The molecular wake-up call

It is 50 years since Arvid Carlsson showed dopamine to be a neurotransmitter. **Alison Abbott** profiles a chemical and its champion.

They were conscious but you wouldn't know it: able to perceive the world around them but powerless to look around, sniff the air or to cry out. So when the young scientist injected them with a chemical called L-dopa, he witnessed what seemed to be a miracle. They stirred, opened their eyes and began roaming around as if nothing had happened.

This may sound familiar from the book *Awakenings*[1] — the true story of how, in 1963, the neurologist Oliver Sacks used L-dopa to spectacularly revive patients with sleeping sickness who had been 'frozen', speechless and motionless, for more than 40 years. But the unwritten and equally startling prequel took place in Lund, Sweden, several years earlier. The protagonists were rabbits; their saviour a young Swedish pharmacologist called Arvid Carlsson.

In his experiment, Carlsson showed that dopamine — the chemical manufactured from levodopa, or L-dopa — acts as a neurotransmitter in the brain, passing signals between neighbouring neurons. Injection of L-dopa restored the propagation of electrical signals in the brains of rabbits that had been rendered catatonic, allowing the animals to move. But the pharmacological establishment was scornful of Carlsson's claim. At a London meeting in 1960, the foremost experts in neural transmission made it clear that they didn't believe him — dopamine was thought to be the metabolite of another neurotransmitter rather than one in its own right.

Within years the critics were silenced. Dopamine was shown to be a pivotal chemical in the neural circuits that drive pleasure and addiction, as well as in illnesses such as Parkinson's disease, for which L-dopa quickly became a first-line treatment. It remains so today. In 2000, Carlsson shared the Nobel Prize in Physiology and Medicine for his discovery. And next week neuroscientists will gather at a meeting in Carlsson's home town of Gothenburg, Sweden, to celebrate the 50th anniversary of his formative paper on the awakened rabbits[2].


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ploid elements a homogeneous population as to their deoxyribonucleic acid content.
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3,4-Dihydroxyphenylalanine and 5-Hydroxytryptophan as Reserpine Antagonists

The depletion by reserpine of storage in the body of 5-hydroxytryptamine ('Serotonin') and of the catechol amines is now well established[1-3]. In reserpinized animals the peripheral part of the adrenergic system does not function owing to lack of the transmitter[4]. This is presumably true also of the central part of the adrenergic system. However, it remains to be proved to what extent the central action of reserpine may be attributed to changes in brain catechol amines and/or 5-hydroxytryptamine.

If lack of amines were responsible for the central action of reserpine, administration of the amines in question should counteract these effects, provided that the amines were capable of entering the brain. However, 5-hydroxytryptamine has been shown not to penetrate the blood-brain barrier readily[5], and this may be true also of the catechol amines. This difficulty may be overcome by administering the amino-acid precursors of the amines. Thus injection of 5-hydroxytryptophan is followed by an increase in the level of 5-hydroxytryptamine in brain as well as by central excitation[4]. Preliminary experiments in this laboratory have shown that in this respect 3,4-dihydroxyphenylalanine, which is the precursor of the catechol amines (dopamine, noradrenaline, and adrenaline), behaves similarly.

Experiments were performed on mice (males weighing about 10 gm.), which received an intraperitoneal injection of reserpine (20–40 mgm. per kgm.). After about 16 hr., when the animals were markedly tranquillized and showed complete ptosis of the eyelid, 5-hydroxytryptophan, 3,4-dihydroxyphenylalanine, or a mixture of both (in the DL-form throughout) [illegible] tonically. In [illegible] 5-hyd[illegible]

A dramatic effect of 3,4-dihydroxyphenylalanine (200 mgm. per kgm. intravenously) was observed also in rabbits which had received reserpine in a dose of 5 mgm. per kgm. intravenously 4 hr. earlier. Within 10–15 min. after the injection of 3,4-dihydroxyphenylalanine the tranquillization as well as ptosis and miosis caused by reserpine had disappeared completely. If the animal had received iproniazid (100 mgm. per kgm. intravenously) about 2 hr. before the 3,4-dihydroxyphenylalanine, the dose of the latter required to antagonize the effect of reserpine was markedly reduced. This supports the assumption that the effect of 3,4-dihydroxyphenylalanine was due to an amine formed from it. (The iproniazid, when administered about two hours after the reserpine as in those experiments, did not *per se* counteract the tranquillizing effect of reserpine.) In normal rabbits 3,4-dihydroxyphenylalanine caused central stimulation, which was likewise markedly potentiated by iproniazid pretreatment.

A full account of these experiments will be published elsewhere.

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June 19.

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Antihypertensive Activity of Hexahydro-1-Azepinepropionamidoxime

Hexahydro-1-azepinepropionamidoxime dihydrochloride (I), designated as *SU*-4029, has been studied for its effect on the cardiovascular system of the dog.

(I)

[illegible] single intravenous dose of 30 mgm./kgm. of this [illegible] and lowered the arterial pressure of neurogenic [illegible] hypertensive dogs while not notably [illegible] the blood pressure of normotensive dogs. [illegible], in normotensive animals 30 mgm./kgm. of [illegible] given intravenously eliminated the severe [illegible] elicited by high doses of amphetamine [illegible] drine and also markedly antagonized carotid [illegible] reflex pressor responses. These antihyper- [illegible] fects were slow in onset and lasted for [illegible] only two to six weeks following single [illegible] *SU*-4029 was orally active and had a [illegible] action when given in small daily doses. [illegible] re the compound, hexahydroazepine was [illegible] ted and gave hexahydro-1-azepinepro- [illegible] b.p. 121–123°/14 mm.; n_D^{20} 1·4710 [illegible] 0·82; H, 10·52; N, 18·53 per cent. [illegible] res: C, 71·11; H, 10·61; N, 18·43 [illegible] eatment of this nitrile with hydroxyl-

Catatonic rabbits were revived by dopamine in a 1957 experiment led by Arvid Carlsson.

During the past half century, Carlsson and dopamine have followed intertwined paths. Researchers now understand that the way dopamine works is subtle and complex, and its mechanisms of action are central to the function of many neurological and psychiatric drugs. And Carlsson, now a sprightly 84-year-old, still spends hours pondering the mysteries of brain chemistry. But he feels marginalized in Gothenburg and, last year, the institute established in his name closed prematurely after bitter feuds about funding.

The field of biomedicine has also evolved during this time, and much has changed. "We used slide rules and manual calculators back then, so statistical calculations were quite time consuming," Carlsson says. But a willingness to embrace new techniques and fight for controversial ideas, as Carlsson has done throughout his career, remains essential to success. "He was one of my heroes when I was a young neurology resident," says William Langston, head of the Parkinson's Institute in Sunnyvale, California. "What impressed me was his brilliant deductive reasoning."

Carlsson is a polite, soft-spoken and humorous gentleman with a hallmark bow tie. He grew up in a family of academics in Lund that focused on the humanities but, in what he refers to as a minor act of rebellion, he chose science over the arts because he thought it more useful to society. He is also single-minded and uncompromising, features that no doubt contributed to his success as a scientist against stiff opposition — and perhaps also to his later problems.

Carlsson calls himself a lucky man, and says his first stroke of luck came when he arrived in the lab of chemist Bernard 'Steve' Brodie at the National Institutes of Health in Bethesda, Maryland, in the summer of 1955. Brodie's lab

pioneered studies with reserpine, one of the very first drugs to be introduced for the selective treatment of schizophrenia, and hence one of the hottest molecules for pharmacologists of the day. Reserpine injections made rabbits catatonic, but how the drug worked was a mystery.

Direct measures

At the time, pharmacologists typically tested the potency of neurotransmitters with assays of their biological activity — for example, applying them to a piece of animal gut under tension in an organ bath to see how much they could contract or relax the muscle. Brodie's team instead developed the spectrophotofluorimeter, a machine able to measure how much neurotransmitter was synthesized from fluorescently tagged precursors. This allowed the researchers to measure the precise level of a compound extracted from tissue rather than an indirect measure of its activity, and later became a standard instrument in biological labs. When Carlsson returned to Lund after his five-month visit to Brodie's lab, the first thing he did was order a spectrophotofluorimeter. "I didn't want to be confined by the indirectness of bioassays," he says.

Carlsson's work with this device showed that reserpine completely drains the stores of several neurotransmitters in the brain. Loss of one of these was causing the rabbits to become catatonic — the question was, which one?

Carlsson reasoned that he could answer this question by adding back the missing neurotransmitter to rabbits that had been frozen with reserpine — the crucial awakening experiment. The blood–brain barrier prevents the neurotransmitters noradrenaline and serotonin from passing into the brain from the blood, so Carlsson instead injected precursors of these molecules that can enter the brain and are then metabolized into the relevant neurotransmitter. One of these precursors was L-dopa, which is converted into dopamine and then, in turn, into noradrenaline.

But when Carlsson examined the revived rabbits' brains after injecting L-dopa he saw a lot of dopamine and very little noradrenaline. At this point it dawned on him that dopamine could be a neurotransmitter in its own right, a memory that still summons astonishment to his face.

Within months his graduate students Åke Bertler and Evald Rosengren had found that dopamine is normally concentrated in areas of the brain known to be involved in movement, such as the basal ganglia[3]. Knowing that high doses of reserpine cause side effects that are similar to some of the movement difficulties experienced by patients with Parkinson's disease, Carlsson proposed that this disease might be caused by a lack of dopamine.

"I won the Nobel Prize 40 years after my discovery. Einstein won one some 20 years after his. So I guess my work was twice as complicated."
— Arvid Carlsson

Sparking opposition

In late 1958, Carlsson travelled across the Atlantic to explain his ideas to a symposium in Bethesda. There, his story was well-received. "But this was not the case when I presented my results at the Ciba meeting in London," says Carlsson, who is still clearly a bit hurt.

The prestigious 1960 Ciba Symposium on Adrenergic Mechanism attracted all the key European players in the field. At the time, a vigorous debate was going on between the 'soups' — who thought that nerve transmission occurred through chemicals — and the 'sparks', who argued that it was all electrical. The soups had more or less won their case for neurotransmission outside the brain but, owing to lack of experimental evidence, the sparks' view still dominated the brain itself. "It's hard to imagine now, but when I was an undergraduate student at Cambridge [in the late 1950s] we were taught categorically that there was no chemical transmission in the brain — that it was just an electrical machine," recalls pharmacologist Leslie Iversen, professor emeritus at the University of Oxford, UK.

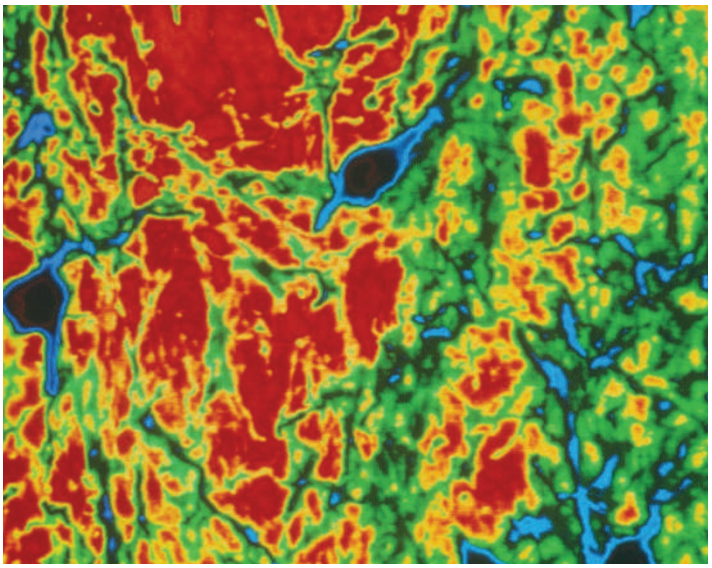
In this setting, Carlsson's ideas went down like a stone. The meeting unceremoniously rejected his interpretation of his data and, to his mortification, the single comment in the discussions praising his work was excluded from the symposium book. "The Ciba meeting might have been the opportunity to tell the world how things really were, but there was uniform hostility from the community," says Iversen.

Carlsson stuck to his guns, and data began to amass to a point that made denial impossible. Later in 1960, for example, the Austrian pharmacologist Oleh Hornykiewicz published studies on postmortem brains from patients with Parkinson's disease, showing the absence of dopamine in the basal ganglia[4]. And a few years later, Annica Dahlström and Kjell Fuxe at the Karolinska Institute in Stockholm, Sweden, showed that in the healthy brain neurons in this region contain high levels of dopamine[5].

In 1961, neurologist Walter Birkmayer, working together with Hornykiewicz, injected the first Parkinson's patients with L-dopa to dramatic effect, allowing previously immobile patients to move freely[6]. Carlsson recalls the penchant of his collaborator Tor Magnusson for testing drugs openly on himself — something that would be regarded with horror today. Expecting to see some kind of neurological effects, Magnusson hooked himself up to intravenous dopamine, but, says Carlsson, "all we saw was emesis!" Many other aspects of practising science were different then. Fax and e-mail did not exist, and all papers were read in the library rather than on a computer. "We certainly wore white lab coats and we addressed secretaries and assistants more formally as Miss," Carlsson says.

Over the next few years, clinicians learnt to start patients on low doses of L-dopa to avoid side effects such as nausea and vomiting. They also noticed other intrinsic imperfections of the drug. After a few years of therapy, its effects temporarily, and unpredictably, switch off in

Arvid Carlsson collects his Nobel prize in 2000, more than 40 years after showing dopamine to be a neurotransmitter.



Degeneration of the nerve cells that secrete dopamine (red) leads to Parkinson's disease.

some patients. In severe cases, patients can suddenly become frozen and be stuck, immobile, for minutes or hours.

Neurologists were also starting to learn that dopamine is involved in much more than the control of movement. They noticed that high doses of L-dopa cause some of the symptoms of psychoses such as schizophrenia, and that the antipsychotic drugs used to treat schizophrenia can cause the same movement problems that are indicative of dopamine deficiency in Parkinson's. This led to the idea that schizophrenia could be related to a disturbance of dopamine neurotransmission in areas of the brain other than those involved in movement.

The molecular multitasker

Carlsson reasoned that antipsychotic drugs could be blocking dopamine receptors, and that neurons might be spitting out more and more dopamine to try to compensate for the blockage. He was right. This type of feedback control of neuronal activity is now a well understood, and critical, phenomenon in neurotransmission. And this way of thinking won Carlsson many fervent admirers. He is "just about the most creative, intuitive scientist I've met", says Solomon Snyder of the Johns Hopkins University in Baltimore, Maryland, who discovered opiate receptors in the brain.

Another side effect of L-dopa treatment is that patients may develop an irrational tendency to gamble. It is now well known that the neural pathways controlling behaviours such as motivation and feelings of reward pivot around dopamine. These pathways drive pursuit of food and sex — and are hijacked by addictive drugs and addictive behaviours such as gambling. In the 1960s, Carlsson was among the first to spot that drugs of abuse work by boosting dopamine transmission in particular brain areas.

Too little dopamine in one area produces Parkinson's, too much dopamine in others can cause psychoses. Over recent decades the importance and complexity of the dopamine system have mushroomed in scientists' eyes. Dopamine acts on many types of receptor, at varying levels and in different brain areas, and in concert with other neurotransmitters.

Another of Carlsson's legacies has been the development of dopamine stabilizers. These are dopamine-like molecules that have been chemically modified so that they activate dopamine receptors to only a certain degree, effectively constraining the level of dopaminergic activation in the brain to within the healthy range. The theory is that a stabilizer could compensate for lack of dopamine in Parkinson's without causing overactivation; or block the overactivity in schizophrenia without too much depletion. Several companies have dopamine stablizers in development, and one, aripiprazole, has been approved by the US Food and Drug Administration for use as an antipsychotic for schizophrenia and bipolar disorder.

When Carlsson reluctantly retired aged 66 — then the law in Sweden — he kept his research going by forming a company called A. Carlsson Research where, among other things, he developed a dopamine stablizer. Then, in 2000, Carlsson's work on dopamine and its control of movement was recognized with a share of a Nobel prize. "I won it 40 years after my discovery," he jokes. "Einstein won his some 20 years after his discovery. So I guess my work was twice as complicated as Einstein's."

> **"When I was an undergraduate student we were taught that there was no chemical transmission in the brain."**
> — Leslie Iversen

Until this time, Carlsson and dopamine had both followed stellar trajectories. But after the Nobel prize, Carlsson's luck began to falter — and the uncompromising side of his nature, which had served him so well in his scientific career, failed to help. Just a few months before winning the Nobel, Carlsson was voted off his own company's board of directors. And in the next few years, plans for his namesake institute also went awry.

Difference of opinion

The Arvid Carlsson Institute was launched in November 2004 with SKr630 million (US$92 million) funding over five years — a tribute from Gothenburg University and the regional authorities to the city's only Nobel prizewinner. Its original mission was to promote health care and neuroscience research in the region, and Carlsson was named honorary chairman of its developmental council. But disagreements began almost immediately about how the money should be divided up. Carlsson wanted a significant proportion to support his field of neuropharmacology, but others argued it should go to neuronal stem-cell research. Scientists decline to discuss details of the arguments, but the hostilities became so bitter that the institute was dissolved in April 2006. Carlsson's daughter Maria, who is also a neuropharmacologist, receives a small proportion of the funds — too small in the opinion of her father, "given that the research was stated to be done to honour my contributions to science".

Carlsson's contributions to science continue. He is closely involved in his daughter's work and they sometimes publish together, although he wishes he could still work in the lab. He also jets around the world to meetings. Much in neuroscience has changed during Carlsson's time, but he believes at least one thing has remained constant. "When I started my career, the most important generator of science was the human mind," he says. "This has probably not changed much during the past half-century." ■

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