Foreword

Sol Snyder: Audacious Scientist, Leader of the Scientific Community, and Remarkable Human Being!

Eric R. Kandel

Sol Snyder, as you will learn from reading this collection of some of his best scientific papers, is an immensely creative person—one of the most creative in my generation of scientists. He has made numerous contributions to neuroscience and psychiatry that are not simply original, but often astonishingly so, because when they first appeared they were so audacious and unanticipated. Each of these discoveries has opened up a new area of understanding, and together they have revolutionized the modern study of the brain.

What I find particularly remarkable about Sol is that he is not only creative in science; he is creative in everything he does. There is a special "Sol" touch to all aspects of his life: an expression of an inner richness. Sol, to put it simply, is a savant of creativity. For example, he is an accomplished classical guitarist, and had he not become a scientist, he could have pursued a successful career as a professional musician. Overall, at every level, both public and private, Sol is a creative, social being, a friend of immeasurable generosity and thoughtfulness to his mentors, his students, and his colleagues.

Sol is very active in his community. He has brought his interest in music (along with exceptional organizational skills) to bear on the Baltimore Symphony Orchestra, on whose board he sits. In addition, he has served as the president of his synagogue, where he recruited its first woman cantor.

Sol was a student of Julius Axelrod, and during the time they worked together they established a wonderful mentoring relationship. Julie, as we all know him, has repeatedly said that one of the great satisfactions of his career was to have Sol as a student. And Sol, in turn, always emphasizes what a privilege it was to work for Julie. Both in word and action, Sol treated Julie as a father.

Sol is a supportive and entertaining husband, an involved father, and a doting grandfather. He recently described his trip to Disney World with his children and three grandchildren in one word: "Heaven!" When his first grandchild was born, every single lecture began with a picture of Abigail.

Sol's strong commitment to people extends naturally to his nurturing of his students, for whom he serves as a mentor par excellence. Sol's students rave not only about his creativity, but also about his thoughtfulness, his generosity, and his attention to their personal as well as professional lives. When meeting a student whom he has not seen for several years, he demands first off to see any baby pictures that might possibly have emerged. Reading letters of recommendation from Sol Snyder is like reading a nomination for the Lasker Award. In each of his students Sol brilliantly pinpoints the unique distinctive strengths so as to make the reader appreciate that he or she is dealing with a very special person—which is exactly how Sol feels about his students.

Sol is an extraordinary leader of the scientific community. At Johns Hopkins he formed the department of neuroscience, which he built into one of the major research groups in the country. In so doing he nurtured his faculty as he nurtured his laboratory and his family. As president of the Society for Neuroscience, in 1980 he launched our major journal, the *Journal of Neuroscience*, against considerable opposition, and established it as one of the premier journals in the field. Sol was also the first neurobiologist to become involved in biotechnology, and his first company, Nova, was a pioneer in the area. With part of the proceeds of this and his other ventures, Sol and his wife Elaine have contributed generously to Johns Hopkins, an institution Sol adores.

Sol has divided his collected papers, which form the body of this book, into ten sections, nine of which represent distinct scientific directions. Here, for more detailed discussion, I have combined some of these and emphasized others. I limit myself to four topics.

First, following up on his mentor Julius Axelrod's interest, Sol began to study neurotransmitter uptake as a mechanism for inactivating transmitters. By labeling receptors using reversible ligand binding, he succeeded in identifying opiate receptors, which are important for understanding pain perception. He then extended this method first to receptors for many of the neurotransmitters in the brain, including serotonin and dopamine, and then to subtypes of these receptors. The isolation of receptor proteins and their subsequent genetic cloning stem directly from the ability to label and thus monitor receptors by these ligand binding techniques. This advance represents a critical step toward understanding the fundamental molecular mechanisms whereby neurons communicate with one another.

Second, Sol has had a key role in the molecular characterization of the inositol 1,4,5-trisphosphate (IP_3) receptor, which is important for calcium

homeostasis. This experience put Sol in a position to explore the role of IP_3 as a potential target for lithium treatment in manic-depressive disorders.

Third, Sol has radically transformed conceptualizations of neurotransmission by establishing a gas, nitric oxide (NO), as a transmitter in the brain and by providing evidence that carbon monoxide may also be a neural mediator.

Finally, he has discovered that D-serine is the normal stimulus for the glycine site of the *N*-methyl-D-aspartate (NMDA) receptor, which provides insight into how this receptor, important for learning and memory in the mammalian brain, is regulated.

Identification of Neurotransmitter Receptors by Labeling With Radioligands

It had long been known that drugs act via specific receptors. The possibility of labeling receptors with radioligands had been explored by numerous investigators, and some preliminary success was obtained in the late 1960s with [^{3}H]atropine and the muscarinic receptor. However, the first successful labeling of neurotransmitter and drug receptors in the brain with reversible ligands began with Snyder's work.

In 1973 Sol Snyder and Candace Pert demonstrated opiate receptor binding in brain tissue. In their very first papers, their elucidation of receptor properties clarified fundamental questions about the actions of opiates. The relative affinities of opiates for binding sites were found to parallel pharmacologic activity, and opiate agonists and antagonists could be distinguished by the effects of sodium ion. The autoradiographic localization of opiate receptors that followed was critical for allowing us to understand how opiates exerted their pharmacologic actions, with receptors discretely localized to nuclei subserving functions influenced by opiates. These initial studies of opiate receptors provided powerful tools for screening new drugs in the pharmaceutical industry, and the discrimination of agonists and antagonists provided a first indication of how the recognition of a neurotransmitter or drug at its receptor might alter cellular events.

Sol next modified these techniques to develop a general strategy to label receptors for the major neurotransmitters in the brain. With Anne Young, he identified glycine receptors utilizing the antagonist [^{3}H]strychnine. Glycine is an inhibitory neurotransmitter in the spinal cord and brain stem that hyperpolarizes motor neurons by increasing chloride conductance (the relative potencies of anions mimicking actions of chlorides being well established).

Labeling muscarinic cholinergic receptors with [^{3}H]quinuclidinyl benzilate, Sol, working with Henry Yamamura, provided insights into important actions of psychoactive drugs. The potencies of antipsychotic neuroleptics

in blocking muscarinic receptors correlated inversely with their propensity for inducing extrapyramidal side effects. Influences of tricyclic antidepressants on these receptors provided a means of assessing the anticholinergic side effects of these drugs.

Sol, working with several students, next identified dopamine receptors in the brain, utilizing [^{3}H]dopamine and [^{3}H]haloperidol. He then showed that affinities of neuroleptics for dopamine receptors labeled by [^{3}H]haloperidol closely parallel their clinical and pharmacological activities, establishing that antipsychotic neuroleptic drugs act by blocking dopamine receptors. With Ian Creese and David Burt, Sol showed that the behavioral supersensitivity following destruction of the nigrostriatal dopamine pathway correlates closely with increased numbers of dopamine receptors. It is fair to say that the modern molecular era of transmitter receptors in the brain—an era that has revolutionized both the theory and practice of psychiatry—had its origin in Sol's lab.

The Function of Nitric Oxide in the Brain

Of the various roles of nitric oxide, its function as a major neurotransmitter was until quite recently the least explored. Sol's work in this area rapidly established a wholly novel concept of synaptic transmission—a gaseous neurotransmitter! Several of the principal insights into the functions and disposition of NO in the brain are attributable to Sol.

Following up on a key report by Garthwaite that brain cultures exposed to glutamate produce a substance that relaxes smooth muscle and behaves like NO, Sol with his Ph.D. student David Bredt sought and demonstrated the formation of NO from arginine. They next showed that glutamate, the major excitatory neurotransmitter in the brain, acting through the NMDA subtype of receptor, triples NO synthase (NOS) activity in a matter of a few seconds or shorter, which could mediate the simultaneous marked increase of cyclic guanosine monophosphate (cGMP) levels. NO formation causes the elevated cGMP, which Sol proved by showing that inhibitors of NOS block increased levels of cGMP formation.

Using antibodies to the purified enzyme, Sol, with Bredt and Ted Dawson, provided the first localization of NOS in the body and demonstrated selective localization to neurons in the brain and peripheral nervous system. The localization to the myenteric plexus of neurons in the gastrointestinal pathway suggested a role in peristalsis, which was demonstrated in numerous laboratories by showing that NOS inhibitors block relaxation of the intestines produced by physiological stimulation. Localization of NOS to penile autonomic neurons surrounding the blood vessels, whose engorgement with blood produces erection, enabled Sol and his colleagues to dem-

onstrate that NO is the physiological mediator of penile erections, as erections in intact animals produced by stimulation of the pelvic nerve are blocked by NOS inhibitors. These experiments establish NO as a neurotransmitter—which overturns classic concepts, since it is not a) stored in synaptic vesicles, b) released by exocytosis, c) bound to plasma membrane receptors, or d) inactivated by receptors or enzymatic hydrolysis.

In the brain, Sol found NOS neurons in discrete sites with a pattern of localization that did not correspond to that of any known neurotransmitters. He showed that the NOS neurons are identical to those that stain for NADPH diaphorase and that NOS activity accounts for diaphorase staining, since non-neuronal cells transfected with cloned NOS display staining for NOS and diaphorase in proportions similar to what occurs in neurons. Because diaphorase-staining neurons are known to be resistant to neuronal destruction following NMDA receptor stimulation in cultures as well as during vascular stroke and in Huntington's disease, Snyder, with Valina and Ted Dawson, postulated that stimulation of NOS by glutamate triggers the formation of NO by NOS neurons, with the NO released to kill adjacent cells.

Much of our understanding of the role of NO in biology has come from insights into properties of the NOS protein. Following NOS purification, Snyder and Bredt achieved the first molecular cloning of NOS, specifically of the brain form. The cloning studies enabled Snyder, in collaboration with Mark Fishman's laboratory, to generate mice lacking neuronal NOS. His demonstration that these animals have grossly enlarged stomachs with hypertrophy of the pyloric sphincter provides insights into the causation of human infantile pyloric stenosis, in which NOS neurons are also depleted in the pylorus.

Reasoning that NO may not be the only gaseous neurotransmitter, Snyder, with Ajay Verma, showed that carbon monoxide functions as a neuronal messenger.

Signal Transduction: IP_3 Receptors and Immunophilins

Snyder, with yet another group of students and postdoctoral fellows, utilized receptor strategies to clarify the actions of inositol trisphosphate, the mediator of the phosphoinositide cycle, which acts by releasing calcium. He identified receptor binding sites in the cerebellum, utilizing autoradiography to demonstrate remarkably high levels in discrete brain areas that greatly exceeded those in any other part of the body. Using biochemical methods, he purified the receptor to homogeneity. His reconstitution of the receptors into lipid vesicles in which the receptor protein mediates the ability of IP_3 to release calcium has established that the IP_3 receptor protein possesses both the

recognition site for IP_3 and the associated calcium ion channel. In the reconstituted receptor preparation, Snyder demonstrated quantal release of calcium, establishing how IP_3 can elicit exquisitely modulated pulses of calcium for intracellular signaling. Molecular cloning of this receptor by others, based on the isolation of the protein by Snyder's group, shows its close similarity to the ryanodine receptor of skeletal muscle, which also triggers calcium release. With Christopher Ross and Jacopo Meldolesi, Snyder showed the IP_3 receptor to be localized to elements of the endoplasmic reticulum including the nuclear membrane, providing a mechanism whereby the phosphoinositide system can enable neurotransmitter and hormonal messages to influence nuclear function. It is likely that the IP_3 receptor is the key intracellular recognition protein involved in communicating actions of intercellular messengers upon calcium within cells.

Sol's attention was suddenly directed to the immunophilins, and he went on to show them to be major signal-transducing proteins in the brain. Immunophilins were first identified as receptor proteins for immunosuppressant drugs. Previously, research on immunophilins had been restricted to lymphocytes and tissues of the immune system. With Joseph Steiner, Snyder demonstrated that the brain contains extremely high levels of the immunophilins, localized to discrete neuronal populations, with levels being 10–50 times greater than in immune tissues. He showed that drugs such as FK-506, by binding to immunophilins and inhibiting calcineurin phosphatase activity, can lead to accumulation of high levels of phosphorylated proteins in the brain. In this way, FK-506 enhances the phosphorylation of nitric oxide synthase. Since phosphorylation of NOS had been shown by Snyder to inhibit catalytic activity, treatment with FK-506 is equivalent to inhibiting NOS activity. Because NOS inhibition blocks glutamate neurotoxicity, Snyder and Dawson evaluated actions of immunosuppressants and showed that they block glutamate neurotoxicity in cultures.

Identification of D-Serine as the Coactivator of the NMDA Receptor

Most recently Sol has turned to the study of D-serine, which not only is an atypical isomer of the amino acid serine, but also occurs in glial cells rather than in neurons. Yet Sol has shown that despite these two improbable characteristics, D-serine acts as a candidate neurotransmitter. Snyder's immunohistochemical maps revealed that D-serine is present in a unique population of glia that surround nerve terminals selectively in a region of the brain that is rich in glutamate receptors of the subtype referred to as NMDA receptors. These receptors were thought to be coactivated by the amino acids glycine and glutamate. Sol found that D-serine is in fact the normal stimulus for the

glycine site of this receptor and that it is released from these glial cells by glutamate and then acts back on the NMDA receptor.

Any one of these four families of discoveries would be a full career for many scientists, but they represent only the core of Sol's remarkable fertile mind and his limitless creativity, energy, and enthusiasm. For these extraordinary contributions, Sol has been widely and importantly recognized. He is listed third in the Institute of Scientific Information list of the twenty-five most influential scientists in the last twenty years. He has received the highest honors America can bestow on its most gifted scientists. Among his many honors, Sol won the Lasker Award in 1978 and was elected to the National Academy of Sciences in 1980. In 2003 he received the National Medal of Science from President Bush.

But, as you will see when you read the papers that follow, none of these recognitions fully captures the range of contributions that have emerged from Sol's laboratory.

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