Serotonin and the Last Great Bioassay

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Abstract: There is little recorded history of the bench work and experimental proof of serotonin as a neurotransmitter, only the third neurotransmitter to be discovered. This review contains an account of serotonin (5-HT) as a neurotransmitter in invertebrates and vertebrates. The studies on invertebrates provided the foundation for our present knowledge of the function of this neurohumor in the mammalian nervous system. The pioneer work of John Henry Welsh before 1954 laid much of the necessary groundwork. Welsh pointed out the opposing actions of 5-HT and ACh on the Venus heart, anticipated and demonstrated the importance of 5-HT as a synaptic transmitter, recommended the concept of the “serotonergic neuron”, suggested and taught the term “neurohormone”, urged that studies be focused on “receptor sites” and showed how molecular configuration affects pharmacological action. An extension of his foresight is a clinical procedure used today, namely the administering to the mentally ill of those drugs which modify serotonin neurotransmission. John Welsh must be included with other pioneer researchers of neurotransmission such as Otto Loewi, Walter B. Cannon and G.H. Parker and recognized as an outstanding futurist in the study of neurotransmitter.

Key words: Serotonin, invertebrates, vertebrates, neurotransmitter

Introduction

Our purpose is to illuminate the discovery of the third of the neurotransmitter, serotonin. This was the work of John Henry Welsh and his student Betty M. Twarog. This was clearly an important neurotransmitter to be discovered yet now, historically, it is difficult to find mention of the critical experiments in the literature. Yet, today some experts in this field will tell you that there are as many as 58 neurotransmitters which have been identified, although they are sometimes called by a different name such as neurocrines.

We carried out a variety of library searches during the year 2001 to find a mention of the discoverer of serotonin (5-HT) as a neurotransmitter. Loewi was frequently mentioned as the discoverer of acetylcholine and Cannon of epinephrine, yet we still could not find mention of the experimental discovery of 5-HT as a neurotransmitter. For example, we read fifty-four pages of the Neuroscience History Archives, still with no mention of the discovery, although there
were numerous article references to 5-HT as a transmitter itself. Also, as usual, there were specific references to Loewi, Cannon, Dale, Eccles, Katz and Hokfelt, for early work on neurotransmitters.

A further purpose of this article is to describe for the sake of young physiologists of today the procedure in a bioassay. For a period, this was as important a technique as the Northern Blot technique is today.

What is a bioassay?

The second neurotransmitter, acetylcholine, (ACh), was discovered by the assay of a frog heart with the vagus nerve intact. There were two reservoirs of Ringers solution with a frog heart suspended in each and the solution from the first reservoir ran into the second reservoir holding the second heart. When the vagus nerve in the first heart was stimulated, the heartbeat was greatly reduced and as Ringers ran from that container to the second, the second heart reduced its beat. This showed that a material provided due to the stimulation of this nerve influenced the second heart. This was the discovery of acetylcholine (Ach). How then would you look for this substance in, for example, certain ganglia of nervous systems in vertebrates? In the early experiments it was defined that if a material from a ganglion or the result of nerve stimulation was diluted to $10^{-6}$M and the response on selected tissue was augmented by eserine and was inactivated by the application of human blood, then the material was ACh. It was found in early experiments that the most sensitive tissue was a strip of leech muscle. This contracted when an ACh-containing solution was diluted to $10^{-12}$M. The scientific name of this worm, Hirudo medicinalis, illustrates why leeches were readily available. They could then be purchased in drug stores and they are even today still part of folk medicine and are still available in drug stores in large cities. They were used and are currently used for drawing blood.

We'll learn that while John Welsh used the leech muscle assay for a number of years in his laboratory, he found that a more convenient bioassay was a mollusk. The reason was that there were no convenient nerves attached to the leech muscle to do the experiment done by Loewi, which was to stimulate a nerve and test material produced at the nerve muscle junction. The assay tissue that John Welsh learned to use effectively was the heart of a common mollusk, referred to as Venus mercenaria. This invertebrate is also known as the hard-shelled clam. The assay was first suggested by Ladd Prosser; at about that time he was the President of the American Physiological Society.

It should not be a surprise to the young physiologist of today that invertebrate tissue is important in classical physiological research. One need only remember the importance of the results obtained from the giant-squid axon to realize that as stated by Krogh, for any experiment one can find in the animal kingdom the most appropriate tissue to make the experiment a success.

Who was John Henry Welsh?

He was on the staff of Harvard University from 1927 to his retirement in 1970 (Fig. 1). In a recent “cited reference search” his cited reference data included cases of individual citation
of 304, 130 and 99 times and different cited references were found 104 times in total. He was the first to publish results showing that serotonin was a neurotransmitter. He is still living in the year 2002. He is 101 years of age and he “works out” each day in his home in Maine. In 1998, John received a cane with a 14 carat gold head from a newspaper, The Boston Post. Later in this article we will discuss his early interests and how certain themes were carried out through graduate school and beyond.

It seems important at this point, before describing the details of the discovery of serotonin as a neurotransmitter, to review why it is significant enough to give credit to the ingenious experiments which first described its action.

The importance of serotonin

In 1948 a powerful vasoconstrictor substance was found in blood serum in the laboratory of Irvine Page. It was named serotonin from sero (Latin serum) and tone (Greek tonic).

Despite the increasing interest and importance of several other neurotransmitters, for instance Glutamate, serotonin still has major pharmacological implications due to its participation in many physiological functions such as feeding, pain, emotion, sleep, circadian rhythms, motor behavior and above all, different neurological and mental diseases. Although serotonin was long assumed to function as a neurotransmitter in the brain, it took several years before it was clearly established which neurons, pathways and circuits were carrying and releasing serotonin. With the method of Falck and Hillarp (1962) that was possible for the first time. The first mapping study localizing serotonin in specific neurons was published in 1965 by Anita Dahlstrom and Kjell Fuxe; they showed that serotonin was restricted to 9 groups of cells (B1-B9) located in the pons and midbrain. The raphe nuclei are the major nuclei projecting ascending serotoninergic fibers to the forebrain (telencephalon and diencephalon) and the more caudal groups descending to medulla and the spinal cord. There are some intermediate groups that send both ascending and descending serotonin fibers.

Fig. 1: John Henry Welch of Harvard University in 1988
Today we know many of the cellular effects of serotonin and many of the serotonin receptors have been identified and characterized. Serotonin receptors not only are classified as 5-HT1, 2, 3, 4, 5, 6 and 7, but as many as 16 different subtypes of those have been cloned in the mammal, each with its pharmacological agonists and localization in the brain, as well as its molecular biology which provides us with knowledge about its functional and structural characteristics. The majority of these receptor subtypes belong to the family of receptors coupled to the G proteins superfamily.

The fact that neurons in the raphe nuclei discharge with a pacemaker pattern of activity prompted the suggestion that this neurotransmitter participates in the timing of many physiological functions as described above. Also dysfunction of the serotonin systems seems to be involved in neurological disease such as Parkinson’s and mental illness such as depression (including suicide), mania, aggression and schizophrenia.

Now that we have reviewed some of the neurological actions of serotonin, let us turn to a description of the classic experiments of John Welsh, who discovered serotonin as a neurotransmitter.

Welsh’s early career

John was a typical college graduate with an interest in science who wanted to go to graduate school but had numerous debts originating from undergraduate tuition fees. In 1924, he took a position as instructor in Biology at the Berkshire School on the Down Mountain Road in Western Massachusetts. Behind this school, where he taught for three years, there was a rocky mountain where rattlesnakes hibernated. Of course, as the biology teacher, he collected some of these and became very interested in animal poisons. He retained this interest until he retired in 1970. At the school, he collected the poison of rattlesnakes and published his first paper about the details of how these animals injected their poison. Even at this time he was interested in the molecular architecture of poisons and we will find that this interest culminated much later in his career in the discovery of serotonin as a neurotransmitter.

The influence of G.H. Parker

The first author of this paper (E.F.) first met John Welsh in 1931. E.F. was a junior at a secondary school and he came to the Department of Biology at Harvard University seeking help on the design of coursework because he intended to apply there to be a Biology major. The Department sent him to John Welsh whom he met climbing some stairs in the laboratory. J. H. Welsh was interested in advising this nervous student and he sat down on the steps and they had a long talk. Later he demonstrated to the student the significant experiment of G. H. Parker, which showed that the change in fish chromatophores was influenced by hormonal stimuli and not nerve stimuli. E.F. later came to Harvard University and was taught in the introductory Biology course by both G. H. Parker and John Welsh. G. H. Parker had a strong influence on John Welsh and was his Ph.D. sponsor. E.F. had one personal experience with G. H. Parker because E.F. was working in the stock room of the Biological Laboratories as a work-study freshman. G.H. Parker strolled in with a boa constrictor in a cloth bag and said abruptly, “Please euthanize this
animal.” It seems that G.H. Parker had wanted a very long nerve upon which to study the passage of the nerve impulse. He had done appropriate dissection to find the long nerve in the snake and was through with his experiments. We euthanized the snake and kept it on display in a bottle for a number of years in the stock room.

The teaching of G.H. Parker and John Welsh influenced E.F. Perhaps it is significant that John Welsh sat down on the steps of the Biology Laboratory in 1931 and was willing to talk to a junior student from secondary school, because that student is now an active fifty-year member of the American Physiological Society.

The atmosphere of Welsh’s laboratory

When the graduate students in his laboratory were having lunch together, John Welsh was apt to join them. There were two things that emanated from his conversation. One was the importance of nerves as secretory glands and their products, neurohumors. Later the more common word was “neurotransmitter”. John Welsh introduced us to the countrywide debate taking place between Eccles and Nachmanson. It was from John that we learned the vernacular referring to the two camps in the world of neurotransmission which he referred to as the “Spark-boys” and the “Soup-boys”, of course referring to Eccles and Nachmanson. He was not alone in making these two divisions; John Cook in 1986 wrote a paper for the journal “News in Physiological Sciences”, with the title “Spark” vs. “Soup”.

The second theme that was apt to emanate from John Welsh during lunch sessions was the importance of the molecular architecture of the active substances in the nervous system. He would remind us to pay careful attention, for example, to the changing positions between molecules of the “carbonyl groups.” A favorite expression of his was “molecular configuration and pharmacological action.”

What was the development in experimental physiology of the debate between the Spark scientists and the Soup scientists? Now there are some 50 or 60 known neurotransmitters, for example nitric oxide and GABA. Note that some neurons contain as many as three neurotransmitters near the synapse. For convenience, we call some of the alike neurotransmitters amines; examples are serotonin, norepinephrine (or “noradrenalin,” the British term) and dopamine (Fig. 2).

What was the result of the long-term debate as to whether transmission at the synapse is by electrical or by neurohumoral means? Both sides were right, although electrical transmission is less common. This last type depends on two adjoining neuronal membranes, so thin that they are almost “open,” one to the other; such a synapse is called a gap junction and provides rapid transmission. It occurs occasionally in four Phyla, including invertebrates and in the central nervous system of some vertebrates. However, most synapses are chemical and are called tight junctions. Their transmission is slower than that of gap junctions.

Techniques in the Welsh laboratory

A study of the publications of John Welsh and his students indicate that from the years 1942-1963 he employed bloassay techniques and most of the papers concerned ACh and 5-HT. There
Fig. 2: This diagram shows the relationship and enzymes concerned in the formation of 5-HT and also the close relationship with melatonin. Melatonin is a hormone from the pineal gland and other tissue; it is associated with jet lag.

is a description above of the technique used in bioassays, especially using the leech muscle. John gave up the use of the leech muscle for the Venus heart which was more sensitive and there were nerves to control the increase and decrease of the beat of the Venus heart (Fig. 3). Using these methods of bioassay he showed, with Ralph Smith, for the first time that ACh is a transmitter substance in crustacea. Later using the Venus heart technique, he did a broad survey of invertebrates and vertebrates and found that ACh was common as a neurotransmitter (Welsh, 1968). His long-range goal was to characterize, in detail, the ACh receptor. John published 27 papers on the distribution of, but especially the mechanism of action of, ACh.

It is important to point out that also in the Welsh laboratory a student, Betty Twarog, had developed another sensitive bioassay which consisted of causing a prolonged contraction of the muscle of another shellfish, Mytilus. As in the case with the Venus heart, using ACh caused a
Fig. 3: Serotonin as a neurotransmitter was discovered by bioassay with the isolated heart of *Venus*, the hard-shell clam (Quahog). An example of its use for a bioassay is shown in Fig. 3, a record of the action of noradrenaline on the isolated Venus heart. (1) $10^{-6}$ M noradrenaline; (2) same; (3) $10^{-5}$ M noradrenaline; (4) $5 \times 10^{-5}$ M noradrenaline

contraction which was potentiated by cholinesterase inhibitors and blocked by ACh blocking agents. Because of Twarog's experiments and those of other graduate students and John Welsh's many experiments, the laboratory workers suspected that there must be another invertebrate neurohormone similar to ACh and epinephrine. A clue came up in discussions and from the literature that this might be serotonin. Meanwhile, in the laboratory of Irvine Page, there had been many studies of a new powerful material; Page suspected it to be an amine and considered it to be a contaminant which interfered with his experiments. Through long and difficult experiments in Page's laboratory, Maurice Rapport proved that the new material was an amine (Rapport, 1949) and so essentially, Rapport discovered serotonin. In the meantime, Reid and Rand carried out other experiments on mammalian tissue. They isolated what they called serum vasoconstrictor substance and later called it "serotonin," which they considered to be a relative of tryptamine. Their substance differed from sympathomimetic amines because it caused contraction of rabbit and guinea pig intestine, rat uterus, pupil of the eye and the bronchi (Reid and Rand, 1951).

When Welsh heard that Robert Cophill, Director, Abbott Laboratories, would provide small quantities of serotonin in the form of creatinine sulfate, he arranged for a sample to be sent in 1953. Using the standardized laboratory procedures, two sets of assays were prepared for tests: three were Venus hearts and three were Mytilus. The serotonin was diluted to $10^{-10}$ M and it excited the Venus heart and relaxed the Mytilus muscle. The same results were obtained in the same dilution from extracts of the ganglion which is on the Venus heart. The workers in the laboratory referred to this as a discovery of the new "invertebrate norepinephrine".
The discovery of serotonin as a neurotransmitter

Using bioassay and paper chromatography, serotonin was identified in the ganglia of both the *Venus* and the *Mytilus* organisms. This series of findings and their publication were so important, we will record here the text of John Welsh’s paper delivered at the Federation meeting in 1954. “Evidence from my laboratory indicate that 5-HT is a mediator of nerve action in certain invertebrates. It has been found to excite the heart of the mollusk *Venus* mercenaria, as does stimulation of its visceral ganglion following block of the cholinergic inhibitor nerves. Agents that block 5-HT also abolish nervous excitation.” There are many details in his abstract of the techniques used and the various blocking agents and inhibitors. His reasoning included in the abstract was that the antagonistic action of the two neurohormones (ACh and 5-HT) on the *Venus* heart was not due to competition for the same receptor substance. Then came his prophetic statement “The addition of 5-HT to the list of chemically-known substances that are produced by nerve cells and that act on effectors or other neurons would appear proper.” He had published earlier the bench-work support for this abstract (Welsh, 1953). Later, he introduced the term “serotonergic” to apply to any transmitter action of 5-HT (1957).

Temporary lack of acceptance

In 1958, Irvine Page still did not accept serotonin as a neurotransmitter. Nor did Garotini and Valzelli in 1965 (Folk and Long, 1988). We presume that the reason for this caution was partly that John Welsh was unable to do the type of experiment accomplished by Loewi, which consisted of stimulating the organ under study with nerves and simultaneously collecting a solution running from the organ. The Welsh laboratory did not succeed in doing this experiment although it was accomplished elsewhere a few years later, confirming the Welsh experiments.

Further logic of Page and Garotini (disclaiming the Welsh discovery) was as follows: although Welsh showed that both serotonin and extracts of *Venus* heart ganglia, when diluted to one thousand million, excited the *Venus* heart maximally, Page claimed that perhaps serotonin was not the transmitter but enhanced some other transmitter. Page and Garotini might have used this same lack of logic by saying that Otto Loewi did not discover acetylcholine as a transmitter because perhaps it really only enhanced some undiscovered second transmitter in the second heart in the preparation.

John Welsh then postulated that serotonin would be found at the nerve endings of mammalian nerves. During the period from 1965 to 1985 work was progressing with experiments using mammals (Folk and Long, 1988). The key experiments in mammals to demonstrate that 5-HT is a neurotransmitter depended upon the development of the technique of micro-loinophoresis. This technique was used in mammalian experiments with the spinal cord where circumstantial evidence had already suggested a transmitter function. The technique demonstrated transmitter function in the spinal cord with evidence that 5-HT could either be excitatory or inhibitory of neuronal function. Furthermore, the evidence of transmitter action was found within the brain by the same technique; it was demonstrated that sites responsive to 5-HT were abundant and were easily demonstrated. As a result of these experiments, modern authors now accept the following: “5-HT serves as a neurotransmitter, a function now established”. Another categorical
statement is “A major function of 5-HT is to serve as a chemical transmitter of neurons within the brain”. Cell bodies of 5-HT containing neurons are concentrated in the raphe-nuclei of the brain stem from which axons project extensively to other portions of the brain stem, to the spinal cord and to the forebrain. Perhaps it is possible to be more categorical about the transmitter function of 5-HT in mammals than it is within the invertebrate group, because when appropriate tests were done on different invertebrate species, in some cases opposite results were obtained from species to species.

Welsh as an inspiring sponsor

Of John’s laboratory, one student was heard to say, “That place is an idea factory.” John was not only a source of many ideas on his own part, but he enjoyed inspiring new concepts as created by his own students. Between 1942 and 1969, he was the sponsor of 36 graduate students. From them, there has been a “cascade effect” of a third generation of graduate students who were well established and productive.

The extent of his inspiration is witnessed by a celebration of his ninetieth birthday. He had left the Department of Organismic and Evolutionary Biology at Harvard approximately twenty years before. You will recall that a bioassay which he frequently used, let us say his work-horse, was the recording of the cardiac activity of the hard shell clam, Venus mercenaria (since renamed). Therefore, John was presented on his ninetieth birthday with a large painting of this mollusk by one of his students who had studied with him in 1970. The frame was of African wengewood made by another of his students who studied with him in 1969. The matte of this splendid framing production was signed by 30 of John Welsh’s graduate students. This seemed like the appropriate presentation for his birthday. John was given other appropriate attention by his academic family; an entire copy of a journal dedicated to his name and work (Schumway, 1988).

The first author (E.F.) should add a final note about John’s relationship with his students. When E.F. asked to be a graduate student with John in 1945 after World War II, John explained his deep interest in neurotransmitters. When it was realized that E.F. was more of a biophysicist than he was a chemist, John suggested that the prospective student go back to an earlier interest of John’s which was that of biological clocks. Specifically, John had published five papers on this topic and E.F. was more than pleased to do thesis work in this important field. When the time came for E.F. to publish the results of the thesis, he automatically placed John Welsh as the second author. A letter soon arrived from John saying, “Please take my name off, I have always considered that the thesis is the graduate students’ own property and have never allowed my name on their publications.”

Welsh’s interest in poisons

In this article we now revert to the early interest of John Welsh before he even entered graduate school which was that of the molecular composition of animal poison. He had become interested in this because of working on the biology of rattlesnakes. To evidence his interest and success with working in this field, here is a sample of his titles published between 1956 and 1967: (1956). On the nature and action of coelenterate toxins; (1963) 5-HT content of some arthropod
venoms; (1964) Composition and mode of action of some invertebrate venoms; (1966) Serotonin and related tryptamine derivatives in snake venoms; (1967) Acetylcholine in snake venoms; (Slater, 1988) Viper venom proteins; molecular probes for neurotransmitter. He summarized these investigations in the obvious generalization that the presence of 5-HT in non-neural tissue such as venom is usually to create pain.

The published description of 5-HT as a neurotransmitter by John H. Welsh in 1953 is a neglected discovery and should be more generally recognized.

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References


