela in our laboratory also made fine contributions as did colleagues from other research teams abroad, especially the Belluardo-Mudo group at the University of Palermo, Italy. The project involved a large number of techniques, including, for example, BRET, co-immunoprecipitation, in situ proximity ligation assay, interface interacting peptides, and biochemical binding assay.

A hippocampal FGFR1-5-HT1A receptor complex was demonstrated (Borroto-Escuela et al. 2012), in which the 5-HT1A protomer enhanced the FGFR1 signaling. Upon combined activation of the two receptor protomers, synergistic increases in neuroplasticity were observed in primary hippocampal cultures. Upon acute and repeated intraventricular treatment with FGF2 and a 5-HT1A agonist, substantial antidepressant effects were observed (Borroto-Escuela et al. 2012). We believe this is a great paper. We had postulated the existence of these effects in 2007 (Fuxe et al. 2007). These results opened up novel strategies for the treatment of depression through the use of brain penetrant heterobivalent drugs built up of FGFR1 agonist and 5-HT1A agonist pharmacophors. The treatment has the potential to reverse the atrophy of the hippocampus found in depression.

In 2015, we were able to present evidence for the existence of FGFR1-5-HT1A heteroreceptor complexes in the midbrain raphe 5-HT system. The fact that 5-HT1A autoreceptors also can be part of heteroreceptor complexes with FGFR1 was of high significance. Thus, in this way, the 5-HT1A autoreceptor may become uncoupled from the GIRK channel and may no longer silence the dorsal raphe and medianus raphe neurons. Instead of reducing the firing of the ascending 5-HT neurons and contributing to depression,

the 5-HT1AR in the raphe cell bodies and dendrites may enhance trophic actions of FGFR1 and increase plasticity and reduce the downstate of the ascending 5-HT neurons with a return of their mood-elevating functions. An enhancement of the FGFR1 protomer signaling of this heteroreceptor complex in the midbrain 5-HT nerve cells was in fact demonstrated after cotreatment with 5-HT1A agonist and FGF2.

Through these exciting findings, it became possible to write a research opinion article in *Trends in Neuroscience* on the integration of 5-HT and neurotrophic factor hypotheses of major depression (Borroto-Escuela et al. 2016). This was based on the demonstrated molecular integration in FGFR1-5-HT1A heteroreceptor complexes through allosteric receptor-receptor interactions.

In 2017, Dasiel and I were happy to be part of a paper by the Mudo-Belluardo group indicating the existence of mAChR-FGFR heterocomplexes enhancing neural plasticity in the hippocampus. Also, activation of such complexes can contribute to counteracting depression-induced atrophy of the hippocampus.

OxytocinR-Alpha2-Adrenoceptor Interactions

In 2000, Dr. Zaida Diaz-Cabiale worked with me as postdoc as part of a collaboration with Professor Jose Narvaez. We started a collaboration with Dr. Kerstin Uvnäs-Moberg and her group at the Karolinska Institutet. Uvnäs-Moberg was interested in understanding the actions of oxytocin on the brain, and so were we. We published a series of papers from 2000 to 2005 in *Neuroendocrinology*, *Journal of Neuroendocrinology*, and *Brain Research*. We found an antagonistic oxytocinR-alpha2-adrenoceptor interaction in the hypothalamus and amygdala involving a reduction in the affinity of the alpha2-adrenoceptor agonist binding sites using receptor autoradiography, which may be of relevance for the ability of oxytocin to acutely reduce the increase in food intake produced by the alpha2-adrenergic agonist clonidine. We also observed antagonistic oxytocin/alpha2-adrenoceptor interactions in the NTS, which is of possible relevance for acute effects on cardiovascular control in the NTS.

The most interesting finding was that subchronic oxytocin treatment could increase the density of the alpha2-adrenergic agonist binding sited in the hypothalamus and the amygdala. We proposed that this increase reflected an increase in alpha2-adrenergic signaling, which could contribute to the antistress actions of oxytocin, including modulation of neuroendocrine and cardiovascular functions. Similar results were observed in the ovarieectomized rat with demonstration also of increases in the alpha2-adrenergic agonist binding sites in the NTS. The mechanism for this increase is still unknown but could involve an increase in the expression of alpha2 adrenoceptors or an increase in the proportion of alpha2-adrenergic agonist

binding sites in the high-affinity state because of an allosteric oxytocinR-alpha2-adrenergic receptor interaction in a heteroreceptor complex.

D2R-Oxytocin Receptor Heterocomplexes and Their Receptor-Receptor Interactions

Professor Insel and his group, through pioneering work, demonstrated the key role of oxytocin and its receptor in social and emotional behaviors. They also found that simultaneous activation of oxytocin receptor and D2Rs in the nucleus accumbens is crucial for pair bond formation. We therefore tested whether integration of oxytocin and DA signals took place in an oxytocinR-D2R heterorocomplex with facilitatory receptor-receptor interactions. Dr. Wilber Romero-Fernandez had a leading role in this project. The oxytocinR-D2R heterocomplexes were demonstrated in cell models with $BRET^2$ and in nucleus accumbens and dorsal striatum with proximity ligation assay (Romero-Fernandez et al. 2013). Using biochemical-binding techniques, oxytocin in nanomolar concentrations was found to produce an increased D2R recognition involving increased D2R density and an increase in the affinity of the high-affinity D2R agonist binding site. As a result, an increase in dopamine-induced Gi/o protein stimulation was observed. All effects of oxytocin were blocked by an oxytocin receptor antagonist (Romero-Fernandez et al. 2013).

Our hypothesis was that facilitatory allosteric D2-likeR-oxytocinR interactions in heteroreceptor complexes in nucleus accumbens may have a role in social and emotional behaviors in rodents and also in humans, including bonding and trust through regulation of the limbic circuits, especially to the prefrontal cortex. This complex may enhance social salience through its presence in a special component of the ventral striato-pallidal GABA pathway.

It is known that oxytocin may exert antipsychotic effects and be a natural antipsychotic. Dysfunction or disruption of the D2-likeR-oxytocinR heteroreceptor complexes may be involved in the social and emotional disturbances found in schizophrenia. By blocking the D2R protomers of these receptor heteromers, antipsychotics may fail to reduce the negative symptoms of schizophrenia, such as social withdrawal and emotionless behavior, by reducing the signaling of the D2R-oxytocinR heterocomplex.

We then established the enhancing effects of the receptor-receptor interactions on the CREB, MAPK, and phospholipase C (PLC) signaling pathways of the D2R-oxytocin receptor heterocomplexes, with Dr. Borroto-Escuela in charge. Oxytocin enhanced the inhibitory D2R signaling in the AC-PKA-pCREB pathway and D2R activation of the MAPK-pELK pathway. D2R enhanced oxytocinR signaling in the PLC-IP3-Calcineurin pathway. In the same paper, their relevance for the anxiolytic effects of dopamine and oxytocin interactions in the central amygdala was found with Professor

Miguel Perez de la Mora in charge (de la Mora et al. 2016). D3R-oxytocin receptor heterocomplexes also may exist.

Professor Malinka recently wrote a beautiful article on ecstasy as a probe and drug for treatment of social behaviors. We propose that the D2R-OTXR heteroreceptor complexes can be an indirect or direct target for ecstasy, which can mediate its actions on social behavior. This will be exciting future work.

D2R-5-HT2AR Heteroreceptor Complexes as Targets for 5-HT2A Hallucinogens: Relevance for Schizophrenia

In 2010, we demonstrated the existence of D2R-5-HT2A heteroreceptor complexes BRET and proximity ligation assays in cellular models and in ventral and dorsal striatum (Borroto-Escuela et al. 2010a). Borroto-Escuela also found that reciprocal receptor-receptor interactions and standard 5-HT2A agonists reduced Gi/o-mediated D2R signaling. In view of the key role of D2Rs in schizophrenia, we proposed it could be a new target for antipsychotic drugs. It was therefore of high interest when we found that the hallucinogenic 5-HT2A agonists LSD and DOI were able to instead enhance D2R signaling and recognition. We proposed that this action could contribute to their hallucinogenic actions. Thus, the hallucinogenic 5-HT2A agonists had the ability to interact with the 5-HT2A binding pocket in a unique way, leading to a conformational change introducing an altered allosteric receptor-receptor interaction, enhancing D2R function.

It seems possible that in schizophrenia, the disease itself could alter the composition and stoichiometry of these heteromers and therefore the allosteric receptor-receptor interactions. Because of such changes, we now believe that the transmitter 5-HT can instead enhance the D2R protomer signaling as found after LSD, which can contribute to the schizophrenic symptoms. The prolonged and enhanced DA release from overactivity in the mesolimbic DA neurons in schizophrenia may contribute to a reorganization of the D2R heteroreceptor complexes or to altered receptor-receptor interactions, as found after amphetamine challenge.

These results are of high relevance for schizophrenia because the 5-HT2A antagonist–inverse agonist Pimavanserin recently was marketed under the trade name Nuplazid as a nondopaminergic atypical antipsychotic for the treatment of PD psychosis and schizophrenia. Professor Meltzer in Chicago made important contributions to its development.

D2R-D4R Isoreceptor Complexes and D4R-MOR Heteroreceptor Complexes

Borroto-Escuela and colleagues in 2011 found the D2R-D4R heteroreceptor complexes in cellular models using coimmunoprecipitation, proximity ligation assay, and BRET. D4.2R and D4.4 R were most effective in forming

heteromers with the D2R. Facilitatory allosteric receptor-receptor interactions were found using assays for MAPK. It was the first demonstration that D4 receptors also can form heteroreceptor complexes.

Rivera and colleagues in 2016 obtained indications for D4R-MOR receptor-receptor interactions in the striosomes and in the zona reticulata of the substantia nigra. These functional D4R-MOR interactions appeared to prevent the MOR-induced activation of the nigrostriatal DA system and take place in D4R-MOR heteroreceptor complexes, as recently demonstrated using in situ proximity ligation assay (PLA) by Borroto-Escuela and Kirill Shumilov in my laboratory. D4R activation also prevented the rewarding effects of morphine but not the analgesic effects. The study suggests a novel strategy to avoid the addictive properties of morphine without interfering with the pain-reducing actions. The paper indicates the high impact of receptor-receptor interactions on modulation of the pathways of the brain.

1990s to Present: Understanding Communication and Integration in the Amygdala, Especially the Role of DA and Other Transmitters in Anxiety and Fear

This work represented a continuation of the work with Professor Miguel Perez de la Mora on the amygdala. He was leading the work. We were interested in understanding the neuromodulation of the intercalated paracapsular GABA islands and the main intercalated GABA islands, known to regulate the input and output regions of the amygdala, the basolateral amygdaloid (BLA) nucleus and the central amygdaloid nucleus (CeA), respectively. We found an overall intense D1R immunofluorescence in all of the nerve cells of the intercalated GABA islands (Fuxe et al. 2003). The density of their DA nerve terminal innervation differed from being high in the rostrolateral paracapsular islands and low to very low in the medio-caudal component of these D1R islands. Likewise, in the rostro-medial component of the main intercalated island, there was a high DA innervation, whereas in the caudal part, DA innervation was very low or absent.

Thus, DA communication was in these islands dominated from extrasynaptic (less than 10 um) and short-distance VT. D1R activation leads to anxiety and D1R blockade to anxiolytic actions as described by Perez de la Mora and colleagues in 2010 and 2012. The mechanism appears to be that D1R activation leads to a hyperpolarization of the GABA neurons through the opening of GIRK channels. This results in a reduction of the activity of the intercalated GABA neurons followed by disinhibition of the BLA and CeA glutamate neurons and production of fear and anxiety as demonstrated by Anne Marowsky in 2005.

Instead, dopamine D2Rs had their highest density within the latero-capsular division of CeA. Microinjections into the CeA of raclopride produced anxiogenic-like effects in the Shock-Probe Burying test. This part of CeA

is innervated by DA terminals (Fuxe 1965a, 1965b). Activated D2Rs may inhibit, via a local interneuronal network, the anxiogenic outflow from the medial division. Thus, the DA projection pathways from the ventral tegmental area and the medial substantia nigra operate in the GABA intercalated cell masses mainly via D1R-mediated VT at various distances to enhance anxiety and fear. Instead, in the CeA they operate mainly via D2R-mediated extrasynaptic VT to produce anxiolytic effects. The balance between these two D1R- and D2R-modulated pathways may have a major role in the regulation of anxiety and fear.

We have studied the distribution of dopamine D1R and mu-opioid receptor immunoreactivities in the amygdala and the interstitial nucleus of the posterior limb of the anterior commissure. Again, there was no correlation between the distributions of D1R and MOR receptor systems with the TH and opioid peptide systems, respectively, indicating that VT was the major mode of communication. It was of substantial interest that strong dopamine and cyclic AMP-regulated phosphoprotein, 32 kDa (DARPP-32) immunofluorescence was observed in TH-D1R match regions but not in TH-D1R mismatch regions. Thus, the D1R signaling becomes altered in D1R-positive mismatch nerve cells, which are reached by lower concentrations of DA. It may mean that these D1R mismatch cells are forced to switch toward other signaling systems and may operate without inhibition of protein phosphatase-1 being part of D1R signaling.

In view of the partial codistribution of D1R and MOR receptor systems in the intercalated cell masses, the existence of receptor-receptor interactions in D1R-MOR heterocomplexes should be considered to exist in the intercalated islands and should be recognized as targets for VT.

In one of the papers in 2010 with Miguel Perez de la Mora dealing with GABAA rho receptor in the amygdala, Professor Ricardo Miledi was a coauthor. Miledi is known for his outstanding work on the key role of calcium ions in regulating neurotransmitter release. It was a pleasure to meet him in Mexico in this period, and he was happy to learn that GABAA rho receptors may have a place in the mechanisms modulating anxiety and fear.

I also had the pleasure to meet Professor Jorge Larriva-Sahd at a Cajal Club meeting in Queretaro, north of Mexico City. Larriva-Sahd is an outstanding neuroanatomist that mastered the rapid Golgi technique. I enjoyed discussing this with him, and our families had a good time together in Queretaro and its surroundings. We got to know this part of Mexico in a splendid way. I appreciated very much their hospitality and believed that Jorge liked my lectures on VT and receptor-receptor interactions at the Neurobiology Center in Queretaro.

We started to collaborate and studied the anterior and rostral paracapsular intercalated islands in horizontal sections along with Miguel Perez de la Mora using rapid Golgi. Our findings indicated that the intercalated islands may gate the information flow of neurons from the contralateral

amygdala and other contralateral parts of the basal forebrain using the anterior commissure for their axons. In this way, the anxiogenic outputs of both amygdalae could be synchronized. In particular, the anterior intercalated islands seemed to participate in decoding neuronal activity produced in the ipsi- and contra-lateral amygdala to achieve a balanced and appropriate output from the two amygdala in the various functional states of the brain. The paper was published in *Neuroscience* in 2012.

In 2014, we published together another fine paper, this time on the tubero-infundibular DA neurons and their relationship to D1R and D2R receptors using not only immunohistochemistry but also Golgi techniques. With the Golgi technique, Larriva-Sahd obtained further evidence that the arcuate nerve cells projected into the median eminence. Their axons formed horizontal axons in the internal layer producing a number of collaterals forming single or multiple strands reaching the external layer. The immunohistochemistry gave evidence for the first time that D1R receptors or D2R receptors exist in the LPZ of the median eminence and may mediate, through dopamine VT in the um range, inter alia inhibition of LHRH release from LHRH-immunoreactive nerve terminals in this region. Our concept on the existence of important integrative mechanisms in the external layer of the median eminence was finally receiving support decades after its introduction. The concept assumed the existence of VT in the um range because no synapses exist in the external layer.

A large number of TH immunoreactive nerve cell bodies in the dorsomedial region of the arcuate region, known to form DA, showed punctate dopamine D1R IR. Thus, dopamine D1Rs may directly modulate the activity or dopamine synthesis in many tubero-infundibular dopamine neurons at the soma-dendrite level. D2R IR was found only in a number of TH-positive neurons in the ventral periventricular cell group of the tubero-infundibular DA neurons. This demonstrated an unknown heterogeneity of this DA neuronal system.

2010 to Present: The Triplet Puzzle Theory: Homologies of Amino Acid Protriplets in the Interface of Receptor Heteromers

I was happy to begin a collaboration with Dr. Alexander Tarakanov, of the Russian Academy of Sciences, St. Petersburg Institute for Informatics and Automation, Saint Petersburg. We both believed that homologies were involved in forming heterodimers and Dr. Tarakanov was leading the work, being an expert in mathematics and bioinformatics. Through a mathematical approach, it was deduced, based on 48 pairs of receptors that form or do not form heteromers, that sets of triplet amino acid homologies may participate in the guiding process or in the receptor interface. This was called the triplet puzzle theory. They formed a kind of code that made different receptors come together and form heterodimers (Tarakanov and Fuxe 2010).

We proposed a "guide-and clasp" manner for receptor–receptor interactions in which triplet homologies represented potential "adhesive guides."

Dr. Tarakanov found the same year sets of protriplet amino acid homologies in the D2R-D4R, GalR1-5-HT1A, and D2R-5-HT2AR heterodimers, which Borroto-Escuela and colleagues recently identified as well in the A2AR-D2R heterodimer. In 2011, the triplet puzzle of homologies also was found in other types of protein-protein interactions.

In 2012, Tarakanov and colleagues obtained indications that the origin of the triplet amino acid homologies found in receptor heterodimers may be from immunoglobulin triplets and Toll-like receptor triplets. It is also of substantial interest that integrin triplets of marine sponges are used in the interface of MHC class I (MHCI) and CD8αβ, representing two immune molecules, and in the interface of neural receptor heteromers. Thus, some triplet amino acid homologies may originate from cell adhesion molecules of the oldest multicellular animals, where they may be used for recognition.

In 2015, we found that the opioid and chemokine receptor subtypes have several sets of amino acid homologies in common, which can be the mechanism for the massive formation of heteromers between chemokine and opioid receptors. This integrative mechanism should make possible a major modulation of pain and neuroinflammation. Thus far, the existence of triplet amino acid homologies in positions relevant to the interface in a receptor pair have turned out to always reflect a heteromer.

The interactions with Dr. Tarakanov had a significant impact on our understanding of how receptor heteromers are formed. To demonstrate the impact of the triplet puzzle theory, I will show its role in understanding the mild neuroinflammation hypothesis of schizophrenia developed by Karl Bechter: We indicated that putative NMDAR-C-C chemokine receptor type 2 (CCR2), NMDAR-C-X-C chemokine receptor type 4 (CXCR4), and NMDAR- interleukin 1 receptor type II (IL1R2) heteromers may develop in the neuronal-glial networks with mild neuroinflammation, in view of the Tarakanov demonstration of triplet Gly-Leu-Leu (GLL), Val-Ser-Thr (VST), and Ser-Val-Ser (SVS) amino acid homologies between these receptor protomers. Tarakanov also found indications for the presence of putative D2R-CCR2, D2R-CXCR4, and D2R-IL1R2 heteromers in mild neuroinflammation because these heteromers also exhibited sets of Leu-Tyr-Ser (LYS), Leu-Pro-Phe (LPF), and Ser-Leu-Ala (SLA) triplet homologies.

In view of the major role of the NMDA and D2 receptors in schizophrenia, these NMDAR and D2R heteroreceptor complexes may cause schizophrenia symptoms in mild neuroinflammation through a putative reduction of NMDA receptor protomer function and an increase in D2R protomer function.

We propose that CCR2, CXCR4, and IL1R2, as well as their ligands, may reach the neuronal cells by being present in exosomes released from astroglia and microglia as part of the inflammatory process and then internalized

into neuronal cells. From recycling endosomes, they may enter the plasma membrane and interact with NMDA or D2 receptors and alter their function. This may lead to symptoms of schizophrenia because of the disturbances developed in the neuronal network.

2007 to Present: Using an Electrophysiological Approach on Xenopus Oocytes to Understand Voltage Sensitivity and Pharmacology of GPCRs and the Actions of Typical and Atypical Antipsychotics

This work became possible through a collaboration with a neurophysiological group at our department led by Professor Peter Århem and Dr. Johanna Nilsson who were in charge of the project. Kristoffer Sahlholm, an excellent graduate student, played an important part in the work. It has been a rewarding collaboration.

The first two papers in 2007–2008 dealt with how H3 receptors couple to GIRK channels and on H4 pharmacology using GIRK coupling in Xenopus oocytes. Then we moved into voltage sensitivity of human D2S receptors. We found this to be agonist-specific. Upon depolarization, the potency of DA was reduced. Instead, the potencies of trace amines beta-phenethylamine and p- and m-tyramine receptors were not influenced by depolarization of the plasma membrane. The differential sensitivity to changes in voltage appeared to depend on properties of the receptor-agonist interactions, and were not linked to changes in the G protein or the GIRK channel. Such a difference in voltage sensitivity among agonists to the same receptor has not previously been observed.

In a subsequent paper in *Synapse*, we presented evidence that the human D2R itself is voltage sensitive using the D2R agonists DA and quinpirole using the same techniques. Such a property of the D2R may play a role in its modulation of synaptic potency and strength and of synaptic plasticity. It is of interest that concentration-response relationships at −80 mV and at 0 mV demonstrated significant rightward shifts for the human D4R but not for the human D3R upon depolarization. Thus, differences exist for closely related receptors.

In 2011, it became possible to demonstrate that the reduced potency of dopamine found at D2S receptors to open the GIRK channels upon depolarization correlated with a reduced binding of the dopamine radioligand. It was also found that the voltage sensitivity was interfered with by a mutation of conserved serines in TM-V, especially S193, or the conserved aspartate residue in TM III (D114). The hydroxyl groups and the amine moiety of dopamine are contacted by these serine and aspartate residues, respectively. Differences were observed in DA receptor agonists used in the treatment of PD. This work on voltage sensitivity of GPCRs is of high relevance for our hypothesis on the molecular basis of learning and memory (see the next section). Thus, changes in the voltage sensitivity and associated changes in

resistance can alter receptor protomer recognition and signaling, and thus can alter the allosteric receptor-receptor interactions in the heteroreceptor complexes building up the presynaptic and postsynaptic membranes. In the postsynaptic membrane, such changes therefore can contribute to learning and memories formed upon consolidation of the altered molecular circuit made up of different types of heteroreceptor complexes in the postsynaptic membrane (Fuxe et al. 2014; Borroto-Escuela et al. 2015).

In 2012, we found that the hH3 (445) receptor isoform had the highest sensitivity to voltage versus the hH3(365) isoform. There was also a difference in response deactivation kinetics between the two hH3 isoforms with the hH3 (365) isoform being slower than the hH3 (445) receptor. Thus, it can be speculated that higher sensitivity to voltage may be associated with more rapid deactivation kinetics.

We were aware that extrapyramidal side effects (EPS) were highly frequent with typical antipsychotic drugs, like chlorpromazine and haloperidol. Instead, they were less frequent with atypical antipsychotic drugs like clozapine. Nevertheless, both typical and atypical antipschotic drugs were mainly blocking D2R to produce their antipsychotic actions. Therefore, the fast-off hypothesis was introduced, which proposed that atypical but not typical antipsychotics rapidly dissociate from the D2Rs. Through this mechanism, an improved dynamics of D2R signaling was believed to be maintained. The reversibility of D2R blockade was not sufficiently examined in functional assays, however. We therefore tested a large number of typical and atypical D2R antagonists on D2R agonist-induced activation of GIRK currents in Xenopus oocytes, which have a high temporal resolution.

The major result of these tests was the demonstration of only small differences between the typical antipsychotic drug chlorpromazine and the atypical antipsychotic drugs amisulpride, clozapine, and quetiapine. Thus, the rate of reversibility of D2R blockade is not the key difference between atypical and typical antipsychotic drugs. Other explanations should be considered, such as the blockade of a distinct population of D2Rs as indicated in the case with remoxipride, which could represent a certain type of heteroreceptor complex with a distinct pharmacology.

In a subsequent paper, Sahlholm and colleagues analyzed in 2016 the maintained D2R antagonism produced by certain lipophilic compounds, such as the typical antipsychotic drug haloperidol. A rather rapid recovery of D2R responsivity from blockade by the antipsychotic drug haloperidol was found. The difference compared with previous work using radioligands may involve increased lipophilic sequestration and rebinding to the D2R. In our study, we used competition with DA and continuity of flow.

Thus, rapid rates of response recovery observed with both haloperidol and chlorpromazine indicate that these drugs remain in the brain tissue for other reasons than slow dissociation rates from the D2R. It may to a large extent be determined by their lipophilic properties.

Instead of the fast-off hypothesis, we should consider that many atypical antipsychotic drugs can target the 5-HT2A receptors with high affinity based on Meltzer's work. We propose that D2R-5-HT2A heteroreceptor complexes can be a novel important target for atypical antipsychotic drugs blocking both D2 and 5-HT2A receptor protomers, leading to synergistic therapeutic effects and allowing low doses to be used. Other D2R heteroreceptor complexes can be relevant targets for atypical antipsychotics. They represent a promised land for drug development in schizophrenia.

2004 to Present: Understanding the Architecture of Central Monoamine Neurons in Wild Rodent Species in South Africa, the Nile Crocodile, the Giraffe, the African Elephant, and the Bottlenose Dolphin

I always wanted to know the structural features of the central monoamine neurons in other species than the laboratory rat. This became possible by having a nice and fruitful collaboration with Professor Paul Manger and his group at the University of the Witwatersrand, Johannesburg, South Africa. Dr. Manger was the leader of the project, and I was happy to come down to his laboratory for a short period now and then. I analyzed the sections with excitement. They were stained using immunohistochemistry.

In the bottlenose dolphin, there was an expansion of the nigral medial and VTA lateral subdivisions forming a large DA cell group visualized with TH immunohistochemistry in the posterior part of the midbrain. Few DA nerve cells were found in the other parts of the substantia nigra. This made the parcellation of the DA cell groups in this area quite different from the rodent species. The coming together of the nigral-VTA DA cell groups may result in rather intense interaction between the nigral DA system involved with motor functions and the mesolimbic DA neurons representing a reward system. It may be speculated that the activity of the DA motor neurons can directly enhance the activity of the mesolimbic DA neurons in the ventral midbrain leading to the "happy" trunk movements of the dolphin.

In the African elephant, the nigral DA neurons instead appeared to form DA cell-rich islands, which are separated from one another by fiber bundles. It is an architecture found only in the elephant. Each DA cell island can form topologically arranged projections to the striatum, where the mosaic of dopamine nerve terminals innervating the striatum can make various trunk movements possible. There is also a medial division of the LC, built up of NA nerve cells, which previously was not observed in other species. It may be related to the demand for wakefulness to maintain a high food intake.

In the sub-adult male giraffe, the most interesting findings were an enlarged ventral component of the nigral DA cell group and an expansion of the NA cell group in the subcoeruleus and its diffuse component in the reticular formation of the rostral pons and caudal midbrain. The overall organization of the monoamine cell groups, however, was similar to that

found in other Artiodactyls in spite of changes in phenotype, brain size, and so on, which is in line with the review of Paul Manger published in 2005.

We also studied the nuclear organization and morphology of serotonergic neurons in the brain of the Nile crocodile. It was exciting to see the marked changes found versus mammals, especially in the 5-HT cell groups in the rostral brainstem. Here I saw the medial and lateral parts of the 5-HT immunoreactive superior raphe nucleus and a widely dispersed group of 5-HT immunoreactive neurons in the reticular tegmentum, known as the superior reticular nucleus.

The parcellation of all the DA, NA, and 5-HT cell groups in various wild-type species of rodents had a similar architecture to the central monoamine neurons of the domestic male rats I had found and characterized in my thesis in 1965. These findings were important because they showed that the fundamental architecture remained the same in the rodent order in terms of parcellation of the monoamine cell groups. Major species differences may instead exist in the monoamine nerve terminal networks and their receptor systems.

Paul and I became friends, and he showed me fine hospitality. Paul took me inter alia to the Pilanesberg National Park around 200 km away from Johannesburg. Paul drove through it in his jeep with me at his side. We found abundant wildlife in this nature reserve giving spectacular views of the South African landscape. Among the Big Five, we spotted the buffalos, elephants, and rhinos. We failed to see the lions, to our great disappointment. I am fond of their golden eyes, beautiful and full of power as they inspect their territory. Paul Manger and I are still interacting, and I hope this adventure will continue.

1990s to Present: The Concept of Allosteric Receptor-Receptor Interactions in Homo- and Heteroreceptor Complexes Give a New Dimension to Molecular Neuroscience and Understanding of the Molecular Basis of Learning and Memory and Brain Disease

The concept of allosteric receptor-receptor interactions in GPCR homo- and heteroreceptor complexes of the CNS gave a new biological principle to understanding brain integration and neuropsychopharmacology. Allosteric receptor-receptor interactions made possible through receptor oligomerization lead to novel receptor dynamics during which the receptor protomers change their recognition, pharmacology, signaling, and trafficking, and novel allosteric binding sites can develop. GPCR heteroreceptor complexes can also involve ion channel receptors, receptor tyrosine kinases (RTKs), sets of G protein-interacting proteins, ion channels, and transmitter transporters.

There is a need to improve our understanding of the molecular organization of the receptor oligomers, their allosteric communication, and the features of the receptor interface. It appears clear that synaptic transmission

and VT (extracellular fluid and CSF) in the brain can become integrated in heteroreceptor complexes in the synaptic and perisynaptic membranes built up of ion channel receptors, GPCRs, and RTKs (Fuxe et al. 2008; Borroto-Escuela et al. 2012; Fuxe et al. 2014; Borroto-Escuela et al. 2016). This enables modulation of synaptic strength and synaptic plasticity in a dynamic way, and receptor plasticity is accomplished. Integration of the molecular receptor circuits in the postsynaptic and perisynaptic membranes, respectively, can take place through integration of their intracellular molecular signaling pathways in the dendritic spines.

It is now clear from the demonstration of the GPCR heterodimer network (GPCR-HetNet) that the allosteric receptor-receptor interactions dramatically increase GPCR protomer diversity and biased recognition and signaling, leading to enhanced specificity in signaling (Borroto-Escuela et al. 2014).

On the basis of this concept, we proposed that the molecular basis of learning and memory was based on the reorganization of the homo- and heteroreceptor complexes in the postsynaptic membrane of synapses, and also was associated with changes in the presynaptic receptor complexes, to facilitate the pattern of multiple transmitter release to be learned (Fuxe et al. 2014; Borroto-Escuela et al. 2015). As discussed earlier, changes in the voltage sensitivity of the receptors and associated changes in electrical resistance also can contribute to the learning process through modulation of the allosteric receptor-receptor interactions. In addition, VT signals like transmitters and growth factors can diffuse into the synapse from surrounding terminals and dendrites and glial cells and target, for example, GPCRs and RTKs of multiple heteroreceptor complexes in the postsynaptic membrane. In this way, the new molecular circuit formed also contains information about the context in which the patterns of synaptic transmitter release was learned.

Long-term memory may be created by the transformation of parts of the heteroreceptor complexes into unique transcription factors, which can lead to the formation of specific adapter proteins that can consolidate the homo- and heteroreceptor complexes into long-lived complexes with conserved allosteric receptor-receptor interactions. Thus, the homo-heteroreceptor complexes are regarded as highly dynamic assemblies formed or disrupted by integrated synaptic and VT signals. These events are regarded as necessary for learning, and they can become transformed into a consolidated rigid state with conserved allosteric communication representing molecular engrams and resulting in a major long-term modulation of the neuronal networks. This molecular plasticity change, whether transient or long term, can then alter the patterns of outflow in the brain circuits and induce transient and long-term changes in behaviors and cognitive functions (Fuxe et al. 2014; Borroto-Escuela et al. 2015). This appears to be a key molecular mechanism for the modulation of the neuronal networks.

We have a concept for how lifelong memories can be formed. The impact of VT signals, including monoamines from terminals of emotional circuits, may

make it possible. They are postulated to act especially on distinct postsynaptic receptor protomers, which otherwise do not become activated. Extracellular-mediated VT also may take place. This can involve internalization of extracellular vesicles containing, as cargo, receptors and transcription factors with a significant ability to contribute to transcriptional activation through, for example, homeoproteins. This can cause, via epigenetic mechanisms, production of adapter–scaffolding proteins with a unique efficacy to consolidate the panorama of various types of heteroreceptor complexes that form the molecular engram in the postsynaptic membrane (Borroto-Escuela et al. 2015).

Dysfunction or disruption of the heteroreceptor complexes can lead to brain disease. Understanding the D2R heteroreceptor complexes and their dysfunction in schizophrenia can lead to new strategies for its treatment and for avoiding side-effects of antipsychotics known to mainly act as D2R antagonists. This includes a way to optimize combined treatment or the single use of heterobivalent drugs targeting distinct D2R heteroreceptor complexes in schizophrenia. This is inspired by current findings on the existence of various types of D2R heteroreceptor complexes in nucleus accumbens core and shell.

It was proposed that novel anti-Parkinson drugs for the future may be heterobivalent drugs, such as a build up of an A2AR antagonist pharmacophor linked to a D2R agonist pharmacophor specifically targeting the A2AR-D2R heterodimer. Other exciting novel targets for anti-Parkinson drugs are A2AR-D2R-mGluR5 heteroreceptor complexes, where A2AR and mGluR5 synergize to put a brake on D2R Gi/o-mediated signaling. Emerging new concepts in PD are that dysfunction of the allosteric receptor-receptor interactions and disbalance of multiple heteroreceptor complexes play a major role. They can be codistributed in the same neuron and synapses of the direct and indirect pathways of the basal ganglia and can contribute to disease progression and levodopa-induced dyskinesias.

Furthermore, the D1R and D2R heteroreceptor complexes in the reward and antireward GABA pathways from the nuc accumbens have become exciting new targets for the treatment of substance use disorder. The changes found in these receptor complexes in cocaine self-administration open up a new understanding of what goes wrong in cocaine use versus cocaine addiction.

Looking into the Past

It all started with our observations of the existence of central DA, NA, and 5-HT brainstem neurons. Their architecture, which often involves formation of global varicose nerve terminal networks in the brain and the spinal cord, made us believe that mainly an extrasynaptic monoamine communication was involved in modulating the panorama of glutamate and GABA neurons building up the CNS. This finding led to the introduction of the concept of the existence of a major mode of communication in the

extracellular fluid and in the CSF. We called this VT, which operates through diffusion and flow of biological signals reaching high-affinity receptors located on nerve and glial cells.

Understanding the function of the central monoamine neurons led us into neuroendocrinology, cardiovascular research, neurology, psychiatry, and neuropsychopharmacology.

Sitting at the fluorescence microscope at a young age, I often wondered how the monoamine neurons and their varicosities releasing DA, NA, or 5-HT could become integrated with the classical neurons and their synapses releasing glutamate or GABA. It seemed to me that the plasma membrane had been neglected as a site of integration of signaling. The dogma was that the synapse was fully isolated from the extracellular fluid and other synapses, with integration of transmitter signals taking place only in the cytoplasmatic signaling pathways. This did not make any sense. Why should integration of transmitter signals exclusively take place in the cytoplasmatic pathways? One way to find out would be to study the possible existence of receptor-receptor interactions in the plasma membrane, which we demonstrated at the recognition level.

We then introduced the concept that the receptor-receptor interactions took place in receptor heteromers. As a follow-up, we proposed that the postsynaptic membrane was built up of multiple homo- and heteroreceptor complexes that represented the molecular basis for learning and memory. Upon consolidation by unique adapter proteins formed in the memory process, molecular engrams were formed from the molecular circuit of homo- and heteroreceptor complexes in the postsynaptic membranes, giving a specific barcode to that synapse. In this integrative process leading to learning and memory, the VT and synaptic signals become integrated in synaptic and perisynaptic heteroreceptor complexes.

This work was accomplished by my own group and through national and international collaborations with outstanding scientists and their groups. Each of them gave their own unique contribution. It is no fun to do research without sharing it with other devoted scientists. The discussions are important to lead the way for new experiments and to be happy together for good results and to be depressed together when experiments go wrong. In the past 10 years, my senior postdoc and scientific laboratory manager Dasiel Borroto-Escuela has made outstanding contributions to the work, and I believe he has a great future in neuroscience.

Looking into the Future

An important area will be to build models of GPCR heterodimers of high relevance like the A2AR-D2R heterodimer. GPCRs can crystallize as homodimers and have a large buried surface area. We have started a collaboration with Dr. Jens Carlsson at the University of Uppsala on the A2AR-D2R

heterodimer. He will have a leading role in developing this model together with us. The dimer model will be generated by protein-protein docking together with competition experiments with transmembrane peptides using BRET to determine the transmembrane domains involved in the dimer interface. Mutations will be performed at the residues believed to be critical based on the docking analysis. A representative molecular dynamic simulation snapshot of the mutated residues will be performed. This work will be critical to reach into the atomic level and establish the dimer interface of the heterodimers we regard as critical targets for the treatment of PD, schizophrenia, depression, and drug addiction. We hope that by mutating critical residues we can identify, for example, the places of transfer for the inhibitory allosteric waves from the A2AR to the D2R binding pocket, reducing the affinity of the high-affinity D2R state.

It will be of highest importance to integrate the new data from the heterodimer model with the results coming out from the triplet puzzle theory introduced by Tarakanov and Fuxe in 2010. It will be exciting to see how mutations of the protriplets will alter the heterodimer model as studied with BRET and protein-protein docking.

By knowing in greater detail the receptor interface from models of receptor heterodimers, we will be able to develop new specific receptor interface interacting peptides targeting and disrupting specific heteroreceptor complexes. We have already started this work with regard to the A2AR-D2R heteroreceptor complex through a collaboration with Professor Malgorzata Filip and her group at the Polish Academy of Sciences in Krakow. We hope to establish the specific role of the A2AR-D2R heteroreceptor complexes in key areas for cocaine addiction by microinjections of A2AR-D2R interface interacting peptides in cocaine self-administration and in cocaine seeking. This principal approach will be used for other heterodimers of relevance for cocaine addiction, after a model of these receptor heterodimers have been developed. Models of PD, schizophrenia, and depression will be employed using this approach. It will be exciting to see how different types of interface interacting peptides targeting distinct heteroreceptor complexes can modulate the learning process and the long-term memories formed in different brain regions.

We also have a plan to develop specific brain penetrant A2AR-D2R heterobivalent compounds that specifically target the A2AR-D2R heteroreceptor complexes. These will be built up of A2AR agonist and D2R antagonist pharmacophors. This work is just starting in collaboration with Professor Katarzyna Kieć-Kononowicz and Professor Malgorzata Filip in Krakow. It will be of high interest to see whether novel treatment strategies for cocaine abuse and addiction can be developed based on our work.

There is a need to improve our understanding of extracellular vesicle-mediated VT in the brain. The exosomes (40–100 nm) are of special interest because they contain proteins, including receptors and homeoproteins, lipids, mRNA, miRNA, and mtDNA, originating from endosomes based on the fundamental work of Simons and Raposo (2009).

Exosomes are postulated to diffuse and flow in the extracellular space of the brain followed by internalization (Borroto-Escuela et al. 2015).

In light of our recent paper focused on putative IL1R2, CCR2, and CXCR4 heteroreceptor complexes with NMDAR and D2R based on the triplet puzzle theory (Tarakanov and Fuxe 2010), it will be important to determine whether exosomes, containing receptors or transcription factors, in fact exist, for example, in the hippocampus operating via VT in models of neuroinflammation. Are the exosomes internalized into neuronal cells after their release from glial cells? Can IL1R2, CCR2, and CXCR4 form heteroreceptor complexes with NMDA receptors as postulated based on the triplet puzzle theory?

The future looks highly interesting moving further into molecular integration and communication in health and disease and understanding the molecular basis of learning and memory. It will be exciting to see how our concepts will survive and be modified by new experimental evidence. The mystery of how the brain works remains for future generations to solve, but at least we are less ignorant than we used to be.

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