25 Nobel Laureates in Neurosciences

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Foreword
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Arvid Carlsson (1923–2018)

Arvid Carlsson's systematic study of the biochemistry of the brain resulted in the discovery that dopamine is a neurotransmitter that plays a critical role in the genesis of movement. This remarkable work paved the way for the understanding of the role of dopamine in Parkinson's disease (PD) and its subsequent use for management.

Carlsson was born in Uppsala, Sweden in an academic family and grew up in the city of Lund where his father was a Professor of History. He chose to study medical science at the University of Lund. A few years before joining the University he went to Germany in the summer of 1939 along with a friend a few months before World War II broke out. During the first year of his clinical training, he was recruited to examine the prisoners of German concentration camps who were mostly Jews and transported to Sweden. He wrote, *"Some of the prisoners were taken to Lund where a big tent was erected in a park to house them... As a medical student, I was given the task to examine several of these prisoners. Many of them were children, suffering from undernutrition. Tuberculosis was not uncommon. However, the most shocking was their mental status. They behaved like wild animals, obviously suffering from severe anguish and suspiciousness and trusting nobody."*

Carlsson received his Bachelor's degree in due time and subsequently, a Doctorate in Pharmacology in 1951. During this period, he was assigned several research activities on different pharmacological agents. He joined the department as a junior member of the faculty and after a few years when he applied for the post of Associate Professor, he was denied. The review committee is reported to have told him that the metabolism of calcium, his chief interest, was not an attractive proposition. Carlsson was left with Hobson's choice of sticking to Pharmacology or making his foray into some new and unexplored field of research work. He opted for the latter option and went to the United States of America for a 5-month fellowship with Bernard Beryl Brodie (1907–1989), a boxer-turned biochemist, considered by many as the father of modern Pharmacology and an outstanding Cardiac Pharmacology at the National Heart Institute, Bethesda.

Brodie was immersed in the study of reserpine, a drug used for the treatment of schizophrenia. Injection of reserpine immobilized rabbits but the reason was not clearly understood. Brodie concluded that reserpine depleted serotonin, a neurotransmitter, which was later linked to the basis of depression. He requisitioned Carlsson to investigate the effect of reserpine on serotonin in erythrocytes and Carlsson exclaimed, *"I can hardly overemphasize enough how lucky I was to get the opportunity to work in Brodie's laboratory."* In collaboration with Alfred Pletscher (1917–2006), an eminent neuropharmacologist from Switzerland, he started working on the antihypertensive effects of reserpine. It was already documented that injection of reserpine lowered the level of serotonin in the brain and it was

speculated that its antihypertensive action was hyposerotoninergic in effect. However, Carlsson felt that the action was brought about by catecholamines and observed that reserpine depleted serotonin in platelets. Stanley Fahn (1933-), a contemporary giant in the field of movement disorders from Columbia, wrote in an exhaustive review article, *"The Medical Treatment of Parkinson's Disease from James Parkinson to George Cotzias"* in the journal *Movement Disorders* in 2015 that Carlsson suggested to Brodie to study the role of reserpine in the depletion of catecholamines in the brain, but the latter was not interested.

On his return to Sweden in 1957, Carlsson was appointed as an Associate Professor and elevated to the Chair of Professor 2 years later. He set up his laboratory at his parent University and continued from where Brodie had left. One observation of great importance was that reserpine depleted another neurotransmitter, noradrenaline. Now, the cardinal question was which agent governed movement: serotonin or noradrenaline? Following his experiments, he was able to show that following the administration of reserpine there was a complete disappearance of norepinephrine from the adrenal medulla and brain, and the akinetic effect of reserpine in rats resembled the clinical picture of parkinsonism, which was quickly reversed by levodopa. The precursor of serotonin, 5-hydroxytryptophan had no such effect. Since serotonin or its precursor did not reverse the picture, Carlsson was delighted that he had solved a riddle. He surmised that noradrenaline is synthesized from dopamine which in turn was metabolized from levodopa, and was surprised to observe that the brains of the animals had little noradrenaline and plenty of dopamine. Till then, scientists in general thought that dopamine was the parent material for the production of noradrenaline but Carlsson hinted for the first time that it itself was a neurotransmitter.

Carlsson collaborated with the histochemist and the notable Swedish researcher on monoamines, Nils-Åke Hillarp (1916–1965), and their works helped to visualize the cellular biogenic amines. The neurons were present in several nuclei in the brainstem and their axons innervated various areas of the cerebral cortex. These works were presented at the symposium on the Biochemistry and Pharmacology of the Basal Ganglia which was held in New York in 1965. Carlsson and Hillarp proposed that the dopamine-containing cell bodies were localized in the *pars compacta* of the substantia nigra and they spread their fibers to the caudate nucleus and putamen. Around this time, Theodore Sourkes (1920–2015) and Louis Poirier (1918–2014), neurochemist and neuroanatomist of great eminence, carried out experimental works in Montreal, Canada, and showed that lesioning of the substantia nigra led to dopamine deficiency. In the meantime, Katherine Montagu (1907–1966) identified for the first time the presence of dopamine in the human brain in August 1957 in Hans Weil-Malherbe's laboratory at the Runwell Hospital near London by paper chromatography and incidentally, this was followed by the work of Weil-Malherbe in November in the same year.

Carlsson and Hillarp acquired an instrument, Aminco-Bowman spectrophotofluorometer, and devised a sensitive fluorescent assay method to measure dopamine in the brain. They observed that the striatum was rich in this substance which was later validated by the experiments of Marthe Vogt, Butler, and Rosenberg, Carlsson's postdoctoral fellows, in the dog brain, while H Sano from Japan confirmed similar results in the human brain. These experiments led Carlsson to conclude that reserpine depleted the dopamine store in the brain and its deficiency was implicated in the development of motor disorders, resembling PD.

At the First International Catecholamine Symposium in October 1958, Carlsson summed up his findings and postulated that dopamine appeared to play a major role in motor functions. He presented his work

entitled, *"On the biochemistry and possible functions of dopamine and noradrenaline in the brain"* at the Ciba Foundation Symposium in London in 1960, but the reception was lukewarm. His presentation generated more heat than light and led to a lot of debate. It was argued whether dopamine derived from levodopa was the precursor of norepinephrine. Furthermore, since many animals died, the question was raised whether levodopa was a poisonous amino acid. Andrew Lees, Eduardo Tolosa, and Warren Olanow, all eminent experts in movement disorders wrote in a scholarly article *"Four Pioneers of L-dopa Treatment: Arvid Carlsson, Oleh Hornykiewicz, George Cotzias, and Melvin Yahr"*, in *Movement Disorders* in 2015, that the much venerated octogenarian Sir Henry Hallett Dale (1875–1968) and the Nobel Laureate in 1936 was not convinced about Carlsson's observations and commented that dopamine had not yet fulfilled the criterion for a neurotransmitter. The actions were possibly attributable to L-dopa being a *"mysterious brain toxin and when combined with a monoamine oxidase inhibitor led to excitation and could be lethal."*

A crestfallen Carlsson proceeded with his investigations of reserpine on brain function and biochemistry. The key innovations were the development of a sensitive fluorescent assay to measure dopamine in the brain, discovering that levodopa, and not L-5-hydroxytryptophan, the precursor of serotonin, reversed the neuroleptic effect of reserpine in rabbits. The other cardinal finding was that reserpine depleted brain dopamine and administering levodopa to the animals replenished it. Å Bertler and E Rosengren, Carlsson's doctoral students, measured the regional concentration of dopamine in the dog brain and found it to be most highly concentrated in the striatum. At the same time, Japanese workers showed a similar distribution to humans. Importantly, Oleh Hornykiewicz (1926–2020), an outstanding scientist with a stupendous *contribution* to the understanding of dopamine and movement disorders identified later that it was concentrated in the highest amount in the striatum.

Below is the set of the famous photographs of the pair of rabbits, reserpinized and rendered lethargic and immobile, while the one below shows the rejuvenated and alert ones with the use of L-dopa by Arvid Carlsson, M Lindqvist, and T Magnusson. The work was published in Nature in November 1957 in a paper *3,4-dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonist.* This photograph was shown by Carlsson in his Nobel Lecture entitled, *"A Half-Century of Neurotransmitter Research: Impact on Neurology and Psychiatry"* in Stockholm in 2000. This has been described by Stanley Fahn (1933-) as possibly the most frequently shown photograph in all research on dopamine.

In a personal note to Andrew Lees and forwarded to Stanley Fahn, Carlsson wrote,

> *"Dear Andrew, Dear Stanley,*
>
> *Thank you for your interest in this picture, which is probably the one I have shown most frequently during my entire research career. I submitted it in 1957 together with my manuscript to Nature and the paper*

was accepted. However, the picture was not published, because the referee (I guess who it was) didn't consider its publication necessary. The earliest publication of this picture is probably the one to be found in the proceedings of a meeting that must have taken place in the fall of 1957, somewhere in a German-speaking country, perhaps in Basel, but for some reason, the proceedings were published as late as 1960, in a Separatum of Psychiatria et Neurologia Basel, 140:220–222, S Karger, Switzerland. A Carlsson, "Zur Frage der Wirkungsweise einiger Psychopharmaka." …Best wishes, Arvid.

When Carlsson's attempt to establish his hypothesis met with acerbic criticisms, Hillarp and his collaborators carried out some histochemical works which confirmed the localization of monoamines in the brain and its pathways. Although in 1963, Hugh McLennan (1927–2004) expressed an identical view that *"no definitely ascribable adrenergic synapse has been detected… in the vertebrate central nervous system,"* an undeterred Carlsson, unfazed at the barbed comments met Lars Leksell (1907–1986) at Lund who was the inventor of radiosurgery along with the arc-centered stereotactic frame and had been treating patients of PD with stereotactic surgery. Like others, Leksell was not fully convinced, while the endeavor to impress others met with similarly frustrating results.

In 1967, Carlsson watched a film that depicted the results of the low and slow increment of levodopa by George Cotzias in New York. On returning to his motherland, he met the geriatrician, Alvar Svenborg, and was finally able to prove the dramatic results of the use of levodopa in severely handicapped patients. Thus, Carlsson stuck to his idea gamely and his pertinacious persistence with his idea and lifelong conviction, despite continual resistance from all quarters, that the loss of dopamine was the chief culprit in the genesis of PD was justified. His work was no fruit of serendipity; on the contrary, it was the outcome of his firm conviction, unabated zeal, indomitable spirit, and use of technological advancement. This was finally vindicated by the improvement in the clinical status of the patients with the use of levodopa, the litmus test of his conviction. His contributions were finally recognized by the conferring of the Nobel Prize in 2000. In his inaugural lecture in Stockholm at the 19th conference organized by the International Parkinson and Movement Disorders Society in June 2014, referring to his own troubled experience and stubborn resistance, his take-home message was ***"Do not give in; stick it out."***

In his Nobel Lecture, Carlsson said,

"… I came to work under Dr Brodie for about five months, … and his colleagues had just a few months before made a breakthrough discovery, namely that the administration of reserpine, caused the virtually complete disappearance of serotonin from the brain and other tissues…

…I proposed to Brodie that we investigate the effect of reserpine on the catecholamines given their similarity to serotonin. But Brodie thought that this would be a waste of time… I wrote to… Professor Nils Åke Hillarp… proposed collaboration, and he agreed… We demonstrated the depletion of catecholamines from the adrenal medulla of rabbits following treatment with reserpine… The same results were obtained when we analyzed the heart and brain… To investigate this, we gave DOPA to reserpine-treated animals and thus

discovered the dramatic reversal of the reserpine-induced syndrome by this catecholamine precursor.

...Later we found the unique distribution of dopamine in the brain... This led us to conclude that depletion of dopamine will induce Parkinsonian syndrome and that treatment with L-DOPA will alleviate the syndrome... All this I presented at the First International Catecholamine Symposium in October 1958.

A year and a half later, ...the debate that followed our paper, ...revealed a profound and nearly unanimous skepticism... Dale expressed the view that L-DOPA was a poison... Marthe Vogt concluded that... the action of serotonin and catecholamines, respectively, in the brain would not have a long life... John Gaddum, an outstanding pharmacologist from Cambridge who discovered the substance P, ...stated that at this meeting nobody has ventured to speculate on the relationship between catecholamines and the functions of the brain..."

It is worth noting that in 2016 he once said, *"Dopamine is involved in everything that happens in our brain, all the important functions... If you look at the number of citations dealing with dopamine, over the decades, it was going up all the time, dramatically, and finally, it was so high the Nobel assembly couldn't avoid me."* One cannot but notice the cry from the heart and a distinct trace of despondency in his lecture that he was not recognized on time and the prize was awarded a bit too late.

No account of Carlsson can be complete without reference to Oleh Hornykiewicz, the brilliant neuropathologist from Vienna, Austria. Like Carlsson, he too trained with Herman Blaschko at Oxford and returned to his motherland in 1956. In collaboration with his laboratory assistant Herbert Ehringer (1932-), he decided to study the dopamine content in the brain of patients with PD, postencephalitic parkinsonism, Huntington's disease, and brains of people without any discernible cerebral pathology. He reported dopamine deficiency in the range of 90% in PD and postencephalitic parkinsonism, while there was no such change in Huntington's disease. Later on, Hornykiewicz demonstrated severe reduction of dopamine in the substantia nigra with little reduction of norepinephrine and serotonin. He postulated that most of the major motor symptoms of PD were related to the deficiency of dopamine in the striatum and the symptoms appear only after an 80% reduction of dopamine in the putamen. As was the experience of Carlsson, the response to Hornykiewicz's works was equally tepid. For instance, Derek Denny-Brown (1901–1981), the eminent neurologist from Boston, and Rolf Hassler, an anatomist from Frankfurt repudiated his claims and denounced the idea of an ascending nigrostriatal dopaminergic system. Thus, despite such brilliant works by Carlsson and Hornykiewicz, the concept of dopamine and PD was thus getting muddled by any clear association between the loss of striatal and nigral dopamine content on one hand and the degeneration of dopaminergic neurons on the other. It must be mentioned that with Carlsson's highly innovative work to localize the biogenic amines by spectrophotometric investigations along with Hillarp, this incongruity was resolved to a large extent. The works and Sourkes and Poirier, as mentioned earlier further bolstered the concept.

Hornykiewicz was turning diffident and beginning to wonder whether dopamine had any future in the management of Parkinsonism. In 2010, he wrote ruefully in the *Journal of Neurology* in an article *"The History of Levodopa"*.

> *"...one would have expected unanimous support... This was indeed the case with the vast majority of clinical neurologists, but not at all the rule among the basic brain scientists... Among those strongly critical of DA and levodopa were the best minds of the contemporary neuroscience..."*

Carlsson's contributions to the understanding of the pathophysiology of PD were immortalized by Oliver Sacks (1933–2015), the British neurologist in his book, "The Awakening" in 1973, which was later filmed in 1990. It dealt with the life of the victims of postencephalitic parkinsonism in the 1920s and the remarkable improvement in their symptoms with the use of levodopa.

Later, when dopamine was found to be a useful agent, the gold standard for the management of PD, the world of neurology found it hard to accept the fact that when the Nobel Prize in 2000 was awarded to Carlsson, Hornykiewicz was overlooked. More than 250 eminent neurologists wrote to the Nobel Committee, expressing their exasperation. However, Hornykiewicz stated politely that scientists take to investigative studies not to win prizes and receive universal recognition. However, he regretted that the Nobel Committee was amiss in their assessment that Carlsson was the chief architect in discovering low dopamine in the brain in PD. True to what he said, Hornykiewicz and his team were the scientists behind this fundamental work. The path-finding works of Carlsson and Hornykiewicz were the inspiration for George Cotzias from Greece, who settled later in the United States of America (1918–1977) and introduced the clinical use of levodopa by low and slow incremental dosage, and Melvin Yahr from the United States of America (1917–2004) who with his colleagues established incontrovertibly the efficacy of the drug by double-blind study.

In his quest for dopamine and neurotransmission in the brain, Carlsson contributed tirelessly and wrote copiously for >60 years in his 95 years of existence. Some of the notable ones are:

- *3,4-dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists, 1957.*
- *On the catecholamine levels in blood plasma after stimulation of the sympathoadrenal system, 1958.*
- *On the presence of 3-hydroxytryptamine in the brain, 1958.*
- *A new histochemical method for visualization of tissue catecholamines, 1961.*
- *A Mg^{++}-ATP dependent storage mechanism in the amine granules of the adrenal medulla, 1962.*
- *Reserpine-resistant uptake mechanisms of noradrenaline in tissues, 1963.*
- *Noradrenaline release by nerve stimulation of the spinal cord, 1965.*
- *Selective protection of 5-hydroxytryptamine stores against the action of reserpine by treatment with 5-hydroxytryptophan, 1967.*
- *Accumulation of 5-hydroxytryptophan in mouse brain after decarboxylase inhibition, 1970.*
- *Distribution of Dopamine in the rat cerebral cortex, 1970.*

Carlsson was critical of fluoridation of water, a method adopted to prevent dental caries. He said that fluoride leads to mottled teeth and there was no way to regulate the amount of fluoride in a person. This indefatigable research worker did not stop once his hypothesis on reserpine was established. He collaborated with the pharmaceutical company AstraZeneca during the 1970s and 1980s and marketed the first selective serotonin reuptake inhibitor, zimeldine, though it was withdrawn from the market at a later date due to the development

of Guillain–Barré syndrome. However, this led to the development of fluoxetine, the first widely used serotonin reuptake inhibitor with a good outcome. Even when he was past 90 years of age his research activities went on unabated and worked with his daughter Lena on the discovery of OSU6162, a dopamine stabilizer for the management of poststroke fatigue.

Carlsson received the Nobel Prize in 2000 along with Paul Greengard, an American neuroscientist (1925–2019), and Eric Kandel, an American-Austrian neuroscientist (1929-), for their work on signal transmission in the central nervous system. This apart, Carlsson received numerous awards in his life and was elected as a Member of the Royal Swedish Academy of Sciences in 1975. He was active in his research activities and was a fluent speaker till his last days. He died in 2018 at the age of 95.

Indeed, Carlsson was the pathfinder in the field of research on levodopa and the management of PD.

25 Nobel Laureates in Neurosciences

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