In Memory of Joseph Knoll (1925 – 2018)

by Thomas A. Ban

In the 1990s the American College of Neuropsychopharmacology (ACNP) became involved in videotaping interviews with "psychopharmacologists" who made significant contributions to the field (Ban 2011a; Sulser 2011a). In January 2001 I had the opportunity to interview Joseph Knoll, whose discovery of deprenyl, the first selective "type B" monoamine oxidase inhibitor (MAOI), and pioneering work on mesencephalic enhancer regulation, was widely recognized by his peers (Ban 2011a; Knoll 2005). Knoll's discovery was based on his recognition that deprenyl differed from other MAOIs by inhibiting, instead of potentiating, the blood pressure increasing effect of amphetamine and tyramine (Knoll, Vizi and Somogyi 1968). Knoll had also shown that deprenyl increased longevity and sexual activity in rats (Knoll, Dallo and Yen 1989).

Joseph Knoll received his MD in 1951, his PhD in Pharmacology in 1955 and his DSc in 1961 from Semmelweis University, Budapest. At the time of my interview he was Emeritus Professor in the Department of Pharmacology, Semmelweis University, the Department he directed over several decades, and was actively in involved in the research that *began* with his development of deprenyl, that was devoid of the "cheese" effect (as tyramine was not a substrate of MAO B); *continued* with his recognition that the enhanced dopaminergic activity following the administration of deprenyl was unrelated MAO B inhibition; and *culminated* in his demonstration that deprenyl-treated rats lived significantly longer and maintained their sexual potency and learning ability for a significantly longer duration than their saline-treated peers (Ban 2011b; Knoll 2005; Sulser 2011b).

Joseph Knoll received many honors for his research: He was a member of the Leopoldina Academy of Natural Sciences and an Honorary Doctor of the Medical Academy of Magdeburg, both in Germany, and the University of Bologna, Italy. He was elected an Honorary Fellow of the Royal Society of Medicine and is a Foreign Member of the Polish Academy of Arts and Sciences. He was also an Honorary Member of the Pharmacology Societies of Poland, Czechoslovakia and Bulgaria; honored with the Award for Distinguished Service from the European Society of Clinical Pharmacology; and received the Award for Outstanding Contributions to Anti-Ageing Medicine from the World Anti-Aging Academy of Medicine. (Sulser 2011b).

An edited version of my interview (Ban 2011a; Sulser 2011a) of Professor Knoll conducted on January 23, 2002, in Budapest is presented below.

TB: This will be an interview with Professor Joseph Knoll for the Archives of the American College of Neuropsychopharmacology. We are at the Department of Pharmacology and Pharmacotherapy of Semmelweis University in Budapest. It is January 23, 2002. I am Thomas Ban. Let's start from the beginning. Please tell us when and where you were born and say something about your early interests and education.

JK: I was born on the 30th of May 1925 in Kassa, a city in northern Hungary, now Kosice in Slovakia, but my parents moved to Budapest when I was three weeks old and where I have lived since. As a matter of fact, I had seen Kassa only 40 years later. As a child, we lived in the outskirts of Budapest, called Kispest, which translates in English as Little Budapest. As a teenager I had to travel a distance by streetcar to high school that was located in the center of the city. In Hungary we have eight years of high school, called gymnasium, after four years of grade school. I was lucky to be admitted to the Jewish Gymnasium. It was an excellent school, where I learned a lot. From my very early childhood, I wanted to be a physician. I can't tell you why; there was no physician before in our family.

TB: When did you graduate from high school?

JK: I graduated in 1943. It was a very difficult year. Hungary was an ally of Nazi Germany, and as a Jewish family we suffered a lot. Although I was at the top of my class in the gymnasium I was not admitted to medical school because I was Jewish. So, I had to take a job instead of going to the university. Then, in March 1944 the German army occupied Hungary. Shortly after, my family, as all Jewish families, was moved into houses marked by the yellow Star of David.

TB: Did you have any siblings or was it just your Mother, father and yourself?

JK: I also had a brother who was two years older.. But, soon after the German occupation he was called for service in the special division of the army for Jews called *munkaszolgalat* that translates literally as "labor service." Before he left home we agreed I would take care of our parents if anything happens. We were prepared for the worst.

TB: So, you stayed with your parents.

JK: Shortly after my brother left I was also called for service, but in May when I learned from the news that all the Jews from the suburb where my parents lived would be "deported," and taken to

a concentration camp, I deserted from the army and joined my parents in Kispest. I felt they were too old to be left alone and I wanted to be with them. So, I managed to be transported with my parents to Auschwitz. But we were not left together for long. As soon as we arrived at our destination we were separated immediately; and they were sent to the gas chambers and killed. There is no way to convey my feelings to anyone who has not lived through that. I was left there alone. For days I was in a daze; I was kept alive by the poems I knew by heart.

TB: You were kept alive by the poems you knew by heart?

JK: It was my Mother who introduced me to poetry. I like books, and by the time I finished high school, I knew by heart about 200 poems. I kept on reciting those poems I knew, and used to recite as a child to fall asleep. But this time I kept reciting them over and over again to keep in contact with humanity, for not losing faith.

TB: When did you arrive in Auschwitz?

JK: I arrived in Auschwitz in June and was barely there for three weeks when I was almost killed. Everyday a few of us had to carry the dinner, usually a dirty vegetable soup, from the kitchen in large wooden containers, to the outside where the other inmates were waiting for the food. The chief of the kitchen, a huge two meters tall sadistic Lithuanian SS, was standing at the door of the kitchen, and, while counting the containers, he struck the back of the man carrying the container who just passed. He knew that those of us who were already weakened by starvation would fall or spill the soup. Those of us who fell or spilled, he dragged to the kitchen and beat to death or until losing consciousness. This is what happened to me and I would have died if Jaksa Wegner had not saved my life.

TB: Who is Jaksa Wegner?

JK: He was another inmate from Kispest; a very strong man, a former boxing champion. He was the leader of a group of inmates working in the Lager's bread and food store for drawing rations. He found me unconscious in the kitchen, carried me to our barracks or "Lager," as they used to call it, and arranged to have me in his group. We had to work hard in the store, but we could eat as much as we wanted.

TB: Did you work in his group all through the time you were in Auschwitz?

JK: No. One morning the commanding officer was looking for an inmate who spoke German. I spoke German with my Mother at home and Yiddish with my father. I was fluent in German so he picked me to become his servant. He treated me well; I think he really liked me. I remember I

always got a taste from the cookies and pastries his wife sent him.

TB: How long were you in Auschwitz?

JK: From June to September. When I was taken from Auschwitz to Berlin I was in good physical condition; I weighed 78kg. Compared to some of the Polish prisoners I was not in Auschwitz for very long. But, it was long enough to see the flames and the fumes of the gas chambers that worked all the time at full capacity, where thousands of Hungarian Jews and others were killed and cremated.

TB: You were transported from Auschwitz to Berlin.

JK: To Berlin first and then to Ohrdruf. I remember that in Ohrdruf I was beaten up and left tied up for 24 hours in the freezing cold for stealing potatoes to curb my hunger. By the time I was untied my hands and feet were frozen.

TB: Were you liberated in Ohrdruf?

JK: No, I was liberated in Dachau. From Ohrdruf we were transported to Buchenwald. When we arrived and were marching towards the Lager I heard guns and people around me were falling to the ground. We did not stop and kept on moving towards the main gate but only a few of us made it. Everyone who turned back to see what was happening was shot, but I will never know what happened. From Buchenwald I was immediately transported to Dachau. It took 21 days to get from Buchenwald to Dachau and only a few of us survived. We were on the train that was called the "Dachau death-train" without any food or water. I was one of the few survivors and weighed 37 kg when we arrived. I was fully conscious but unable to move. The day after our arrival at Dachau on April 29, 1945, our "Lager" was liberated by American soldiers. Most of them were black. Each of us was given a loaf of bread and a can of meat; thousands died after eating the first food from being starved for weeks. It took me several weeks to get back my strength and learn to walk again. Since my English was fairly good I became a clerk in Captain Schlenker's office, who was very helpful to me. He even offered to get me a scholarship in the medical school at Zurich. I turned it down because I firmly believed my brother was alive and I wanted to return to Budapest to meet him. Although Captain Schlenker warned me of the slim chance of my brother's survival, I still decided to return. Unfortunately, he was right, I lost my brother as well as my parents

TB: When did you arrive in Budapest?

JK: On September 8, 1945 and I soon learned from my family only an aunt survived with a niece, who was to later become my wife. I was alone and had to rebuild my life. I wanted to enter medical

school immediately, but it was too late to register. So, I applied and was admitted to the Műegyetem, the Technical University in Budapest, where they trained engineers. After the first semester, in February 1946, I managed to transfer to medical school. In the summer of 1946 I was given the opportunity to take the courses and examinations from the first semester I had missed. I graduated from medical school in 1951, summa cum laude. I wanted to become a neurologist or a psychiatrist, but I also thought I should get some research experience before becoming a specialist. As a good student, I was one of the best in my class, I had no difficulty in getting a job as a demonstrator in a basic science department while still a student. I was very impressed with the famous scientist Géza Mansfeld our professor of physiology, and my intention was to apply for a job in his department. But he became seriously ill and passed away. In February 1949 I took my final examination in pharmacology and Professor Bela Issekutz, our professor asked whether I would like to work in his department. I was happy to have this opportunity and agreed. It was important that the Hungarian Academy of Sciences was reorganized just about the time this happened so I was able to get a stipend from the Academy to live on.

TB: So, you joined the Department of Pharmacology in February 1949.

JK: And I have never left the department since. At the time it was still on the second floor of the old building in the medical school. I fell in love with my work, and did not become a clinician. But, I always kept in close contact with the clinical faculty of the university. I loved my work so much I even gave up chess to spend all my time in research.

TB: So, you were playing chess in your free time.

JK: I loved to play chess and was on the chess teams of the Jewish Gymnasium and the University but I gave up playing because it distracted me from work.

TB: What was your first project in the department?

JK: I was studying cholinesterase, the enzyme involved in the metabolism of acetylcholine. My research dealt with morphine and cholinesterase. I studied the synergism between morphine and prostigmine, a cholinesterase inhibitor. By the time I graduated from medical school I had seven papers published on this topic.

TB: What was your next project?

JK: In about 1951 I started the project I was to become engaged in for the rest of my life. It is concerned with the physiological basis of life, the brain and its self.

TB: How did you get involved in CNS pharmacology?

JK: I never worked in any other field. I entered pharmacology when neuropsychopharmacology began and it became the center of my interest. I was a member of the team in Hungary that was involved in studying chlorpromazine, imipramine, and desipramine; it was in the early 1960s that I developed deprenyl.

TB: Before moving to the 1960s could you tell us something about your research in the 1950s?

JK: When I started to work in the 1950s I had to find a method that would link CNS physiology and pharmacology. I became interested in the "activating system" of the brain. In the early 1960s experimental tools specifically influencing the operation of the catecholaminergic and serotonergic systems in the brainstem were developed and I thought they might provide a key to understanding the operation of the brain. I became especially interested in what is responsible for what we call drive.

TB: You used the term "activating system." Were you referring to Moruzzi and Magoun's "reticular activating system"?

JK: Let me give you a simple example of what I mean when I refer to an "activating system" in the brain. A rabbit is eating cabbage in a relaxed manner and an eagle comes with lightning speed to capture the rabbit. The rabbit, to survive, has a split second to change the activation process in the brain. In that split second, it must change from a relaxed situation to maximum activity, to use all its capacities to escape. I'm referring to the system that makes it possible for the rabbit to escape. There is a mechanism, I call the "enhancer mechanism" responsible for this activation, in which endogenous monoaminergic substances, such as noradrenaline, dopamine and serotonin are released. I have been interested in the regulation of "enhancer mechanisms" and in developing substances involved in that. From the different agents that have an effect on enhancer regulation, so far only ß-phenylethylamine, (PEA), and tryptamine have been analyzed. I developed drugs for enhancer regulation. In the early 1960s I had found deprenyl, a synthetic phenylethylamine derived enhancer, and in the late 1990s, I developed (-)-BPAP, essentially a tryptamine derived selective and highly potent enhancer substance.

TB: You mentioned you did research with some of the newly introduced psychotropic drugs in the 1950s but did not say what you did.

JK: When chlorpromazine was introduced in the mid-1950s I developed two tests for the differentiation between classical sedative-hypnotic drugs and the new tranquilizers. One was based on a jumping reaction, and the other on hunger motility. We found using these tests that the new

tranquilizers selectively blocked the conditioned reflex, whereas the old "hypnosedatives" blocked both unconditioned and conditioned reflexes. I presented my findings in a paper at the first CINP congress in Rome. Do you remember that congress?

TB: I know of that congress from my activities on the CINP's history committee. It was held in 1958, about a year after the CINP was founded. Emilio Trabucchi, the professor of pharmacology in Milan, organized it.

JK: Daniel Bovet, one of the founders of the CINP, invited me to participate in that congress. It was my first trip to the West.

TB: So, you were invited to participate in that congress by Daniel Bovet, the Nobel Laureate?

JK: Yes. He was President of the first CINP Congress and had won the Nobel Prize in 1957. Bovet was interested in my research on the "active focus." He recognized its importance; later on we became friends and collaborated in research projects. I used to send my assistants to spend some time in his laboratory in Rome.

TB: So, Bovet was interested in your research in the "active focus." When you say, "active focus," could you tell us what you are referring to?

JK: I refer to a special form of excitation in a particular group of neurons that provides the basis of an acquired drive. I developed, in the 1950s, a rat model to follow changes in the brain during the acquisition of a drive from the start of training until it becomes manifest.

TB: Didn't you write this up in your first book?

JK: Yes, I did, with the title, *The Theory of Active Reflexes: An Analysis of Some Fundamental Mechanisms of Higher Nervous Activity*. It was published in 1969 jointly by the Publishing House of the Hungarian Academy of Sciences in Budapest, and the Hafner Publishing Company in New York.

TB: Could you elaborate on your theory summarized in this monograph?

JK: According to my theory, the appearance of the mammalian brain with its ability to acquire drives ensured the development of social life and ultimately led to the evolution of human society. This most sophisticated form of organized life on earth is still in the trial and error phase of its development. It seeks to outgrow the myths-directed era of its history, and arrive at its final state, a rationally organized human society. Furthermore, in the mammalian brain capable of acquiring drives, untrained Group 1 cortical neurons possess the potential to change their functional state in response to practice, training, or experience in three consecutive stages, getting involved either in

an extinguishable conditioned reflex (ECR,) in case of Group 2 neurons, in an inextinguishable conditioned reflex (ICR,) in case of Group 3 neurons, or in an acquired drive, in case of Group 4 neurons. The activity of the cortical neurons belonging to Group 3 and 4 is inseparable from conscious perception. At any moment of life 'self' is the sum of those cortical neurons that have already changed their functional significance and belong to Group 3 or 4. In the early period of my work I wanted to show by EEG that there is a difference between an extinguishable and inextinguishable conditioned reflex, but our laboratory was poorly equipped in the early 1950s. It's a very long story.

TB: Tell us the story.

JK: The story began with my interest in drives.

TB: What is your definition of a drive?

JK: In behavioral studies "drive" is the force that activates the mammalian organism. There are innate drives of a limited number in the service of indispensable, vital goals. The analysis of innate-drive-dependent functions, such as maintenance of homeostasis, fight for survival, feeding, sexuality, progeny-care, etc., constitutes the main body of literature on behavioral physiology and endocrinology. Though innate drives are primarily based on sub-cortical regulations, none of the goals can be reached without the participation of the cortical neurons. Exclusively innate drives keep the majority of the mammalian species alive.

TB: What about acquired drives?

JK: The capability to acquire an irrepressible urge for a goal, which is not necessary for survival of the individual or species, represents the most sophisticated function of the telencephalon. Though the development of an acquired drive always originates in one way or another in an innate drive, this relation becomes later unrecognizable. Humans are the only living beings on earth whose life is predominantly based on acquired drives. To a certain extent, a minority of the mammalian species; the monkey, dog, horse, dolphin and rat possesses this endowment, which, under natural conditions, remains unexploited. Nevertheless, humans obviously discovered thousands of years ago, probably through a kind of serendipity, that the behavior of such animals can be modified by proper training, and this started the development of the domestication of various species. The ambition to be in a permanent state of activity is a natural endowment of the human brain, which acquires drives with utmost ease. In goal-seeking behavior, which is the essence of life; the nature of the drive determines the goal and determines the fixation of millions

of chains of inextinguishable conditioned reflexes, the ‘knowledge’ needed to reach the goal. The mechanism is simple, always the same, but the drives and the goals determined by them are immensely different. Thus, the essence of my theory is that an immortal poem is created by essentially the same mechanism as a pair of shoes. Since the basic mechanism operates also in animals capable to acquire drives, I studied it from the early 1950s in the rat and summarized my findings and conclusions in my first monograph. The acquisition of proper drives in the most sensitive developmental period of life, from weaning until sexual maturity, will thereafter be the determinant for the lifelong basic activity of the individual. It is obvious that since the fate of most individuals is still governed by the position in the society into which they are born, only a minority is lucky enough to acquire professional drives in full harmony with natural endowments. The majority forms, under coercion, work-related drives that will ensure the place of the individual in society. Conformity between one’s innate abilities and acquired work-related drives is of key importance for lifelong equilibrium. However, not only the desire to be permanently active is a natural endowment of the human brain, but there also is a need for a new challenge to one’s drives in due time. Even the most satisfying professional drive becomes boring after its permanent, continuous use and there is a need to continue to keep the brain in a satisfyingly active state. Inexhaustible forms of supplementary activities serve this aim. Absolute dominance of a fully satisfying professional drive and the acquisition of well-chosen supplementary drives are the conditions for a harmonious, well balanced life. Lack of full satisfaction in one’s acquired professional and supplementary drives generates an urge to flee from frustration and seek salvation in smoking, alcohol, drugs, and so on.

TB: How did you study acquired drives in animals?

JK: In the early 1950s we developed a method to show the development of an acquired drive, a “glass-cylinder-seeking drive,” in the brains of rats that was stronger than the animal’s innate drives. It was based on an unconditioned avoidance reflex, escape from a hot plate to the sound of a bell that played the role of a high priority conditioned stimulus. The cylinder was open at the bottom and on the top, and the animals were trained to search for the glass-cylinder, manage to get into the glass-cylinder through an opening in the side and jump to the upper rim. In properly trained rats, the acquired cylinder-seeking drive was so strong that it suppressed innate drives. When such a rat was deprived of food for 48 hours and then was offered food within the usual setup, that included the glass-cylinder, it looked for the glass-cylinder and left the food untouched at the sound

of the conditioned signal. Similarly, when a receptive female was offered to a glass-cylinder trained male rat, the male looked for the glass-cylinder at the sound of the bell and neglected the receptive female. With the employment of this method it became obvious to me that cortical neurons have the innate potential to acquire a drive. With the help of our training method the rat activated a group of cortical neurons that kept the animal active until the goal, the upper rim of the glass-cylinder, was reached. The essence of both, innate and acquired drives is a selective activation of a special population of subcortical neurons, that I refer to as an active focus in the case of innate drives, and of a special population of cortical neurons, in case of acquired drives.

TB: Did you succeed in developing acquired drives in all animals?

JK: The faculty for acquiring a drive is uncommon in the animal kingdom. It was shown by Berta Knoll in the late 1950s that the mouse, a rodent closely related to the rat, was unable to acquire the glass-cylinder-seeking drive. She has found that, in striking contrast to the rat, the mouse was unable even to fix the inextinguishable form of the conditioned avoidance response, the functional stage that preceded the acquisition of the glass-cylinder-seeking drive in the rat. It seems that the appearance of mammals with the ability to acquire drives was the last step in the development of the mammalian brain. Vertebrates can be divided into three groups according to the mode of operation of their brain. One, a large group, which includes the majority of vertebrates, operates with innate drives only. Another, a small group, which includes some vertebrates, has an ability to acquire drives. And, the third group which includes only one vertebrate, Homo Sapiens, operates almost exclusively on the basis of acquired drives. Thus, the appearance of the mammalian brain with an ability to acquire drives ensured the development of social life and ultimately led to the evolution of human society. This most sophisticated form of organized life on earth is still in the trial- and- error phase of its development. It seeks to outgrow the myths-directed era of its history and arrive to its final state, the rationally directed human society.

TB: Coming back to your earlier remark, how did you record the corresponding EEG activity to an extinguishable- and an inextinguishable conditioned reflex?

JK: My coworker Károly Kelemen spent half a year in Rome in Bovet's laboratory and finished the work showing the difference between the short lasting EEG activation to an extinguishable conditioned reflex, and the prolonged EEG activation to an inextinguishable conditioned reflex. The paper co-authored by Kelemen, Longo, Knoll and Bovet was published in 1961.

TB: How did Bovet learn about your work?

JK: I published about six papers on my findings by 1956. Bovet read some of those papers and became interested in the research I was doing. At that time, Hungary was a communist country. We had the rats but no sophisticated machinery, not even EEG. We could only do the EEG studies in a laboratory like Longo's in Rome that was properly equipped.

TB: Vincenzo Longo, one of Bovet's collaborators?

JK: Yes. He is a very nice man; a good friend of mine. I have not heard of him for a long time. He has probably retired by now.

TB: He retired from the Institute but still works as a consultant.

JK: Longo understood very well what we were doing, and he had the necessary EEG technology to show the expected functional difference between an extinguishable and an inextinguishable conditioned reflex.

TB: Did you yourself do any work on the neurophysiological and molecular level?

JK: I didn't at the time but now I do, and measure, for example, the enhancer effect of drugs on noradrenaline release from the locus coeruleus, dopamine release from the substantia nigra, tuberculum olfactorium and striatum, and serotonin release from the raphe.

TB: So, by the 1960s you became interested in the enhancer mechanism and enhancer regulation by drugs?

JK: I was interested in understanding the physiological characteristics of an acquired drive. It was only later, in the course of my research with deprenyl, that I ultimately recognized the operation of an enhancer regulation in the brainstem. This finding initiated the working hypothesis that the enhancer regulation operates also in the cortical neurons and determines ultimately the learning capacity of the individual.

TB: Could you elaborate on that?

JK: According to this approach the naïve cortical neuron, which is born with the ability to perceive one of the senses, color, light, pain, sound, smell, taste, or touch, also has the ability to synthesize its own specific enhancer substance. PEA and tryptamine or their long-acting synthetic analogues, deprenyl and BPAP respectively, enhance the activity of the enhancer-sensitive brainstem neurons; the natural cortical enhancer substances act similarly on the proper cortical neurons. Since this working hypothesis was an outgrowth of deprenyl research, it would be more expedient to come back to this approach after discussing the deprenyl story in more detail.

TB: I see. According to your present view deprenyl is a synthetic, phenylethylamine derived

enhancer?

JK: Yes. Deprenyl, the therapeutic agent now in use, is the minus isomer of phenylisopropyl-methylpropargylamine, a close relative to methamphetamine, thus a derivative of PEA. Long-acting PEA derivatives, like amphetamine and methamphetamine, release catecholamines from intraneuronal stores, as their parent substance, and produce aimless hyperactivity and inhibit goal directed activity of innate and acquired drives.

TB: Does that mean that by releasing catecholamines these substances instead of enhancing, are inhibiting innate and acquired drives?

JK: Amphetamine and methamphetamine are of course enhancer substances but their releasing effect completely covers up the enhancer effect of these amines, which were classified as the prototype of indirectly acting sympathomimetics. I am not going into details but I was interested in that. In 1960 I developed, with a good friend of mine, Zoltán Mészaros, the director of research at Chinoin, a Hungarian drug company, a new family of analgesics. When I told my friend I would be interested in finding someone working with amphetamines he brought me together with Zoltán Ecsery, one of the leading chemists at Chinoin. At the time iproniazid and monoamine oxidase inhibitors in general were at the center of interest as experimental tools because of their antidepressant effect. I worked with iproniazid as soon as it became available and I had the feeling that maybe somehow we have to combine amphetamine-like effects and MAO inhibition. So we started work on that. As I was hoping, Zoltan Ecsery presented me with a series of about 60 compounds and I selected, as the best candidate for development, the compound we now call deprenyl. At that time it was E-250. I selected it because I was fascinated by the finding that E-250, in contrast to the other monoamine oxidase inhibitors known at the time, did not potentiate the blood pressure increasing effect of amphetamine by releasing norepinephrine from stores in noradrenergic terminals. In fact when I gave E-250 it inhibited amphetamine's blood pressure increasing effect. That was new to me. It showed me we had something new.

TB: To what did you attribute the uniqueness of E-250?

JK: in 1963 a large number of clinical reports, demonstrating the occurrence of dangerous hypertensive attacks in patients treated with MAO inhibitors were published. In accordance with Blackwell's suggestion, the metabolism of tyramine was inhibited by the MAO inhibitors and therefore cheese and other foods containing tyramine provoked the hypertensive episodes in patients treated with MAO inhibitors. This 'cheese effect' restricted the clinical use of MAO

inhibitors. We analyzed the peculiar behavior of E-250 and as I expected, the studies revealed it did not potentiate the effect of tyramine but inhibited it. This was first demonstrated in a study performed on cats, and on the isolated vas deferens of rats which was published in 1968. We proposed in this study to use deprenyl as an MAO inhibitor free of the cheese effect.

TB: How did you know that it applied also to humans?

JK: In 1965, after we found that deprenyl in contrast to other MAO inhibitors, inhibited the tyramine releasing effect of amphetamine, the psychiatrist Ervin Varga, who worked in the Psychiatric Clinic of our university, checked it out for me. He administered deprenyl and tyramine to normal volunteers and found that deprenyl did not potentiate the effect of the tyramine. But he did not publish his results. As a matter of fact the validity of my proposal that deprenyl is an MAO inhibitor free of the cheese effect was demonstrated in humans by my good friend Merton Sandler and his coworkers in London in 1978.

TB: When did you publish first on E-250?

JK: The first paper appeared in Hungarian in 1964 and the English version in 1965. The paper was co-authored by my collaborators at the time; Ecsery, Kelemen, Nievel and Berta Knoll. Then, in 1968, I published a second important paper on E-250 that was co-authored by Vizi and Somogyi, my other collaborators in this project. As I mentioned, it was in this second paper that we noted that the hypertensive reaction seen in some MAO-inhibitor-treated patients after cheese consumption is absent with deprenyl. We suggested that deprenyl, an MAO inhibitor without the cheese effect, might be highly valuable for human therapy. But no one cared. Unfortunately even the leaders at Chinoin did not dare to develop further E-250 because of its MAO inhibiting property.

TB: Didn’t you already have in the title of your first paper that E-250 is a psychic energizer?

JK: We did. Actually the first clinical trial with racemic deprenyl in depression was done by Ervin Varga, my childhood-friend, my schoolmate in gymnasium and class-mate at the university. The preliminary results were presented at a Conference in Budapest in 1965. The study was extended and was published by Varga and Tringer in 1967. The first clinical trial with the minus isomer, the drug now in use, was published by Tringer, Haits and Varga in 1971. In spite of their favorable findings the possibility of introducing deprenyl as an antidepressant remained unexploited for many years after.

TB: One wonders why. Am I correct we are before the time you discovered that E-250 is a selective

MAO-B inhibitor?

JK: Yes, we are. Varga and Tringer published their first in extenso paper in 1967, and we discovered E-250's selective B type monoamine oxidase inhibiting effect in 1970.

TB: Could you elaborate on that discovery?

JK: In 1968, the same year our second paper was published, Johnston reported on a substance to be named clorgyline that preferentially inhibited the deamination of serotonin. He proposed the existence of two forms of the monoamine oxidase enzyme, a type A enzyme that is selectively inhibited by clorgyline, and a type B enzyme that is relatively insensitive to clorgyline. Thus, a selective MAO-B inhibitor was a missing pharmacological tool for further research. Because of the peculiar behavior of deprenyl I expected my substance might be the missing link. It was about two years after that, in 1970, that we were lucky to prove that deprenyl selectively inhibits the enzyme that was insensitive to clorgyline. Since János Nievel, who was responsible for the biochemical techniques in my laboratory, did not return from the study-tour I organized for him in London, where he spent a year in 1965, a young medical doctor, trained in biochemistry, Kálmán Magyar joined in deprenyl-research. I published our finding that deprenyl is the selective inhibitor of MAO-B with Kálmán Magyar in 1972. This paper became a citation classic ten years later, in 1982.

TB: So, the paper was published about two years after the discovery.

JK: Yes. This paper was an in extenso publication of my lecture presented in the first international MAO meeting in Cagliary, Sardinia, in 1971. I first presented evidence at that meeting that deprenyl is a selective type B monoamine oxidase inhibitor. Then I presented a lecture about the pharmacological effect of selective MAO inhibitors in 1975 at the Ciba Foundation Symposium in London, with title, '*Monoamine Oxidase and its Inhibition.*'

TB: Would it be correct to say that after you discovered deprenyl is a selective inhibitor of the type B monoamine oxidase your interest shifted to this particular effect of the drug?

JK: For several years the selective MAO-B inhibitory effect was at the center of our interest. It was the selective MAO-B inhibitory effect of the compound that led to the first clinical application of deprenyl.

TB: What was the first clinical application of deprenyl?

JK: In the light of the serious side effects of levodopa in Parkinson's disease Birkmayer and Hornykiewicz tried to achieve a levodopa sparing effect by the combined administration of

levodopa with a MAO inhibitor. As such combinations frequently elicited hypertensive attacks they were soon compelled to terminate this line of research. After we found deprenyl is a unique MAO inhibitor that does not potentiate the catecholamine-releasing effect of indirectly acting amines and thus free of the cheese effect my claim was corroborated on human volunteers by Sandler and his group in London. The results of that study came to the knowledge of Birkmayer before it was published, so he finally dared to combine levodopa with deprenyl in the treatment of Parkinson's disease. The trial was successful. The levodopa-sparing effect was achieved with deprenyl in parkinsonian patients without any hypertensive reaction. His report triggered a development that lead to the world-wide use of deprenyl in Parkinson's disease.

TB: When did this happen?

JK: The Birkmayer paper which triggered this development was published in the Lancet in 1977.

TB: Deprenyl is still extensively used in the treatment of Parkinson's disease.

JK: Today, the most evaluated effect of the drug is its ability to slow the progress of Parkinson's disease by retarding the rate of functional deterioration of the nigrostriatal dopaminergic neurons. It is obvious now that this effect is unrelated to the MAO-B inhibitory potency of deprenyl.

TB: So, your research on the type B monoamine oxidase inhibiting effect of deprenyl has paid off in the treatment of Parkinson's disease?

JK: Yes. The real progress in the clinical history of deprenyl was the establishment of the indication to use it in de novo parkinsonism. This was the conclusion of the famous DATATOP study in the USA performed between 1989 and 1993. This indication was further supported by important multicenter studies between 1991 and 1999 in France, Finland, Norway and Denmark. The authors of the DATATOP study expected deprenyl to be efficient in their trial because of its MAO-B inhibitory effect. Their hypothesis was that the activity of MAO and the formation of free radicals predispose patients to nigral degeneration and contribute to the emergence and progression of Parkinson's disease. In accord with their working hypothesis, they expected the combination of deprenyl, the MAO inhibitor, with tocopherol, an antioxidant, would slow the clinical progression of the disease because MAO activity and the formation of oxygen radicals contribute to the pathogenesis of nigral degeneration. They selected patients with early untreated Parkinson's disease and measured delay of the onset of disability necessitating levodopa therapy. When the DATATOP study started I already knew from my studies that only deprenyl would be efficient because of its peculiar stimulatory effect on the catecholaminergic system, which tocopherol was

devoid of. Nevertheless, at the time I was only at the beginning of fully understanding the enhancer mechanism, but more and more experimental evidence accumulated in favor of the concept that deprenyl induced activation of the catecholaminergic neurons is unrelated to its MAO-B inhibitory activity.

TB: What is the evidence that deprenyl's enhancer effect is unrelated to MAO-B inhibition?

JK: With the development of 1-phenyl-2-propylaminopentane, (PPAP), the deprenyl analogue free of MAO-B inhibiting property, we furnished direct evidence that the enhanced dopaminergic activity following administration of deprenyl was unrelated to the inhibition of MAO-B. I published this paper in 1992; my coworkers in the study were Berta Knoll, Zoltán Török, the chemist, who synthesized the compounds, Julia Timár and Yasar Sevil. Because PPAP, like deprenyl, inhibited the uptake of tyramine in isolated smooth muscle tests, we first assumed that the drug-induced enhanced dopaminergic activity was due to an uptake inhibiting effect. Further studies revealed that this interpretation was false. The availability of HPLC to measure catecholamines and serotonin in physiological quantities allowed a new approach. The thorough analysis of the dose-dependent effect of deprenyl on the release of catecholamines and serotonin from isolated, discrete rat brain regions, dopamine from the striatum, substantia nigra and tuberculum olfactorium, noradrenaline from the locus coeruleus, and serotonin from the raphè, pointed to enhancer regulation in the mesencephalic neurons. Ildiko Miklya, a young talented pharmacist was my coworker in these studies which demand much hard work. We treated rats with five different doses of deprenyl, between 0.01 and 0.25 mg/kg, once daily for 21 days, isolated the discrete rat brain regions 24 hours after the last injection and measured the biogenic amines released during a 20 minutes period from the freshly isolated tissue samples. The amount of dopamine released from the substantia nigra and tuberculum olfactorium clarified that the dopaminergic neurons worked on a significantly higher activity level even in rats treated with the lowest, 0.01 mg/kg, dose of deprenyl. As this small dose of deprenyl leaves the MAO-B activity and the uptake of amines practically unchanged, this study was the first unequivocal demonstration of the operation of a hitherto unknown enhancer mechanism in dopaminergic neurons stimulated by deprenyl in very low doses. We published this work first with Ildiko Miklya in 1994. This work was of crucial importance for the further development of enhancers. Further studies clarified the operation of an enhancer regulation in the catecholaminergic neurons in the brainstem and proved that PEA is a natural enhancer substance. Since PEA, in higher concentrations, is a highly effective

releaser of catecholamines from their intraneuronal stores, this effect covered up completely the enhancer effect of the endogenous amine, which was classified as the prototype of the indirectly acting sympathomimetics. Amphetamine and methamphetamine are PEA derivatives with a long-lasting effect which share with their parent compound its catecholamine releasing property. Deprenyl was the first PEA, methamphetamine derivative that maintained the enhancer effect of its parent compounds but lost completely the catecholamine releasing property. This peculiar change in the pharmacological spectrum of the PEA-derivative ultimately enabled the discovery of enhancer regulation, since the enhancer effect of deprenyl was not covered up by the release of catecholamines from their intraneuronal stores. In the light of this knowledge we realized that clinicians who used deprenyl in the belief that the therapeutic benefits observed in patients treated with this drug were due to the selective inhibition of MAO-B in the brain, were mistaken from the very beginning. It is clear by now that besides the levodopa-sparing effect of deprenyl due to its MAO-B inhibiting property, the clinical benefits are due to the enhancer effect of the drug.

TB: I see. We keep on talking about "enhancer regulation." Could you tell us what the term "enhancer regulation" means?

JK: I define enhancer regulation as the existence of enhancer-sensitive neurons capable of changing their excitability and working on a higher activity level in a split second, due to endogenous enhancer substances. Of these substances, PEA and tryptamine are currently being experimentally analyzed, and their synthetic analogues, deprenyl and BPAP are the specific experimental tools for studying enhancer regulation in the brainstem.

TB: Where are those enhancer-sensitive neurons located in the brain?

JK: We usually refer to mesencephalic enhancer regulation because even if enhancer sensitive neurons also exist outside the mesencephalon, the mesencephalic dopaminergic neurons are of key importance in enhancer regulation. These most rapidly aging neurons of the brain are primarily responsible for the progressive age related decline of behavioral performances.

TB: Did you say that the mesencephalic dopaminergic neurons are the most rapidly aging neurons?

JK: According to our present knowledge the nigrostriatal dopaminergic neurons are. The dopamine content of the human caudate nucleus decreases steeply at the rate of about 13 percent per decade over age forty-five. We know that symptoms of Parkinson's disease appear if the dopamine content of the caudate nucleus sinks below 30 percent of the normal level. The age related decline of the nigrostriatal dopaminergic brain mechanisms play a significant role in the decline of performance

with passing time. Safe and effective prophylactic medications are needed to slow these changes. I suggested the use of deprenyl for this purpose after we found that treating rats with 0.25 mg/kg of deprenyl three times a week, prolonged their life significantly. It was my lecture at the Strategy in Drug Research, the 2nd IUPAC-IUPHAR Symposium held in Noordwijkerhout, The Netherlands, in 1981 when I first presented this new strategy. The lecture was published in the volume of this symposium in 1982. We also revealed that deprenyl-treated rats lived not only longer than placebo-treated rats, but also that males maintained their ability to ejaculate for a significantly longer period and remained better performers in the shuttle box than saline treated pairs. With my coworker János Dallo we followed through decades the sexual performance of male rats and found that a daily dose of 0.25 mg/kg deprenyl slowed significantly the age-related decline of this function. In one of this series we worked with 90 male CFY rats and treated half of the group with saline and half with deprenyl from the 25th week of age until they loss their ability to ejaculate. The saline-treated rats reached this stage at an average of 112 weeks, whereas the deprenyl-treated rats reached it at an average of 150 weeks. That deprenyl is capable of slowing the rate of functional deterioration of the nigrostriatal dopaminergic neurons was shown not only in rats but also in patients with early, untreated Parkinson' disease. Age related deterioration of the striatal machinery is a continuum and any short segment of it is sufficient to measure the rate of decline in the presence or absence of deprenyl. Tetrud and Langston were the first to publish in Science in 1989 that deprenyl delays the need for levodopa therapy. In their study, the average time that elapsed before levodopa was needed was 312.1 days for patients in the placebo group and 548.9 days for patients in the deprenyl group. This was clear proof that deprenyl, which enhances the activity of the surviving dopaminergic neurons, kept these neurons at a higher activity level for a longer duration of time. Today the most evaluated effect of the drug is its ability to slow the rate of the functional deterioration of the nigrostriatal dopaminergic neurons in patients with early, untreated Parkinson's disease, and thus to slow the progress of the disease. The indication to use deprenyl in de novo Parkinsonian patients was established in the USA by the Parkinson Study Group and corroborated by a French Study Group in 1991, a Finnish Study Group in 1992 and a Norwegian-Danish Study Group in 1999.

TB: Let us get back to PEA for a second. You refer to it as a natural enhancer substance. Now, PEA is usually classified as an indirectly acting sympathomimetic drug.

JK: As we discussed already, since PEA, in higher concentration, is a highly effective releaser of

catecholamines from intraneuronal stores, this effect covered up completely the enhancer effect of the endogenous amine. Deprenyl was the first PEA derivative that maintained the enhancer effect of the parent compound but lost completely the catecholamine releasing property. It was this peculiar change in the pharmacological profile of a PEA-derivative that ultimately enabled the discovery of enhancer regulation in the catecholaminergic neurons in the brainstem, since the enhancer effect of deprenyl was not covered up by the release of catecholamines from their intraneuronal stores.

TB: You mentioned that not only PEA but also tryptamine is a natural enhancer substance.

JK: It was in 1994 I first published that tryptamine is an endogenous enhancer. It is a natural enhancer like PEA, but not a releaser. The discovery opened the way for a structure-activity relationship study aiming to synthesize a new family of enhancer compounds structurally unrelated to PEA and the amphetamines. It was on the basis of the results of that study that benzofuran-propylaminopentane, BPAP, was selected as a tryptamine-derived synthetic mesencephalic enhancer. Because I couldn't get the work done in Hungary, I found a small Japanese private company, Fujimoto, to develop it. Professor Yoneda, an excellent chemist, led the group which synthesized about 60 compounds and I selected the highly potent and selective enhancer, needed for my further work. BPAP was 100 times more potent than deprenyl as an enhancer. My first paper, co-authored by Yoneda, Berta Knoll, Ohde and Miklya was published in the British Journal of Pharmacology in 1999. A new world was opened! This substance stimulates, activates, and enhances the activity of noradrenergic, dopaminergic and serotonergic neurons in femto to picomolar concentrations, in a very special manner with a bell shaped curve. This indicates that very specific enhancer receptors exist because otherwise we cannot explain a compound acting in femto to picomolar concentrations. And now something came about which I have to tell you. Towards the end of the last year, they found a gene for a totally independent family of receptors that are activated by PEA and tryptamine, the two endogenous enhancers I described. It was published in the proceedings of the neuroscience meeting of the United States. This might be very important for the future. I think BPAP, the new compound, which is at present a highly specific and highly potent experimental tool for studying the enhancer regulation in the brainstem, might also become very important clinically as an antidepressant, an anti-Parkinson drug, an anti-Alzheimer's agent, and also a safe and effective compound to slow the age-related decline of the catecholaminergic system in the brainstem, thus prolonging life span.

TB: What you are saying is that enhancers might have a broad range of clinical indications.

JK: Absolutely. In my view the only reasonable hope to fight off the two main neurodegenerative diseases, Parkinson's and Alzheimer's, is prevention. In case of Parkinson's disease there is no doubt that age-related irreversible deterioration of the nigrostriatal dopaminergic neuronal system has already surpassed a critical level and the disease is incurable; prevention remains the only chance for the future to fight off Parkinson's disease. The daily administration, from sexual maturity until death, of a small dose of a synthetic enhancer substance acting on the dopaminergic neurons in the brainstem suggests a proper and safe method for this aim. In case of Alzheimer's disease the only reasonable hope to fight off the disease is to keep the cortical and hippocampal neurons at a higher activity level as long as possible by the prophylactic administration of a proper enhancer substance. It is remarkable in this regard that BPAP protected cultured rat hippocampal neurons from the deleterious effect of β-amyloid$_{25\text{-}35}$ fragments in as a low as $\mathbf{10^{-15}}$ M concentration.

TB: What about your longevity studies demonstrating that deprenyl treatment extended significantly the lifespan of rats?

JK: We performed two longevity studies in rats, the results of which were published in 1988 and 1994. If you compare the average life expectancy in 1900 to the average life expectancy in 2000 in developed countries, there was at least, a 25 year extension. Average life expectancy at birth increased from 55 years to 80 years. Why? The reason is that many people died earlier before the introduction of immunization, before the development of antibiotics, lack of hygiene and many other factors. But, regardless of life expectancy each species of animal has a natural life span that cannot be exceeded. You remember that according to the Old Testament Moses lived 120 years. This is by chance in accord with the human Technical Life Span (TLS_h), which is in fact about 115 to 120 years. It did not change from 1900 until 2000. Why? Because we had no knowledge about what regulates it. What I'm proposing is that the age-related decline in the enhancer regulation of the catecholaminergic system in the brainstem is of key importance to natural life span and to slow this process by the preventive administration of a proper synthetic enhancer will extend lifespan. As I summarized the physiological and pharmacological evidence in an invited paper, '*Memories of my 45 Years in Research'* in Pharmacology and Toxicology, in 1994, there can be little doubt that the maximum level of activation of the CNS via the catecholaminergic system, decreases progressively with aging. The blackout, natural death, of the integrative work

of the CNS, signaled by the disappearance of EEG, occurs when the catecholaminergic system's ability to activate the higher brain centers sinks below a critical threshold and the CNS can no longer be activated to the required extent. This would explain why a common infection, a broken leg, or any other challenge that is easily surmountable in young age, while the catecholaminergic machinery is working at full capacity, may cause death in old age. My hypothesis is that the quality and duration of life rests on the inborn efficiency of the catecholaminergic brain machinery. A high performing longer-living individual has a more active, more slowly deteriorating catecholaminergic system, than its low performing peer; a better brain engine allows better performance and a longer life span. We demonstrated in rat experiments that the age-related decline of the catecholaminergic system in the brain stem which starts immediately after sexual maturity was reached, plays a key role in the natural aging of the brain and the rate of decline can be slowed by the life-long daily administration of 0.25 mg/kg deprenyl. Deprenyl-treated rats lived significantly longer and maintained their sexual potency and learning ability for a significantly longer duration than their saline-treated peers. Thus, it is feasible to transform a lower performing, shorter living rat into a better-performing, longer-living one. It follows that the duration of life beyond the "technical" life span, with a yet unpredictable upper limit, must be possible in all mammals, including the human species, by keeping the catecholaminergic system in optimal operation with the administration of a very small daily amount of a proper enhancer substance.

TB: Was deprenyl the first substance in the literature shown to prolong life span?

JK: Deprenyl was the first compound described in the literature that by curbing the age-related deterioration of the nigrostriatal dopaminergic neurons in the brainstem, prolonged the lifespan in the rat significantly, so that in some rats it exceeded the technical lifespan. I presented these findings in two papers published in Mechanisms of Ageing and Development: The title of one of the papers, published in 1985 was, '*The Facilitation of Dopaminergic Activity in the Ageing Brain by (-)Deprenyl: A Proposal for a Strategy to Improve the Quality of Life in Senescence.'* The title of the other paper, published in 1988, w*as, 'The Striatal Dopamine Dependency of Lifespan in Male Rats: Longevity Study with (-)-Deprenyl.*' After publishing our first longevity study with my coworkers Janos Dallo and Tran Ty Yen in 1989, I became interested to see whether the highest performing rats selected from a huge population live significantly longer than their lowest performing peers and whether deprenyl treatment would evenly extend the lifespan of both groups. Thus my second study lasted over four years, published with my coworkers Tran Ty Yen and

Ildiko Miklya in 1994, had 1600 male, healthy rats from a special strain. We tested their sexual activity by bringing them together with receptive females in four consecutive weekly mating tests. On the basis of their sexual performance we separated the lowest and highest performing individuals. The selection from such a huge population was extremely tiring, boring work. Then, we measured the learning performance of the selected two groups of rats in five-day training in the shuttle box. We found that the sexually high performing rats were significantly better learners than their sexually low performing peers. In the four year study we also found the low performing rats lived 134 weeks, while their high performing peers lived 151 weeks. In both low and high performing rats, deprenyl, an enhancer of the release of catecholamines in the brain, significantly increased sexual performance and longevity. The lifetime of deprenyl-treated low performing rats increased from 134 to 152 weeks, and of high performing rats from 151 to 185 weeks. The increases in longevity were statistically highly significant. So the enhancer increased sex, learning ability and duration of life. This applies also to man. In Hungary, for example, millions die at age 62 or 63 now but if you compare that with the age of the members of the academy you will see that their average age at the time of death is 81.5 years. What I'm saying is that a man who works, who is active, lives longer than a passive one. I work a lot although I am now retired. I could just look at television. So people ask me why are you going at 8:00 in the morning to your laboratory and coming home at 6:00 in the evening and then you work until 2:00 a.m. on your papers at home. Are you crazy? I'm not crazy. The conclusion of my lifework is that the longer you keep your brain at maximum activity, the longer and better you live. What I'm saying is that what we have shown in the rats, applies also to humans. I'm 77 now. In the 20th century we have seen a highly significant increase in average life expectancy. By enhancer regulation we should be able to prolong life span further, and sometimes in the future surpass significantly the TLS_h. Enhancer regulation is the key to life and death.

TB: Would it be legitimate to hypothesize that if one would get a bunch of 30 years old guys, measure their sexual activity and, if it is high, predict they would live longer?

JK: Man is complicated. It is optimal for the human brain to work under the influence of an acquired drive which is in harmony with one's natural endowments. It is reasonable to assume that for a human being the optimal condition is to be in a state in which a group of cortical neurons are permanently maintained by their specific enhancer substance at the highest level of excitability. The essence of this mechanism is detectable even in animals capable of acquiring drives. An

acquired drive in the brain of a dog is coupled with the animal's extreme joy in exercising the acquired goal-seeking activity and the animal spares no effort to reach that goal. Humans know from experience they prefer to be in an active state that is pleasant, amusing, that makes them happier and more satisfied than to be in a vigilant leisure state. It is natural for humans in possession of a proper work-related drive that their preferred activity never makes them tired. Creative minds demonstrate this physiological endowment of the human brain most convincingly. Mozart wrote once to his father that to compose music is a rest for him and the inability to do so immediately tires him. Millions and millions in possession of a proper work related drives could have written this letter.

TB: Where do you measure enhancer effects in the brain?

JK: Since catecholaminergic and serotonergic neurons are enhancer sensitive neurons and we demonstrated that PEA and tryptamine are natural enhancer substances acting on these neurons, their long-acting analogues, deprenyl and BPAP, are the proper experimental tools to study enhancer regulation in the brainstem neurons. In contrast to deprenyl that is an enhancer of the catecholaminergic neurons and almost ineffective on the serotonergic system, BPAP is a highly potent enhancer of the serotonergic neurons too. As a matter of fact BPAP is at present the most selective and potent experimental tool to investigate enhancer regulation in the catecholaminergic and serotonergic neurons in the brainstem. I just finished a paper, co-authored by Ildiko Miklya and Berta Knoll, which I am sending to Life Sciences, analyzing in more detail that a bi-modal, bell-shaped concentration effect curve is characteristic to the enhancer effect of both deprenyl and BPAP. BPAP acted, for example, on the isolated locus coeruleus of rats in a manner where we found a peak-effect at [10^{-13}]M concentration and a second peak at [10^{-6}]M concentration. It is obvious that the specific enhancer effect is the physiologically relevant one. Interestingly, at [10^{-10}]M concentration we were unable to detect the enhancer effect. We measured also the BPAP-induced enhancement of noradrenaline release from the locus coeruleus 30 minutes after the subcutaneous administration of a single dose of BPAP and found the same characteristic dose-dependency of enhancer effect. For example, the most effective dose of BPAP, 0.0005 mg/kg increased the release of noradrenaline from 4.7 nM/g in controls, to 15.4 nM/g, but a 100 times higher, 0.05 mg/kg dose of BPAP, did not change it.

TB: This a very strange, unusual form of dose-dependency, isn't it?

JK: It is. But, it seems to me that this peculiar form of dose-dependency is of high physiological

significance. It allows giving a reasonable explanation for the substantial individual differences found in behavioral performances. Since an optimum concentration of the enhancer substances was needed for the optimum performance, I postulate that the substantial individual differences found in behavioral performances are due to the peculiar dose-dependency of the still unknown natural cortical enhancer substances. This approach grants us new perspective on the results of our two longitudinal studies on rats. As an example, let me analyze our second longitudinal study on rats from this perspective. This study was performed between 1990 and 1994. As I mentioned earlier we started working with a random population of 28-week old male rats and tested their sexual performance once a week. Rats representing the two extremes in performance were selected for the study; ones that did not display a single intromission during the four consecutive weekly-mating tests used for selection, and ones which showed full scale sexual activity with mounting, intromission, ejaculation, in each of the four tests. Out of 1600 sexually inexperienced 28-week-old Wistar-Logan male rats that met a receptive female once a week for four consecutive weeks, 94 did not display a single intromission during the selection period and 99 displayed at least one ejaculation in each of the four tests. The former were taken for the sexually lowest performing (LP) rats and the latter for the highest performing (HP) ones. Considering the unique dose-related effect of an enhancer substance, it is reasonable to assume that out of the 1600 rats the 99 HP rats produced their endogenous enhancer substances at the peak of the bell-shaped concentration/effect curve, while the 94 LP rats produced them at the least active part of the curve; and the production of the overwhelming majority of the population, 1407 rats, would fall between the two extremes.

TB: Are enhancer substances neuroprotective agents?

JK: It is obvious that an enhancer substance acts as a neuroprotective agent on enhancer-sensitive neurons. To illustrate it, let us analyze our first study on the neuroprotective effect of BPAP on cultured rat hippocampal cells. To elicit cell death the cultured hippocampal neurons were treated with ß-amyloid$_{25\text{-}35}$ fragment. BPAP exerted its enhancer effect in its characteristic bipolar manner with bell shaped concentration-effect curves. The peak effect was reached at 10^{-14}M in the low femto/picomolar concentration range and at the high 10^{-8}M concentration. Because of the neurotoxic effect of ß-amyloid$_{25\text{-}35}$, no more than 20 per cent of the cells, obviously the high performing cells, survived this attack. As BPAP significantly enhanced the performance of the neurons in the culture, in the presence of the optimum concentration, i.e., 10^{-14}M of BPAP, about 70 percent of the cells survived. We published these findings in 1999. We also published that

BPAP enhanced the activity of the catecholaminergic and serotonergic neurons in isolated discrete midbrain regions in exactly the same bipolar manner and in the same concentration range. The studies with BPAP performed on noradrenergic, dopaminergic, serotonergic and hippocampal neurons proved unequivocally the operation of a highly specific, complex form of enhancer regulation in sub-cortical neurons. This is very much in keeping with the ascription of a commanding role to midbrain neurons in goal seeking behavior.

TB: So, there is a highly specific form of enhancer regulation in sub-cortical neurons.

JK: The sub-cortical system is the place of the innate drives in the service of the limited number of vital goals, sexual activity, feeding, nurturing. But, as I told you, humans are the only living beings on earth whose life is predominantly based on acquired drives.

TB: Didn't you test the effect of your enhancer substances on cultured cortical neurons?

JK: The first study of the enhancer effect on cultured cortical neurons was performed with BPAP on a primary culture of rat cerebral cortex. It was done by the Japanese and showed that BPAP significantly protected cortical neurons against serum-free-condition induced cell death in the high concentration range. However, in striking contrast to the finding on cultured rat hippocampal neurons, BPAP did not exert an enhancer effect on the cultured rat cortical neurons in the femto/picomolar concentration range.

TB: So, BPAP in the low concentration range has no effect on cortical neurons.

JK: The reason for this finding is now clear. BPAP acts on the enhancer-sensitive sub-cortical neurons, but it is ineffective on cortical neurons.

TB: What is the experimental evidence for your statement that BPAP has no effect on cortical neurons?

JK: To test a compound's ability to enhance the acquisition of a conditioned avoidance reflex (CAR) in the shuttle box, it is necessary to select proper training conditions. In the case in which the rat was trained with 100 trials per day, the acquisition of CARs reached an 80% level. To demonstrate the highly significant enhancer effect of BPAP on sub-cortical catecholaminergic neurons in vivo, we trained the rat with 100 trials per day, blocked the acquisition of CARs by pretreating the rats with tetrabenazine, and restored the learning ability with the simultaneous administration of BPAP. Learning is a cortical function. In the series of experiments aiming to test the effect of BPAP on cortical neurons we trained the rats with 20 trials per day in order to have a chance to detect the drug-induced improvement in the learning ability realized via the direct

stimulation of cortical neurons. The percentage of CARs in rats trained with 100 trials per day was 77% on the 5th day of training. In contrast, it was only 8.5% in rats trained with 20 trials per day. Thus, in case BPAP had possessed a specific enhancer effect on cortical neurons, we could detect it easily in the form of a significant, dose-dependent increase in the percentage of CARs in rats trained with 20 trials per day. Because of the bell-shaped concentration effect curve, characteristic of the enhancer effect of BPAP, we used 10 doses of the compound, ranging from 0.000001 to 10 mg/kg, to clarify the effect of BPAP on the cortical neurons. None of the applied doses of BPAP was capable of changing the learning performance of rats in the shuttle box. Thus, in accord with the findings on cultured rat cortical neurons, the in vivo experiments confirmed that BPAP, the presently known most potent enhancer of the sub-cortical catecholaminergic neurons, is devoid of a specific enhancer effect on cortical neurons.

TB: I see. You say that BPAP activates the cortical neurons only via enhancement of the catecholaminergic system in the brainstem?

JK: Exactly. And now I'm coming back to my unexplained working hypothesis, catalyzed by the discovery of the enhancer regulation in the brainstem, that learning is a cortical enhancer-regulation-dependent function. My concept is that learning only needs the concurrent operation of functionally different groups of cortical neurons under proper conditions. In vertebrates, learning, the modification of behavior through practice by training or experience, is the main physiological function of the cortex. Modification of behavior rests on the inborn ability of cortical neurons to get acquainted with each other through training, learning to influence each other's function, and cooperate thereafter according to need. The mechanism of this important process is still unknown. The discovery of enhancer regulation offers the following interpretation of learning. Each member of a population of naïve, Group 1 cortical neurons, born to perceive a specific quality of stimuli originating outside or inside the body, synthesize the same enhancer substance. It is also supplied with enhancer receptors to which this enhancer substance is the highly specific ligand. The stimulation of the neurons with their enhancer substance leads to enhanced excitability. On the other hand, each cortical neuron is able to activate under proper conditions, by training, an enhancer receptor to any of the cortical enhancer substances. Thus, neuron A is born with its specific enhancer receptor ER_A and with the ability to synthesize its own enhancer substance ES_A. Neuron B is born with ER_B and synthesizes ES_B, and so on. Whenever a cortical neuron gets excited, its specific enhancer substance is synthesized in an increased amount, and its sensitivity

toward other enhancer substances is significantly increased. When neuron A and B are simultaneously stimulated, both are continuously bombarded with a higher amount of the enhancer substance of the other neuron and at the same time also sensitized to activate a receptor to the alien enhancer substance. As a consequence, the concurrent stimulation of neurons A and B time after time in training, ultimately leads to the fixation of a new functional constellation. Neuron A acquires sensitivity toward ES_B, and neuron B acquires sensitivity toward ES_A. Thus, learning means that a neuron acquires the ability to respond to originally alien stimuli. As a consequence of this change we experience the training induced modification of behavior.

TB: Am I correct that your neuronal inferences are based primarily on behavioral findings?

JK: Using the shuttle box technique, there is a reasonable possibility of testing the validity of this concept on rats. The shuttle box is a simple and useful setup for following the development of a two-way CAR. The box is divided inside by a barrier with a small gate. The rat is trained to cross the barrier to a flashing light, the conditioned stimulus (CS). If the rat fails to do so, the animal is punished with an electric foot shock, the unconditioned stimulus (US). The rat is trained to respond to the CS with 100 trials per day. One trial consists of a 15 seconds intertrial interval, followed by a flashing light for 15 seconds that overlaps with a foot shock for 5 seconds. The rat learns to avoid punishment, acquires the CAR, and escapes in response to the flashing light within 10 seconds. This is automatically counted. According to present views, the rat, driven by fear, tries to prevent punishment and learns by trial and error to escape in due time. The efficiency of learning is thought to be proportional to the number of the successful crossings in response to flash light within 10 seconds. According to our new concept the efficiency of learning depends on the repeated simultaneous operation of functionally different populations of cortical neurons. In light of this approach we need to weigh carefully the series of events in the cortex during the training procedure. The concept predicts that development of a stable CAR in the shuttle box signifies the acquisition of a special cooperation between the groups of cortical neurons born to perceive the US foot shock and the CS flashing light. Nevertheless, other groups of cortical neurons, stimulated for example by the setup as a whole, are also involved in the special modification of the rat's behavior. In the course of training numerous groups of cortical neurons, A, B, C, born to perceive special information only, are synchronously active and influence each other. Furthermore, each group of neurons has a chance to develop sensitivity toward each of the enhancer substances belonging to the simultaneously activated groups of neurons. Thus, during the training procedure

a network of co-operating groups of cortical neurons develops, which operates thereafter as an entity. The training-induced cooperation between the groups of neurons can be transient in nature, such as a chain of extinguishable conditioned reflexes (ECRs) or a chain of inextinguishable conditioned reflexes (ICRs), or to the development of the most sophisticated form of excitatory state in a group of cortical neurons, an 'active focus' that will operate thereafter as an acquired drive. However complicated the cooperation developed between different group of neurons during training may be, it is their common feature that they work thereafter as an integral whole, and this entity can be activated via a few decisive groups of neurons. Thus, my approach is that the modification of behavior of the rats trained in the shuttle box depends on the synchronous activation of different groups of cortical neurons in the brain for a proper period of time.

TB: I see. Could you say something about how these behavioral findings were influenced by drugs with known pharmacological actions?

JK: Treatment of rats with 1 mg/kg tetrabenazine, which blocks selectively and reversibly the reuptake of the catecholaminergic transmitters into their intraneuronal stores, depletes noradrenaline and dopamine from the end organs of the catecholaminergic neurons in the brain stem. Since the operation of the catecholaminergic brain engine is the condition sine qua non for the trial and error mechanism, the success in reaching a goal, the acquisition of a CAR in the shuttle box, cannot be detected in tetrabenazine-treated rats because of the blockade of the animal's ability to cross the barrier. Nevertheless, the activation of cortical neurons via the unconditioned and conditioned stimulus remains unchanged in tetrabenazine-treated rats. These experiments are now in progress; let me mention my first results. I treated rats with tetrabenazine which blocks the catecholaminergic engine of the brain without acting on cortical neurons. I am using a strain of rats with exceptionally low learning capacity and work with females which are lower performers in the shuttle box than their male peers. I am testing the rats daily in the shuttle box from Monday until Friday with 100 trials daily. One group is treated subcutaneously with saline the other group with 1 mg/kg tetrabenazine. The saline-treated rats developed a stable conditioned avoidance reflex. Because we work with a dull strain of rats, on the first day of training, the flashing light, the conditioned stimulus was only an average of 10% effective in eliciting escape to the other part of the compartment within 10 seconds. On the 5th day of training 79% of the rats escaped in response to the flashing light. However, even on the 5th day of training less than 5% of the tetrabenazine-treated rats escaped in response to the flashing light. After the 5-day-training period

both the saline-and tetrabenazine-treated rats had a rest on Saturday and Sunday. This resting period is enough for the complete elimination of tetrabenazine. On Monday we tested again the animals and found that 81% of the saline-treated
rats and 65% of the rats treated with tetrabenazine during the training period, escaped in response to the flashing light. You remember that only 10% of saline-treated rats of this dull strain escaped on the 1st day of training in response to the flashing light. Now, despite of the fact that the tetrabenazine-treated group of rats did not show any sign of the acquisition of a CAR during the 5-day training, in fact they fixed the CAR in their cortex since after the elimination of tetrabenazine 65% of the rats escaped in response to the flashing light. This finding is in accord with the concept that learning needs only the concurrent operation of functionally different groups of cortical neurons under the proper condition.

TB: The results of this experiment are really thought provoking and seem to be supporting your working hypothesis that learning might be an enhancer-dependent cortical function. But be that as it may, it will for sure initiate much work in this new direction. What about the recent finding that BPAP exerts an enhancer effect also on neuroglial cells?

JK: Neuroglial cells play an important physiological role in the brain and modulate the function of neurons in a complex manner, but they do not participate in the realization of drive-dependent, goal-seeking behavior. Our Japanese collaborators used astrocytes in their research and measured the rate of synthesis of three neurotrophic factors, the nerve growth factor (NGP), the brain-derived neurotrophic factor (BDNP) and the glial cell line-derived neurotrophic factor (GDNF). They found that BPAP increased significantly the synthesis of neurotrophic factors in the micromolar concentration range, but we found in a series of experiments, now in progress, that BPAP is ineffective on glial cells in the low from femto to picomolar concentration range. Thus, the specific form of enhancer regulation is not detectable in the glial cells. These findings support the view that the specific form of enhancer regulation stimulated by BPAP in the extremely low concentration range is the behaviorally important form, whereas the enhancer effect of BPAP in the micromolar concentration range is insignificant in behavioral terms. Nevertheless the finding that BPAP induced enhancement in the synthesis of neurotrophic factors in the micromolecular concentration range is a remarkable pharmacological effect that deserves further analysis in the future.

TB: Am I correct that you have done all your research in the Department of Pharmacology at

Semmelweis University in Budapest?

JK: Yes. I started my career in the department as a medical student in February 1949 and I never left during my lifetime.

TB: Since the time you published your first book on *The Theory of Active Reflexes* more than 30 years have passed.

JK: As a matter of fact by the end of 1953 I already developed and studied in detail the technique to analyze in rats the acquired drive and my theory, that I summarized in this monograph, was basically ready 16 years earlier. I needed thereafter 30 years to get to the core of the acquired drives and realize that the root of the matter is enhancer regulation in the brainstem and the cortex. As we already discussed, enhancer-sensitive neurons in the brainstem and in the cortex are in my view capable of changing their excitability and working according to the need on a higher activity level in a split second. I have already started to summarize my neurochemical concept of innate and acquired drives in a new monograph.

TB: You mentioned earlier that the antidepressant effect of deprenyl was shown but not fully explored. Could you elaborate on that?

JK: It was Varga who first described the antidepressant effect of deprenyl in 1965 and published with his coworkers two more papers in 1967 and 1971 extending their results. Then, later in the 1980s, Mann and Gershon, Mendlewicz and Youdim, Quitkin and his associates, and McGarth and his collaborators provided substantiation that deprenyl is an antidepressant. Unfortunately no big drug company picked it up and deprenyl was never registered as an antidepressant. It might happen in the future and BPAP is also from this aspect a promising compound.

TB: Do you think that enhancer substances have antidepressant effects? The diagnosis of major depression refers to a clinically and pharmacologically very heterogeneous population.

JK: BPAP, which is a selective enhancer substance, stimulates the catecholaminergic and serotonergic neurons in the brainstem via a previously unknown mechanism. Because it is a highly potent compound there is good reason to believe it will be used sometime in the future as a valuable antidepressant.

TB: Regardless what happens with BPAP and enhancer regulation, you developed deprenyl, the first MAO-B inhibitor and this alone is a major contribution to the field of neuropsychopharmacology. Was this research followed up? Are there any other MAO-B inhibitors?

JK: There are, but none of them is comparable to deprenyl in its effect in Parkinson's disease.

TB: When did you start with the development of BPAP?

JK: It started in the early1990s.

TB: How did you get the idea to develop BPAP?

JK: I wanted to develop a selective enhancer substance which is unrelated to phenylethylamines and is devoid of MAO inhibiting properties. I firmly hope that in the long run BPAP will convince the scientific community that enhancer regulation in the brain is a mechanism of key importance and drugs which stimulate selectively this mechanism are of significant therapeutic value.

TB: Is there any relationship between your anti-aging drugs and the late Giurgea's nootropics?

JK: Nootropics have nothing to do with enhancer regulation. Since we have the specific method for measuring quantitatively the enhancer effect of a compound on the locus coeruleus, striatum, substantia nigra, tuberculum olfactorium and raphè, we recently tested piracetam in a wide dose range on these isolated discrete rat brain regions. We found this prototype of nootropics, Giurgea's original substance, is devoid of an enhancer effect.

TB: Could we switch to more personal matters in your life. You told us you joined the department of pharmacology after your third year in medical school.

JK: I started to work in February in 1949 as a student and graduated from medical school in 1951.

TB: Tell us about the Department of Pharmacology at Semmelweis University. Isn't it one of the oldest pharmacology departments in the world?

JK: Since the first pharmacology department in the world was founded in 1849 in Dorpat Germany, now Tartu in Estonia, and our department was founded in 1872, it is really one of the oldest. Its first chairman was Kalman Balogh. He was followed by Arpad Bokay, Zoltan Vamossy, and by my predecessor, Bela Issekutz. I succeeded Issekutz in 1962. I was the fifth chairman of the department. I retired from my chair in 1993, after 31 years. But I remained fully active as a member of the Hungarian Academy of Sciences and continued with my research. I was chairman of the department longer that any of my predecessors.

TB: So, you are an active member of the Hungarian Academy of Sciences.

JK: Yes. Each of my predecessors was a member of the Academy and I have continued in that tradition. I became a corresponding member of the Hungarian Academy of Sciences in 1970 when I was 45 years old, and a full member in 1979. In 1970 I was the youngest member of the Medical Class of our Academy and now I'm one of the oldest.

TB: I remember the celebration of your 60th birthday by the Academy.

JK: In 1985 I received the National Prize, the highest honor given for scientific achievement in Hungary. The birthday celebrations at the Academy and in the Institute, were touchy events. I was honored with a Festschrift, "*Neuropharmacology 85,"* edited by Károly Kelemen, Kálmán Magyar and Szilveszter Vizi, published by the Hungarian Academy of Sciences. It included 49 papers by distinguished scientists from all over the world: I was honored also on my 75th birthday with a Festschrift, "*Milestones in Monoamine Oxidase Research: Discovery of (-)-Deprenyl."* This one was edited by Kálmán Magyar and Szilveszter Vizi, and published by Medicina Publishing House, in Budapest.

TB: What about your relation to foreign Academies and Universities?

JK: I was honored in 1974 to become a member of the Leopoldina Academy of Natural Sciences, one of the eldest academies in the world. In 1984 I became honorary doctor of the Medical Academy of Magdeburg and in 1989 I was honored with a honorary doctorate by the Bologna University at the occasion of its 900th year anniversary. Since the University of Bologna was the first university in the world, I feel this honor a privilege. In 1990 I was elected Honorary Fellow of the Royal Society of Medicine, London, and in 1995 I became a foreign corresponding member of the Polish Academy of Art and Science.

TB: Have you been active in professional societies?

JK: Traditionally, pharmacologists everywhere in the world were members of their national physiological societies which were, in turn, members of the IUPS. The rapid development of pharmacology made it clear by the end of the 1950s that time was ripe for the creation of independent national pharmacological societies and the IUPHAR. But it was neither on the national level nor on the international level easy to break with tradition. In 1958 I started, in Hungary, the fight to attain our independence and we succeeded to establish, in 1962, the Hungarian Pharmacological Society; I was the first executive secretary and after Bela Issekutz, the second president. Since 1983 I have been Honorary President of the Society for Life. IUPHAR was established in 1965; I was member of the Executive Board from 1982 until 1984 as councilor and from 1984 until 1987 as First Vice President. I was elected, in 1980, an honorary member of the Pharmacological Society of Poland, in 1985 of the Czechoslovakian and Bulgarian Pharmacological Societies, and in 1986, of the Austrian Parkinson Society. I was honored in 1999 with the Award for Distinguished Service in European Pharmacology, and in 2001 with the Award

for Outstanding Contribution to Anti-Ageing Medicine..

TB: Would you like to mention people you trained?

JK: I'll mention just those who worked with me through decades; Károly Kelemen, Berta Knoll, János Dallo, Kálmán Magyar, Szilveszter Vizi, Zsuzsanna Fűrst, Tamás Friedman, Klára Gyires, Huba Kalász, Valeria Kecskeméti, Julia Timár, Zsuzsa Gyarmati, and Ildiko Miklya.

TB: Is there anything else you would like to mention?

JK: You can see two-large leather bound volumes on my bookshelf. I received those on my 50th birthday in 1975 from my co-workers. Reprints from our numerous publications during my first 13 years as head of the department are bound in them. One would need at least 10 such volumes to include a reprint of all our publications from the 31 years I was chairman of the department.

TB: And you are still fully active.

JK: I am fully active in research, but I retired from my administrative positions. Zsuzsanna Furst, one of my pupils, is now head of the department.

TB: Besides being chairman of the department of pharmacology did you have any other administrative position?

JK: I was, from 1964 until 1970, the Vice President of the University responsible for research, and I was Vice President of the Medical Class of the Hungarian Academy of Sciences from 1967 until 1976. After that, apart from the Hungarian Pharmacological Society and IUPHAR, I never accepted any administrative position.

TB: What would you consider your most important contribution?

JK: From a practical point of view, the discovery of enhancer regulation and the development of synthetic enhancer substances, and from a theoretical view, the discovery that with the evolution of brains capable of acquiring drives, species appeared whose members could manipulate each other's behavior and act in concert. This was the sine qua non for the evolution of social living, a form of life that enabled the species to surpass, qualitatively, the performance of any individual.

TB: On this note we should conclude this interview with Professor Joseph Knoll. Thank you for your contributions to neuropsychopharmacology and for sharing this information with us.

JK: I feel honored by having this interview. Thank you very much.

References:

Ban TA, editor. An Oral History of Neuropsychopharmacology .Volume Three. (Sulser F, editor.

Neuropharmacology). Brentwood: American College of Neuropsychopharmacology; 2011a.

Ban TA. Preface. In Sulser F, editor. Neuropharmacology. (In Ban TA, editor. An Oral History of Neuropsychopharmacology The First Fifty Years Peer Interviews. Volume Three). Budapest: Animula; 2011b, pp. ix – xxx.

.

Knoll J. The Brain and Its Self. A Neurochemical Concept of The Innate and Acquired Drives. Berlin/Heidelberg/New York: Springer; 2005.

Knoll J, Vizi ES, Somogyi G. Phenylisopropylmethylpropinylamine (E-250), a monoamine oxidase inhibitor antagonizing the effects of tyramine. Arzneimeittelforschung. 1968; 18: 109-12.

Knoll J, Dallo J, Yen TT. Striatal dopamine, sexual activity, life span. Longevity of rats treated with (-) deprenyl. Life Sci 1989; 45: 525-31.

Sulser F, editor. Neuropharmacology . Volume Three. (In Ban TA, editor .An Oral History of Neuropsychopharmacology The Firs Fifty Years Peer Interviews). Budapest: Animula; 2011a

Sulser F. Introduction and dramatis personae. In Sulser F, editor. Neuropharmacology. Volume Three. (In Ban TA, editor. An Ora History of Neuropsychopharmacology The First Fifty Years Peer Interviews). Budapest: Animula; 2011b, pp. xxxix – lx.

November 8, 2018