Excitatory Amino Acids: From Basic Science to Therapeutic Applications
JEFF WATKINS

How did you get into the field

Well I was a chemist, a 'pure' chemist, interested in chemistry for its own sake. I didn't have the opportunity to go to Medical School in Australia, in Perth. It didn't have a medical school but in any case I doubt if I would have been attracted to medicine. I did science because all my friends did science and I also liked mixing things up in the lab. So I ended up as a chemist and then the thing to do was a Ph.D. Luckily I got a scholarship to Cambridge, and then what many people did in those days was to go to America. In the mid-50s, you could usually get a post-doc position just by writing to whoever you wanted to work with. I went to Yale for a couple of years. All this was automatic, before I really started to think what I ultimately wanted to do or be.

I wasn't exactly 'mixed up' but I wasn't quite certain what I wanted to do with my life. I was reading everything - philosophy, religion, that sort of thing - and it just came to me, rather "Eureka-fashion", in the middle of the night, that you couldn't know anything fundamental about oneself until you knew something about the brain. Young people think in these terms - what constitutes 'I'? 'I' do this, 'I' do that - but what is 'I'? Basically what came to me was that the sense of self can only be in the brain, somewhere. Not very original, you might say, but new to me, to be thinking in those terms. So that was quite a conclusion to come to - that I was actually interested in the brain itself - how it worked, not in just using it for some purpose or other like doing chemistry, for its own sake, for example. Of course, LSD was around at the time and this was a drug that altered 'perception', that is how people, I mean, how their *brain* interpreted the sensations they received - how it produced in itself the experience of what they saw, heard, felt, etc, and that made me think that there must be chemical elements at work there. What was known about the chemistry of the brain at that time? Virtually nothing! Nothing anyway that overtly related to the special functions that the brain performed and the mechanisms underlying such activity.

Glutamate curiously had been mentioned - long before noradrenaline or 5HT. There was a report from Derek Richter about glutamate and schizophrenia in the 1950s which seems early and prescient.

I worked in his unit later on. I think the evidence underlying this speculation was pretty flimsy and quite probably irrelevant - but I would need to look at those reports again. Even before that, in the late 40s, Heinrich Waelsch and others in the States were looking at glutamate as a possible cognitive enhancer. They thought it increased intelligence in mentally handicapped children. So glutamate was already in the field but I believe that was just coincidental and whether or not it has any relevance to what is known about glutamate today is debatable, but shouldn't be dismissed.

Were they in anyway thinking about it as a neutransmitter at the time?

No. Except for Hayashi. At that stage the idea of chemical transmission wasn't even universally accepted for the brain. It was generally accepted for the peripheral nervous system and some people said that if neuromuscular transmission worked like that then probably synaptic transmission also worked like that in the brain, and bit by bit, between

the 20s and early 50s, everybody came over to the chemical theory for brain synaptic transmission except the chap I went to work for eventually - J.C. Eccles.

It wasn't glutamate that interested me at that time, it was chemistry of the brain in general. Now, luckily enough, in Yale I was living in a medical school hall of residence, and even though I went to the chemistry department on the other side of town everyday, I was actually living with medics. When I got the idea I would like to do something on the chemistry of the brain, one of my medical friends said "well, you ought to write to this chap Eccles in Australia and tell him", which I did. I wrote to him and said "look, I know its a bit ridiculous, I know nothing about the brain, or any physiology or even biochemistry, nothing biological at all, let alone neurophysiology", but, I said "surely the brain is made up of chemicals".

Not the man to say this to

Well he was by that stage, 1956, although he was almost the last man to have come across from stout adherence to the electrical transmission theory, except for a chap called Lloyd. He did that in the early 50s, as I recall.

But then he managed to make it look like he had discovered the whole idea of chemical transmission

Well, there's nothing like a convert for enthusiasm. He had to prove it to himself scientifically and he did this by being the first person to record intracellularly in neurones in the brain. The spinal cord, actually, because it was easier there but he correctly surmised that what happened in the spinal cord would happen in the brain as well. When he found that stimulating the nerves from antagonistic muscles produced a different post-synaptic potential change, one depolarizing, the other hyperpolarizing, in the same spinal motoneurone, he said well, obviously, it can't all be done by the same *electrical* influence of one cell on another, so, overnight, he said it must be done *chemically,* a complete volte-face. The different pathways stimulated must result in the release of different transmitters, one excitatory and one inhibitory producing opposite potential changes in the neuronal membrane of the same motoneurone. He had just done all this three or four years before my letter arrived saying "surely you must have a place for a chemist". His reply bounced back saying that it couldn't be more appropriate to have a chemist in the department at this stage and that I must come and work with a young chap there who was just completing his Ph.D called David Curtis. Even though he, David Curtis, had no real chemistry background, he had recently been trying - or thinking about trying - to extract chemicals from the brain. He suggested we should work together, which we subsequently did. I began there in January 1958.

The idea was that I should take over from David and try to extract transmitters from the brain. I said "Well there must be a thousand compounds at least in the brain and they're all mixed up in there. To get any one out individually will be difficult and how are you, David, going to test each of them to see if they might be transmitters". He said "I'll put each one in these little micro-electrodes and pass them out electrophoretically on to single neurones in the spinal cord and monitor the electrical activity of that particular neurone". I said "But if I'm taking a chemical from the brain, and you're testing it on just a single cell in the spinal cord, how will you know you're testing it on the same sort of

cell that it might act on as a transmitter in the brain. Can you provide me with a broader screening system?". He said "Well, there's the isolated toad spinal cord that some Japanese have used, and also Eccles a few years ago. He said we could take the spinal cord out of the Queensland toad - an enormous thing - bathe the cord in artificial CSF and then just wash in all my compounds or crude extracts and if we see any gross activity, as reflected in recordings from ventral roots, we can then test them further, and in more detail with the microelectrophoretic method in mammalian spinal cord. The toad spinal cord will allow us to monitor the activity of a large number of neurones simultaneously, and it is likely that neurones in the spinal cord, and their transmitters, will be similar to those in the brain. Although the toad spinal cord will obviously have differences from the mammalian spinal cord, there is a good chance that there will be many similarities as well".

This was fairly early to be doing micro-electrophoresis.
He was not the pioneer of the technique itself but he was the first to use it in the CNS. Rose Eccles, his daughter, and David Curtis, did it first I think, on Renshaw cells in the spinal cord because they surmised that the synapse formed between motoneuron axon collaterals and Renshaw cells was likely to be a cholinergic system. Fatt and Katz had used the technique in the neuromuscular junction, a couple of years before. I'm not sure but I think Nastuk or Del Castillo and Katz actually pioneered the method.

This early work was in Australia?
Yes. Eccles had been at Oxford with Sherrington but he moved back to the Antipodes and after a couple of prior posts in Sydney and Dunedin, I think, ultimately took the chair in the Physiology Department of the new John Curtin School of Medical Research at the Australian National University, which had been created and generously endowed by the government to be a place of excellence. Conditions were extremely good and Eccles attracted people from everywhere. It really was the most intellectually exciting period of my life because there were all these good young people coming to work with Eccles. I was working down the end of the same corridor and I knew enough at this stage to know that this work of his really was at the very forefront of brain research.

What was he like, Eccles?
He had one overriding interest and that was his research. He didn't really want to talk much about anything else actually - at least, that was my impression at the time. In retrospect, I'm sure he would have talked enthusiastically about 'consciousness' and the nature of 'mind'. I didn't get to talk to him very much because I wasn't one of his group. I virtually only saw him at seminars and in the tea room or corridor and whatnot. He obviously had a very sharp mind and dominated all the seminars - most of which he gave personally. It was nice to meet him again in 1993, after twenty-eight years, on the occasion of his 90th birthday celebration[1]. He was still working more or less until about 4 or 5 years ago, mainly on philosophical topics and has written a number of books - none of which I've read yet, I'm afraid. I did read a very early book, The Neurophysiological Basis of Mind, based on a series of lectures he gave at Oxford in the mid-50s. The last chapter, as I recall, was on the nature of mind and I remember a

[1] he died on May 2, 1997

neurophysiologist I was friendly with saying that the book was very good except for the last chapter. But I must say I enjoyed this chapter most of all, though I'm not sure I accepted all his assertions.

Everyone in his group was well organised. His students could be there at 3 in the morning but they were nevertheless expected to be there again at 9 a.m. or before, on the next day, to process the results of the night before. The students I think also prepared the animal for each experiment and on those days Eccles himself would come into the lab about 11 a.m. to take charge of the experiment. The students took notes, I believe, while Eccles manipulated the microelectrodes.

He was keen on 'wholesome' activities such as square dancing, 'social' tennis - he had a tennis court - and walking. I was invited only once for Sunday afternoon tennis. Partnering Lady Eccles in doubles I let rip one of my erratic cannon-ball services which whistled dangerously past her ear. She displayed considerable nervousness thereafter. Also, my performance seemed not to fit the description of 'social'. To make matters worse, at that time I smoked. That was definitely unwholesome. My further participation was not requested.

I can tell you a story about "The Nobel Prize that wasn't". We all used to work at nights. One night a reporter on the Canberra Times rang up to say that it had just come over on the 7 p.m. BBC news, from London, that Eccles had won the Nobel Prize. She then came to see him in the lab. Aged about 18 she said "Could you tell me, Sir John, what exactly is the Nobel Prize"? Somewhat incredulously he replied: "Young lady. if you don't know that you had better go back and send someone who does!" I went off to purchase suitable celebratory beverages, returning well stocked. Eccles - virtually a teetotaller - allowed himself half a glass of sherry, then went off home to celebrate with family and friends there. At 9 p.m. the BBC repeated the report, and the party at the lab gathered steam. By 11 p.m. most experiments had been abandoned. But in the 11 p.m. news the BBC corrected the error. Another Australian, not Eccles, but Macfarlane Burnet of Melbourne, had won the Nobel Prize, shared with Medawar. Eccles immediately came back to the lab to resurrect what he could of the experiments, staying presumably till 3 or 4 am as was customary. The next day, by chance, the real winner of the Nobel Prize turned up in Canberra while Eccles, David Curtis and I were having lunch together. Eccles leapt out of his chair, rushed over to the chap, congratulated him warmly, indeed most effusively, and showed none of the great disappointment and embarrassment he must have been suffering. Luckily he did win the Nobel Prize two years later, which he shared with Hodgkin and Huxley.

There are lots of other little anecdotes. One of the biochemists at the tea break one day said "I am finding it difficult to keep up with the literature these days. How do you cope, Sir John?". Eccles replied that "People tend to send me reprints if they want me to know of their work. If they don't send me reprints they don't deserve to be read!" These were unsolicited reprints of course. An Eccles tea break usually consisted of two cups of tea, swamped with cold milk, the quicker to be able to consume them. Both cups were gulped down while still standing at the counter, giving a total tea-break of approx. 15 secs plus travel time to and from his lab, one floor up. If you wanted to talk to him you

had to chase after him as he left the tea room and complete your discussion, often from two paces behind, before he got back to the lab.

What gave you feeling it was a breakthrough area because the oddity is that all the fuss had been about the catecholamines and 5HT. The glutamate story has been a slow burner as it were.

Well, the slow take-off of the glutamate story was because we were somewhat negative in the first paper we wrote about the possibility of it being a transmitter. This was understandable in the light of what we knew at the time. We had got onto glutamate because I had said that I wasn't keen to go to the great trouble of extracting brain to get all of these compounds out individually when I could get some of them, already in pure form, straight from the laboratory shelf. There was a big bottle of glutamate up there and I knew that glutamate was one of the main chemicals in the brain, so I said lets try this first and we did. In a sense, our discovery of its excitatory activity came *too easily* for us to regard glutamate as the transmitter we were mentally prepared to spend years searching for. And its action was shared by several structurally related compounds, some endogenous, others not. We tested many other known brain constituents as well but it was the excitatory amino acids, and also the inhibitory ones, related to GABA, that had the most obvious effects, so we concentrated on those.

Following a paper on GABA by Curtis and John Phillis, his Ph.D student, published in Nature in 1958, we published a letter to Nature on glutamate in 1959, and a full paper in the Journal of Physiology in 1960, but *we* unfortunately slanted it that, although these were extremely interesting effects, glutamate wasn't likely to be a transmitter for a long list of reasons: for example, because it had important other roles as a protein constituent and as a metabolite involved in energy metabolism and there's so much of it there, whereas you don't need much if its a transmitter. Its overall concentration in the brain is about 10 millimolar, which is a huge concentration compared, for instance, to acetylcholine which is there in only nanomolar concentrations. Also there was one crucial experiment from which the wrong conclusion was drawn, an experiment to see if the reversal potential for glutamate was the same as the reversal potential for the natural transmitter when you stimulated the pathway to the particular neurone under observation which was responding to glutamate. The 'wrong' answer was obtained mainly because the techniques weren't developed enough at the time. Our feeling then was that, whatever the mechanism of this interesting action of glutamate, it seemed to be different from that of the excitatory transmitter to this particular neurone because the reversal potentials for glutamate and the natural transmitter appeared to be different. Therefore glutamate probably wasn't a transmitter. It was to be another ten to fifteen years before techniques were improved sufficiently for others to show that in fact the reversal potentials are indeed similar.

What was David Curtis' background

I think he was originally a neurosurgeon who decided he would rather do neurophysiological research than practice medicine. If I remember correctly, he got a scholarship in Eccles' department, did a PhD with Eccles and then more or less went off on his own projects. I arrived at that point and my background nicely complemented his chemical interests. I stayed for 7 years and we got a nice series of papers out. Also

there in Canberra at this time was Kris Krnjevic, who was a post-doc I think at that stage, and he and David's student John Phillis later got together in England, at Babraham, and took the view that the Canberra attitude to glutamate was possibly overly negative. Eccles himself felt that glutamate looked promising despite the 'contra - indications'. Krnjevic and Phillis repeated in the cerebral cortex everything we had done in the spinal cord and got more or less the same answers - not exactly the same which is interesting now, in retrospect, with the range different receptor types that have recently been recognized. As I recall, they also failed to show a reversal potential for glutamate similar to that of natural excitatory post synaptic potentials.

I did a little cartoon a few years ago in the form of a schematic electrophysiological response to illustrate the history of the glutamate story. There was an initial burst of interest - 'excitation' - followed immediately by 'inhibition', which kept the field depressed. Not many people were interested for 20 years or so after our initial discovery of the excitatory action, mainly I suppose because of our initial pessimism. Then there was a series of 'spikes' in the 80s following the discovery of antagonists and the excitation has continued since then with the recent breakthroughs in molecular biology. The early period of 'inhibition' was due to all sorts of factors. The main one was this thing about the wrong reversal potential but the other major prejudice was that there's 'too much' glutamate in the brain and it obviously had other roles and why would a transmitter need to have other roles. And people became more interested, as you say in 5HT, noradrenaline and dopamine - all the Americans went off in that direction and there was just a few of us left interested in glutamate.

Who was left interested - you and..

David Curtis kept it up after I left in 1965 with a younger colleague Graham Johnston, a chemist like myself. Later - about 1968 - there was Hugh McLennan in Vancouver. In the mid 60s there was also a spattering of interest elsewhere, Krnjevic, of course, in Babraham and then Montreal, and Takeuchi and Takeuchi in Japan, working on crayfish muscle, following the pioneering work in this tissue by Robbins in the USA, and later Usherwood in England with his proposal that glutamate was the transmitter at the insect neuromuscular junction. McIlwain pioneered some combined electrophysiological-biochemical studies on brain slices. But there were only a few of us consistently involved from the electrophysiological angle over a period of 20 years or thereabouts -1960-80 - certainly as far as the mammalian CNS was concerned. Krnjevic remained active for a while in the 70s but his interests were broader and the definitive pharmacological tools had not yet been developed. Donald Straughan, a colleague of Krnjevic in Babraham, also maintained an interest for a while, though I think he was more interested in amines. John Davies of course began collaborating with me in 1970, as did Tim Biscoe and Dick Evans in 1973. David Lodge joined David Curtis in 1974 having done his PhD with Tim Biscoe, an ex-Krnjevic associate at Babraham, who had later also spent a year at Canberra with David Curtis. Lodge has been a stalwart in the field ever since. Other people publishing in this area between 1970 and 1980 included Shinozaki, who introduced kainic acid and continues to make a major contribution to the present day, Barker and Nicoll, Zieglgansberger, Engberg, Stone, Macdonald, Nistri and Constanti, and later on Krogsgaard-Larsen with Curtis, but there weren't many, to be sure. Throughout the 1970s, however, an increasing band of neurochemists became

interested - Aprison, Bradford, Cotman, Balazs, Roberts, Fonnum, Cuenod, the McGeers, Wenthold and others.

But how does this fit with the point you made earlier that you had felt that this was breakthrough stuff

Eccles, of course, with his straight neurophysiology, elucidating the electrical responses of neurones rather than the chemistry of these responses, was involved in major advances in understanding of the excitatory and inhibitory synaptic activity and connections in spinal cord and brain. I'm not sure I really thought our work was necessarily 'breakthrough stuff', because we were lacking the definitive evidence of its real importance. The reason it went so quiet was because there were no antagonists. You couldn't tell one way or the other if the glutamate action was important. You just made up your mind on the basis of other factors. But I, personally, not being trained in neurophysiology, was restricted to a chemical approach so I kept on doing chemistry related to glutamate. Luckily the MRC here supported me from 1973 on to make new compounds until we eventually did get some antagonists. Only then was I able to convince myself that there might be something in the glutamate transmitter theory. I wasn't trying to prove people wrong or anything, I just didn't know myself whether glutamate was or was not a transmitter and I did not want to give up until I knew, one way or the other.

How did the NMDA angle come into the picture?

Curtis and Johnston had something to do with that, following a lead from one of David Curtis' students called Arthur Duggan and before them Hugh McLennan. They looked at the relative potencies in the brain of a few amino acids and saw they were different depending on which cell and in which region of the CNS they were tested, and they thought, therefore, that there might be more than one glutamate receptor. That was in the late 60s - early 70s.

Had you made NMDA before that?

Yes, I made it 10 years before that. I made NMDA in order to see if there was any difference in stereoisomers. In the spinal cord there wasn't much difference in potency between D- and L-glutamate or between D- and L-aspartate and for a receptor you'd expect there to be marked enantiomeric differences. So I thought maybe if you introduced larger groups at the asymmetric carbon atom, steric differences might show up better, and they did. That was in the early 60s.

What did you think you had produced though when you made NMDA - just another chemical

Yes, to begin with, but it proved to be by far the most potent agonist we had tested at that stage and very interesting just from that point of view. We didn’t know why it was so potent but originally assumed that it had a higher affinity for the receptor than glutamate. Now we know the NMDA potency is actually due to its lack of rapid uptake. This knowledge is due mainly to Graham Johnston and his student Vladimir Balcar. Glutamate is taken up by the cells when you try to test it, and therefore you don't see the full effect at the receptors, whereas the NMDA isn't taken up, so you do get the full effect. But we didn't know that in 1960, we just knew that in some tests, NMDA was up

to a thousand times more potent than glutamate. We now, of course, know that glutamate is actually about ten-fold more potent than NMDA at the receptor level. Harry Olverman in my group showed that in the mid 80s. Of course, we were only thinking of a single glutamate receptor at the time I first made NMDA.

Was there any feeling at the time, that there might have been any commercial applications for NMDA.
No, not really. It was of peripheral interest to investigators of a murder in Sydney. A couple of well-known scientists suddenly and mysteriously died after a party and one of their friends clutching at straws rang me up asking whether NMDA could have caused their deaths. I said it wasn't likely to have been that, whatever it was, because NMDA wouldn't be likely to cross the blood-brain barrier sufficiently - according to our tests on mice. The murders are still unsolved, I think. No, there wasn't any immediate commercial interest and we weren't interested to search for any possible commercial application.

How did McLennan get involved? Did he have any links with the original group?
No. It was quite independent work, as far as I know. He was one of the original GABA investigators, working with Florey on Factor 1. I think it was after he saw our results that he swapped over to glutamate. I believe he was working on the crayfish stretch receptor with regard to GABA actions. Robbins in the States had independently published similar results to ours on the actions of excitatory amino acids on crustacean neuromuscular junctions, so I think McLennan came via those two areas, deciding to look at these substances also in the mammalian CNS. This was the mid 60s but once he was in the field he stayed in until he retired about 7 or 8 years ago. It was he who first got the idea at the end of the 60s that there was more than one glutamate receptor.

Three things came together in the multiple receptor story. First there was McLennan suggesting it. Then Arthur Duggan, a PhD student of Curtis', on his own initiative, looked at glutamate and aspartate potencies on Renshaw and other interneurones in the spinal cord and found they were different suggesting to him the possible existence of two different types of receptor - maybe a 'glutamate' and maybe an 'aspartate' receptor. Finally Curtis and Johnston said "well, if that's the case, some bulkier molecules might show this better and they chose NMDA and kainate to test on the same types of interneurones as Duggan had used and did indeed get bigger differences, so they supported the idea of two different receptors, one more sensitive to NMDA, and one more sensitive to kainate.

At this stage, I was making substances that were beginning to look like they might be antagonists. In view of the multiple receptor possibility, I decided to test them on a range of agonists. I soon found that my first antagonists were all NMDA selective and didn't touch some amino acid agonists at all. That led me to propose the NMDA receptor as one easily identifiable receptor type in the late 70s.

Did you run into the problems that the monamine receptor people ran into which was a certain scepticism about whether these had functional relevance whether they were any more than binding sites?

No, because we had started on the functional side. There was skepticism in relation to whether they had a functional synaptic role, but they clearly could influence synaptic activity. Harry Olverman introduced binding here, in Bristol, later, and gratifyingly got parallel results to those had obtained in our electrophysiological systems. I would usually be more swayed by the electrophysiological-functional tests rather than the binding data if only one set of results were available - except where uptake or enzyme degradation may be expected to obscure the electrophysiological effect. It is like the present day situation with cloned receptors. We can differentiate several different subtypes of EAA receptors in our functional assays and sometimes get activity with compounds that don't seem to have any action on some of the cloned receptors.
When we pass our compounds on to our electrophysiological colleagues, Graham Collingridge here, and Tom Salt previously here and now at the Institute of Ophthalmology in London, to see what our compounds do in the hippocampus or the thalamus, respectively they find that the same compounds also usually work in those areas similarly to their actions in the spinal cord. So we believe in our functional receptors. I don't necessarily believe the importance of everything you find from a neurochemical or binding assay or even from studies on cloned receptors but one needs evidence from as many sources as possible of course.

Is there a sense in which the whole NMDA field compared to the monoamine field has always remained more functional. With the monoamines in the mid-70s when people were beginning to radiolabel these receptors for the first time, the field had a choice between electrophysiological work showing that the antidepressants improved signal-to-noise ratios and changes in receptor number. The scientific market-place as it were took a view that receptors rather than electrophysiology were the way forward.
I haven't kept up with the monoamine field very well but there was a lot of work in the mid 60s finding out where these monoamines were active in the brain and then later with labelling the neurones containing the amines, and, finding out where there were concentrations of monoamine receptors via binding studies. I think there is probably a fair amount of functional correlation for dopamine, 5HT, noradrenaline, etc, as regards location of receptors and behavioural effects so I'm not quite sure I agree with you that the two have been divorced, except with regard to demonstrable effects at single synapses or on discrete populations of neurones. I think in the drug development field they just want to screen as many compounds as possible, as quickly as possible with the screens that have previously thrown up successful drugs, albeit somewhat empirically. As to the benefit of looking in the brain to see exactly what's going on, synaptic effects of amines for example and the possible function of such effects, the drug companies seem to have been less interested but between receptor binding studies and the behavioural effects there seems to be quite a good correlation. I think all the required conceptual connections will be made in due course - you get shifts in emphasis don't you? It would in any case, be well nigh impossible to screen all new compounds produced by pharmaceutical companies for electrophysiological effects of functional relevance in brain.

You proposed the NMDA receptor idea in 77. Had the field begun to pick-up speed at that stage or was it still a small field.

It had picked up a bit but was still a relatively small field. Once we'd produced specific antagonists things changed. The first antagonists were just longer molecular-chain glutamate analogues such as D-α-aminoadipate, the 'unnatural' form of the next higher acidic amino acid homologue to glutamate, which was something McLennan indirectly first got onto as a possible antagonist but without reference to possible selectivity. At this stage we already had similar compounds and by then already knew of their selective actions. When I saw McLennan's results, I felt I knew in advance what D-α-aminoadipate would do in our system and could confidently predict that it would be selective. We had already made a range of similar compounds and McLennan didn't have the D-form of α-aminoadipate, having made his logical prediction of the glutamate/aspartate antagonist activity of the D form on the basis of the activity of the DL- and L-forms. I made the D-form of α-aminoadipate and found that it was NMDA selective like our earlier compounds and that convinced me of the existence of a specific NMDA receptor. Then I made many more related compounds and there were other types of NMDA antagonist as well such as magnesium and HA-966, which we had actually characterized as selective NMDA antagonists prior to the D-α-aminoadipate group. They all had a similar spectrum of antagonist action against the same range of agonists.

Did they all come on stream at the same time and how did magnesium appear?
Yes they did. Magnesium had been used at the neuromuscular junction to block transmission on the basis that it inhibited calcium influx into presynaptic terminals. We tried it for this purpose in the frog spinal cord and then found unexpectedly that the presence of magnesium had a differential antagonist effect against a range of exogenous excitatory amino acid agonists. It so happens that the medium that you use for frog spinal cord preparations *in vitro* doesn't have magnesium in it, and that was lucky because it meant our preparations were very sensitive to NMDA agonists and antagonists. When we added magnesium to the medium, in quite low concentrations, much lower than needed for presynaptic depression of transmitter release, we got both a strong depression of synaptic activity as well as this selective depression of excitatory amino acid agonist actions. The depression produced by magnesium turned out to be similar to that which we saw a little later with organic compounds. So it looked as though magnesium was involved in the activity of this new receptor type that we were identifying as the NMDA receptor, and importantly, that this receptor had functional significance, since transmission and NMDA receptor activity were reduced in parallel by low concentrations of magnesium.

About the same time we also had our similar results with this other compound known as HA-966 - later it was found by Lodge to be an antagonist at the glycine site of the NMDA receptor, after this had been described by Ascher in the late 80s, but we didn't know that in the late 70s. This was the first glutamate/aspartate antagonist we had got onto in the early 1970s when I first began working with my colleague John Davies. We didn't know how or why but when we got the new antagonists later in the 70s we found that HA-966 had a similar spectrum or activity - they were all acting at the same receptor.

How did you get onto HA-966 and how did John Davies come into the picture?

John and I first got together in early 1970. I'll tell you later how this came about and also the beginnings of the HA-966 story. The background is that I had left Canberra to try my luck in England in 1965, to broaden my horizons, so to speak. I didn't do any further electrophysiological work on glutamate for a few years. I went to Babraham and worked for a while on liposomes. The head of the Institute when I went there was one of the foremost cholinergic pharmacologists, John Gaddum.

I actually went to Babaham first on sabbatical leave from Canberra in1963/64 as a trial for a possible future longer term move. I went there to work on model membranes. I thought why do all this work on animals when you might be able to do it on model membranes. They were doing lipid membrane work there and I thought I'd like to try it out. I became involved with Alec Bangham in the development of liposomes.

How did liposomes come into the picture?
Well, I was interested in the interaction of amino acids with neuronal membranes, and biological cell membranes were considered to be protein-coated bimolecular lipid layers, so that's how I got involved. I had written rather a speculative paper that had proved popular on the possible involvement of bimolecular lipid/protein membranes in glutamate/GABA action. Liposomes were just coming into the picture. I was originally more interested in the Mueller-Rudin bimolecular 'black film' that you get when you take a plastic beaker, poke a hole in the side of it, immerse it in an aqueous fluid and paint a solution of phospholipids over it, whereupon, if you're lucky, it forms a *bimolecular film* over the hole with the lipid parts of the molecules meeting *in the middle* of the film and the polar parts extending in the fluid on each side of the hole. So you can have an 'internal' medium and an 'external' medium and can find what happens to electrical potentials created between the two with different solutions on either side. That's what I was interested in doing but Alec Bangham was more interested in the structure and permeability of liposomes per se. The liposomes were also easier to work with and so I spent three years doing that eventually. I did try GABA and glutamate on liposomes - with some vaguely encouraging results - but not very seriously.

Was there any feeling at the time that some of the compounds you were using were membrane stabilising in some sense
Membrane active but not membrane stabilising, more membrane destabilising really. The receptor idea took root because of the differences in the actions of a range of molecules of different shapes. If they were all just dissolving in the membrane, you wouldn't expect such big differences. But we had found that even a single methyl group on glutamate, for example, could practically abolish activity altogether. The question was, were these receptors just chance orientations of groups on extracellular proteins or whatnot, coating the lipid core. Just chance, without functional meaning. Pure coincidence. I mean, if you threw glutamate onto the floor you might find some wood, sand or clay particles that would absorb it 'specifically' just because the shape of parts of the molecular surface of some of the component substances just happened to be complementary to the acidic and basic parts of the amino acid molecules.

That's not a receptor idea?

It would be a ‘receptor’ in the sense of an arrangement of atoms on the surface of a macromolecule that is complementary to a pattern of atomic sites on the foreign molecule. It doesn't mean to say its a functional receptor in any sense. It is certainly a molecular entity, but only fortuitously resembling a so-called physiological or pharmacological receptor. We would now say our compounds are acting on a functional glutamate receptor. By this we would mean part of a macromolecule that plays a specific physiological role in this case being specifically located within synapses, and playing a discrete role in transmission. The active sites of the protein interact with complementary sites on the glutamate molecule so as to induce a change in the conformation of the protein and allow ions in or out of the cell that wouldn't normally be able to get in or out so easily and so change the electrochemical potential across the cell membrane. If the protein was serving some other purpose, for instance, as a structural component of the membrane matrix, the glutamate action could be regarded as an interesting Pharmacological Phenomenon but not necessarily related to synaptic physiology. When we eventually obtained the antagonists and were able to antagonise both the synaptic event and the glutamate event and show that the pharmacology was the same, that's when people began to believe that the glutamate receptor was indeed involved in synaptic transmission.

John Davies unfortunately died about 5 years ago, a great loss to the field. As mentioned earlier, in the late 60s I was working on model membranes. This was more a fundamental biophysical than a truly physiological project and I felt I would like to get back to working on neurotransmission. First I left Babraham to work in Richter's MRC Neuropsychiatry Unit at Carshalton, where I began working again on amino acids, but mainly on the metabolic side. I subsequently arranged to go back to Canberra for three months and I took a few compounds with me and worked with David Curtis for three weeks at the beginning of 1970. David felt I was somewhat 'wasted' working on model membranes and metabolism and that I should work preferably with someone like Donald Straughan, Professor of Pharmacology, at the School of Pharmacy, London, who had worked with Krnjevic at Babraham and who was doing microelectrophoresis. I got in touch with Donald Straughan who said he had a young lecturer in the Department, John Davies, and suggested they both come over to Carshalton to talk about the possibility of collaborating. They came over, John got interested, and it began from there.

At this stage, when I was working in Richter's unit at Carshalton, I was looking at amino acid and energy metabolism as influenced by the actions of extracellular glutamate and other excitatory amino acids. Richter wasn't particularly happy with what I was doing and neither was I. So we agreed I could spend one day a week at the School of Pharmacy working with John. I went in with a lot of speculative compounds to test, the third one of which was HA-966. We got onto that fairly quickly since I had seen a brief report on its central action and thought it had an 'amino acid-like' structure related to glycine, which turned out to be an important factor. It proved to be a glutamate/aspartate antagonist, and we got a note into Nature, which was very nice. Iversen wrote a little note in the 'News and Views' section hailing the discovery and emphasising its possible importance. John Davies' appetite for that sort of work was truly whetted, such that we then worked together for another twenty years, even though we were geographically separated for most of that time.

When that association first developed, David Curtis wrote to me and suggested that John Davies should come out and work with him for a while to learn more about the electrophoretic technique. John went out for two years from 1972 to 1974. David Lodge also went out there about the same time. That was a continuation of a Bristol/Canberra link which began with Tim Biscoe who had been at Babraham working in association with both Krnjevic and Straughan. Biscoe wrote to David Curtis, after he had become interested in this sort of work through Krnjevic and Straughan, and subsequently worked with him in Canberra for a while. Then when Biscoe came to Bristol, the Professor of Physiology here at the time was Arthur Buller who had also worked with Eccles in Canberra - so there's a long connection between Canberra and Bristol. Buller had worked on nerve-muscle interactions. There were slow muscles and fast muscles and what Eccles and Buller did, as I recall, was to cross the nerves to these muscles over, whereafter fast muscles became slower and slow muscles became faster, correlating with corresponding changes in the activity of motoneurones. That was all going on in Canberra when I was there. Then Arthur Builer came back to England and eventually got the chair here in Bristol and when Tim Biscoe came back from Australia he also came to work here, eventually succeeding Buller as Professor. Biscoe had two students - Max Headley, who worked in Canberra later with Duggan and who is back here again now, as a Professor in the Physiology Department , and David Lodge who became Professor of Veterinary Physiology in London and is now a Research Director with Lilly.

So John Davies was out there at the same time and on his return we resumed our collaboration. He became a long term colleague. John did the really crucial experiment that finally convinced me, after 20 years, that glutamate receptors were indeed involved in neurotransmission in the CNS. He showed that D-α-aminoadipate, the specific NMDA receptor antagonist, selectively blocked transmission through non-cholinergic pathways to Renshaw cells, for which no previous antagonist was known while nicotinic-type acetylcholine antagonists selectively blocked cholinergic activation of the same cell through the motoneurone axon collateral pathway. This was the real turning point in the field.

At this stage I must also mention Dick Evans. When I came to Bristol it was with the idea of collaborating with Tim Biscoe in the Department of Physiology by supplying substances to him for testing microelectrophoretically in spinal cord *in vivo,* as in Canberra and London. We did this - David Lodge, Max Headley, Mike Martin and I - for several months but, as in Canberra and London, progress was very slow. Dick Evans, a relatively young lecturer in the Department of Pharmacology at the time, expressed a keenness to collaborate and, thinking of less labour-intensive techniques, suggested we try the isolated spinal cord of the local frog *(R. temporaria or R. pipiens)* as a screening method, as the Queensland toad spinal cord had proved so useful in Australia. This was in 1974. The system worked very well and we made a lot of our original discoveries on magnesium, HA-966 and diaminopimelate, leading on to D-α-aminoadipate, using it. Later, Dick adapted the isolated spinal cord of the neonatal rat as described first by Konishi and Otsuka in 1974 and this had several advantages over the frog spinal cord. It was mammalian, it gave a more elaborate dorsal root-evoked ventral root potential

which could be differentiated into several components. It also responded to more endogenous transmitter candidates - acetylcholine, amines, peptides. We are still working with this technique today, and much credit must go to Dick for providing us both with his electrophysiological expertise and a magnificently useful screening preparation. We definitely could not have made the advances we have done without his crucial input.

You were still a small group at this time? What kind of impact did you have at scientific meetings at the time - were there any symposia on glutamate
Our 'group' at that time comprised just Dick Evans, a chemical technician, Dan Oakes, and myself. In 1975 this was expanded with MRC help to include a pharmacological technician, Alison Francis, and later a post-doctoral chemist Keith Hunt, who was succeeded in 1978 by Arwel Jones. Arwel was with us for 6 years and was involved in our big leap forward with competitive antagonists.

No, there were no glutamate symposia then. The first glutamate symposium as distinct from general amino acid symposia was in 1979. Neuroactive amino acid symposia actually began in the late 50s. They were then mainly about GABA with glutamate sometimes thrown in. The glutamate component in later symposia grew gradually throughout the 70s and the first specifically glutamate symposium was co-organised by Graham Johnston from Canberra and yet another chap originally from Bristol, Peter Roberts, who is also back here now as a Professor of Pharmacology, working on the neurochemistry of excitatory amino acids. He was here as a PhD student when I first arrived here, working on excitatory amino acids under James Mitchell, another ex Babraham man, who had originally been mainly interested in acetylcholine. Peter is one of the originators of the binding technique as applied to excitatory amino acids and he also published early neurochemical results suggestive of metabotropic glutamate receptors.

The room Peter and Graham had arranged, in Jerusalem, would have taken at most about 30 to 40 people, I would say. It was a session within a Meeting of the International Society for Neurochemistry, a fairly big affair, but not one that we thought would have necessarily attracted many people who were interested in glutamate. But we greatly underestimated the number. The room was full and overflowing, with standing in the aisles and doorways. This was very encouraging. Even so, it has since developed much beyond what we could ever have imagined. You only have to look at the abstracts for the Society for Neuroscience now - there are 20 odd sessions that are somehow related to glutamate, through epilepsy, neurodegeneration, and all that, not to mention psychology and psychiatric conditions in addition to basic neurophysiology and neuropharmacology.

Did it take 2 or 3 years more for the next symposium?
Yes, the next one I can recall was on Excitotoxicity in Stockholm in 1982. For a while they continued to be linked to those on GABA but by the mid to late 80s, they became increasingly resticted to excitatory amino acids as a topic in its own right.

Somewhere in the early 80s, the MK-801 story began to impact as it became clear that this drug *also* acted on the NMDA receptor. How important was that?

Well, it was important in advancing the ketamine/PCP story and the recognition and elucidation of the channel-blocking mechanism. The ketamine story began in the early 80s through the work of David Lodge, and later, also Max Headley here. MK-801 was the most potent compound showing a similar action, identified as such by Iversen and colleagues at Merck, Sharpe & Dohme in the mid 80s.

Did you have any feeling before 1980 that ketamine was acting through the NMDA receptor?
No, in fact at that stage I knew nothing about ketamine. It was David Lodge who found that out. He's a vet and I believe it had a use in veterinary medicine. He tested many known medical and veterinary drugs for actions in excitatory amino acid systems and found several correlations. Interestingly, we had earlier, in 1977, found that chlorpromazine and diazepam in relatively high concentrations, had NMDA-selective depressant actions. These have never been further investigated but are quite probably channel-blocking effects.

How did the ketamine angle get missed then? Was it because there were so few people working in the area?
Yes, there weren't many people at that stage thinking that they should test clinically used CNS drugs on glutamate receptors. It just didn't figure in people's thinking until the early to mid-80s. Remember the NMDA receptor had been recognized as a functional glutamate receptor only a short while before. Lodge's work on ketamine and PCP was the first to establish the principle and then the work on MK801 followed.

What impact did David's work have on you here?
Not much, really. Its mechanism of action was originally problematical, though it was clearly non-competitive. Harry Olverman, within our group, found it did not displace labelled AP5 from brain membranes. David was interested in it, I think, mainly because it was clinically effective. But its molecular structure didn't fit into our previously elucidated structure-activity relations for competitive NMDA antagonists and I didn't really want to get involved with a more complicated phenomenon shown by a range of chemically disparate substances. All these channel-blockers as they ultimately turned out to be were a problem 'structure-activity-wise' because they all had different molecular shapes. And we had our hands full already with competitive antagonists.

Did the sheer complexity of the NMDA receptor hold things back? Its such a complicated beast with glycine sites and all that.
No, it didn't. We weren't originally aware that it was so complicated. We were able to make very potent NMDA receptor antagonists, which were competitive and completely conventional, pharmacologically. I got involved with Sandoz on this and helped develop a compound that may mitigate brain damage in cerebral ischaemia. Such conventional antagonists were all developed before we knew about glycine and the channel-blocking story, or the spermine-spermidine modulatory site on the NMDA receptor or the various subtypes of NMDA receptor that have since emerged. We didn't know about this complexity. If we had done we might have been a little put off, though I don't think so, really. At the time it all seemed rather simple. You take an amino acid molecule a bit longer than glutamate, put a phosphonate group on the end, bend it round a bit forming

various cyclic groups, arrange for the amino acid end to be of the D configuration - in the earliest antagonists, anyway - and you ought to have a good NMDA antagonist. That much was easy, and although somewhat simplistic, produced many a good new NMDA receptor antagonist.

Other things weren't so easy. Similarly specific and potent antagonists for kainate and AMPA were not as readily developed. You could get compounds that were somewhat active although they weren't very potent or selective. But they did depress the synaptic responses which weren't depressed by pure NMDA antagonists. That was important because it implicated non-NMDA receptors also in synaptic transmission. But, no, ketamine didn't make too much impact on me, although I'm sure it impacted more on anyone involved in clinical medicine and on the drug companies, who were also looking at other ways of getting at the NMDA receptor. Ketamine is one of the few substances in the field that passes the blood-brain barrier readily.

When did the long-term potentiation story, the LTP story, impact?
The LTP story, pharmacologically speaking, started here in Bristol as well, in a sense, Graham Collingridge was one of our first pharmacology students here. He came out of the second year that the course was run, graduating in 1977. It was a famous year. He and Mark Mayer, now at NIH, and two others all got firsts. In fact there was a fifth one who should have got a first but the external examiner said we can't possibly have 5 out of 9 getting firsts. After completing his first degree here, Graham went to work with John Davies as a PhD student and then to Hugh McLennan in Vancouver as a post-doc. It was when he was working with Hugh McLennan that he decided to see if this LTP phenomenon was susceptible to modification with excitatory amino acid agonists or antagonists. That led him to the discovery that the NMDA antagonists were able to prevent the generation of LTP. He came back here as a Lecturer, then went off to Birmingham for a while but came back here again 2 or 3 years ago as Professor in Anatomy.

LTP has become a subject in its own right now. You could almost be an LTP-ologist. It has become one of those central phenomena that pulls thinqs into it.
Yes, his MRC programme grant is simply for investigating LTP now. The original findings pulled in Richard Morris, an experimental psychologist, who I guess was originally interested in any pharmacology that related to memory and learning. He tried some of these compounds behaviourally, testing them on memory and learning, and found that there a specific deficit developed if you put NMDA antagonists directly into the hippocampus - the rats couldn't learn as well as control animals how to get to a submerged platform under the influence of the drug.

We gave ketamine to healthy volunteers recently with a rationale that it was going to affect frontal lobe function and that it would mimic schizophrenic psychoses but the biggest effects showed up in memory.
To my way of thinking it is surprising when some functions aren't affected with these drugs because the NMDA receptor appears to be involved in practically all central synaptic events. Quite probably it is just that some forms of memory acquisition are particularly sensitive rather than uniquely affected.

Synaptic plasticity is one of the things that fall out of the NMDA story but this is not part of the usual lock-and-key way of viewing things.
But that's getting all tied up now with increasing knowledge of the involvement of the metabotropic glutamate receptors in longer term effects. That's one of our main interests now, in collaboration with Graham Collingridge.

How did the metabotropic idea come on stream?
I can't claim anything to do with the beginnings of that except that we had probably the first specific agonist without knowing it, which was the phosphonic acid analogue of glutamate - what we called L-AP4. It is just glutamate with a phosphonic acid group rather than a carboxyl group at the end of the molecule. Our first group of NMDA antagonists were all the D-forms of glutamate analogues. Normally, we made racemic forms and separated the D and L forms, so we ended up with both. We tried the D-form of AP4 and it was a weak NMDA antagonist but not very selective. The next longer one, the equivalent of D-alpha-aminoadipate but with a terminal phosphonic acid group, has become the standard specific NMDA receptor antagonist now - D-AP5.

Carl Cotman, who has also been in the excitatory amino acid field for a long time, some 30 years or so now, and was one of the first people to work on hippocampal pharmacology, wanted some of this compound and I sent some of both forms to him, D- and L-AP4. He wrote back and asked if we had the labels mixed up because they found L-AP4 to be much more potent than the D form at depressing synaptic transmission in the perforant path of the hippocampus. We had expected the D-form to be the most active, based on EAA receptor antagonism. He sent the sample back and we checked and said no, we hadn't got them mixed up. At about the same time we were finding that L-AP4 was unexpectedly depressing monosynaptic transmission in the spinal cord. This was mainly Dick Evans' discovery using his *in vitro* preparation, but John Davies confirmed it also *in vivo.* We didn't know how it caused this depression. It didn't fit in with any of our - by now - quite neat structure-activity relationships for excitatory amino acid antagonists. In fact it didn't antagonise any of our excitatory amino acid agonists - NMDA, quisqualate or kainate - at all but it still depressed transmission.

We now know that its action is via a metabotropic effect. At the time we thought it was probably working pre-synaptically because it wasn't working postsynaptically on any known excitatory amino acid receptors. Cotman and colleagues did some elegant work on miniature excitatory post-synaptic potentials that definitely suggested the L-AP4 effect was presynaptic, but we didn't know the exact mechanism. Now we know its action fits into those of Group 3 metabotropic glutamate receptors, mGluRs. Indeed. this activation characterized Group Ill mGluRs. They are activated by L-AP4 and some of them at least mediate depression of synaptic transmitter release. Their action is associated with a depression of cyclic AMP synthesis.

But when did the metabotropic idea emerge?
From the mid-80s, via neurochemistry, glutamate-stimulated inositol phosphate formation from phosphatidyl inositol initially. These results led up to the idea that there was a particular kind of glutamate receptor that caused metabolic changes. Nicolletti,

Costa and colleagues were the original proponents. As I recall, it wasn't till late 87 that Sugiyama, a Japanese neuroscientist actually showed an electrophysiological effect in of such receptors in oocytes. They expressed a cloned receptor in oocytes that was coupled to a G-protein, and associated with glutamate induced effects on certain channel currents. They suggested that these receptors be called metabotropic because they were obviously coupled to metabolic changes. This idea was already prevalent for amines.

But the word metabotropic isn't used in the amine field.
No. I think the Sugiyama group suggested it to differentiate the function of these EAA receptors from the ionotropic actions of the amino acids at certain receptors. I am not sure whether there are purely ionotropic receptors for amines. Possibly all are of the metabotropic type.

When did the implication take hold that one was neurotransmission related and the others were metabolic related with longer term effects on synaptic efficacy.
Well, I suppose the implication was there from the mid-80s, when people were working with brain slices and getting significant neurochemical effects that were initiated by glutamate and a restricted number of analogues, but not by the standard excitatory amino acid agonists, NMDA, kainate or AMPA, and not antagonised by any of the usual antagonists of the then known transmission related glutamate receptor sub-types. The molecular biologists began to clone all the different ionotropic and metabotrophic receptors and characterized a range of sub-types within each major group in the late 80s and early 90s. Then Collingridge showed the involvement of one particular group of mGluRs in the generation of hippocampal LTP, while Salt showed a particular association with the transmission of pain responses in the lateral thalamus. I suppose the Collingridge work in the early 90s brought the possible role of mGluRs in longer term electrophysiological effects to the fore though metabolic cascade effects would probably imply that mGluRs participate in longer term changes than the rapidly reversible ionic effects induced by ionotropic receptors.

Before we leave the Japanese completely, some credit for kicking off the area seems owing to another Japanese - Hayashi. He had a first report on glutamate in 1954. Who was he?
His first important publication in English was in 1954, as far as I know, but there were earlier reports in Japanese. I think he was doing his work mainly in the late 1940s and early 50s.

Was this an isolated effort?
If you mean was Hayashi alone in investigating glutamate as a neuro-excitatory agent well, yes, I believe so, but he had a number of students working with him. He seems to have been the first one to cotton on that glutamate was a convulsant substance. He tested a whole series of both glutamate-related and glutamate-unrelated compounds. His work was mostly reported in Japanese until he wrote a book in the late 50s on amino acids related to glutamate and their possible role in epilepsy. He was the first one to put his finger on the excitatory action of glutamate but the mechanism was not clear. You could, of course, cause convulsions by putting any number of substances into the brain,

so the glutamate action couldn't necessarily be assumed to be caused by a direct synaptic effect.

I think he died in the late 50s. He was an experimental neurologist, I think, who had these interesting ideas of endogenous convulsant substances and put students to work on them. There are all sorts of little bits in the abstracts of meetings in Japan in the early 50s. But he gets the credit quite rightly for being the first one to report the excitatory action of glutamate as manifested in convulsions, and his speculation that this might be due to an effect on sodium permeability proved essentially correct, though based on ion fluxes occurring during electrical conduction in nerve fibres as elucidated by Hodgkin and Huxley, rather than on known synaptic events.

When did John Olney's name begin to come into the frame?
In the late 1950s there was a paper by Lucas and Newhouse who found some neurodegenerative effects in retina when they injected large quantities of glutamate sub-cutaneously, I think. It seemed to be destroying retinal cells. Olney picked this up in the late 60s. I believe he began his work completely independently of our results, originally, and only later became aware of our earlier work on excitatory amino acids in general. I know he did write to David Curtis in the early 1970s and got some compounds from him. Irrespective of how and when he came across our series of amino acids, his series turned out to be practically identical, potency-wise, in its ability to cause neuronal damage when given in excess, as we had found to be excitatory. It fitted in very nicely. The excitotoxic story, though obviously related to excitatory amino acid actions, is a separate story and deserves recognition as such. He tells the story that the first time he postulated at a conference that endogenous glutamate may cause the degeneration seen in certain medical conditions, the hall guffawed with scornful laughter. I'm sure his ideas are taken more seriously now.

He halted MK-801 when he reported on the vacuolation following its use.
You can see why. I don't know the full story but if you do find things like that in drugs that are in the clinical trial stage, I think you're duty-bound to report it.

When did you become aware of interest from either the clinical people or the drug companies?
Well, I first became aware of this unexpectedly and in a rather unfortunate manner. I was never interested in those sorts of things. I was only interested in finding out how the central nervous system worked. But, anyway, I developed these phosphonic acid compounds and I would talk to anybody about them. To cut a long story short, some unpublished results of ours were seized upon by others, developed along the lines we were already following, and patents applied for.

This told me that some people were keen to take out patents in this area, so I had better be more careful in future. The next time I had a good compound I got in touch with the British Technology Group which was a government department at the time - since privatized - and the appropriate authority for taking inventions in universities and getting patents on them. That culminated in the drug that Sandoz is now developing. Because of all that, however, we were held up with publications on that compound and on related

analogues and we didn't get into press until some four years later and then with a lot of extra names on the paper, relative to our initial group. By then we were experiencing competition from Ciba, and not long after by Lilly, Parke-Davis and others. But the early to mid-80s was the time when people began to think there might be some drug potential in the area.

The clinical indications were..?
As anticonvulsants first and antispastics. But with Olney and other 'excitoxicologists' coming in and stressing all the brain damage that could be caused by glutamate and analogues, it became obvious that antagonists might also be useful to prevent or alleviate the progress of some neurodegenerative conditions. This idea came out of excitatory amino acid meetings in the early-80s. I wasn't all that drawn in this direction myself, and was somewhat sceptical. When the research director of a large continental drug firm got in touch with me and asked if they should be looking towards excitatory amino acids as possible drugs to prevent brain damage, in stroke, heart failure, or head injury, for example, I said quite honestly I doubt whether there's any future in it. If you antagonise the main transmitter receptors in brain, God knows what's going to happen. They dropped the idea and did something else. They probably saved a lot of money because that was 15 years ago and there's nothing on the market yet. But traumatic head and spinal injury are the best current indications since the anticonvulsant possibility went by the board due to unacceptable side-effects. Neuroprotection via glutamate antagonists looks a better possibility now.

What about schizophrenia and nervous conditions generally?
I wouldn't have thought so yet. But with all the subtypes of amino acid receptor now known there's bound to be some that are more involved in particular medical conditions than others. Huntingdon's chorea and ALS are possibilities. Maybe some aspects of Alzheimer's. Parkinson's too - L-DOPA for instance is an excitatory amino acid. There are, of course, any number of neurological and psychiatric syndromes in which there may be some particular involvement of the NMDA or other glutamate receptor system susceptible to drug modification. Maybe even in schizophrenia.

I'll tell you something I just learned two days ago. David Lodge is now Research Director at Lilly's lab in Windlesham. In an open lecture a couple of days ago he told us about this new compound they have, which is a potent agonist at one of the metabotropic glutamate receptor sub-types. It passes the blood brain barrier well which is unusual for these compounds. It turned out to be quite potent at the cloned receptor level but they didn't know what to look for in terms of therapeutic usefulness, so they put it through a whole range of behavioural tests and it turned out to be anxiolytic. It was as potent as the benzodiazepines but it was without the benzodiazepine side effects. So I think it is only a matter of time now before glutamate receptor agonists and antagonists make it to the clinic. If you can find a substance with a discrete action at a specific type of amino acid receptor, I think a drug use will be found for it eventually, provided it can be got to the target site, one way or another.

Curiously the glutamate field has evolved in a completely different way to the monoamine field, which grew up in order to explain the effects of drugs we already had and as a means of producing more. The amino acid area has grown without much input from clinical compounds, other than ketamine, PCP and MK-801, and with relatively little support from the companies originally
Yes, it has been more oriented towards fundamental research. All the compounds you've mentioned clearly had their side effects which made them generally unsuitable clinically. But there was big debate whether the side effects had anything to do with NMDA receptor antagonism. Unfortunately, the early phase-1 clinical studies with pure competitive antagonists also brought on these effects. In epileptic patients they came on at much lower doses. As they were nice pure drugs which acted on very little else, it definitely looked like the NMDA receptor was involved in the unwanted side-effects, although there are so many NMDA receptor subtypes that we may eventually be able to control for some of these effects by developing more subtype specific drugs.

How many years before there will be an impact clinically.
Maybe even within the next couple of years. I have a feeling that it will soon take off. Maybe not for five years, but its only a matter of time. The trouble with these drugs is the difficulty getting most of them through the blood-brain-barrier. If that can be overcome - ketamine gets through quickly and this latest anxiolytic one also - it will be interesting. So far none of them have much effect on the peripheral nervous system, or on the major physiological systems so even if only a small proportion of a drug gets through, you could afford to give a large dose in order to get a therapeutic dose.

Its a unique position to have been associated with the development of a field.
I've been lucky, that's the first thing to say - lucky in being at the right place at the right time and being able to contribute an expertise that had been largely lacking in the field at that time. Profoundly lucky also in the people I have worked with over the past 40 years in Canberra, London and Bristol, not to mention many other outstanding collaborators in other institutions. The second thing to say is that the field has now grown beyond the point where I feel I can continue to contribute much to it. Its gone well beyond my ability even to keep up with the literature. In fact I don't even try now. I still have a programme grant which goes on for another 12 months but I decided a couple of years ago that I would now begin to retire. I don't feel the need to stay personally active in the field. I'm confident that my present team under David Jane will continue to make a major contribution - they are an excellent group.

I gave up talking about the subject 3 or 4 years ago. I'm happy to have done my bit. I wanted to make a contribution. More than anything I had wanted to find out something about how the brain worked and I've accumulated enough knowledge about that now - poor as this is in relation to what remains unknown - to be able to begin to think about its relationship to the phenomenon of mind and the idea of 'self', which was the original motivation. Maybe I'll do this in retirement but I suspect that I will not be able to contribute anything new or original.

Has the area got the recognition it deserves in terms of honours etc.

Pretty well. Who contributed what in the past is difficult to disentangle, but modern advances are less confused. I've had three international awards in the last few years, each shared with other people, which is quite right. Olney, Choi and I shared a Wakeman Award, for contributions towards the treatment of spinal injury. Another was the Dana Award for pioneering achievements in health. This one was shared with Olney again, and with Philippe Ascher, for his glycine and magnesium contribution. The last one was a Bristol-Myers Squibb Award for outstanding achievement in Neuroscience which I shared with the molecular biologists Heinemann and Nakanishi, who had cloned, expressed and characterized glutamate receptor subtypes. There will probably be many more awards in the field in due course. One problem has been the rather slow build-up and the number of individual discoveries made that contributed to our present understanding. It is difficult to single out individual people or individual papers. There are so many people who have made or are currently making important contributions. My particular contribution has always been as part of a team. Indeed our collaborators have done much more with our compounds than we could have ourselves, to charactise their actions and demonstrate their potential usefulness in the field. People like Graham Collingridge, Mark Mayer, Carl Cotman, Dan Monahan, Shigedada Nakanishi, Tom Salt, Peter Roberts, Brian Meldrum and Astrid Chapman, Peter Cook and many others, Harry Bradford, Martin Croucher, Richard and Robert Miller and all their colleagues, not to forget our industrial associates.

A lot of people wouldn't see it the same way. In science clearly there can be the co-operative approach, which you seem to have, but there *is also* the competitive approach which many people have.

Well, while I felt there was nobody else interested, I was more open but once people began to patent compounds based on our discoveries it changes one's attitude. It was more fun in the early days. I'm obviously not against the development of compounds which will be of a therapeutic benefit, and these would seem to be on the way now, I'm glad to think. But the commercial element changes the field and for the academically orientated person, not for the better. Despite this there is still plenty of scope for co-operation since neuroscience is multi-disciplinary by nature and any one group will probably not progress as fast as they could by combining forces with other groups with complementary disciplines. I personally derive more satisfaction from such a collaborative approach and I feel our work has benefited greatly from it.

A full reference list is available in:

Watkins JC (1986). Twenty-five years of excitatory amino acid research. in Roberts PJ, Strom-Mathisen J, Bradford HF, MacMillan Press, London, pp 1-39.

Watkins, JC (1994) The NMDA receptor concept: origins and development. in: The NMDA Receptor. Eds. Collingridge G, Watkins,JC, Oxford University Press, Oxford pp 1-30.