A Paradigm Shift in Brain Research (1955-1970): Opportunities and Challenges*

Seventy Years of Brain Research - Looking Back and Ahead

Arvid Carlsson¹

When science began considering the brain from a chemistry rather than electrical standpoint, this marked the beginning of new perspectives on how this complex organ operated. Seminal work relating to dopamine helped to introduce the breakthrough concept of neurotransmitters leading to the understanding of the numerous circuitries within the brain. The observance of decreased dopamine levels when anti-psychotic drugs were administered helped to launch new medical treatments especially for patients suffering from Parkinson's disease. Today stabilizer compounds are being tested to provide beneficial therapies for both elevated or decreased levels of dopamine as well as for other medical conditions.

Keywords: Paradigm Shift; Brain; Dopamine; Neurotransmitters; Stabilizers.

Although the title of my talk for today indicates that I will be discussing my 70 years of research on the brain, I have reduced this claim by ten years. This because my first ten years of scientific work were dedicated towards training as a pharmacologist at the University of Lund. It was a good training which pretty much focused on the biochemical aspects of pharmacology. However, it was not until a decade later in 1955 when I went on sabbatical that things really started to happen in my research. A paradigm shift in our understanding of the brain was underway and perhaps I should first explain what I mean by this, before expounding on my work.

It was at the time when people first really started to think of the brain in terms of chemistry. Before that, the brain had been considered as an electrical device. And as such, of course truly a black box. And when the brain began to be considered from a chemistry angle it opened up entirely new

perspectives. And it was towards the realm that I gravitated. In my opinion this was when the whole early understanding of the brain began. Shortly prior to my entering this field, I was involved in a discovery that would eventually lead to unraveling the role of dopamine as an independent neurotransmitter and along with this, new ways of considering the brain. At the time however I was unaware that this was a breakthrough discovery. And what did this involve? Well, we gave animals in the lab an anti-psychotic drug called Reserpine, not much used anymore, and following this analyzed their brains for serotonin levels, only to find that after a few hours the serotonin had disappeared. At the time serotonin had been identified as present in the brain and speculation relating to its role was that it was important to preserve sanity.

What happened next is that some very clever people, especially in the field of organic chemistry, attempted to explain

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why the elimination of the serotonin had occurred. Among these was Professor Bernard Brodie, a very remarkable individual who ran one of the top labs in chemical pharmacology at the National Institutes of Health in Bethesda. Work was done to try to explain the pharmacological action of Reserpine in terms of the disappearance of serotonin when the drug was administered, and to make it short, they were unable to explain this. I was there in the Bethesda lab during this time, and as a result was introduced to what were for me entirely new methodologies as we tried to work out the explanation to the mode of action for Reserpine. After this I was slated to return back to Lund, my home university, however even prior to my arrival back I had worked out an agreement with a clever histologist, Nils-Ake Hillarp that we would join forces and continue to work on solving the mechanisms relating to Reserpine. We decided to take a look not only at serotonin but at some other closely-related chemicals – catecholamine, adrenaline and noradrenaline. So upon my return, we began immediately working on this. As a result of this research we discovered that when Reserpine was administered not only serotonin was impacted.

Figure 1. Reversal of reserpine's effects by DOPA. (Top) Rabbits treated with reserpine (5 mg/kg intravenously). (Bottom) The same rabbits 15 min after D-L-DOPA (200 mg/kg intravenously). [From (41)]

At the top you can see some rabbits. They have been given the anti-psychotic drugs, and it is obvious that there is not much in terms of wakefulness or motility in these animals. We can place them in these unusual positions and they remain so. However after giving them an IV injection of the precursor of noradrenaline, L-DOPA, they were within a short period up and running again. All of the symptoms brought on by the administration of the Reserpine had seemingly reversed and we realized that it was not the serotonin that had caused this. We thought that it was the noradrenaline. But when we analyzed

these brains, we observed that there was still noradrenaline there. So we thought that it had to be dopamine and so we developed methods for analyzing dopamine levels and sure enough, it proved to be dopamine. Dopamine disappeared after this anti-psychotic drug was administered and returned when L-DOPA is dispensed. So that's how we discovered that dopamine occurs normally in the brain as it was not known before.

So that was how the whole notion of Dopamine and the unraveling as to its role began. We were, of course very excited about this discovery. And I remember especially one meeting in London in 1960 with Sir Henry Dale, a Nobel laureate and the top man in the field of neuro transmitters at that time, along with a number of other illustrious scientists. We presented our story and Dale and the others all agreed that our results were in error. They unanimously agreed. And so Hillarp who had also attended the meeting, and I had to return home. We were adamant that we had to do something to convince the scientific community that we were correct in our findings. So Hillarp, an especially skillful histochemist, developed a method by which it was made possible to visualize these compounds – dopamine, noradrenaline and serotonin in the brain at the cellular level, at the microscopic level. To do this fluorescence microscopy was used and sure enough, we found after florescent treatment, that there were dopamine cells in the brain stem of a rabbit. And with this information we could then map the neural pathways for noradrenaline, dopamine and serotonin. At the synaptic level we were able to come up with a model where we could explain the effect of Reserpine as acting in a storage vesicle. In the transection of the nerve there could be either a dopaminergic, noradrenaline or serotonergic transmission and we could show how Reserpine acts to block this uptake. There was another uptake from this, with another anti-psychotic drug called chlorpromazine, it did not work at all at this location. However, it worked on the other side of the synaptic cap at the receptor. This work proving our earlier claims was done in 1965, five years after the London meeting. Another international meeting was set up this time in Stockholm to consider the findings and everybody agreed indeed these compounds were important. And we were right, these were the neurotransmitters in the brain.

Then something happened that in parallel with the basic work that we were conducting. When a Viennese neurologist named Walther Birkmayer, who was working in a geriatric hospital in Vienna, heard about the effects of the antipsychotic drugs, in particular that Reserpine caused the depletion of serotonin, he knew from his numerous neurology patients that treatment with Reserpine exhibited a side effect representing symptoms that very much mimicked Parkinson's Disease. And so he came up with a great idea which was to analyze the levels of serotonin in the brain of diseased Parkinson people and see whether or not it had been depleted.

Birkmeyer went to the pharmacological society of Vienna and tried to persuade them to do this, but it was deemed a bad idea. Therefore nothing came from this initiative. After we had published our dopamine data however, the pharmacological society changed their minds. An analysis of dopamine levels was conducted and sure enough, the findings showed that the levels of dopamine had depleted. However, if they had conducted the serotonin analysis earlier when Birkmeyer had suggested this, they would have found that serotonin too, although not completely gone, was very much reduced.

So an opportunity was missed, but none-the-less it was this fellow Birkmeyer who first came up with the idea of a link between these mechanisms and Parkinson's Disease, the relation to movement control and all that. As a result, he collaborated on a lot of important work in this field and also gave, for the first time, L-Dopa to Parkinson' patients and it worked. He had movies proving that it was effective, however there were a number of other clinics around the world who tried this but the outcome was variable. We really don't fully understand why the treatment didn't work everywhere. Before I go on with a story, I would like to mention how the world responded to all these findings. Take a look at the number of citations, from 1950 through 2000 and beyond. The green curve here is the number of annual citations for dopamine. What you can see is that during the late 50's the years when we did most of the crucial work that was rejected in 1960 in London as well as in the following years, during the period when we conducted additional work to further substantiate our findings, there were hardly any citations at all. This even though there was work being done in Vienna as well as some similar research in Japan, all of which substantiated that we had discovered something terribly important. It took a very long time for widespread acceptance of dopamine as anything significant however, in part as the pharmacologists knew that dopamine as an intermediate in peripheral organs, measured as inactive and therefore it was supposed, not of

consequence. This perception was maintained until the late 1960's.

In 1967, a Greek-American neurologist, by the name of George Cotzias had an idea to change the process by which dopamine was administered. Until then this had been administered by intravenous injection, however Cotzias introduced an oral procedure, which slowly increased the quantity of the dosage and this method proved to work tremendously well. The new therapeutic approach was so effective that within a few years, the treatment of Parkinson's Disease with L-DOPA had successfully spread all over the whole world, which was of course a dramatic event. It was then that the citations started to increase. This fantastic medical story also caught the imagination of the general public through the book written by Oliver Sacks that became the movie titled "Awakenings". In the film the audience is treated to "awakenings" in patients similar to those that we had demonstrated in our rabbits, an exciting experience to witness.

Once the concept of a neurotransmitter had finally been accepted, a number of other neurotransmitters were identified allowing for the making of all kinds of circuitries. I am not going into details here but of course it is very important to really get a better understanding of how the brain works. Here you have the cortex and the striatum where the Dopamine is very much located and you also have the GABA receptors and further down is the brain stem with the dopaminergic and other monoaminergic cell bodies. When trying to understand these complex systems a number of pharmacological mistakes were made early on. These are understandable as what had been discovered at that time was that anti-psychotic drugs act by blocking Dopamine. There was some evidence that there actually is an elevation of the dopamine function within the cervix, however the dopamine present there represents more of the psychosis. This has been confirmed in various ways later on. On the other hand, in Parkinson's Disease you have the depletion of dopamine and other chemicals and then by means of administering agonists, you can reverse this. So initially for treatment of psychosis, antagonists were used. These dopamine antagonists provided very powerful anti-psychotic action. Before this time when we began to first understand the mechanisms for how these were working, the effects of treatments such as Chlorpromazine had been discovered mostly by serendipity.

So you had these findings and the pharmaceutical industry started to work on these. Both agonists and antagonists were very powerful, the idea was to screen for the most potent, the most selective agonists or antagonists that could be found. For those low in dopamine the thinking was that they should receive agonists. Whereas for those with higher dopamine levels, they should be treated with antagonists. This concept was utilized when we began dealing with other neurotransmitters as well. New powerful in vitro screening

technologies were being put to use to help with the development of anti-psychotic drugs. With these you could target a specific molecule, a receptor, such as a dopamine receptor or D2 receptor, that you believed could be important for a certain disorder. Using molecular biology one could get these transected into cells or have them multiply. Very powerful screenings were conducted looking for the most powerful antagonists and agonists using this wonderful technique and what resulted was that the strongest of these were indeed found. These developments were useful and led to a number of new compounds. However, the mistake that was made was that the primary screening involved looking for the most powerful option and of course this was the outcome. Certainly progress was made, however if you compare the degree of pharmacological progress compared to the scientific knowledge that was accumulating in this field, the bedside utility was a rather modest one must say. By and large the premise to look right away for the most powerful remedy was a great mistake and I think one of the reasons why the pharmaceutical industry in developing new drugs, insofar as the brain is concerned, hasn't been doing so well lately. Of course, there are other reasons as well for that but the perspective involving the search for the most potent application has been an influence. So, what should be done in order to improve this?

Another thing before I address this question is that what we have learned over the years relating to neurotransmitters is that if you manipulate neurotransmitters you can generate acute, very powerful effects. Either therapeutic effects or side effects, but in addition to this, there are long-term effects. Very strong effects that can really alter the brain and I am now coming in to the concept of plasticity which, of course, has been known for a long time. There is however a growing awareness that this plasticity aspect is enormously important. I will give you one example. Such an experiment or study cannot be done in humans for ethical reasons, so these are being conducted in macaws and monkeys. What we see here is an experiment where you have S that stands for sham control, you have H that's for haloperidol, a powerful anti-psychotic, such as Olanzapine, a more modern type of anti-psychotic, but both of these represent anti-dopaminergic compounds.

They drugs were administered to monkeys in a very careful, controlled way and a series of studies were conducted over about a year and a half and following this their brains were analyzed. One can see that there were even microscopic changes. Evidence that the brain is being rebuilt by these very powerful compounds. So what we see here in this one example, is that there is a reduction of gray matter in certain cortical areas. This is the result of treatment from the drugs administered in comparable protocol to those used in humans. And what we know is that changes such as those that you see here can also be seen in schizophrenia patients

and the question is, are these the results of a disorder or is it the drug that causes the change? And it's very hard to tell the difference as this kind of experiment cannot be conducted in humans, of course. A human cannot be given a substance for a year and a half to see if their brain shrinks or not.

So this is the information that we have. This series of studies conducted in Pittsburgh, indicated that when you block these particular receptors a profound change occurs. Agonists overall have been abused as drugs. They do terrible things to human beings and, therefore, should be avoided. Although we cannot stop using the anti-psychotic drugs that are available this time, we must try very hard to get rid of them. What we have come up with and I will try to make this short, is the concept of stabilizers. So this is a stabilizer, these are very important. There are the pros to stabilizers, of course, as you can stabilize something like dopamine for example if it is high, you can bring it down and if it is low you can bring it up. However, there are also cons as of course you are dampening the system and that can change these levels. This compound that is being used to test the stabilizer hypothesis has a preferential action mechanism for receptors that lie along the dopaminergic nerve. Semiological techniques conducted by electron microscope allow for the actualization of the location of the receptors such as the D2 receptors. Some of the receptors are sitting on the dopaminergic neuron itself, while others are in other neurons. Here are located the auto receptors but there are also hetero receptors. As mentioned the stabilizing compound that we are trying now has a preferential action mechanism. In very low doses it starts to act which means that now in a very mild physiological manner you are increasing the dopamine activity very differently from what centrum stimulants, such as what amphetamine and the like are doing. So this activity represents a very smooth physiologic stimulation, a well-controlled stimulation of the dopaminergic system. These compounds are antagonists, in principle. So if you are able to antagonize auto receptors preferentially, you can stimulate dopaminergic activity. But if you give a higher dosage, it starts to work also on the other dopaminergic symbols that you have as well. These will want to be stimulated so that they can operate in the opposite direction. There is a balance effect here.

In animal testing, animals that were placed in a cage with few distractions, do not exhibit much activity or stimulation after they have explored the cage. At this point there is limited activity and when you give an increased dosage of the stabilizer you see in these animals that the dopamine level starts to rise. On the other hand, if you have animals that are introduced into a big arena with lots of things to explore, they start out with very high levels of dopamine and if now you give the same drug in increased dosages, this starts to go down. So this is the stabilizer function, if you a situation that is in between, neither high nor low levels of stimulation, there

is almost no effect from the compound. If levels are high, they go down, if they are low, they go up. That is the stabilizer concept. We have demonstrated this repeatedly in a number of animal studies, and this has also been shown to occur in humans as well. And before going there, I just want to just to say that if you conduct a study with the drug in healthy volunteers, where you have a 0.2 micromolar concentration in the blood you have about 20–25 per cent displacement of radioactive erythrocytes you are able to see that there has been a binding to the D2 receptors. In addition to the binding by the drug itself, you also have the displacement in use by the released Dopamine that has been stimulated by the blocking of the auto receptors. So we have a very good understanding as to how these drugs act. It has also proven effective for patients experiencing mental fatigue. For this study people were selected following a stroke. They are in a poor condition, exhibiting an extreme kind of fatigue. The original study and we have conducted several since then, was published

in 2012. We found a very clear cut effect. Those who are on the high columns here, are the ones who said that they felt much better on drugs. There was only one fellow who felt the opposite and we think that he was a responder as well. So, there is a very high percentage of respondents amongst these mental fatigue patients. All the studies that have been done all show statistically significant effects. The studies have not been very big but still large enough to demonstrate statistical significance. This also includes one study where you do have not a deficiency of Dopamine, but rather an agonist effect on the Dopamine due to alcohol intake. The study conducted at the Karolinska Institute also considered effects on alcohol cravings upon treatment with this drug and reduced cravings were confirmed as statistically significant. I think we have reached a proof of concept here and that these drugs and this principle of using stabilizers rather than full blown agonists and antagonists has a great future, and I thank you so much for listening.