Fridolin Sulser: From the Presynaptic Neurone to the Receptor to the Nucleus
David Healy Interviews*

You started in psychopharmacology with Brodie at the NIH.

Yes, I personally owe everything to Brodie in terms of my career. I was a very young postdoctoral student from Switzerland who barely spoke English. Brodie would even teach me English or correct my "Swiss English." He emphasized that three things are essential to be successful in science: (l) You have to have an idea, (2) You have to be able to develop your own methodology to explore the idea and, (3) You have to be lucky.

Brodie was conceptually enormously influential. He had a large group of collaborators and he put biochemical pharmacology on the map, there is no question about that. His laboratory was a Mecca of Psychopharmacology that facilitated the development of new psychotropic drugs, and of sound methodology to measure drugs and their metabolites in plasma, brain and other tissues. I was very fortunate to be associated with colleagues there, who ideally complemented my own scientific background. I was privileged to learn solvent - extraction procedures and fluorometric methodology long before isotopic procedures and HPLC became fashionable.

It was the application of these techniques that led to the discovery of the secondary amine desmethylimipramine (DMI) in the brain of rats chronically treated with imipramine. Since DMI was a more potent antagonist of the reserpine - like syndrome than imipramine - Brodie called it "reserpine depression" – and it had a longer biological half-life, when it was shown to be clinically effective in depression in man, Brodie developed the notion of imipramine being a pro-drug. I vividly remember Brodie traveling around the country with a movie showing how rats treated with DMI and reserpine displayed a compulsive motor hyperactivity instead of the "depression" after reserpine alone. They jumped off of a high board only to resume the compulsive motor activity when put back on the platform.

Brodie had a very quick mind. He was enormously creative and imaginative. He used to tell me that it is important to see what everybody else has seen but to think what nobody else has thought. As a person though he was somewhat egocentric. He liked to put his name first on manuscripts, which originated from his laboratory. So, it was Brodie et al. I remember reading about a scene in "Apprentice to Genius" that illustrated this point. It was, I believe, during a staff meeting when the discussion focused on the preparation of an important manuscript and Brodie said, "Let's publish it alphabetically." Though Julius Axelrod was one of the prominent contributors, the manuscript went out under Brodie et al.! I think this was perhaps part of the reason why he didn't end up with a Nobel Prize, though in my view, he fully deserved it. His egocentric behaviour "messed up" the politics. He got very hurt when Julius Axelrod, his former technician, received the Nobel Prize. I remember how everybody was smiling and happy at a

ceremony in Axelrod's honour - except Brodie.

While Axelrod's research was very focused, Brodie's approaches were always more global conceptually. He opened up whole new fields such as Biochemical Neuropsychopharmacology. He inspired young people to pursue a scientific career. His laboratory of biochemical pharmacology produced the leaders in the field: Julius Axelrod, Sidney Udenfriend, Park Shore, Sydney Spector, James Gillette, Bert LaDue, John Burns, Allen Conney, Erminio Costa, to mention a few. If you look at the people in Europe who have made major contributions to biochemical neuropsychopharmacology, they all have spent some time in Brodie's laboratory. For example, Arvid Carisson from Sweden who proceeded me by a few years, Alfred Pletscher and Marcel Bickel from Switzerland, Rudolfo Paoletti and Luigi Gessa from Italy, Norbert Matussek, Kari Netter and the late Eric Westermann and Hans Dengler from Germany. The list goes on and on.

Why did he take so many people from Europe to his lab?

Reputation. Impact of his scientific output. How did I come to Brodie? Well, Brodie made such a splash with a paper, which he published together with Pletscher and Shore in Science, demonstrating that reserpine depletes brain serotonin. Remember, this was in 1955. It had an enormous impact on the field of psychopharmacology and biological psychiatry. First, there was the development of a fluorometric method to measure quantitatively the concentration of serotonin in brain. That was a first. The first spectrophotofluorimeter had been developed at that time by Bowman and Udenfriend and this greatly facilitated the study of biogenic amines and our understanding of their function in the central nervous system.

The demonstration that the "depressive" action of reserpine was associated with a change in the concentration of endogenous serotonin in brain opened up the entire field of biochemical neuropsychopharmacology and contributed to the pharmacological basis of the serotonin hypothesis of depression. I remember reading that article in Science. I could just read the summary; I couldn't read the whole paper because my English was too poor, but I thought it was a terrific discovery with an enormous heuristic potential.

I went to see Alfred Pletscher, who had just come back from Brodie's laboratory. I mentioned my desire to go for a year or so to the United States. He said I should go to Brodie. As I mentioned previously, his laboratory was a Mecca of biochemical pharmacology. Julius Axelrod was there. Sidney Udenfriend was there. Park Shore was there. It was a wonderful place for young postdoctoral students who found in Brodie's Laboratory of Biochemical Pharmacology a nurturing environment for their scientific growth. The Swedes and the Germans came, the Swiss came, the Italians came, the French came, a few British and then there was the Japanese invasion. It was an incredible place where scientific collision-coupling occurred in an atmosphere of total openness.

When did you leave the NIH and what happened then?

I came as an exchange visitor and I couldn't get employed at the NIH because I wasn't a U.S. citizen. But I didn't want to go back to Switzerland because at that time, the "Geheimrat System"

was still operative and there were no attractive jobs available for somebody who had just experienced the freedom of working independently. I could have gone back to my old job as an assistant professor in Bern, but this was not appealing to me. I had to change my visa status because I couldn't get employed with a visitor's visa in the United States. I was supposed to go back for two years after which I could apply for a permanent visa. The circumstances were fortunate however, and I was able to change my status.

It was an interesting story. Jim Dingell who was a graduate student in Brodie's laboratory, with whom I did some of the early work with tricyclics, had a brother in congress, congressman John Dingell who was a powerful Democrat from Michigan. I learned that his mother was of Swiss origin, circumstances which must have contributed to our getting along so smoothly! He facilitated my obtaining a waiver of the foreign residence requirement. My visa got changed. I still remember the joy when I opened the letter from the Justice Department stating that "my departure would not be to the best interests of the United States and therefore, I, Robert F. Kennedy, Attorney General of the United States waive public law..." I was an immigrant!

What was the Geheimrat system?

At that time, we had one professor, the "ordinarius" who dominated the scene. There was no way for a young person to pursue his or her own independent research. Now, this situation has changed, the system got "Americanized" and there are now independent research positions available for those who return from their training in the States.

Extraordinary.

Yes, I became an immigrant and I could get legally employed in the U.S. John J. Burns who was the director of research at Burroughs Wellcome and Company and who previously was deputy chief of the Laboratory of Chemical Pharmacology at the NIH, persuaded me to join him and other NIHers at Burroughs Wellcome. So, I went for a short time as Head of Pharmacology to the Wellcome Research Laboratories in Tuckahoe, New York. Though the particular industrial environment at Burroughs Wellcome was conducive to basic research in pharmacology, I nevertheless decided to return to academia when Vanderbilt University had an opening in 1965 for a Professor of Pharmacology with particular emphasis on psychopharmacology. It was the late Daniel Efron, Director of Psychopharmacology at the National Institute of Mental Health and a former colleague of mine in Brodie's laboratory, who facilitated the transition from industry to academia by awarding Vanderbilt one of the first Center grants in psychopharmacology.

Okay, for a person like you going into psychopharmacology in a university at that point in time, what was happening?

Though there was little activity in psychopharmacology at Vanderbilt at that time, there were superb people in related scientific fields one could interact with. One of those was Earl W. Sutherland who, as you probably know, won the Lasker Award followed by the Nobel Prize in physiology and medicine for his work on cyclic AMP.

Can you fill me in on his background?

Earl Sutherland was an M.D. who received his scientific training with the Cori's at Washington University. Prior to his appointment at Vanderbilt, he was chairman of the Department of Pharmacology at Case Western. He felt that administration interfered too much with his research endeavours and consequently, he accepted a position of Professor of Physiology at Vanderbilt. His research philosophy was somewhat similar to that of W. R. Hess, one of my teachers in Zurich – that is it was functionally oriented. With the discovery of cyclic AMP, he defined for the first time the action of hormones at a molecular level and opened up the field of second messengers.

We all know the impact of Earl's discovery on essentially every field in biology. Perhaps his most outstanding characteristic was his fantastic intuition. To quote Alan Robison, one of his postdoctoral fellows, and later a collaborator of mine, "it almost seemed at times as he could peer into a living cell and see exactly the sense of what occurred and why." A precept by which he lived and which he often cited, was that you should never fall in love with your hypothesis. When I came for my job interview with Alan Bass in Pharmacology in 1965, I met Earl and immediately cherished his strong belief in the value of open scientific communication and his concept of what science should be - an open, honest idealistic society of people searching for new truths. Vanderbilt was a very small University in 1965. Everything was - and still is - on one campus from medicine to law, to physics and chemistry, to philosophy and even divinity. I loved the place and the independence and the lack of distractions inherent in my new position and as you know, I have been at Vanderbilt ever since.

What were you doing between 1966 when you went there and '74 when the beta receptor story began to unfold?

This period was not the most exciting one from the point of view of generation of new concepts. We studied the role of storage and synthesis of brain noradrenaline in the action of psychotropic drugs. We found that the action of amphetamine depends on the availability of newly sensitized catecholamines whereas a rapid release of noradrenaline from its storage sites is essential for the behavioural stimulation elicited by tetrabenazine in DMI pretreated rats. We also used Gaddum's - push - pull cannula to demonstrate that DMI blocked the reuptake of noradrenaline in the hypothalamus of rats in vivo. We played around with the amphetamine model of Larry Stein and found in due course that tricyclics are potent inhibitors of the aromatic hydroxylation of amphetamine thus explaining the enhancement and prolongation of the action of amphetamine. Also, during this time, Elaine Sanders-Bush and I demonstrated the irreversible inhibition of tryptophan hydroxylase in brain by chlorinated amphetamines which explained the prolonged reduction of central levels of serotonin and its major metabolise 5H1AA observed by others.

During these early years at Vanderbilt, Alan Robison, Gene Palmer and I began to develop the brain tissue-slice system to study the modification by psychotropic drugs of the cyclic AMP response to noradrenaline in vitro. The choice of brain slices over homogenates was necessitated by the fact that homogenization of brain tissue led in most cases to an almost complete loss of hormonal sensitivity to noradrenaline. In many ways, these studies paved the way for the

conceptually more exciting studies that were initiated when Jerzy Vetulani from the Institute of Pharmacology of the Polish Academy of Sciences in Krakow, arrived in my laboratory in 1974.

When I came to do pharmacology first with Brian Leonard in 1980 we were looking at alpha-2 receptors on platelets and beta-receptors on lymphocytes and your name was the dominant name in the field then because of the Beta Receptor hypothesis. There was still then a feeling, however, that even though you could radiolabel receptors, not everybody was absolutely convinced that they were there. How did the receptor begin to come into your thinking?

This is an interesting story. You know, we actually looked at the function of the beta receptor rather than the receptor itself because of my exposure to the philosophy of both W. R. Hess and Earl W. Sutherland. Both of them stressed that function is more important than levels of proteins, including receptor proteins. When Jerzy Vetulani was in my lab in I974, we were struck by the fact that all clinically effective antidepressants increased the availability of noradrenaline or serotonin within minutes, while the therapeutic action is delayed for three or more weeks. So, we started to treat rats with various antidepressants chronically, that is on a clinically relevant time basis.

Since Earl Sutherland was at Vanderbilt at that time and he had discovered how the beta receptor agonist adrenaline makes cyclic AMP by activation of adenylate cyclase, we decided to study not the receptor per se but the function of the receptor. We used isoprenaline and noradrenaline as agonists of the beta receptor and measured the accumulation of cyclic AMP in brain slices prepared from animals chronically treated with various antidepressants. Incidentally, at that time the beta adrenoceptor was considered to be a site at the adenylate cyclase molecule. How things have changed since the discovery of G proteins! We were hoping that if we treated animals chronically, we would detect increased beta receptor responses. We expected increased cyclic AMP production in response to noradrenaline or isoprenaline. We were quite surprised when it turned out the opposite way. Acute administration of antidepressants increased the formation of cyclic AMP in response to noradrenaline or isoprenaline and chronic administration decreased it. This is what we called the desensitization of the beta adrenoceptor coupled adenylate cyclase system.

We saw this first with monoamine oxidase inhibitors and then with tricyclics and also with ECT. The measurement of the actual beta receptor number came about one or two years later. This was done by Banerjee from Sol Snyder's lab, who demonstrated that this desensitization of the beta adrenoceptor system is linked to a decrease in the density of beta adrenoceptors. It all fit nicely together but antidepressant induced desensitization of the beta adrenoceptor coupled adenylate cyclase system can occur without a change in the number of receptors - you can uncouple the receptors from the adenylate cyclase and have a decreased responsiveness to agonists such as noradrenaline.

It is important to distinguish desensitization and down-regulation of the beta adrenoceptor system. Desensitization refers to the functionally important deamplification of the beta signal while down-regulation refers to the reduction in the number of beta adrenoceptors in the membrane. Naturally, I prefer the term desensitization.

It took a little while for people to accept the implications of our findings, which were quickly confirmed all over the world. The beta adrenoceptor desensitization hypothesis helped to move research on the action of antidepressants from acute presynaptic to delayed postsynaptic adaptive processes occurring at and beyond the receptors and their link to effector systems such as adenylate cyclase. The elucidation of the mechanisms underlying beta adrenoceptor desensitization and-or down-regulation had to wait for the work of the Lefkowitz group at Duke and of others demonstrating the role of receptor phosphorylation via activation of various protein kinases.

What role did Jerzy Vetulani play in this?

Jerzy played a crucial role. How did he come to me? At the 1972 CINP Meeting in Copenhagen, I met Jerzy Maj from Krakow. He mentioned that Jerzy Vetulani would like to come over to my lab. After the Copenhagen meeting, I travelled to Krakow, met Jerzy Vetulani and learned about his interest in antidepressant action and we arranged the exchange visit. As previously mentioned, we initiated studies on chronic treatment with antidepressants and ECT and - the beta adrenoceptor down-regulation hypothesis was born! Jerzy was an extraordinarily gifted laboratory scientist and hard working too. He shared my enthusiasm for function and he greatly contributed to the new conceptualization.

The fascinating part of the story is that we now know that the deamplification of the second messenger system results in a net deamplification of the beta adrenoceptor cascade. Is it reflected beyond the protein kinases at the level of gene expression? This is precisely where the action is today - 25 years after the birth of the beta adrenoceptor desensitization hypothesis.

In the early 1970s when they began to radio-label the receptors first, my impression was that people got the idea that the receptor is this solid little chunk of protein that you have got to chisel away at for about two weeks before it changes.

That was the early view, but it obviously was not correct. We know now that beta adrenoceptors display considerable plasticity. They are regulated at various levels, at the level of transcription, translation and post-translationally by phosphorylation via protein kinase A and beta adrenoceptor kinase. The plasticity is evidenced for example in the downregulation and-or desensitization following chronic antidepressant treatment and reflects adaptive processes at various levels of the beta adrenoceptor cascade. A considerable amount of this knowledge on the regulation has been generated by Bob Lefkowitz's group at Duke.

How did people see it then? What did they think they were dealing with?

Well, this was long before the discovery of G proteins and our understanding of how receptors are linked via G proteins to effector systems, either enzymes such as adenylate cyclase or ion channels. I still have one slide, which shows the beta receptor as a site on the adenylate cyclase enzyme. We saw the beta receptor as essentially a part of the adenylate cyclase enzyme. The desensitization mechanism by antidepressants was not understood at that time. All we knew at that time was that antidepressants failed to desensitize the beta adrenoceptor system in the absence of noradrenaline. The field has since moved on dramatically due mostly to the work of

Gilman who cloned the trimeric G proteins and of Lefkowitz and collaborators at Duke on the isolation and the cloning of the beta receptor. Once the receptor was cloned, everybody agreed that the receptor exists!

Can I ask you about the early work of the Duke group? When did they begin to influence you? They were very early into radiolabelling hormones to look at receptors.

In the mid '70s, Robert Lefkowitz and his colleagues at Duke made important progress toward a definition of the molecular properties and the regulatory mechanisms of aminergic receptors. The key step in their work was the development of specific radioligands with high affinity such as tritiated dihydroalprenolol (DHA) for the beta adrenoceptor. The Duke group worked on the desensitization phenomenon in the cardiovascular system and other in vitro systems. They elegantly demonstrated that beta adrenoceptors are not static entities in the membranes but are subject to very dynamic regulation by catecholamines. They demonstrated that the receptors are integral membrane proteins distinct from the enzyme adenylate cyclase thus invalidating earlier concepts of Sutherland.

When did you begin to realize that you had stumbled on something important - not just in the sense of scientifically important but almost socially important in that it was going to be important to the industry and important to biological psychiatry? It was going to be a symbol almost.

It's amazing what the 1975 *Nature* report on beta adrenoceptor desensitization by chronic antidepressants triggered. It essentially shifted the research emphasis on the action of antidepressants and on the pathophysiology of affective disorders from acute presynaptic to delayed receptor mediated adaptive phenomena. This finding was I believe important because it dealt with a physiological mechanism. It was functional in the Hessian sense!

I can see the scientific importance but when did you begin to appreciate that something else was also happening, that you'd hit the right note for other reasons?

Well, again if you think functionally, it was evident in the mid-'70s that in some way this discovery provided a gateway to events beyond the receptors all the way to the nucleus. The demonstrated deamplification of the beta adrenoceptor - cyclic AMP cascade had to have consequences on the net effect of signal transduction at the nuclear level. Now, 20 years later, we are arriving at the nucleus. I'm still fascinated how molecules on the outside of the membrane can talk to molecules on the inside of the cell. This is through G protein coupled receptors. So, if you modulate receptor function, you modulate the way a molecule on the outside communicates with a molecule on the inside of the membrane. You change the amplitude of the signal. In that sense I think it was an important discovery with great heuristic value. What was gratifying for Jerzy and myself was how quickly the data were replicated, except when people started using the SSRIs, but we will come to this later on when we chat about the convergence of aminergic signals at the level of protein kinase mediated phosphorylation processes.

But it didn't just get replicated by other labs, it ended up in the textbooks extraordinarily

quickly. It ended up being used as a screening model by the industry to produce new compounds.

I believe that the reason why our findings were so well received is the fact that sensitivity changes of the receptors were only observed when the antidepressants were administered chronically, that is on clinically relevant time basis. People started to realize that, maybe, acute studies don't tell us very much. Then came the period when people tested various prototypes of antidepressants and all of them with exception of the SSRIs caused this deamplification of the beta adrenoceptor coupled adenylate cyclase system. Even non-drug antidepressant treatments such as ECT and sleep deprivation shared this action with that of antidepressant drugs.

This common action of antidepressant treatments provided the first link for new hypotheses on the psychopathology of affective disorders, to be followed in later years by the "serotonin/noradrenaline/glucocorticoid" - link hypothesis of affective disorders. We suggested that the antidepressant sensitive, serotonin linked and glucocorticoid responsive beta adrenoceptor system in brain functions as an amplification-adaptation system of vital physiological functions including mood, sleep, arousal, pain, neuroendocrine and perhaps even immune functions. I think this is why industry set up beta receptor screens to detect new antidepressant drugs. Though new drugs got discovered, in my opinion, there is not a single one that is more efficacious and faster acting than the original antidepressants discovered almost 40 years ago by serendipity. If you set up screens to discover a certain type of drug, you find by necessity more of the same, though the side effect profile may somewhat vary.

Is there a sense though in which, while you yourself were interested in a functional response, that one of the side effects of the beta receptor hypothesis was to lead people away from function and just on to binding? They lost sight of function and just asked was the drug producing beta receptor down-regulation.

Yes, you are right. It's easier to measure binding. The receptor assays are simple, quick and cheap whereas the second messenger function is more complicated to measure. This was particularly so in the early '70s. When I came to Vanderbilt, the adenylate cyclase and second messenger assays were very complicated, tedious and time consuming. Cyclic AMP had to be eluted from Dowex - 50 columns, lyophilized and determined enzymatically by the conversion of inactive liver phosphorylase to the active form of the enzyme. So, it is perhaps not surprising that beta adrenoceptor binding studies where the preferred assays to determine drug actions on the beta adrenoceptor system though they did not shed light on changes in the function of the receptor system.

In the 1960s clinicians like Paul Kielholz were saying that the antidepressants which act on the noradrenergic system are more drive enhancing than other antidepressants which we now know have an action on the 5HT system. These were doing something else functionally he said. It was this that led to the SSRIs, which we only got because all antidepressants aren't all the same. But once we got a beta receptor hypothesis, which all the antidepressants seemed to affect the same way suddenly the thinking was that all the antidepressants are essentially all the same.

Well, this view needs some correction. While it is certainly true that all antidepressants or antidepressant treatments with a strong noradrenergic component desensitize the beta adrenoceptor coupled adenylate cyclase system, the SSRI's such as fluoxetine, fluoxamine, citalopram or paroxetine, do not consistently down-regulate beta adrenoceptors or desensitize the cyclic AMP response to noradrenaline. However, there is more than one way to go to Rome and recent studies, particularly with dual uptake inhibitors such as venlafaxine, suggest that the signals generated by noradrenaline and or serotonin receptor interaction converge beyond the aminergic receptors at the level of protein kinase mediated phosphorylation processes.

So, a final common action for mood alteration may indeed occur but beyond the aminergic receptors. In other words, beta adrenoceptor down-regulation or desensitization of the beta adrenoceptor coupled adenylate cyclase system is not a prerequisite for antidepressant activity since an increased activation of 5HT receptor cascades can affect the final link of the aminergic transduction cascade without altering beta adrenoceptor density or sensitivity. This realization has now directed research on the convergence of the aminergic signals beyond the receptors and their consequences at the level of gene expression. So, what is emerging now is indeed some type of a unified hypothesis on the mode of action of all antidepressants with regard to mood alteration though the side effect profile of the various antidepressant drugs shows considerable variation and will determine the overall therapeutic index.

Let me bring in Hess, your teacher, who characterized these two two brain systems, the noradrenergic and the serotonergic as ergotropic and trophotropic systems. He wouldn't have expected drugs which are selective on these systems to be just the same as each other.

Well, the ergotropic system obviously is functionally a noradrenergic system. The trophotropic system was characterized by Hess as a central cholinergic system. It was Brodie and not Hess who suggested at the first catecholamine symposium in 1958 that the trophotropic system may be a serotonergic system. You could argue that a deamplification of the noradrenergic system by antidepressants could lead to a predominance of the serotonergic system, if the two systems are opposed as Brodie and indeed Hess thought they were and so, I don't think there would have been any conflict.

What was Hess like?

I think he was terrific both as a scientist and a teacher. As a medical student in Zurich in the ‘50s, I was always a little afraid of him, because of his often sarcastic comments and sharp arguments. He made a point of the functional importance in biological research - almost pathologically so. He thought in terms of biological relations and considered single facts only in their context with functional systems. Hess explained to his students that single facts mean nothing for CNS physiology unless they are *leistungsbezogen*, that is, related to function or to the biological goals of the behaviour. For example, when in vitro experiments were conducted in tissue culture, Hess would come by and comment "you are studying monolayers of cells; how do you think you will learn from such studies why you fall in love with a girl or why you can't remember the name of your grandmother?" Very sarcastic, but he had a point you know. He contrasted his systems-oriented physiology with the fact-oriented British physiology.

Single cell systems didn't appeal to him. I think, we need somebody like him today when the momentum of molecular biology threatens to neglect the very functions so dear to him. Jim Black has commented similarly as Hess did when he predicted the progressive triumph of physiology over molecular biology. I think they are both right. Molecular biology per se - no matter how technically sophisticated - operating in a functional vacuum will not contribute substantially to our understanding of emotional and cognitive functions of the brain. I think it is very important to get this philosophy across and I hope someone like W. R. Hess would emerge in the next few years. Otherwise, I am afraid we just become technocrats. It worries me when I see today's pharmacology graduate students. They don't know Hess; they don't know the history of science. One meets Ph.D. candidates in pharmacology today that cannot distinguish the front end from the back end of a rat; but they can make a point mutation in the 5t' intracytoplasmic loop of a receptor and if you ask them what it means, they don't know. We need to resurrect the research philosophy of W. R. Hess!

What was Hess' background?

He was a physician who specialized in ophthalmology before he went into physiology. Hess has written an autobiographical sketch. From medical practice to theoretical medicine, published in *Perspectives in Biology and Medicine*[1]. His system - oriented physiology made a lasting impact on the field and earned him in 1949 the Nobel Prize for Physiology and Medicine. He did not underestimate the importance of new methodology, but it had always to be applied and considered in the context with functional systems of the organism.

When Tom Ban and yourself were at Vanderbilt, Tom's view would have been that these drugs aren't all the same and there are going to be some groups of depressed people who will respond to different antidepressants or antipsychotics and we need to able to predict who is going to respond to what.

Well, if you look at antidepressants, I think you are correct. Rick Shelton and I have investigated protein kinase A activity in fibroblasts from depressed people. When we broke the code, three groups emerged. The group with the statistically highly significant decrease of PKA activity belonged to what DSM-IV designates as major depression of the melancholic type. Then we had one group with PKA activities, which were statistically closer to control values. These were subjects with atypical depression. A third group which could not be classified as either major depressive or atypical depression was intermediate. Now when you come to drug responses, it looks to me that monoamine oxidase inhibitors and tricyclics are therapeutically effective in both subgroups and perhaps more efficacious in major depression of the melancholic type whereas SSRIs might be more effective in patients with atypical depression.

So, I think Tom is probably right. However, I wouldn't go so far yet as to say that you can subtype patients based on PKA activity and predict which drug may be more effective. What is becoming apparent, however, is that dual uptake inhibitors such as venlafaxine seem to be more effective in severe depression and may be of value in treating therapy-resistant depression. Perhaps, this would be expected from drugs with double signalling via noradrenaline and serotonin receptor cascades!

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[1] Perspectives in Biology and Medicine 6, 400-423 (1993).

I personally have serious doubts about the overall effectiveness of available treatments, particularly if you take into consideration the rates of remission which are rather disappointing. I was involved in the first clinical trial with desipramine after we isolated it from the brains of rats chronically treated with imipramine.

Was this the one that was Kline, Brodie and Simpson?

Yes, and the late Fritz Freyhan was involved. I still remember, as a young postdoc, I was driving Brodie down to St. Elizabeth's Hospital where Fritz Freyhan had just completed a small placebo-controlled study with desipramine. Only a few patients though, but you could clearly see, even if you were not a psychiatrist, that some of them were very cheerful and they chatted with us and smiled and others looked sad, unhappy and even cried. When the code was broken, all the ones who seemed to be much improved were the ones on the placebo! A small sample though, but it impressed me as a pharmacologist. I have witnessed at Vanderbilt enormous placebo responses, not just 20% but 40% or 45%. I think we should seriously study the "pharmacology" of the placebo or more precisely the physiological and molecular mechanism of the therapeutic action of the placebo. There was a recent meta-analysis study published in the *British Journal of Psychiatry* comparing antidepressants with active placebos - anticholinergic agents. Only two out of the nine studies examined showed a consistent significant difference in favour of the drug.

Well, we don't know that anticholinergic agents aren't anti-depressant.

This is true. There are no good double-blind, placebo-controlled studies on this issue. You don't imply that the reason why the tricyclics are perhaps more efficacious antidepressants is because of their anticholinergic component?

Not an impossibility.

A possibility okay. Though I think there will never be a comprehensive study on this because the peripheral side effects are so unpleasant. I sometimes wonder whether these side effects in some way generate expectations in patients resulting in some therapeutic efficacy.

Have the side effects been overestimated? Anti-cholinergic drugs are to some extent euphoriants.

Well, there are probably some mechanisms involved in the therapeutic action that are totally unrelated to aminergic and or cholinergic receptor interactions. I always thought that the action of drugs like DMI depends totally on noradrenaline and indeed a large number of pharmacological effects including desensitization and down-regulation of beta adrenoceptors are dependent on the synaptic availability of noradrenaline. However, experiments conducted in our laboratory have recently shown that a noradrenergic tricyclic drug such as DMI can exert potent effects in the brain independent of the availability of noradrenaline. Chronic administration of DMI can significantly increase the steady state levels of the glucocorticoid receptor MRNA in the hippocampus to the same degree in the presence and absence of brain noradrenaline. These results are consistent with previous in vitro studies by Barden's group in Canada demonstrating

that DMI significantly increased GR promoter activity of a CAT reporter gene in the absence of noradrenaline.

So, we have to be prepared for direct actions of drugs, including anticholinergics, on post-receptor events, on transcription factors for instance and the translocation of these transcription factors from the cytoplasm to the nucleus, as has been so elegantly demonstrated by Pariante and Miller at Emory. I strongly believe, that in order to get more efficacious antidepressants, and antidepressants with a truly shorter onset of therapeutic action, we will have to pursue these new avenues and we have to advance our understanding of the molecular psychopathology of depression that alone will facilitate the development of novel pharmacotherapeutic approaches. More studies on the action of drugs in normal animals and in tissue culture in vitro will not shed light on the psychopathology of affective disorders. Again W.R. Hess is right!

But going into new areas is very expensive and you have no guarantees that you will succeed. It's easier to make a me-too drug, which is what we have been doing for the last 40 years. If you can get 20% of fluoxetine's market you can make $400-500 million a year with very little investment in R&D. The pharmaceutical industry has generated more SSRIs, fluvoxamine, sertraline, citalopram, paroxetine. I don't know how to solve this dilemma in an industrial society that is so heavily driven by profits. Perhaps, large foundations such as the DANA Foundation will help to break the impact.

Let me take you back to the mid-1970s. The receptor story has begun to role. But in order to make sense of the data you have, it seems to me, invent, at least from the point of view of receptor theory, a whole new concept, down-regulation. Was it you who invented the concept or where did the concept actually come from - the idea that it takes two weeks to change the receptor system? This is not anything that the receptor theory before that would have proposed.

No, I don't think so, at least not in the brain. The terms down-regulation and or desensitization of the beta adrenoceptor coupled adenylate cyclase system in brain got coined when it was demonstrated in our laboratory in I975 that antidepressants, given on a clinically relevant time basis, reduced the sensitivity of the beta adrenoceptor system to noradrenaline and or decreased the number of beta adrenoceptors. Since we know now that the phenomenon depends on the availability of noradrenaline, it reminded us of the phenomenon termed "tachyphylaxis", a term that refers to the fact that treatment of tissues or cultured cells with beta adrenergic agonists leads to a reduction in the tissue's subsequent responsiveness to beta aminergic agonists. As I have previously mentioned, the term desensitization is related to the functional deamplification of the beta adrenoceptor system with decreased responsiveness to the physiological agonist, in this case noradrenaline. Our group did not measure receptor numbers early on for reasons previously emphasized.

I think it was Cuatrecasas who distinguished between receptors and acceptors. He demonstrated high affinity, stereospecific and reversible binding of certain ligands to glass walls and millipore filters. This is what he called binding to acceptors since there was obviously no function involved. Binding to receptors means coupling to effectors such as enzymes or ion channels. Fortunately, we did study the function of receptors i.e., formation of cyclic AMP via activation

of adenylate cyclase.

With the beta receptor story, you appeared for a long period of time to have put a nail in Brodie's idea that 5HT was important for antidepressant actions.

Yes, in the late '70s perhaps, but the 5HT idea got resurrected as we began to better understand the action of serotonin beyond the receptors. Obviously, we had to face that problem with the SSRIs, which do not affect the beta adrenoceptor system. A great deal of the work to solve this problem was done in my laboratory by Elaine Sanders-Bush. She discovered that drugs, which increased the availability of serotonin by inhibiting its reuptake enhance PI hydrolysis with the formation of two second messengers, DAG and IP3 both of which activate protein kinases, PKC and calcium/calmodulin stimulated protein kinase, respectively.

This then led to the notion that the aminergic signals converge beyond the receptors at the level of protein kinase mediated phosphorylation that can be synergistic at the level of e.g., phosphorylation of transcription factors such as CREB. One implication of this is that dual action agents such as venlafaxine might be more potent because the 5HT and noradrenaline signals are synergistic further down in the cascade. Studies by Craig Nelson from Yale would be supportive of such a view. He has combined desipramine and fluoxetine and found that the antidepressant action was not only enhanced but the onset was faster as compared to that of desipramine or fluoxetine alone. So, we know that serotonin has a role in antidepressant action and Brodie was not at all wrong though in the early '60s before the SSRIs appeared on the scene, noradrenaline was the relevant neurotransmitter for antidepressant activity.

I remember an experiment Marcel Bickel and I did that almost cost us our jobs. We found that the reserpine - like depression was not reversed by desipramine in rats if you depleted brain noradrenaline. This was the first demonstration that the drug's action depended on noradrenaline. We told Brodie this on the board walk in Atlantic City, at the spring meeting of FASEB. Lovely board walk. Boy, he got so mad at us. He said that we are planning experiments to disprove his hypothesis. He felt we had sabotaged his idea. This was of course not true. All we wanted to know is if desipramine's action in reversing the reserpine - like depression depended on brain serotonin or noradrenaline. We had depleted serotonin and the drug still worked. We depleted noradrenaline, and desipramine didn't work anymore. So, it was fair to conclude that the desipramine exerted its antidepressant action via noradrenaline and not serotonin.

I think in science you have to be prepared to revise hypotheses and concepts as new information is generated. The truth of today maybe the error of tomorrow. I think this is the beauty of science, unlike in philosophy or theology, you can experimentally prove or disprove hypotheses. Scientific truths are very relative because they depend on methodology. I would not have embraced this notion when I was young and thought that scientific truths are absolute truths.

Absolutely, I agree completely. We have focussed on the interaction between the two systems, the noradrenergic system and the 5HT system, but lately you have been concerned with the concepts like synaptic plasticity, which seems to go beyond these neurotransmitters and may require newer methods.

Yes, this new direction in antidepressant drug research had something to do with the arrival of a new postdoctoral student, Paul Rossby. It was almost like the Vetulani story. Paul was a molecular biologist with keen interests in neurobiology and brain function. I felt that his background and expertise would ideally complement my own research interests. So, we started chatting about the brain and the delayed therapeutic action of antidepressants and other psychotropic drugs. We thought that we should investigate if chronic treatment with antidepressants could affect gene expression in brain because that would be a process that takes time. It was during these informal chats that the idea of testing the "5HT/NA/glucocorticoid" link hypothesis of affective disorders at the level of gene expression was generated. The move to drug action beyond the neurotransmitter receptors had started, conceptually at least.

We know that approximately 20,000 genes are expressed exclusively in the brain. So, we argued that mental and emotional phenomena could involve the regulated expression of some or ail of these genes in extremely complex temporal patterns. Paul argued that the best analogy is perhaps a symphony played by a 20,000-piece orchestra! Using this analogy, the pathogenesis of any CNS disorder would involve some number of instruments (genes) producing "dissonance" by playing their parts at the wrong times, or for the wrong durations or at the wrong amplitudes in comparison with the same instruments in a normal healthy CNS.

From a clinical perspective, the development of methodology to detect impairments in the transduction cascade beyond the receptors leading to full expression of programs of genes should enable us to design a novel generation of antidepressants, which restore harmony by regulating not only the amplitudes of gene products but also their rhythms. We know now from experiments conducted in many laboratories, including our own, that antidepressants such as DMI do indeed affect the expression of various genes after their chronic administration - tyrosine hydroxylase, beta adrenoceptors, CRF, glucocorticoid receptors, BDNF and trKB, to mention a few. Moreover, drugs such as DMI influence the transport of transcription factors such as GR from the cytoplasm to the nucleus. Through activation of the aminergic receptor cascades they affect protein phosphorylation via PKA, PKC and calcium/calmodulin dependent protein kinase and in doing so, they influence the cytoplasmic - nuclear trafficking of transcription factors, their dimerization, affinity to responsive elements in promoters of genes and ultimately programs of gene transcription.

To use the analogy with the orchestra, they turn on various sections in the symphony, the first violins, the woodwinds, flutes, oboes, bassoons, etc. So, the stage is set for the detection of the action of these drugs on various targets between the receptors and the double helix in the nucleus. To hasten progress in this new and exciting area, we do need new methodology. The methodology which we use in our laboratory at this time is the differential display reverse transcriptase polymerase chain reaction methodology, developed by Peng Liang at Harvard and now at Vanderbilt. This differential display technique is capable of producing the complete pattern of all of the genes, 10-1 5,000 expressed in a particular cell. Differentially expressed genes can be recovered, reamplified and cloned for sequencing and characterization.

In your chapter in "Antidepressants at the Dawn of the Next Millennium" you say the drugs we have at the moment compensate, they don't cure. They are not putting things right. Do

you envisage new drugs making a radical difference?

Yes, I do. I believe that as we begin to test the "5HT/NA/glucocorticoid link" hypothesis and determine how these molecules act in concert to affect signal transduction pathways, gene expression and ultimately, emotions and behaviour, we will be in a much better position to develop the next generation of antidepressants which will meet the 3 as yet unmet criteria - increased efficacy, a truly faster onset of action and efficacy in therapy-resistant depression. It will cost money to get there but if you have a potential for a $5 billion market, it seems to me worthwhile to invest a few millions.

Your chapter in that book, which is supposed to be about the future, is the only one that really looks to the future.

I think it is gratifying and refreshing to speculate where we will travel in the future. To quote Joel Eikes, "traveling is more fun than to arrive." At Vanderbilt, we are trying to put together a consortium on the molecular neurobiology of depression, with the best people in the world participating, no matter where they reside. I could have retired last year but I stayed on to help implement this scientific collision-coupling of ideas. We have over the years travelled all the way from presynaptic events to the receptors, to second messengers to the protein kinases and now we have arrived at the nucleus. The work and the fun have just begun!

You've hinted at a whole new theme. Your receptor hypothesis moved us from the pre-synaptic area to the post-synaptic area. Now the pre-synaptic area is involved with learning, conditioning, behaviourism that kind of approach but the post-synaptic receptor is much more the potential site of a lesion. Were you aware of any impacts of your work in this area?

Important as the post-synaptic receptors are, I believe that what you call a potential site of a lesion is located beyond the receptors. For example, our findings of an impaired PKA activity in human fibroblasts from patients with major depression are indicative of a potential site of a lesion beyond the receptors. It will affect up-stream the regulation of receptors and down-stream the phosphorylation of transcription factors and consequently the rate of programs of gene expression. The receptor mediated signal transduction cascade represents a biologically important amplification process with small changes occurring at the receptor level being amplified via second messengers and protein kinase mediated phosphorylation processes, with a maximal amplification occurring at the level of gene activation and/or repression. It is too early to measure an impact of our recent work in this area.

Well, that's very much back to issues of plasticity and behaviour but there was a period in the 1970s and 1980s because of the dominance of receptor views, perhaps not your receptor views but receptor views, when it did seem almost as though what people expected was there would be a lesioned receptor which drug treatment puts right. Would you agree that that's how things were seen?

Yes, if you imply that the receptor lesion meant an inability of the receptor to adapt, say to an increased concentration of noradrenaline. I think it was Eric Stone at NYU who actually proposed that all that antidepressants do, is help to adapt to stress by down-regulation of the beta

adrenoceptors and or desensitization. Today we know of course that it is not the beta receptor per se that is someway faulty, but it is more likely the regulation and or fine-tuning of the receptor's sensitivity by protein kinase mediated phosphorylation that is impaired.

The beta receptor hypothesis became the place where the antidepressant action was at.

Yes, it had a relatively long half-life. Ten years or more. It has opened the gate for research on second messenger mediated cascades by noradrenergic and serotonergic receptors and their multitude of subtypes. Perhaps, if the desensitization and or down-regulation of the beta adrenoceptor coupled adenylate cyclase system following chronic administration of antidepressants would not have been discovered, the journey to the nucleus with its 10,000-piece orchestra would not have been undertaken or at least, it would have been delayed.

Can I ask you this? Despite having a functional view, you seem to think that antidepressant signals should converge at some point. Why is there such a powerful need, do you think, on all our parts to have some common mechanism of antidepressant action? For example, I have often used an antipsychotic to get depressed patients well and there's good clinical trial evidence that these drugs work for some patients. There's also a good functional reason to explain how they work - they reduce levels of agitation. Drugs acting on noradrenergic systems seem to be doing something that is functionally very different. Is there any need for these functionally diverse effects to converge to some common physiological mechanism?

I don't know about you David, but what I refer to as the common site of antidepressant action is the final step beyond the receptors at the level of altering programs of gene expression resulting in improvement of mood. There are multiple ways to get there, however. Through the NA receptor cascade (secondary amines of tricyclics; reboxetine, ECT, REM sleep deprivation), the 5HT receptor cascade (SSRI's), both cascades (MAO inhibitors, tertiary amines of tricyclics, dual uptake inhibitors such as venlafaxine) and perhaps sunlight. Some antipsychotic drugs such as chlorpromazine increase the availability of NA at post-synaptic receptor sites as a consequence of alpha 2-adrenoceptor blockade and inhibition of noradrenaline reuptake and thus share the beta adrenoceptor down-regulating action with antidepressants. In addition, as you mentioned, the reduction in the level of agitation may be beneficial in "agitated" depressions. The ultimate proof of the common mechanism of all antidepressant treatments - pharmacological and nonpharmacological - will have to await the identification of the individual genes in the altered programs of gene expression i.e., the identification of the individual players in the string, wood wind or brass sections of the symphony!

Let me take you back through two or three other people whom you have mentioned. Alfred Pletscher for instance, what kind of a role in the field did he have?

Alfred Pletscher had an enormous influence in shaping the field of biochemical psychopharmacology. It was Alfred Pletscher who together with Park Shore demonstrated in 1955 in Brodie's laboratory that reserpine's tranquilizing action was associated with a dose - dependent depletion of brain serotonin. This was approximately 20 years before neurotransmitter receptors became fashionable and it opened up the entire field of the neurobiology of biogenic amines in the central nervous system. Pletscher was also one of the

first investigators to use spectrofluorimetic methods to quantitate the small amounts of serotonin in the brain.

When he returned to Switzerland to become Director of Research at Hoffmann LaRoche, he developed the synthetic benzoquinolizines - tetrabenazine and RO41284, which I used later in Brodie's laboratory to produce the reserpine - like syndrome. As you may remember, its reversal by chronic treatment with impramine led to the discovery of the N-demethylated secondary amine, desipramine (DMI). So, a Swiss facilitated the research of another Swiss! Besides contributing substantially to the birth of biological psychiatry, Alfred Pletscher was a mentor for a generation of younger psychopharmacologists and, as President of the Swiss Academy of Medical Sciences, he shaped research policies that put Switzerland's biomedical research on the map. Finally, without Pletscher's wise advice, I probably would have been practicing general medicine somewhere in the Swiss Alps!

Before him the big figurehead was Hugo Bein.

Hugo Bein was at Ciba when I was a post-doctoral fellow with Franz Gross in 1954. He was the one who did the early work with reserpine. Schlittler and Muller had isolated the alkaloid from Rauwolfia Serpentina Benth and Bein was the one who worked on the pharmacology that catalysed the development of reserpine as a drug for the treatment of hypertension and schizophrenia. I think the studies on reserpine from Hugo Bein's laboratory and then the clinical reports that reserpine can cause depressive reactions in patients treated for hypertension influenced Brodie's thinking. That's why we used in the late ‘50s the "reserpine model of depression" to detect antidepressant effects of drugs. The reserpine story represents another milestone in the history of psychopharmacology with both Hugo Bein and Alfred Pletscher playing significant roles.

Can you tell me anything about a period, which you inaugurated? Because of the beta receptor hypothesis and the alpha-2 receptor hypothesis from Crews and Smith, a generation of psychiatric researchers, Gene Paykel and me included, looked at alpha-2 receptors on platelets and beta receptors on lymphocytes etc. Did you at any point think we were doing the right thing or did you always think well...?

I always thought that measuring changes in the number of aminergic receptors in either platelets or lymphocytes reflected fluctuations in circulating catecholamines and the density of these receptors e.g., beta adrenoceptors in lymphocytes were an indication of the severity of stress. This is perhaps why your studies in depressed patients showed an increase in beta adrenoceptors, downregulated by antidepressants, while studies from New York reported a reduced density of beta adrenoceptors in lymphocytes from depressed patients which perhaps indicates that there is more stress in New York than in the Irish countryside! Maybe, if there wouldn't be a blood brain barrier, a stress induced increase in circulating catecholamines would have an antidepressant effect as it would mimic the action of antidepressant drugs? You remember from our earlier discussion that Eric Stone postulated that antidepressants facilitate the adaptation to stress.

So, you thought that we were all wasting our time?

No. Platelets and lymphocytes display aminergic receptors linked to second messenger systems and, with the caveat previously expressed, can be used as model systems in psychopharmacology. Functional approaches such as the one used by Kay and his collaborators are, however, preferable. These workers have demonstrated that lymphocytes from bipolar depressives have a reduced capacity to down-regulate beta adrenoceptors in response to isoprenaline.

Did you at any point feel that your hypothesis did a lot to make psychiatry biological? It seemed to give people a view of what was happening that they thought they could understand.

Well, the only thing I felt, was as Kety would say, it had heuristic value. Without it, a lot of us would not do what we are doing right now. The hypothesis generated new hypotheses and catalysed the scientific journey from presynaptic to nuclear events.

Why are you so focused on the future? What is it about scientists that make them anticipate what's going to happen? You and Julius Axelrod and a few other people have worked into your 70s and 80s.

Yes, Julius Axelrod is still working. He is in the high 80s! I think it is the passion to know and the joy of discovery that keeps scientists going. Horace Judson in the Search for Solutions has said it probably best: "Science has several rewards, but the greatest is that it is the most interesting, difficult, pitiless, exciting and beautiful pursuit that we have yet found. Science is our 'century's art.' The deeper we see into nature, the more beauty we find!"

I believe that neurobiology and biological psychiatry in particular, are truly the last scientific frontiers, with complex processes such as the molecular basis of perception, the true basis of emotions and the nature of thoughts, still eluding us. It is the future that holds the key for our search for solutions!

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*Adapted from The Psychopharmacologists, Volume I. Interviews by David Healy. London, Weinham, New York, Tokyo, Melbourne, Madrid: Altman. An Imprint of Chapman and Hall; 1996, pp. 239 - 258.

April 7, 2022