Paul Greengard: Signals underlying moods, addictions, and brain disorders

Nowadays, whether people suffer from depression, mental health disorders, or addictions, medication can help. Researchers have learned how to fine tune the brain's functions, often by targeting chemicals called neurotransmitters and the many signaling pathways through which they act.

Although certain neurotransmitters had been identified more than 40 years ago, scientists knew relatively little about how these chemicals actually functioned. The basis for much of what neuroscientists know today about brain signaling was shaped by National Academy of Sciences member Paul Greengard's 1972 PNAS Classic paper, "Dopamine-sensitive Adenylate cyclase in caudate nucleus of rat brain, and its similarity to the 'dopamine receptor'" (1).

In the PNAS Classic paper, Kebabian et al. (1) identified enzyme activity in the brain that was triggered by the neurotransmitter dopamine. This understanding of how dopamine works has formed our basic understanding of brain signaling and helped discover therapies for brain disorders. Dopamine continues to be the subject of much research and has been implicated in addictions, attention deficit hyperactivity disorder (ADHD), mood disorders, schizophrenia, and movement disorders such as Parkinson's disease.

Greengard realized that the enzyme activity that he discovered probably indicated the presence of a dopamine receptor that bound to the neurotransmitter. Subsequent identification and purification of the proteins that form this receptor revealed that Greengard had figured out the critical first steps of neurotransmitter signaling. He went on to show that the receptor-mediated enzyme activates a second messenger, which then triggers a cascade of other signaling events that regulate complex neurological traits and affect how nerve cells grow and function.

Dopamine's role as a neurotransmitter was first uncovered by Arvid Carlsson, who shared the 2000 Nobel Prize in Physiology and Medicine for this discovery (2–4). Carlsson et al. (2, 3) noticed that patients with Parkinson's disease have particularly low dopamine concentrations in certain parts of the brain. Dopamine depletion in the brains of animals also caused Parkinson's-like movement symptoms, and Carlsson was able to treat these symptoms using L-DOPA, which gets converted to dopamine in the brain. His experiments helped develop L-DOPA as a drug for Parkinson's disease, and it remains one of the disease's most important treatments.

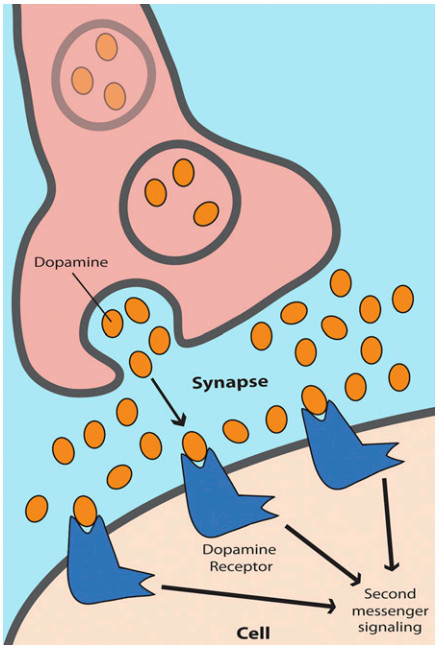

Fig. 1. Dopamine release and binding to receptor. Dopamine is released by a neuron into the junction between two nerve cells called the synapse. There, it can bind to dopamine receptors on neighboring neurons, and the receptors activate the second messenger and downstream signaling pathways.

Greengard's discovery of the dopamine receptor opened up many additional avenues for clinical treatments. Many antipsychotic drugs, particularly those drugs used to treat schizophrenia, act by blocking dopamine receptors. Greengard's results helped explain how these drugs acted and how they could be improved. Dopamine signaling pathways have also been implicated in the reward mechanisms of the brain, and drugs that modulate these pathways can help with mood disorders and addictions.

However, the implications of Greengard's work extended beyond clinical treatments. They also helped lay the foundations of modern neuroscience. His use of classical biochemistry to study the brain influenced many other researchers and helped discover new signaling pathways and regulatory proteins. In recognition of his work's wide-ranging influence, Greengard was awarded the Nobel Prize in Physiology and Medicine in 2000, the same year that Carlsson was honored.

Despite his contributions to neuroscience, Greengard's initial interest was in mathematics and theoretical physics. It was only after he served in the US Navy in World War II and after the dropping of the atomic bombs that he changed his career path. "I didn't want to go into theoretical physics and risk making more potent bombs," Greengard said. Instead, he entered the nascent field of biophysics and focused on trying to understand the brain.

During his graduate research at Johns Hopkins University, Greengard used electrophysiology, or the study of electric currents in nerve cells, to try to understand how the brain functioned. He started with this approach, because it was the major tool of neuroscientists at the time. As he progressed through his postdoctoral work, however, Greengard increasingly turned to biochemistry, believing that it could provide unique insights. His research led him to pioneer many biochemical approaches prevalent today.

These approaches soon bore fruit. In 1968, Greengard became a professor in the Department of Pharmacology at Yale and "within a year to a year and a half, generated papers that still have continuing impact today," said Angus Nairn, a neuroscientist at Yale who did his postdoctoral research in Greengard's laboratory. "It's really quite remarkable how much they generated in a short time."

Huge Conceptual Barrier

By the 1970s, many scientists had studied neurotransmitters, but no one had a clear idea of how these chemicals exert their myriad influences on the brain. The concept of receptors that neuroscientists take for granted today was "still mostly a black box," said Snyder, a neuroscientist at Johns Hopkins University and author of the PNAS Classic Perspective on Greengard's paper, "What dopamine does in the brain" (5).

"Receptors were still a conceptual thing," said Richard Huganir, a neuroscientist at Johns Hopkins University and former postdoctoral researcher with Greengard. "It wasn't even clear if receptors were proteins, believe it or not." Researchers studying other signaling molecules such as hormones had revealed that they bound to receptors on cell surfaces, which triggered other signaling events inside the cell. One of Greengard's insights was that neurotransmitters might work in the same way in the brain.

See Classic Article "Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the 'dopamine receptor'" on page 2145 in issue 8 of volume 69.

See Classic Perspective on page 18869.

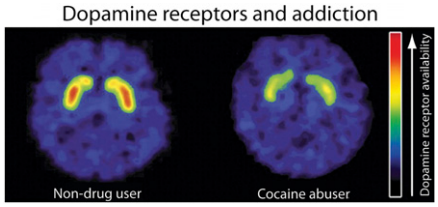

Fig. 2. Dopamine receptor availability in the brain. Brain images show a decrease in the available dopamine receptors in the brain of a person addicted to cocaine compared with a nondrug user. A similar decrease is seen with many other addictive substances, including alcohol and heroin. The lower level of receptors is thought to occur because of their repeated overstimulation, and it can lead to a reduced activation of natural reward pathways that contributes to the tendency to abuse these addictive substances. Adapted from Volkow et al. (9).

As Greengard started putting various pieces of evidence together, he was inspired by the discovery that cAMP mediated the action of certain hormones in the liver (6). cAMP acted as a second messenger that transmitted a signal from a hormone's receptor to other proteins within the cell, triggering downstream signaling pathways. Greengard started looking for a similar interaction in the brain mediated by adenylate cyclase, the enzyme that converts ATP into cAMP.

However, this search was far from an obvious train of thought. Most neuroscientists still believed that any signaling in the brain was electrical and not biochemical. "There was a huge conceptual barrier," said Nairn. adenylate cyclase took many seconds to minutes to generate a signal, a seemingly lethargic process compared with the rapid electrical signaling that neuroscientists were familiar with in the brain. That Greengard pushed past this paradigm and explored such a counterintuitive theory was a major factor in his receipt of the Nobel Prize, Nairn said. "He kept pushing at it, gathering biochemical evidence for it."

Gathering the biochemical evidence to support this theory brought its own challenges. The only neurotransmitter receptor discovered until then had been in the electric eel, where it constituted 20% of the weight of the eel's electric organ (7). This breakthrough result only made scientists more convinced "that it would be impossible to isolate receptors in the human brain, where they would constitute a millionth of its weight at most," said Snyder.

However, Greengard was careful in his selection of the neurotransmitter and the target location to study. He picked dopamine and looked for its effects in a brain area where Carlsson's previous studies had shown that it was not only abundant but where its loss elicited a syndrome similar to Parkinson's disease (2–4). "If he had looked at the whole brain, he would probably have missed this phenomenon, because everything would be diluted out," said Nairn.

The researchers had to carefully extract specific brain regions from multiple rats and pool them together, while ensuring that they did not destroy the enzyme activity in the process. Isolating the appropriate brain area using the techniques of the time required "heavy slogging," said Snyder. "They used very classical biochemical techniques that required big time careful work to perform successfully," he said.

However, the hard work paid off. When Greengard and his graduate student John Kebabian added dopamine to specific brain fractions, they saw a production of cAMP as a second messenger, indicating that the neurotransmitter was signaling through an adenylate cyclase. "To actually find an adenylate cyclase that was coupled to neurotransmitter—nobody had done that," said Snyder.

Kebabian said that Greengard and he had anticipated that this research would be an important piece of work, and they were pleased when they succeeded. However, the importance of their results was cemented by what was to come. "The paper sets the stage pretty well for what followed," said Nairn. "The details got more complex, but the direction was really set up by this paper."

Greengard, Kebabian, and others went on to characterize dopamine receptor activity in more detail, finding that dopamine actually had multiple different receptors to which it could bind. They and other researchers used similar approaches to study the receptors and signaling of other neurotransmitters, such as serotonin or noradrenaline. Their work led to more studies of the slow, biochemical transmission of signals by these neurotransmitters, which has prolonged effects on nerve cells and helps regulate basal nervous system functions, including moods. This slow transmission can also open ion channels to mediate the fast transmission of nerve signals. As Greengard and others were to find out, slow, chemical nerve signaling is an extremely important and complicated process, involving a whole cascade of downstream agents.

Setting the Stage for Modern Neuroscience

As a follow-up to the Classic paper, Greengard's laboratory systematically tracked down the events that occur after dopamine binds to its receptor and activates an adenylate cyclase enzyme. In other organs, cAMP had been shown to regulate enzymes called kinases, which add phosphate groups to molecules in a process called phosphorylation (8). Phosphorylation can change the structure and function of proteins and cause them to trigger signaling events. "[Greengard] figured out that neurotransmitters could work in the same way," said Huganir.

Greengard showed that, after dopamine acted through its receptor to increase the amount of cAMP in a nerve cell, the cAMP activated a kinase, which phosphorylated other proteins in the cell. Some of these proteins could form ion channels in the cell membrane, thus controlling the nerve cell's ability to send electrical impulses. Greengard also found a phosphorylated protein, Dopamine- and cAMP-regulated phosphoprotein (DARPP)-32, which is a central regulator of neuronal function and affects a large number of other proteins. DARPP-32 has been implicated in several neurological disorders and the action of multiple addictive drugs.

Greengard's work showed that phosphorylation was an important signaling mechanism in the brain. The discovery that neurotransmitter signals are mediated by the addition and removal of phosphate groups from proteins in nerve cells continues to have an impact today.

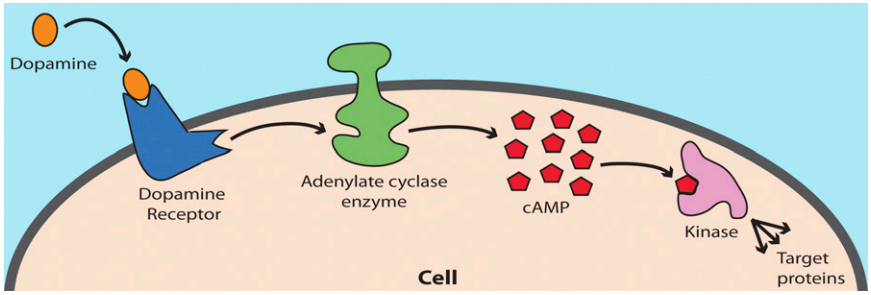

Fig. 3. Dopamine signals through a cAMP second messenger. After dopamine binds to its receptor, the receptor then triggers the activation of an adenylate cyclase enzyme, which produces cAMP from ATP. cAMP acts as a second messenger that transmits a signal from the receptor to other proteins within the cell by binding to them and altering their activity. After dopamine increases the level of cAMP in nerve cells, the cAMP activates a kinase that adds phosphate groups to other proteins in the cell in a process known as phosphorylation. Phosphorylation of these other proteins triggers downstream signaling pathways.

Many current drug discovery efforts involve studying the phosphorylation of different proteins in nerve cells.

However, when these results were first published, their novelty made them controversial. "There was a point when I thought they would not be accepted in my lifetime," said Greengard. "There was a lot of skepticism." He remained undeterred. "After we got the first data, I was positive I was correct," he said. The rapid rise of modern molecular biology in the 1980s helped validate his convictions. "Real acceptance came with the development of new techniques," Greengard said. "Gradually, the evidence became so overwhelming that half the people in brain science today study signaling."

With this Classic paper and his follow-up work came the realization that "signaling pathways are cell function," said Greengard. "It's at the heart of how all cells function, and researchers now use more and more sophisticated analyses of signaling to understand how nerve cells function."

As researchers have developed a better understanding of the various molecules, receptors, and second messengers involved in signaling pathways, their research has begun to focus on the interactions between these components as well as between different pathways. As it turns out, these interactions are not only critical to understanding how the human body works but vital to our understanding of many diseases. Disruptions in normal cell signaling are features of both cancer and chronic diseases such as diabetes and heart disease, and many pathogens hijack the body's signaling mechanisms to cause infections.

Broader Impacts

Apart from changing the way that neuroscience research is done, Greengard has also mentored and trained many now prominent neuroscientists. For several of them, Greengard's Classic dopamine receptor paper inspired their choice of field and approaches to research. "I read the paper when I was 19," said Huganir. "It particularly struck a chord. It cemented my commitment to neuroscience."

Nairn said he first read the paper as a postdoctoral researcher, and he appreciated its logical, straightforward approach and clear data. "It was very typical of Greengard," he said. Having reread the paper recently, Nairn said it was also quite striking how it "really got into translational medicine. It has great relevance to Parkinson's disease as well as in other areas such as ADHD and the addictive actions of drugs of abuse."

The principles and approaches in Greengard's Classic paper helped discover many nervous system drugs used today. The paper had major applications to drug discovery, said Huganir. As a result of it, "now we could do pharmacology," he said. "It led to the ability to look at antipsychotics and other drugs."

According to Greengard, "the fact that it was possible to work out entire pathways used by nerve cells to communicate made it implicit that one could find potential new targets for drug development." Researchers could identify chemicals that modified brain signaling by targeting the regulatory proteins involved. Additionally, as more signaling proteins and pathways were discovered, therapeutic agents could more finely modulate the brain, Greengard said.

Four decades later, Greengard remains fascinated by the intricacies of the brain's signaling and continues to expand on the ideas introduced in his 1972 paper at his research laboratory at Rockefeller University. "That's part of the thrill of it all," he noted. "Testing things and hoping you uncover new worlds." His Classic paper certainly opened up new worlds, with new avenues of research and experimental approaches for neuroscientists to follow.

Sandeep Ravindran, *Science Writer*

1. Kebabian JW, Petzold GL, Greengard P (1972) Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the "dopamine receptor." *Proc Natl Acad Sci USA* 69:2145–2149.
2. Carlsson A, Lindqvist M, Magnusson T (1957) 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature* 180:1200.
3. Carlsson A, Lindqvist M, Magnusson T, Waldeck B (1958) On the presence of 3-hydroxytyramine in brain. *Science* 127:471.
4. Hornykiewicz O (2010) A brief history of levodopa. *J Neurol* 257(Suppl 2):S249–S252.
5. Snyder SH (2011) What dopamine does in the brain. *Proc Natl Acad Sci USA* 108:18869–18871.
6. Sutherland EW, Rall TW (1958) Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles. *J Biol Chem* 232:1077–1091.
7. Cohen JB, Changeux JP (1975) The cholinergic receptor protein in its membrane environment. *Annu Rev Pharmacol* 15:83–103.
8. Krebs EG (1972) Protein kinases. *Curr Top Cell Regul* 5: 99–133.
9. Volkow ND, Wang GJ, Fowler JS, Tomasi D, Telang F (2011) Addiction: Beyond dopamine reward circuitry. *Proc Natl Acad Sci USA* 108:15037–15042.