VISUALISING RECEPTORS – AND MORE
SOL SNYDER

Could we begin with where you were born, your early training and how you thought you might go into medicine?

I was born in Washington DC in 1938. I am the second of five children. I went to regular public schools and didn't really know what I wanted to do. There was no powerful direction from my parents. My father worked at the US cryptanalytic agency, called The National Security Agency and so he admired science. He used to say that science was very nice and he majored in chemistry in college. When I was in High School I was influenced by what my friends were doing. My own predilection was for philosophy and but that's not much of a job for a nice Jewish boy. In those days in the 50s everybody was going into engineering – the military industrial complex was developing. But I hated that sort of thing.

Then I noticed some of my friends thought that they would go to college to be doctors which I hadn't thought about very much. I wondered about psychiatry as a profession akin to philosophy, which was sort of stupid. So I became a pre-med like my friends. The other major influence in my life then was that I played the classical guitar and concertized. My teacher was the best friend of Andres Segovia. So a career in that was a possibility, something my mother, an atypical Jewish mother actually was advocating. After High School I could have spent the summer in Sienna, Italy, in master-classes with Segovia, but I wanted to go to college and there wasn't enough money to go to Sienna also. So I just went to college but the guitar became important because I worked my way through college giving guitar lessons at night.

One of my students was in the first class at the NIH of the Research Associates, a program the NIH had set up in which you could do your military service at the NIH. In those days every single male medical school graduate in the United States had to do two years military service and they could be sent to Korea - which was terrible. What was incredible was that the National Institute of Health was part of the Public Health Service, which was a military commissioned corps, so positions there were highly desirable. A fellow named Donald Brown was in the very first group - Irv Kopin was in the same class. He was a guitar student of mine and needed some help in the lab the summer before I started Georgetown Medical School. I was just 19 years old.

So I worked in the lab. I had never liked science in college – memorising books but research was fun and I got really involved. At the same time as I was working there in the summer, my sister's husband was a psychologist who had done studies of perceptual closure - one of the Gestalt phenomena. He got me interested in giving tests to the psychiatric patients as part of research with schizophrenic patients at the NIH. So I did that on the side. This was in the Laboratory of Clinical Science under Seymour Kety. He had set up research wards with schizophrenics and wards with normal controls. All that got me interested in mental illness, especially schizophrenia, trying to understand what was going on. It worked out very well and I published a number of papers while I was a medical student.

Meanwhile I was working in the summers at the NIH with Donald Brown who was a wonderful mentor - we've stayed close friends ever since - and I liked it

a lot. I just knew I wanted to be a psychiatrist. I never wavered since High School. But I thought gee, maybe I can also dodge the draft and maybe after two years of psychiatry residency I could come to the NIH and get the third year of residency while I was doing my military obligation. What happened is when I was in the last year of medical school I met the lady who would be my wife. It was clear that after interning I would have to come back to Washington for her to finish college. That was a complicated time because I had a matching program and it was too late to get positions at the NIH right after internship. I was panicked. I wasn't sure what to do. I started walking around the NIH, talking to the people that I knew because I had worked all my summer and elected periods at the NIH. Now, Julie Axelrod had a lab right across the hall from the lab of Marion Kies, the lady who was the head of the lab, in which Donald Brown was my direct supervisor. Julie Axelrod had done some experiments together with them in one of the summers. The matching program was complete but the fellow that had matched with Julie had dropped out and Julie said to me you know I have this opening. He said normally Sol, its so hard to get these positions - there are only 10 of them in NIH and they have thousands of applicants, all from Harvard and Yale - but you know, he said, I don't have anyone else for the slot and I thought you did good work so you can come. That was the key event of my life.

Of course as Julie Axelrod was doing research on neurotransmitters and drugs, which made very good sense from a psychiatric point of view, this made me very very happy. The training with Julie was the key event in my life because he was an incredible mentor. At that time in the lab there were just a few people - his lab never had many people. There was Jacques Glowinski, Leslie Iversen, myself and Dick Wurtman was there for the first year I was there. I was a very close friends with Leslie Iversen and Jacques Glowinski. We did a lot of things together and stayed close friends. My lab was right next to Julie's. He had a lab bench in one half of the lab and mine was the other half. I was very physically close to him, much closer physically than the others. And during the summers that I had worked in the NIH, in Marion Kies's lab, I was doing biochemistry, so I actually had more real biochemical background than most of the other people in the lab. Now what Julie liked to do was biochemistry and purifying enzymes, so we had a scientific link. Julie was a lot like my father in personality - just one year younger. We just clicked and he and I became very close. He was a wonderful mentor to everybody and he especially influenced me.

How much do you suppose the fact that he'd used radiolabelled agents to make some of the breakthroughs that he made, influenced you in due course to use radio labelled agents to get the receptor? Do you think there's a link there?

Yes there's a certain link in that. I became well versed in the use of radio-activity at a time when there wasn't that much use of it. Actually in my work as a summer student, starting in 1958, I was already using radioactivity right across the hall. At that time it wasn't used a great deal. The introduction of radioactive labels into biochemical research is one of the biggest revolutions in biomedical research. Very few people realise that.

Can you pinpoint the origins of this?

I’m not a great historian of science but I read a little about it. What happened was, after the development of the cyclotron and all the events of World War II out at Berkeley, one of the people there, Martin Kamen, realised that this Carbon14 they were making could be used for biomedical research and he started using it as a tracer. I think one of the first people to use it most effectively was Melvin Calvin, also at Berkeley. He used Carbon 14 to work out photosynthesis. There were some others in the late 40s but even into the 50s, there was not much use of radioactivity because there weren’t a lot of radiolabelled drugs or biochemicals available. People mostly made them themselves. One of the biggest advances was the commercial development of radiolabels.

There were at first a couple of companies that were sort of Mom and Pop garage operations. The first one that made a real business of it was New England Nuclear and they only began in the mid-1950s. They had a catalogue and a salesman, a fellow named Howard Novich. I remember meeting him when I was 19 years old when he would come around. I had used radio-lables, while I was still working as a medical student, but when I was with Julie, as a Research Associate and had resources to order lots of things, I could immediately see the power of the technique. Julie’s major breakthroughs involved radiolabelled activity. The other people in neuropharmacology at the time were not using this. The other major lab, Brodie’s would measure endogenous levels of neurotransmitter, using the fluorometric technique, which they developed, to measure serotonin and other things. Brodie and Sidney Udenfriend, using this could measure endogenous levels of neurotransmitters, but Julie Axelrod was shrewd enough to see you could do much better with radio labels. You could do things faster, you could monitor metabolism and using radioactive norepinephrine and epinephrine he discovered the uptake phenomena, which never would have been found otherwise. So that was the thinking set of the laboratory and I got very much involved with that. I probably became much more facile with the use of radioactivity than most young scientists.

The other thing was that there was carte blanche - you could order anything you want. Nobody ever checked an order. You could order almost infinitely. I once asked Julie was there a budget for the lab and he didn’t even know. You could order anything you wanted. There was at a higher level some sort of budget but people didn’t pay much attention. It seemed to be a flexible bid. So I’d go to the New England Nuclear catalogue, look for reactive chemicals that I hadn’t thought about before, and I’d get ideas for experiments from what was in the catalogue. Then, I’d order them and do things with them, which I don’t think many other people would have thought of doing. Of course the key thing was having an unlimited budget because they were very expensive.

Before we move on to the receptor story, how much does the availability of the new technique drive the field?
Well in all science, Julie always said a new technique is an important thing. Science is driven by new technology, which is proved over and over again but many scientists don’t realise it.

They like to think it’s the great ideas, the great insights.

Well everything is important but techniques probably generate more ideas than vice versa. People are starting to appreciate this at least in molecular biology. In this morning's symposium, it was pointed out that the revolutions in microbiology had included three Nobel Prizes strictly for techniques. One for restriction enzymes, one for DNA sequencing and one for PCR. All in the last 20 years, just for pure techniques.

Julie would say, for instance, in measuring something you could go to the literature and look up how people used to do it and follow it like a cook book or sometimes you could figure out something yourself that would be better than people used to do and you'd have developed something novel. That came up for instance when we were working on serotonin in the pineal gland. Basically Julie had seen a paper by fellow named Wilbert Quay showing that the serotonin level in the pineal gland was enormous, a hundred times the level in the brain and it was 10 times higher at midnight. Julie said you know that's incredible, if one could just figure out what's going on there. However, with the technique used in that paper, just to measure the day night cycling would require 200 rats. It was a very insensitive technique. Now Julie had noticed a paper in the literature where somebody had just heated serotonin with ninhydrin, a reagent used to stain amino acids, and they got a fluorescent product that seemed to be about 10 times more fluorescent than what was used for the standard method for measuring serotonin. This fellow had published in a chemistry journal, so it hadn't been used to actually measure serotonin. So Julie said to me, you know Sol maybe that would make a new technique and we could measure the serotonin in the pineal gland and actually do something with this system. Sure enough I used that and I developed it into a new method of measuring serotonin that was roughly 10 times more sensitive than the standard method. This meant I could measure the serotonin in two or three pineal rats rather than 200 and that made a big difference.

When did you move from Julie's lab over to Hopkins?
I worked with Julie for two years 1963 to 1965. In 1965 I went to John's Hopkins for a psychiatry residency because I had never changed my mind about that. The arrangement that was worked out was that in the first year I would be a full time psychiatry resident and in the second year I would have an appointment as full time Assistant Professor in Pharmacology with a lab and I would do teaching and research but I still would continue as a psychiatry resident for nearly 3½ days a week. And then in the last year of residency may be two days a week residency and three days in the lab. But I still got credit for full time residency while I was on the faculty.

Joel Elkes was the Chair at Hopkins at the time and the Hopkins Department of Psychiatry had a number of people in it who've since become major stars in the field. It must have been a very exciting place.
Oh it was nice. I actually wasn't spending a lot of time at psychiatry. There were no researchers in psychiatry. When I came to Hopkins I was the only person in the whole medical school who did any research on drugs or neurotransmitters. In the psychiatry department a fellow named Jerry Frank was a very eminent psychiatrist in figuring out how psychotherapy worked. There was a team across different schools studying that. Some of people at

Hopkins collaborated with Karl Rickels at Penn, in studying anti-anxiety drugs and there was a medical liason service.

After completing residency, there was a laboratory for me in the psychiatry department lab areas and but I was already an Assistant Professor of Pharmacology and had a laboratory in the pharmacology department where there were other people you could borrow a cup of sugar from. The psychiatry labs were in a clinical building where there wouldn't be many fellow spirits so I kept my primary appointment in pharmacology with the secondary appointment in psychiatry.

Okay so let's move into the early 1970s before the receptor story began to open up. One of the things that from the outside looks as though it would be important in later receptor work was the radio labelling of hormones. Did this play any part in your thinking?
Yes. What happened is that my staying in pharmacology was relevant because in 1970 there came to Hopkins pharmacology a fellow named Pedro Cuatrecasas. He had been at the NIH working with Christian Anfinsen, who invented affinity chromatography, a major advance in purifying proteins. He had just started at the NIH on attempts to measure the insulin receptor. Pedro was doing a lot of this kind of work in the lab next to me at Hopkins and he and I were close friends and still are. I thought his work was fascinating. I tried to think of ways to make it relevant to the kinds of things I was interested in. Now at that time there was a lead article in Science on the full amino acid sequence of nerve growth factor. They didn't have DNA Cloning - they just took the protein and sequenced it amino acid by amino acid. The punchline of the abstract was that the amino acid sequence of nerve growth factor had many similarities to the sequence of proinsulin.

I said, ah ha now nerve growth factor stimulates nerve growth at very low concentrations, there must be a specific receptor for it. Maybe its sequence is similar to the insulin receptor and maybe you could find it in similar ways to the way they found the insulin receptor. I had a brand new post-doctorate fellow named Shailesh Banerjee joining me then so I asked Pedro if maybe we could collaborate and he could show Shailesh how to use his filter machine. He had a manifold filter machine, where you could filter a large number of samples simultaneously with a strong vacuum. This way things could be filtered so rapidly that the fluid wouldn't just pool above the filter and let the ligand bound to the receptor wash away. Because it filtered rapidly you could also wash it many times and get rid of non-specific binding and you could do many samples simultaneously so that you wouldn't have variability.

How long did it take to work out that you needed to wash these things this way. I mean this has to have been very hit and miss at the start.
Well Pedro had been doing that with the insulin receptor and he had the filter manifold developed by the machine shop in the NIH before he came to Hopkins. So he had worked out the basics of how to do it.

How many years do you think it took him to get things to the point where he could show clear and specific binding?
Oh for the insulin receptor, I don't think it took very long. He was a very efficient guy, very talented. I think he worked most of it out in a year or so.

He had a competitor, a fellow named Jesse Roth, who was also at the NIH when he was there, who independently identified the insulin receptor. They were competing with each other but I have no idea of the details because I wasn't there. But this gave him quite a bit of incentive to work things out fast.

Anyway we worked on the nerve growth factor receptor and of course we had to work out conditions for that as well but with Pedro's expertise it was very easy. We identified the NGF receptor. It was fascinating, we wrote a couple of papers about it. This was in 72/73. Around about that time, Richard Nixon declared war on drug abuse and appointed Jerry Jaffe as the Drug Czar with enormous amounts of funds under his control. Jerry was a friend. He is a biological psychiatrist. Another mutual friend, Arnie Mandell, had the idea that there was a lot of money there and we should talk to Jerry and get him to put some money into research. One way or another money was allocated to have drug abuse research centres and we at Hopkins applied for one.

All of those factors came together plus the fact I had long talks with Jerry Jaffe, who needed help immediately. He had been appointed Drug Czar to take care of all the heroin addicts in the army and co-ordinate all Federal research on drug abuse and he had no resources. The only thing he had was the ability to tap into anybody in the military service at a moment's notice. He called me and asked if I had any ideas. Now the first year I started at Hopkins, whilst still a second year resident on the faculty, two second year medical students came to work with me. They were the beginning of my lab. One was named Joe Coyle and the other was named Alan Green. Just at the time Jerry Jaffe had been appointed as Czar of drug abuse, both of these fellows started as Research Associates at NIH in the military. Joe Coyle with Julie Axelrod. Alan Green with Erminio Costa. Alan wasn't all that happy, he was always interested in politics. Jerry told me about his dilemma and I said I have an idea. I have this medical student Alan Green, who he is doing very good research and he's in the military public health service. He might be interested in some work in this area. Within 24 hours, Alan Green was Research Director for all drug abuse for all America under Jerry Jaffe and he was my student. So that didn't hurt.

Meanwhile Jerry was pestering me with Sol what are you guys doing for drug abuse there. Why don't you do something on opiates - what's wrong with you? So that got me thinking about things to do and here we were working on receptors anyway. Then the final thing was this. In one of the summers, at a Gordon Conference, 71 or 72, Avram Goldstein had given a talk about the concept of identifying opiate receptors. One strategy might be to look for stereospecific binding of radioactive opiates to the receptor. He'd done some experiments but only 2% of the binding was stereospecific. As it turns out that was to a lipid, it wasn't to the opiate receptor. But he got me thinking that indeed of all the problems in the opiate field that was the key thing. There were many ways of studying opiate addiction, all sorts of research one could do, but if one was to ask what's the most important question, the most important question was what is the opiate receptor. So I started thinking about the opiate receptor and the fact that we would need to get a radioactive opiate.

I had a new graduate student, Candace Pert, and she was working on something to do with choline uptake, which was drawing to a close. So I said I have this idea, lets make some radioactive opiates. The only commercially available radioactive opiate was dihydromorphine. We bought some and tried it out on the binding assay. It didn't work. Subsequently we know it didn't work because it's light sensitive and we didn't know you were supposed to turn off the lights.

Small things like that are make or break aren't they?
Yes. Then we said, well maybe it doesn't work because it's an agonist. Maybe you need an antagonist. You could make up a reason why that should be. But in any event we contracted with New England Nuclear to get radiolabelled naloxone which we tried out and the first experiment worked.

This has to have been a huge breakthrough in the sense that nerve growth factor wouldn't have meant anything to the public but the opiate receptor had a huge resonance, didn't it? How much did this help to really put the area on the map not only the area of the opiates but the also the area of receptors?
Yes. One way of even thinking about looking for the opiate receptor is this. Back in 1970 the nicotinic cholinergic receptor in the electric organ of the electric eel had been identified by several different labs. That was a unique situation because in the electric organ of the torpedo, 20% of the membrane protein is the acetylcholine receptor. Now 20% is hard to miss. Whereas you could do armchair calculations that the opiate receptor ought to be one millionth of the brain.

The second point is that alpha bungarotoxin was a tool that the various labs used. It's extremely potent, pseudo-irreversible and we labelled it with Iodine 125, which has a very high specific radioactivity. Now even with all of those things going for it, it was hard to make the breakthrough and that discovery was a big accomplishment. I remember speaking to Julie Axelrod in 1970 when this was coming out at a meeting in Paris where there were a number of Julie's students, Jacques Glowinski, Leslie Iversen, Lincoln Potter and myself and others - it so happened we were all together at a relatively small meeting sponsored by the Roussell drug company – and we were talking about this because it was the biggest discovery of the last couple of years. Now when we were thinking about the opiate receptor, I was thinking that maybe it would be impossible. Maybe it couldn't be done at all but we'd try anyhow.

Did the fact that they'd been able to show there was a nicotine receptor, free you from a handicap that these receptors were still somewhat mythical beasts in one sense in that no one had ever seen one. Did the fact that you knew that the nicotine receptor had been actually found and you were reasonably confident with the work mean that you had the mindset which said well look lets actually go for this one it is there and we can find it.
Well you could take it both ways. From one perspective you could say oh somebody had done binding studies and found a neurotransmitter receptor, isn't that nice now we know it can be done. Receptors weren't biblical entities. I mean there were still people saying a receptor could be some lipid bla bla bla – a lot of nonsense. But most people knew they had got to be protein

receptors because the extraordinary specificity of drug action and neurotransmitter action fits very much with the specificity of enzymes. Everything about the behaviour of receptors is a lot like enzymes. They should be proteins, just like enzymes. So, there were a lot of perhaps not so reasonable people who would say oh no it's just some conformational change or lipid binding and not a real receptor protein. So in that sense the discovery of the nicotinic cholinergic receptor helped but on the other hand it hindered in that it was such a tour de force to be able to find something that was 20% of the protein of the electric eel that it was a hopeless exercise. And indeed it is interesting that nobody jumped into the field as soon as nicotine cholinergic receptor was published in 1970. Nothing happened. We didn't see anybody else doing anything with other receptors in the brain.

People often say they knew where they were when they heard that President Kennedy was shot or when they say the Challenger explode. In the psychopharmacology domain, many people seem to have had the same experience when they heard of your opiate receptor work. I was talking to James Woods the other day who was able to tell me what plane flight he was on going where when he heard the news. That's a measure of the impact on outsiders, it must have been very exciting from the inside.
Well in the very first experiment, Candace was measuring the binding of labelled naloxone to membranes from the guinea pig intestine. To ascertain whether any binding would involve the pharmacologically relevant opiate receptor, we include the plus and minus isomers of levorphanol and a few other opiates of known potencies. We obtained a few thousand counts of total binding. The active isomer of levorphanol lowered the binding by two-thirds while the inactive isomer was inactive and all the other drugs bound with the appropriate potencies. So we knew after the very first experiment that this was the "opiate receptor". Everybody in the lab was quite excited. The experimental technique was sufficiently simple and the result so robust that it was clear that it was very likely that we could answer many important questions in a matter of weeks. Candace and I immediately sat down and thought out exactly what experiments to do. Things moved forward smoothly so that we were able to put together a paper fairly quickly.

Many people could immediately see that the identification of the opiate receptor opened up a possibility of finding answers to many of the key questions of opiate pharmacology that had been mysteries for a hundred years or more. For instance, simply by dissecting a monkey's brain into many small areas we were able to show extremely high concentrations of opiate receptors in discrete areas of the thalamus which are involved in the affective component of pain perception, fitting in with what was known about the clinical effects of morphine. Other localisations could explain why morphine causes nausea or constricts the pupil of the eye.

How did people respond? Well we submitted a paper describing these results, our second paper on the opiate receptor to Nature. The editor sent it back by return mail, without sending it to referees at all. He indicated "There has already appeared a publication on this opiate receptor and one paper for such an entity seems quite enough". I telephoned someone at Nature and

managed to convince them that field might warrant more than one paper. The journal agreed to send the paper to referees and it was published.

A number of others were unenthusiastic. More than one scientist serving as a referee of submitted papers argued that we weren't dealing with the opiate receptor at all. A pharmacologically relevant receptor must include a functional response. A binding site had no biological significance. A substantial number of people also couldn't replicate our findings. I remember receiving a call from my good friend George Aghajanian who was very worried. He said "Sol people have been telling me that there is no opiate receptor. Everything you did is an artefact because they can't reproduce the findings". What was going on was that many laboratories didn't appreciate the importance of rapid filtration when washing brain membranes. If the washing procedure is too slow, there is time for the radioactive ligand to dissociate from the receptor. The vacuum filtration device we had obtained from Pedro Cuatrecasas enabled us to filter a large number of samples simultaneously with very rapid washing. Extensive studies for receptor binding for opiates and other drugs didn't emerge until a company began to market an apparatus like ours.

One of the things that you got into fairly quickly after this was the issue of the dopamine receptor and the dopamine hypothesis for schizophrenia. A range of people like Les Iversen, for instance, say that you were the person who for the first time put the elements of this story together in a way that really made sense. You can trace back the dopamine story to people to the work of people like Arvid Carlsson but it only really took clear shape in the early 1970s when people like you actually began to put it together and began to talk about a dopamine receptor hypothesis. Is this the way you see it or how did that evolve?
Well how it evolved was this. In research, methodology is important but strategy is also very important. So when we identified the opiate receptor, I remember my head of pharmacology, my boss Paul Talalay, who was a very important adviser in my lab, saying now Solly a good biochemist when he had an enzyme he would purify that protein. You've found that opiate receptor now all your resources should go into purifying it. I know that as a membrane protein it must be dissolved with detergents and that hardly anyone has ever isolated a membrane protein but this will be a real challenge - you'll do real protein chemistry. That's one strategy that could have been taken.

That would have been a mistake because nobody to this day has really purified an opiate receptor from the membrane. Instead I said no I think what I would like to do is this. The binding technology can be used for two big things fast. Number one through binding technology we can ask important questions about how opiates work. Number two if this worked for the opiate receptor, we can use it for other neurotransmitter receptors if we're just clever enough to get the right ligand that will bind specifically. It has to have real high affinity and it has to have slow dissociation rates so it doesn't wash off. So I put most of my energies into figuring out how to label receptors, getting the right drug, getting other students working on different receptors.

Dopamine was one of the ones we attacked and we said well lets just try tritiated dopamine itself. Now at that point most of our energies went into

looking for antagonists because our original work with the opiate receptors said the agonists don't work - but that was light sensitivity. So I kept looking for antagonists and the most potent commonly available dopamine antagonist was haloperidol. We got that tritiated and we threw in tritiated dopamine at that point because we were looking for agonists anyhow. I had a new postdoctoral fellow named David Burt, who worked on that. I had another postdoc named Ian Creese who was going to work on opiate pharmacology but when he saw that David Burt was going to work on a dopamine receptor he got very upset because his PhD thesis had been at Cambridge with Susan Iversen as a psychologist doing behavioural ramifications of lesioning the dopamine system. He said "my life is dopamine, how can you let somebody else work on it". I said well you know we'll have two ligands – tritiated dopamine and tritiated haloperidol and David Burt will use the dopamine and you use haloperidol. It turned out that Ian Creese was the more energetic one. He took control of the project and was very good at it, although they collaborated on most of these things.

But dopamine gives a good example of a project in which we used identification of the receptor as a tool for answering questions. Independently Phil Seeman of Toronto found dopamine receptors with tritiated haloperidol at the time. So a number of things became evident. First both dopamine and haloperidol were labelling the receptor but there were relative potencies of drugs in competing with the binding side. And by the way, using receptor binding as a tool to evaluate the relative potencies of drugs became very important because that opened wide pharmacology and it provided a strategy for the drug industry to use to screen new drugs that act on receptors. Coming up with relative potencies of drugs at the receptors labelled with dopamine and haloperidol was different.

At that point, we'd seen different potencies with different ligands for the opiate receptor. Candace had read a lot about allosteric enzymes and Jacques Monod's theories. I knew nothing about it but she taught me about the allosteric properties of enzymes. Jean-Pierre Changeux had adapted the allosteric concept of enzymes to the receptor. The theory said that there's an agonist state of the receptor and an antagonist state of the receptor. With the antagonist we were labelling the antagonist state and with the agonist, the agonist state, so of course the drug potencies were different. That's how we were thinking and we were all wrong.

Actually had we been a little smarter we would have known on theoretical grounds that that was wrong, that dopamine was labelling what we now call the D1 receptor and that haloperidol was labelling what we now call the D2 receptor. We had that in our hands but we didn't interpret the data correctly. But anyway we found that once we looked at the relative potency of drugs it became evident that there was an extraordinarily close correlation between antipsychotic potencies and blocking dopamine receptors as labelled by haloperidol, which we now know to be the D2 receptor. This was remarkable because here we were comparing potencies in a test tube versus potency in man and there were all these intervening variables of absorption, metabolism, penetration into the brain and yet it worked out real good and Phil Seeman independently found the exact same data. Those data provided the first clear data that neuroleptics exert their therapeutic effects on dopamine receptors.

The first biochemical monitoring of dopamine receptors had been done a year or two before by Paul Greengard, who identified a dopamine sensitive adenylate cyclase and showed that neuroleptics blocked that. However, he was measuring was what we now call the D1 receptor and actually relative potencies of neuroleptics at blocking that don't correlate with antipsychotic potency. Haloperidol, the most potent neuroleptic in those days, was in fact quite weak in this assay. He said oh well maybe it isn't soluble when it really was soluble. In contrast, with the dopamine D-2 receptor the results were extraordinary and you could be convinced.

Just about that time, we were having success labelling other receptors. With another MD/PhD student, Steve Peroutka, we compared neuroleptic potencies at histamine H1 receptors, where they are very potent, and at serotonin 5HT-2 receptors, where they are also very potent and at some other sites. We could show that neuroleptics could bind at 10 nmolar at all these other receptors but the relative potencies at these receptors had no correlations with therapeutic effect whereas with dopamine there was a correlation. And that really tended to clinch the argument.

There were two things that seemed to fall out of that. One was as you say it provided the industry with a screening program. They could look and see did their drug displace radio-labelled haloperidol for instance and in this way they could get an idea if this drug was going to be useful clinically or not. How much influence did that have on the way the industry went about trying to actually develop new drugs for schizophrenia?
Well what happened is that the drug industry in general up till that point in time had only one way of screening drugs, which was in intact rats. And that requires that a chemist must synthesise say 25 grams of drug and a lot of rats got used. It became obvious to me that now you could do hundreds of tests easily. We'd got the methodology such that one of my best technicians Adele Snowman, who still works with me, could knock off 500 test tubes in a day, using a fraction of a mg of drug. From a chemist's perspective this means you can synthesise it in a day rather than in a month. And there would be so many things they want to screen.

I felt very strongly that the whole drug industry should change how it developed drugs in general. Very shortly this became evident to people in the drug industry and the first company I became involved with was Sandoz when the Head of Chemistry, a Dr Stephan Guttman, made a special trip to Baltimore to talk to me about this. He saw this from reading our papers on opiate receptors and a couple of other papers. He really felt strongly that at Sandoz the pharmacologists were sort of stupid and the chemists were smart and he didn't want to be dependent on the pharmacologists. He wanted to incorporate into the chemistry division the screening of drugs on receptors so then they would have a rapid feedback. Chemists could synthesise something, check them on receptors and get immediate feedback, modify the chemical and escalate the potency very rapidly. This was easily 10 times more rapid than drug development could have been done before and he didn't want to have to deal with the pharmacologists.

I became a consultant to Sandoz and people from Sandoz would visit our lab and learn the receptor technology. My friend Pedro Cuatrecasas left Hopkins in 1975 to become head of research and development for Burroughs Wellcome and he asked me to be his senior consultant and we did the same thing there. At Dupont pharmaceuticals, I became an ongoing consultant doing the same thing, trying to convince people to do extensive screening. It was actually slow. Although the heads of research in the company would say this is the way to go, when we'd then talk to a scientist at lower levels, chemists especially, they unwilling to advocate screening things - why do it? What I was advocating was to take the big library of chemicals that most drug companies have in the basement, hundreds and thousands and put a massive screen on. Listen I would say put 10 drugs in one test tube mix them all so in 100 test tubes you'd screen 10,000 drugs in one day. Don't worry about it, you'll get a hit and then you worry about which drug was active. But it was very hard to get through. Gradually it started sinking in especially as robotics came along and the ability to make up all of these solutions. Simply weighing out 10,000 drugs takes a lot of time until the technology to simplify the techniques was adopted and fortunately is now routine.

How much did the beta receptor story begin to feed into the momentum. The fact that a similar model for antidepressants came out - the idea that what we want to do is to get all the antidepressants to down regulate the beta adrenergic receptor – which made it feasible to screen for drugs that did this. How much did the fact that this was not just something that applied to the antipsychotics but looked like it could actually apply to all the psychotropic drugs help?
Yes. Well downregulation of beta receptor is just one example. Just measuring drugs that compete in receptor binding, either as agonists or antagonists, became the main thrust of receptor screening in the drug industry then and now. Downregulation of beta receptors was really one example, which wasn't necessarily important in that a lot of people weren't sure that that was the mechanism of action. Beta receptor labelling was done by Bob Lefkowitz initially who did a very nice study of this just about the time or a little bit after we did opiate receptor.

The other thing that was opened up by this radio labelling of the dopamine system was the idea that there are D2 receptors in people who have got schizophrenia and clearly the drugs act on those, so conceivably this is the site of the lesion as well and we can now begin to look at it.
Right the theory that something is abnormal about dopamine in schizophrenia which people call the dopamine hypothesis is based on two things. One neuroleptics act therapeutically by blocking dopamine D2 receptors. That's pretty solid. The other was amphetamine psychosis and that's I think fairly solid but people would argue about it still. Amphetamine at high doses causes an acute paranoid psychosis. Some people would say its like acute paranoid schizophrenia and others would say no. But put it all together and you get too much dopamine is bad for you and less dopamine is good for you. But of course the thing to bear in mind is that that doesn't tell you that dopamine is fundamentally abnormal in schizophrenia at all. It just means that you can manipulate schizophrenic symptoms by manipulating dopamine. I think saying there's a dopamine theory of schizophrenia, which I never ever

said is taking it too far. As a betting person, I would never put any money on an abnormality in dopamine systems as the cause of schizophrenia. I just never expected that to be the case. But we did do a nice study when Ian Creese was in the lab of getting schizophrenic brains and normal brains and we didn't really find any difference in dopamine receptors.

No and you clearly put the point of view that you just put to me now in an article in Science that appeared around 1982 or thereabouts I think but your earlier work around 1976 was being read as indicating the dopamine receptors might be the site of the lesion and what we should be doing is receptor brain work etc. Would you agree?
Oh yes. I mean, it would have been criminal not to exploit the possibility that abnormalities in dopamine receptors are the cause of schizophrenia because if that were true and you could show it just by measuring techniques in post-mortem samples and you just didn't bother doing it you'd feel pretty stupid if you missed a discovery of that magnitude. So that's why we yes definitely did that very early on.

You say you also moved on to look at histamine receptors and 5HT receptors. Now one of the big breakthroughs, at least looking at it from the outside, was the radiolabelling of 5HT receptors and being able to distinguish between 5HT1 and 5HT-2. You said Stephen Peroutka was involved with that.
Yes. Steve was an MD/PhD student in the lab. He was very bright. About that time, we were working on alpha adrenergic receptors. We had an agonist including tritiated clonidine that Boehringer Ingelheim had just sent me and an antagonist WB4101, which I stumbled on by reading a journal that nobody reads The Journal Pharmacy and Pharmaognosy. I used to leaf through it because I thought there's sometimes nice papers in there. I saw that this drug was very potent and I wrote away and got a sample and New England Nuclear tritiated it, so we had the alpha adrenergic receptor labelled two ways.

Then we got tritiated epinephrine which was used by David U'Prichard, another postdoc. Now the receptor labelled with the agonist had different drug specificity than the receptor labelled with the antagonist. So we initially said "ah agonist state of the receptor - antagonist state of the receptor!" Steve Peroutka was just a summer student working, hadn't even started his PhD, and I said okay you'll work on that with this other fellow David Greenberg. Steve started looking at it and reading about this allosteric theory of the receptor and he said this is wrong, this doesn't make any sense. I said what do you mean its the Allosteric Theory, Jacques Monod's theory, Jean-Pierre Changeux. He said listen this is ridiculous - I can't remember the reasoning – but he said no, it cannot be, look at the data, it has to be two separate receptors. Finally I understood and I said oh my God you're right. There are actually two receptors.

So we wrote a paper separating them. Independently some other people, on pharmacological grounds, had distinguished alpha 1 and alpha 2 adrenergic receptors, which was exactly what we were looking at. But then Steve started to look at serotonin receptors. Another student in the lab had labelled serotonin receptors with tritiated LSD and Steve utilised tritiated 5HT and tritiated spiperone, which we were already using to label dopamine receptors.

If you used the caudate nucleus, it labels dopamine receptors but if you use the cerebral cortex it labels serotonin receptors. Again, we found that the properties of the drug labelled with the agonist, serotonin, were different from properties labelled with the antagonist, spiperone. But because Steve was smart, he said this was not agonist state and antagonist state but these were two separate receptors number 1 and number 2 and then he proceeded to go get functional and even behavioral correlates. He did a brilliant job. A wonderful student. That was probably the first instance of differentiating sub-types of receptors by ligand binding at least for biogenic amines.

We had the same situation with opiate receptors when enkephalin was isolated by John Hughes and Hans Kosterlitz and tritiated enkephalin became available. Actually we got some even before the structure of enkephalin was known because some chemist guessed what the structure might be. So we were comparing the binding of tritiated enkephalin with tritiated naloxone and they had different specificities. I said well you know that's not surprising because enkephalin is a peptide - it has five amino-acids that can bind in five different places whereas poor little morphine or naloxone are only equivalent to say the tyrosine of the enkephalin peptide. You could make all kinds of hand-waving explanations that would fit in beautifully with why the drug specificities were different.

Hans Kosterlitz, a 75 year old man, got the exact same data, doing the exact same experiments but he said "ah two sub-types of receptors, the enkephalin one I'll call, delta, the morphine one I'll call mu. And he did functional studies in the guinea pig ileum and the mouse vas deferens and of course he was right. We were wrong because we had this stupid theory of agonist and antagonist states or multiple points of binding. I was trying to use Occam's Razor, the principle of why invoke two receptors when you can invoke one. If one receptor explains everything why use two receptors. I have kicked myself many times because we had all this data and just interpreted it wrong.

One of the things about the 5HT 1 and 2 story, however, is that it came at just the right time. A time when the pharmaceutical industry had begun to produce drugs which were active on the 5HT system. Your interest and theirs coincided and this gave rise to the huge explosion in 5HT receptor pharmacology - we now have what 17 different 5HT receptors or whatever. If the industry hadn't had the SSRIs and drugs like that coming on stream, would the radio-labelling of the 5HT receptors have taken off to quite the same extent?
Well the proliferation of serotonin receptors of course also came with the molecular cloning revolution. But there was a lot of interest in the binding studies and one reason for looking for sub-types in serotonin receptors had to do with looking for anti-emetic drugs and of course some of these sub-type specific serotonin antagonists have been very valuable anti-emetic drugs. And I suppose the Prozac Story heightened all general interest in serotonin.

You may want to pass on this one but I want to ask you how much the receptor story fitted into something else. I have just been talking to Karl Rickels, who as you know was interested in non-specific aspects of therapy. But the discovery of receptors feeds into the story about specificity in therapy. The idea that we want to be able to get a bullet

which is just going to hit one target highly specifically and it's going to cure the disease without doing anything else to the person and it won't actually be important if you have good bedside manner or not. It's going to be a bit like trying to hit a bug with an antibiotic. How much, do you think, your work fed into that one way or another for good or for bad?
Well I mean one of the points of the receptor work that we did was to focus the drug industry on developing drugs in this fashion that enabled you to get much more potent and much more selective drugs. Now that's been a boon for drug development in general. But also there was the ability to understand more about how neurotransmitters and other things act. The ligand binding techniques could be applied to many other things. Speaking of Prozac for instance, antidepressants inhibit neurotransmitter uptake and I always figured there must be uptake receptors. Some other labs, including Steve Paul at NIMH had looked at the binding of tritiated imipramine and that seemed to label an uptake site - maybe for serotonin. But it was sort of fuzzy because as it turns out the imipramine was labelling 4 different things. We got interested in that and were able to selectively label the different uptake sites as uptake receptors.

Interestingly in terms of drug development, neurotransmitter uptake is another example where screening technology can be important. When Julie Axelrod developed uptake, he was injecting radioactive norepinephrine into the whole rat. Then the ability to do it in vitro developed - several labs first of all incubated brain slices with tritiated norepinephrine. Then when Joe Coyle, was a medical student working in our lab, he found that he could take isolated nerve endings, synaptosomes, and incubate them with neurotransmitter and get physiologic uptake. This was notable because people had tried this in the past and failed. The reason being that to prepare a synaptosome, which is actually a remarkable phenomenon, is very difficult. The credit for it goes to Victor Whittaker in Cambridge. You homogenise the brain but you must do it with a Teflon pestle very gently and you must do it in slightly hypertonic sucrose, no salt is allowed or else it won't work. But if you do it under the proper conditions, you homogenise the brain, nerve terminals break off from the axons and instead of falling apart and spilling out their contents, they seal off into round balls, becoming isolated nerve-end terminals, synaptosomes. You can purify them, not perfectly but pretty well.

Now you could study the mediation of neurotransmitter uptake in these but if you do that in sucrose, there won't be any because you need sodium. But if you added sodium and you homogenise it, you won't have any isolated nerve endings. Joe Coyle systematically looked at all the possibilities and he found if you homogenise a brain in sucrose with a Teflon pestle to make the synaptosomes and then put them in a test tube and add sodium, the nerve endings stay intact and you can study neurotransmitter uptake just like you would study an enzyme. What's more you can do 50 test tubes at a time and so you can screen agents. That technology became very valuable in studies of neurotransmitter uptake in general - for amino-acid neurotransmitter uptake and lots of other transmitters.

Interestingly I only discovered after I read a book by Peter Kramer called Listening to Prozac that this screening technology was important in their developing Prozac. A fellow named Dr Wong at Lilly had been in a lecture I

gave when I went to Lilly explaining what I have just described about neurotransmitter uptake and synaptosomes and he said you know we could use this to screen and get drugs that would selectively inhibit the uptake of one or another neurotransmitter. So they said, we already have desmethylimipramine that's selective for norepinephrine, lets see if we can get a drug that would be selective for serotonin. And there you are. Simple minded technology in this case and just being able to do enough test tubes with isolated nerve endings to screen for uptake enabled them to get neurotransmitter specific uptake inhibitors.

Is there a risk though that with this kind of approach which makes it easy to focus in on just beta receptors, or reuptake sites or the dopamine receptor without really understanding the functional impact of all these or the role of that function in the illness, that you'll produce a whole lot of me-too drugs. It becomes awfully easy for the industry to produce Me-Too drugs without really making the breakthroughs.
Right. What you're getting at is that if we decide that the mechanism of therapeutic action of neuroleptics is the blockade of dopamine D2 receptors, then through screening we can get the world's best dopamine D2 receptor blocker. Now you say well that's just another neuroleptic, it's a Me-Too drug. But in the case of neuroleptics, and this is true for many other drugs, the original neuroleptics had lots of side-effects. In the case of neuroleptics they are very sedating because they block alpha adrenergic receptors, which we were able to find once we could measure the alpha 1 receptors in binding studies and show that the sedative potential parallels the blockade of that receptor. David U'Prichard in our lab published a paper in Science on that.

It was clear that the anticholinergic activity of the drug was because they were blocking muscarinic receptors and we could measure them in the test tube. One of the first receptors we labelled, which today is used in the exact same form was tritiated QNB, which Hank Yamamura, a postdoc in my lab identified. That was an interesting story because QNB was actually one of the army's chemical warfare agents which was develop at Edgwood Arsenal outside of Baltimore. It wasn't legally a secret but it was still a secret and Hank Yamamura was in the army and when I was giving a talk on the opiate receptor at Yale. George Aghajanian, a very close friend from Yale who himself had been at Edgwood Arsenal for the army, said you know you were just telling me that you want to find new drugs to measure new receptors and get them labelled. When I was in the army there was this thing that we used to give to the soldiers. They were told they were so called volunteering and we gave them this stuff that would drive them crazy. It was a muscarinic antagonist of unbelievable potency called QNB. If you could get some of that that would be fantastic. I said wow I have a new postdoc who is coming to work with me named Hank Yamamura, and he's at Edgwood right now, I'll call him up. I called him up and asked have you got this QNB there. He said how do you know? I said don't worry about it. I said look you're going to come in July, do you think you could bring a little sample with you. And he did and that was the muscarinic receptor, which enabled us to identify anticholinergic effects of drugs.

Anyhow using those things, what drug companies did was to screen for dopamine D2, to get a potency screen and for muscarinic or alpha-2 to get rid

of the side effects. So you could say it's a Me-Too drug. However Prozac, Zoloft and Paxil are just Me-Too's with fewer side effects. Fewer side effects is nothing to sneeze at.

Well, we could chase that - I'm not sure they're either as effective or have fewer side-effects. But for example, we went down the route of producing purer D2 receptor blockers and we got to Remoxipride which seemed to be extremely pure and clean but possibly not as potent and then all of a sudden there's the re-emergence of Clozapine which is a dirty messy drug but somehow seems to be more potent. So what's actually going on here?
Right the technical definition of potency is potent at a lower dose. Clinically potent means more efficacious, more effective. And that's a very interesting concept - that maybe we want dirty drugs because the old dirty drugs did things that we just don't understand. Indeed, when I was a consultant in Sandoz where clozapine was developed, the concept was to say we don't know how clozapine works. We know that it does do something different and everybody agrees that its not just blocking D2 receptors. Clozapine is active on lots of receptors and we're not sure which is most important. Lets just measure tritiated clozapine binding and screen drugs and see if they act like clozapine. That's what a lot of the drug companies have done. In our own lab we've tried to tease apart what clozapine was binding to.

Some drug companies are now saying lets go back and start using really dirty drugs like plant extracts and I know Bayer is developing more standardised preparations of St John's Wort. They are considering developing standardised preparations of some other folk medicines that seem to be really effective and we just don't know what the active ingredient is. So instead of tearing your hair out and finding the active ingredient lets just make standardised preparations so it will be helping people much more than getting the garbage they get in Health Food Stores.

Is this though from your point of view, a certain retreat from rationality – this giving up trying to find out just what's the active bit?
Oh no.   I think it's smart. I mean you have to go where the money is. That's why Willy Sutton would rob banks - that's where the money was. In this case, we want to make sick people better. Clozapine makes them better. As long as we don't know exactly how clozapine works we'll just try and mimic it while simultaneously we try to figure out why it works. Of course, we want to find out the active ingredient of St John's Wort but till then we can do other things.

Now you moved on from the receptor story during the 80s when you got into nitric oxide.   Do you want to fill me in on how that story goes?
Certainly. Well all along I'd always been interested in the notion that there are lots of different neurotransmitters. I had a review article in Science talking about 100 different neurotransmitters in the mid to late 80s. These included the peptides. But even before that in the 60s, when Joe Coyle developed the technique of measuring neurotransmitter uptake in synaptosomes, we could see that reuptake inactivation is probably the rule for inactivating neurotransmitters.

Now at this time people were suggesting that amino-acids could be neurotransmitters like glutamate, even though glutamate does a lot of other things. Ditto for glycine. That was a radical concept. How would you get evidence that there's something neurotransmitter specific about certain amino-acids. Lets say you believe it, still there's so much glutamate that goes into protein and amino acid metabolism, how could you even study the possibility that it's a neurotransmitter. You'd have to focus on the neurotransmitter pool. We said if neurotransmitters were inactivated by reuptake then we should see if there would be a unique high affinity sodium requiring uptake system for those amino-acids that were neurotransmitter candidates. If this theory was worth anything, then amino-acids that weren't likely to be neurotransmitters wouldn't have high affinity sodium requiring uptake. When we did such studies, sure enough glutamate and glycine were unique. One other amino acid worked - proline. We still don't know why - for all I know proline may be a neurotransmitter. It acts a lot like GABA but we don't know.

So the notion of novel neurotransmitters was something we were working on even in the 60s. Then there was the identification of enkephalin. We competed with Hans Kosterlitz on this. Once we'd identified the opiate receptor we tried to find the endogenous morphine whatever it might be, if it existed at all, by looking for something in brain extracts that would compete with radioactive naloxone in binding to opiate receptors. Hughes and Kosterlitz independently looked for something in brain extracts that would mimic morphine's effects on smooth muscle, which was their expertise. Gavril Pasternak got that working in our lab and then he went back to medical school and a postdoc named Rabi Simantor purified the extract till he had something that looked like it was pretty pure. Just at that time I received in the mail galley proofs from Hughes and Kosterlitz and they had gotten the sequence of enkephalin. They had the same amino acid composition as we had but their paper was out. We finished sequencing ours a few weeks later. So we were very interested in enkephalins and peptides. At that point I felt there must be other peptide neurotransmitters. Substance P had already been isolated by Susan Leeman and she suggested it was a neurotransmitter based on other evidence. So we began with lots of peptides from different realms and checking them out for neurotransmitter candidacy.

This was a long term interest. So then my reaction was this is fantastic when I read what I think was the first paper I read on nitric oxide - Salvador Moncada's paper in Nature definitively showing that it was endothelial derived relaxing factor. This is fascinating. Shortly thereafter I think John Garthwaite wrote a paper in Nature – in 1987 or 1988 - saying that there was something in brain extracts that had activity that was like endothelial derived relaxing factor, which might be nitric oxide. At any event I thought that's really cool and we should have a look at it.

I had a new MD PhD student named David Bredt who had been working on something else and was getting a little bored. I said why don't we study this nitric oxide. If it's a neurotransmitter, it will be so different than anything else. Most neurotransmitters are stored in synaptic vesicles, so that only 1% is released on a nerve impulse. But if this is a neurotransmitter, gas can't be stored - it would have to be regulated by the enzyme that makes it. We

already knew that arginine gave rise to nitric oxide. So the arginine to nitric oxide step, the nitric oxide synthetase enzyme, must be extraordinary. We could work out the neurotransmitter of that enzyme should be in neurones in the brain, localised to a specific neurones, and the enzyme should be a very important regulating factor because every time you want to release a molecule of nitric oxide you have to activate that enzyme, which would make it a remarkably regulated enzyme.

So lets try and get that enzyme purified, which will be hard to do but lets see what we can do. The first thing was to get technology. At that point people were measuring NO synthase activity by a colorimetric technique called the GRETS technique. This was very insensitive. We couldn't detect enzyme activity in the brain with it. Besides being insensitive it was very tedious. Doing 10 samples was a big deal and that wasn't my style. In receptor technology we knocked off hundreds at a time and we got lots of data so you can ask lots of questions and get lots of answers. If you do it real fast the science moves more rapidly. Dave and I talked about it and we said you know this is ridiculous. A colorimetric reaction is looking at whether nitric oxide is converted to nitrite but we knew that the arginine was converted to citrulline, so why not take tritiated arginine and measure its conversion to tritiated citrulline. We could take a tiny little ion exchange column and a tiny little pasteur pipette, which cost nothing. Arginine would adhere to the column, citrulline would run through to the counting block, it would be just as easy as receptor binding. And sure enough it worked.

This meant we could really study this enzyme. We had heard that people were trying to purify it but never could because it was very labile. Sure enough, you would run a brain extract through a column and it would lose all its activity right away. So either it was very labile or maybe it wasn't so labile and maybe the column was separating it from a crucial co-factor. Well David said we can test for that possibility my taking all the tubes and mixing them back together again. That restored activity. We said we'd better find this co-factor. We could use that technique of mixing things together and looking for what was active to isolate the co-factor or we could just make some guesses. We said well lets see - we know that calcium is important for enzyme activity - it was already in the literature. So lets try calmodulin, the calcium binding protein, and sure enough that was it.

What was interesting was we actually didn't start trying to purify the enzyme. Purifying the enzyme is a big challenge. I wouldn't have taken the risk of leaping into that if we didn't already have some other data telling us that we really want to work with nitric oxide. What preceded purifying the enzyme was we decided to see if we could get any evidence for nitric oxide being important in the brain. We developed this arginine to citrulline assay and then we knew that nitric oxide, when it relaxes blood vessels, does it by stimulating guanyl cyclase to cGMP - that was well known. So we said maybe in the brain cGMP might be the second messesger and where do you get that in the brain? Well it was very well known in the literature that if you take the excitatory amino acid neurotransmitter, glutamate, that acting through the NMDA receptors in the cerebellum, it stimulates cyclic GMP formation. You do it with standard techniques.

David said well lets take that standard system and see if nitric oxide synthase activity changes. Lo and behold, if you take those cerebellar slices and add glutamate and NMDA, as fast you can measure it - half a minute - the arginine to citrulline conversion is tripled and cGMP levels go up. We could show that the increase of NO synthase activity caused the increase of cGMP because we just took an arginine derivative, N-methylarginine, that would inhibit NO synthase and then you didn't get arginine converted to citrulline and you also didn't get the increase in cyclic GMP. We published that in 1989 in PNAS and that told us that nitric oxide is doing something important in the brain that's mediated in the actions of the major excitatory neurotransmitter. This is exciting lets go for it.

Just as isolating the opiate receptor was an important question to be addressed in opiate pharmacology, getting that enzyme was clearly the most important issue of nitric oxide research, so we said okay now lets go get it. David did that work. He was a very talented fellow and he figured out a beautiful affinity chromatography step to purify the enzyme 5000-fold in one step. Basically, we could purify the enzyme to homogeneity in a day. So we could make antibodies to it and localise it. Lo and behold its got very discrete neuronal localisations and just knowing where it was told us lots of things. Having the pure protein, we could get amino acid sequences and clone it.

Once we got the structure of the cloned enzyme, that told us an enormous amount about how it worked. The enzyme was a fusion of two enzyme proteins, the nitric oxide synthase part and a cytochrone P450 reductase part, which donated electrons. For most other oxidated enzymes, cytochrome P450 reductase is a separate enzyme protein, but here they were fused together. We found it had an FAD, flavine adenine dinucleotide, and an FMN, flavine mononucleotide, which was important because those must be co-factors and indeed they are. This told us that the oxidative step of this enzyme used more electron donors than anything else. There's a heme binding site and a tetrahydrobiopterin site, so clearly by cloning and isolating it, we found that it is indeed perhaps the most regulated enzyme in biology. This is extremely important because everything about nitric oxide is that enzyme - we don't have any vesicle system, there's no exocytosis, there's no receptor - nothing – this one enzyme does everything.

There's a few things that fall out of this. One is the whole idea that a gas could be a neurotransmitter. I mean if you told this to people even in the late 80s and early 90s, after it had all been published, they still wouldn't have believed you. The world cannot be this mysterious. We just had neurotransmission worked out and this is just a totally different kind of concept.

Yes it changed all the rules about neurotransmission. No reuptake, no storage vesicles, no release by exocytosis, no receptor - very very different. Actually the rules are changing even more now. Subsequently we obtained evidence in our lab that a d-amino acid, d-serine, is a neurotransmitter. It is the endogenous ligand for the so called glycine site of NMDA receptor. What's remarkable about that is that it really overturns rules like all amino-acids in mammals are l-amino acids. Only bacteria have d-amino acids. We didn't discover that, some Japanese workers did but we read the paper and then decided to go to see what it does.

We made antibodies to d-serine, localised it and it was localised just where NMDA receptors were. The next remarkable thing we discovered is when we looked under high power, we found it's exactly where NMDA receptors are, in the grey matter of the brain, but it's not in neurones at all. It's in glia that ensheath the nerve terminals, a special kind of glia, called the type 2 astrocyte, that is also called a grey matter glia. Then we found that those type 2 astrocytes, that have all this d-serine, have glutamate receptors of the kainate sub-type.

What happens is that glutamate is released from the nerve ending. Some of it goes to the postsynaptic NMDA receptor. Some of it binds to the kainate receptor on the type 2 astrocytes, which releases the d-serine, which then also goes to the NMDA receptor to the glycine site where d-serine acts somewhat better than glycine. We've just recently figured out, after several years, how d-serine is made. Herman Woloster a very good postdoctoral fellow in the lab discovered that its very straightforward. There's an enzyme that converts l-serine to d-serine, which we called serine racemase. He purified, cloned it and localised it. Interestingly it has localised right where NMDA receptors are but it's not in glia, it's in neurones, it's in nerve terminals. What happens appears to be that the d-serine is actually made in the neurones. When it leaves the neurones, it's taken up by the glia. It is not metabolised there, so it accumulates in glia. When we did our immunohistochemical staining, we weren't seeing any in nerves which was a puzzle because its made in nerves but then seems to leave them immediately and is taken up by the glia.

So d-serine is even more bizarre than nitric oxide. I think what we are learning about all of this is that at every step of the way the rules for neurotransmitters keep changing. With acetylcholine, the rule was the key aspect is enzymatic degradation of the neurotransmitter. We must eliminate the neurotransmitter from the synaptic cleft by an enzyme that rapidly degrades acetylcholine. Well Julie Axelrod gave the lie on that theory when he showed that uptake is more important. Then that became the rule rather than the exception. The peptides are presumably inactivated by enzymes, like acetylcholinesterase. Nitric oxide is presumably inactivated just by diffusing away. I don't know about D-serine - it's too early to know.

It's really made the whole thing much more mysterious again hasn't it? Things had become very predictable during the 80s - the monoamines were there and maybe the peptides and we were close to working out what was going on. Now it's all begun to seem much more mysterious again would you agree?
Yes, I'd agree. Interestingly these weirder neurotransmitters are actually doing more things and more important things than some of the conventional ones. Nitric oxide terminals ramify so that it influences in fact every single neurone in the whole brain. D-serine, being involved in the area of NMDA receptors, given that glutamate is the major neurotransmitter, D-serine is part of the number 1 neurotransmitter system in the brain which accounts for at least half of all the neurotransmission in the brain.

So in due course we'll have psychotropic agents, which act on these systems.
Right in the case of D-serine, you could say well a drug that would inhibit serine racemase would stop the formation of D-serine and that would be the equivalent to blocking NMDA receptors, maybe even better.

Which would do what behaviourally?
Well the major research in the glutamate field looking for therapeutic drugs is looking for blockade of NMDA receptors to block stroke damage. Its well established that a massive release of glutamate in stroke causes a major part of neural damage and an NMDA antagonist in animal models in stroke are quite therapeutic.

How about the nitric oxide story?
Nitric oxide, there is abundant evidence that Ted and Valina Dawson began in our lab, which indicates that when glutamate acting through NMDA receptors causes stroke damage. Now in our initial work, the very first work showing that nitrate oxide did anything, David Bredt found that glutamate acting through NMDA receptors acted on nitric oxide neurones to stimulate NO synthase to make nitric oxide and that accounted for the increase in cGMP. Now we know that a massive excess release of glutamate in stroke causes neural damage, could it also be via nitric oxide?

The evidence consisted initially of cerebral cortical cultures in which neurotoxicity produced by glutamate could be modulated by nitric oxide. How? Well many things turn it on – new protein synthesis - and it cleaves the heme ring to biliverdin that's almost immediately reduced to bilirubin, releasing a one carbon fragment as carbon monoxide. I didn't know about this but Ajay Verma, an MD. PhD student did. I said that's a very clever idea - if its a neurotransmitter then maybe the enzyme should be in neurones.

Just a little while before we had started talking about that, there had been papers published showing that while the inducible heme oxygenase, called heme-oxygenase no 1, was concentrated in the spleen where all those red blood cells go, in purifying that enzyme Mahin Malnes in Rochester had found a second peak on her columns, which she purified and she called HO2. Now HO2 had a completely different tissue localisation. It was not in the spleen at all, it was only in the testes and brain. It wasn't inducible and not many people cared about heme-oxygenases and fewer people cared about the non-inducible form. HO1 is mostly studied by haematologists. But it was sitting there in literature, so we said lets look at this HO2 and lets see if it's in the brain. Since it had been cloned, it was trivial to get oligonucleotides and do in situ hybridisation and it was there in very discrete neuronal populations in the brain, whose localisations were exactly like that of guanylyl cyclase, the enzyme which makes cyclic GMP, which it resembled much better than nitric oxide synthase.

So that was pretty cool. In some neural forms but not in others, in olfactory neurones, if we added an inhibitor of heme-oxygenase we could deplete cGMP levels, whereas an inhibitor of NO synthase wouldn't. That got us very interested. We got antibodies to this HO2 and we found that in the peripheral nervous system, its localisations were just like neuronal NO synthase in very

specific places. The peripheral nervous system is a better place to study something as a neurotransmitter. I don't think anyone has really proven that NO is a neurotransmitter in the brain or that serotonin or dopamine really are – its very hard in the brain to satisfy all the criteria. Whereas in smooth muscle you can do it much more nicely. For instance, we found that the myenteric plexus neurones in the small intestine stained for NOS and for HO2. 70% of the neurones would stain and the exact same ones stained for NOS and for HO2.

Now in that system we can take the myenteric plexus with the intestine and put it in an organ bath. It is well known in that system, if you block adrenergic and cholinergic transmission and stimulate the myenteric plexus you get relaxation. This relaxation is involved in intestinal peristalsis. It's been called nonadrenergic, noncholinergic relaxation. This is very well known but the neurotransmitter had never been identified. People had suggested that peptides like VIP might do it, but there was no proof, even though this was perhaps the most important component of intestinal motility, of peristalsis. So here we could test whether NO or CO were involved. We had knockout mice who had deficiencies in all the systems we wanted to measure and we could just measure the transmission. If we electrically stimulated and measured the relaxation, in the NOS knockouts half the transmission was abolished and in the HO2 knockouts half was abolished. And in the NOS knockouts, the half that was remaining could be abolished by drugs that inhibit HO2 and vice versa. Basically its pretty clear that CO and NO are both neurotransmitters.

Absolutely fascinating. We don't have any drugs, which act on these systems at the moment do we? I mean you hear about clozapine and all the antidepressants and things like that and we're told that they act on X receptor or Y receptor but now you find they also act on most other things as well. There's no conceivable way that any of the drugs we've been using act on any of these systems is there?
That's a very good point you raised. When you find a new system you should always say nobody has ever studied the old psychotropic drugs on this. Nobody cared up till this. We should think about every drug in the world, not just psychotropic drugs, you've gotta spread the net widely, any drug that you can think of that nobody really knows how it works might be interesting to look at its effects on these systems. Will it inhibit HO2, will it stimulate it? We haven't done that systematically but that's the kind of thinking that we do and we really ought to do it. One of the things that slowed us a little bit in the heme-oxygenase field again was techniques. That NOS assay got things going. There was no good assay for heme-oxygenase. We tried a lot of things but we couldn't get anything going until we finally figured out, with the same company, New England Nuclear, which is now called NEN-Life Sciences, who have made for us heme with a radioactive iron in it. After a lot of trials and tribulations, to make a long story short, we finally got that to work. It's extremely easy because all you have to do is separate radioactive heme from radioactive iron and they are of course very different so a trivial column does it. It's even easier than the NOS assay now that we've eliminated various hurdles. So quite recently we got to the point where we had enough of the iron-labelled heme to do lots of routine screening for drugs. We have not done yet what you suggested but we should.

To come back, where does that leave receptors because they don't really particularly figure in this story at all do they.
Well there's no classical receptor for CO or for NO so there's no receptor story. It raises the question of what are the targets of NO and CO. Now CO like NO won't stimulate guanylyl cyclase. But that may not be the only target. We have to bear in mind that there may be other targets that we can call receptor targets but they're not conventional receptor targets.

In a sense then, during the early 1970s you helped create the receptor story as we now know it but during the 1990s you've changed it, destroyed it almost, in that what you appear to be saying is that drugs which act on things like D-serine, CO, NO are going to be just as important if not more important than the drugs which act on the classic monoamines and their receptors.
Yes. My philosophy of science is that the way to make discoveries is always have an open mind and don't stay in your own little cubby-hole. Had I said I'm a neuroscientist I only read papers about the brain, I would have never seen Salvador Moncada's paper and would never found nitric oxide as a neurotransmitter. Had I never paid attention to the insulin receptor, there wouldn't have been anything to do with receptors. So you have to keep your mind open and don't pay attention to catechism. It says in the rule book this must be that, or this is this and that is that. The best way to read things is to be a rebel. Something about my own psychology always was to rebel against authority, to get in trouble in school. Psychologically when I read papers that say A is B, I sort of say maybe you're full of shit maybe A isn't B.

Of course that's what scientists are trained in graduate school to do. I didn't get a PhD, I never went to graduate school. I just have a psychological thing to be dubious against anything I read, which is of course what all scientists are supposed to do. But, of course, its important not just to be critical but also to come up with new ideas. PhDs are very well trained to be critical. They're trained to be so critical that they criticise all their own thoughts, so they're never creative. That's one big difference between MDs and PhDs. PhDs are so rigorous. They're trained never to do artefacts, don't make a mistake. Don't do something stupid and make a finding that was really an artefact - you didn't have a control sample, you didn't have a blank value. They are trained to be very careful and ruthlessly tear everything apart. They tear apart their own ideas so they don't come up with new ideas. People without such fancy training, MDs like myself, don't know about that and they just try and think new thoughts. Not necessarily to overturn what they've read but my own psychology is if somebody said day is day, I might say maybe day is night and that attitude always helps.

I'm struck by the fact that your early receptor work seemed to confirm the views that go all the way back to early the idea that there must be some receptor substance but your later work has closed the book on it. In a sense you brought the early model to fruition but then you undercut it as it were.
You could say it that way but I wouldn't. I'd just say biology is fascinating and there are a lot of different things and don't worry about old paradigms. If you get a new paradigm, it doesn't mean the old paradigm was bad it just means that was great but there are other things to think about now – we should

always look for new stuff. As you can tell from the story, after we work in an area for a period of time, I can get bored and want to go on to new things. I don't forget the old things because you know they could come back again. But sometimes you get to a plateau and you say well we've got about as far as we can get with this project and let's not put too much in it. Let's look for something else. People say "oh that means you're not a serious scientist you're skimming the cream off the top and not doing the hard plodding work". But sometimes you get to a certain point and that's as far as you can get with that project. For instance had I followed Dr Talalay's advice and said "well I will not move one inch, until I have purified the opiate receptor protein" - I'd be still doing it.