Review Article

Ferdinand Hucho*

Personal View – The Evolution of Neurochemistry

Two questions – one answer

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Abstract: This esssay is a personal account of the evolution of Neurochemistry in the past century. It describes in parallel the authors way from chemistry to biochemistry and finally to Neurochemistry and the progress of a most exciting chapter of the Life Sciences. It covers the successful time period of reductionist research (by no means comprehensively), which lay the ground for the recent and future systems approach. This development promises answers to fundamental questions of our existence as human beings.

Keywords: Chemistry; Biochemistry; Life Science; Neurochemistry

Zusammenfassung: Dieser Essay ist ein persönlicher Bericht über die Entwicklung der Neurochemie im vergangenen Jahrhundert. Er beschreibt parallel den Werdegang des Autors vom Chemiker zum Biochemiker und schließlich zum Neurochemiker, und den Fortschritt eines der aufregendsten Kapitel der Lebenswissenschaften. Er behandelt den erfolgreichen reduktionistischen Forschungsansatz (keineswegs umfassend oder lückenlos!), der Grundlage für den systembiologischen Ansatz unserer Tage und der absehbaren Zukunft ist. Diese Entwicklung verspricht Antworten auf fundamentale Fragen unserer Existenz als menschliche Wesen.

Schlüsselwörter: Chemie, Biochemie, Lebenswissenschaften, Neurochemie

Neurochemistry is a branch of Biochemistry. Biochemistry in turn originates from Chemistry and Physiology. The latter branched off from Medicine. As a whole this sequence describes in brief the reductionist pathway of life science research in the time span from the late 19th century up to our times. Reductionism was hoped to solve two of the most fundamental riddles which were central to human thinking since antiquity: The world, believed to be composed of ‚mind and matter', poses the question: What is life? The neurochemist goes one step further and asks: what is mind (conciousness, cognition, free will)? The physiologist Emil du Bois-Reymond (1818–1896) included these questions in his ‚seven riddles' (*Finkelstein 2013*) and summarized his answer in 1880 in his famous "*ignoramus et ignorabimus" ("*we don't know and we never will know"*)*. Never say 'never', because this could be the end of human curiosity and research, preventing discoveries including new methods of investigation.

In the 20th century the question *What is life* was most vividly posed by physicists like the Nobel laureates Erwin Schrödinger (*Schrödinger 1944; Fischer ed., 1987*) and Max Delbrück (*Delbrück 1986*). Why physicists? In times of Quantum Physics they suspected a novel matter/mind dualism in living matter similar to the wave/particle dualism of light (Delbrück) or they were waiting for the discovery of still unknown physical laws (Schrödinger). Neither proved to be right.

I found among my notes of a biochemistry lecture presented by Kurt Wallenfels, my teacher and PhD supervisor at the University of Freiburg/Brsg. a quotation by Linus Pauling (1962): "*Life is a property between molecules and not a property of any molecule*". In other words: There is not a single molecule (or at that a group of molecules) defining life. Rather life is a system of interacting molecules which makes matter live. Similarly, there is no *vis vitalis* (a "living force", still postulated by some in the first half of the 20th century; see also the last significant dualist treatise: (*Popper and Eccles, 1977*). Rather life is a special state of the known existing forces, – it is a set of physical parameters embedded in the laws of physics, especially of thermodynamics, which define the living state. The 2nd Law of Thermodynamics postulates an increase of entropy of a system for any exothermic process. Life is a highly unlikely, extremely ordered state of matter, a state of reduced (negative) entropy (Schrödinger,1944). This state can be maintained only as an open system far away from equilib-

***Corresponding author: Ferdinand Hucho**, Institut für Chemie und Biochemie, Freie Universität Berlin, Thielallee 63, 14195 Berlin, Tel. +49 30 3248693, e-mail hucho@chemie.fu-berlin.de

rium (for *non-equilibrium thermodynamics see (Prigogine and Stengers, 1981,)*. Today we would say: This open system extends to the environment of the living organism, and by that includes signal processing and epigenetics. If all processes, metabolic reactions, transport phenomena, information exchange, etc. in living matter like cells and organisms reach equilibrium it means the matter is dead.

So thermodynamics come close to a convincing definition of life and death. We have to admit that this definition leaves many questions unanswered. Our textbooks of biochemistry list in their opening chapters many properties of living matter: metabolism, identical self replication etc. But none subscribes to dualism, none even postulates a special *"mind stuff"*, as the philosopher/neuroscientist Daniel Dennett names it ironically (*Dennett 1991*). The answer to the two questions, the topic of this essay must be found within the laws of physics and chemistry. No serious scientist disagrees.

Given the complexity and the seemingly unsolvability of the problem, it is not surprising that the interest of many researchers faded away and the still unanswered question "What is Life" was set aside for the time being. Molecular Medicine seemed more urgent and rewarding. Recently we observe a certain resurgence of the fundamental question in connection with the growth of a new branch of molecular biology with the rather diffuse denomination 'Synthetic Biology' (*Köchy und Hümpel, eds., 2012)*. Molecular biologists in this field construct among others living matter from organic ('dead') building blocks. They tinker with genomes and genes and try a top down approach asking how few genes are necessary and sufficient to maintain life. If this will be successful, will it answer our question?

How to become a Biochemist

'*What is Life*' is not the main topic of this essay. Here I would like to ponder over the development of Biochemistry into Molecular Biology and Cell Biology and to one of its most exciting, though mysterious fields: Neurochemistry. This includes the second "*ignoramus, ignorabimus?"*, the most fascinating fact that there is matter in this universe which is not only alive but is conscious and able to think. As was mentioned above, biochemistry has its roots in medical physiology and chemistry of the beginning of the 20th century. I follow this evolution from the viewpoint of a biochemist who started out as a chemist at a time when biochemistry curricula at German universities were still rare. Two impulses started this evolution: One came from basic science asking fundamental questions like the two questions posed in the introductory paragraph above. The other was triggered by the urgent need to get a better understanding and treatment of the many devastating human diseases. I describe this evolutionary pathway following the pathway of the scientific life of a typical biochemist:

Up to the sixties of last century there was only one curriculum of biochemistry offered by a German university, established by Günther Weitzel at the university of Tübingen and started in 1962.

Outside Tübingen therefore one possible way to become a 'biochemist' was to study chemistry (an awfully lengthy and tedious curriculum) and finish this with an experimental diploma work and a subsequent PhD thesis under the supervision of an 'Organic Chemist' interested in the chemistry of life. My choice in Freiburg was Kurt Wallenfels who together with his group synthesized among others homo- and heterocyclic aromatic compounds with strained π-electron systems. This topic is not too far away from electron transfer molecules used by most aerobic organisms in a variety of coenzymes. The Wallenfels group developed more and more interest in biocatalysts (enzyme proteins with and without coenzymes. Of course nothing was heard of RNA- or DNA enzymes at this time).

For the young chemist this offered an attractive entry door from dead chemicals to the chemistry of life: catalysis is an important topic in chemistry and biophysics. The same laws executed with different means and mechanisms, this was enzymology! Enzyme kinetics analyzed with appropriate assays was quite a challenge, promising insight into the structure and function of biocatalysts. Biocatalysts on the other hand are essential for metabolism. One prime difference compared to non-bio catalysts makes enzymes even more interesting: they are flexible; they are able to adjust their efficiency to the needs of an organism. Enzyme regulation is another most important research topic of enzymology. But at the beginning of all biochemical research in 'pre-cloning times' was purification. "*First purify than think! Don't waste pure thinking time on dirty enzymes*" was a statement by an unknown biochemist pinned to the door of my lab. Wisdom like this set the stage for another development essential for the evolution of biochemistry: One could write a history of biochemistry as a history of methods and machinery. Centrifugation, electrophoresis chromatography developed their specific applications in preparative biochemistry. On the structural side progress in protein and DNA/RNA sequencing, X-ray crystallography, mass spectrometry, electron microscopy and the tremendous variety of light microscopic techniques secured advances, just to name few.

To sum up what I as a biochemist see in retrospect: *Biochemistry came a long way, originating from physiology. One could say simplistically: it dealt with organs and organisms, i. e. with systems, proceeded to reduced cellular entities and molecules. It returned to molecular systems and moved on to Systems Biology as part of Life Science in our times. The most complex and challenging* ***system*** *known so far is the thinking matter of mind. Let me go on in my description of my personal way from chemistry to Neuroscience.*

From Lifescience to Neuroscience: The reductionist approach

With regret we see that more and more specialist fields develop in science. On the other hand definitions of those specialties become more difficult and less useful: what is biochemistry? It used to be the chemistry of life. Not knowing what life really is this definition does not make much sense. Some colleagues in the field prefer to call their field molecular biology. This implies that we reduce our focus to molecules rather than cells, organs and organisms. And today the term *Life-science* makes all previous definitions oblique and reunifies scientist from so diverse fields as informatics, ultra structure research, immunology, development, molecular genetics, and many others. It enables specialists to enter new fields and allows them to join teams and cooperations in their effort to add partial answers to our persisting and unanswered questions. As we have seen, by this re-definition the biochemist could move in the middle of the previous century from chemistry, via catalysis and enzymology to metabolic regulation and finally to – Neurochemistry. This latter step needs explanation: what made me, the chemist, a neurochemist?

The obvious answer could be: The obligatory structure of the academic career in Germany of that time: To grow up to an independent researcher required a 'Habilitation'. This was a major step, which after completion allowed to ask oneself: what now? Shall I continue as before or shall I grasp the opportunity to channel my curiosity to an entirely new field? In the early seventies of last century James Watson at Cold Spring Harbor/New York initiated courses aiming at young scientists from various fields who like me were looking for exciting new challenges. This was a wonderful opportunity: three immensely intensive course weeks at a world center of research with great teachers, a Nobel laureate or somebody of similar caliber flown in every other day, W. M. (Max) Cowan slicing three human brains, explaining the overall architecture of the CNS and its development, Thorsten Wiesel elaborating the visual system and its columnar structure, – a constant flow of information from 11.00 a. m. to midnight, 'brain food' for a scientist's life span.

These were the heydays of reductionist Neuroscience. Neuroscience had gone molecular decades before, exemplified by the cholinergic system, my central field of research over the decades 1973–2005: In 1929 Otto Löwi discovered the 'Vagusstoff', identified by Sir Henry Dale as *acetyl choline* shortly thereafter (Nobel prize in Physiology or Medicine in 1936). Sir John Eccles (Nobel Prize in 1963), Sir Bernhard Katz (Nobel Prize 1970), Bert Sakmann and Erwin Neher (Nobel Prize 1991) among others opened the door to our present-day picture of *'chemical neurotransmission'*. The latter three names are linked to the most important development of electrophysiology in the twentieth century, proceeding from wholesale measurements of membrane potentials of excitable cells to single channel currents, i. e. to the molecular events underlying nerve impulses. The *cholinergic system* became my playground starting at Cold Spring Harbor in 1973. After these exciting (and exhausting!) weeks organized by John Nicholls (Neurophysiologist at the Biocenter of the Basel University/ Switzerland) and his team I went to the laboratory of Jean-Pierre Changeux at the Institute Pasteur in Paris to apply experimentally what I had learned just in theory: I learned to purify (remember: "first purify then think...") the *Nicotinic Acetylcholine Receptor,* a protein which Changeux had identified as an entity different from the acetylcholinesterase, the enzyme catalyzing the removal of the '*Vagusstoff,'* the neurotransmitter acetylcholine, from cholinergic synapses. I solved the quaternary structure of the receptor from the electric tissue of the electric eel *Electrophorus electricus*. Work on a protein in the stimulating atmosphere of Changeux' laboratory, the discoverer of allosterism as a fundamental mechanism of regulation of oligomeric biocatalysts, gave a good start. I was able to contribute to an entirely new field, on the basis of what I had learned as a chemist/biochemist before.

Meanwhile Neurochemistry became molecular throughout: Shosaku Numa was first to clone and sequence a cDNA coding for the α-subunit of the nicotinic acetylcholine receptor from the electric ray Torpedo spc. (1982), with many others to follow. Ion channels, transporters, components of what became the new field of signal transduction and intracellular signaling emerged from various experimental approaches and were characterized on the molecular level. More and more 3D structures were elucidated with atomic resolution.

In brief: What is reductionism? The final aim of neuroscience is to understand the (human) brain, presumably

the only substance in the universe able to think, to be conscious, and to exert a free will (of course the human brain shares these capacities with other animals to varying degrees). One method to understand it is to analyze its functional elements. Among these elements ion channels, receptors, transporters, membranes, synapses play important roles. Reductionists hope to reconstruct complex functions of nerve systems from their functional components. Of course this implies already the limits of this method: The whole (the system) is always more than just the sum of its parts. A word is more than the addition of letters. Even more so are complex texts, composed of words, sentences, language. A meaningful text requires more than letters and words: Without grammar and semantics there is no useful text in any language.

Fortunately, the functional molecular components in the nervous systems throughout the animal kingdom follow similar principles: Therefore reductionism on the organismic scale was (and still is) useful. To understand signaling of nervous systems does not require investigation of the human brain. Nerve impulse propagation was elucidated with squid giant axons, simple nervous systems underlying behavior were successfully investigated with invertebrates like the leech and the sea snail aplysia, just to mention two examples. This type or reductionism is called *methodological reductionism*. Philosophers (*Wuketits 1989*) add to it a strategy which is called *"Episthemiological Reductionism"* which as a matter of fact is an expansion which helps to discover more comprehensive principles. A third definition of reductionism, called "*Ontological Reductionism",* has to be discarded at least for the Life Sciences because it postulates to reduce a complex phenomenon to its 'smallest parts'. Methodological Reductionism can be exemplified by molecular genetics: *"What is true for Escherichia coli is true for the elephant",* this is a famous definition of the reductionist belief by the micro biologist Jacques Monod (Nobel prize 1965). It is still valid today with respect e. g. to the DNA structure, the genetic code and some features of gene expression, but the limitations of its meaning for the genetics (incl. epigenetics) of 'higher' organism is obvious. First of all, the genome is a *system,* a network of *about* 20.000 genes (in humans) the expression of which is regulated manifold, *via* expression factors, gene products and signaling cascades, just to name a few regulators. The expression pattern, not the genes as such make the species and the individual. It is trivial to state: both the reduction of genetics to its molecular elements *and* the sum of the molecular interactions in the system must be elucidated to understand molecular genetics. It is not *either* reductionism *or* systems biology; both are fundamental to understanding complex phenomena as life and mind.

In a thoughtful essay in this journal Martin Heisenberg covers this subject, albeit limited to animal *(Drosophila)* life (*Heisenberg, M. 2018)*. In this essay Heisenberg narrows the gap between Mind and Matter: Mind (german *Geist, Seele*) is an important result of evolution which increases the fitness of an organism for survival and reproduction. Heisenberg in his essay calls this beneficial property *behavior* and he observes in the insect's behavior "indirect, mental" elements as for example'intentionality', 'attention', 'formation of hypotheses', 'emotions', 'motivation' 'sociality' etc. All living organisms 'behave' (if we accept the definition that behavior is the interaction of an individual organism with others and with its environment). To improve and sustain behavior signal reception and processing, signaling cascades and finally nerves and neuronal systems evolved. All living organisms accordingly possess mind (again: german Geist, Seele). Note that this definition does not include consciousness, free will! It describes an increase of complexity, at the end of which stands the most complex matter known, the human brain. Philosophers coined the term *emergence:* the conscious mind is emerging from dead matter *via* life matter and unconscious nervous tissues. Consciousness is located in the cortex of the brain; most of the human brain is unconscious. But what is the difference in structure and functional mechanism of the unconscious and the conscious? This is the other 'ignoramus', the fascinating property of thinking matter: The human brain is not only alive, it can dream (passively), actively plan, intend, want, – but how? *ignorabimus?*

Neuropharmacology

The prime beneficiary of reductionist neuroscience was – and still is – molecular pharmacology. Molecules involved in nerve impulse propagation and transmission are targets of drugs and therapeutic treatments. This was one of the justifications of *receptorology*: Neurotransmitter receptors are key protein molecules pivotal in signal transmission and processing. They were postulated to exist by J.N. Langley (1878) long before being isolated in the test tube. Langley coined the term 'receptive substance' in 1905. Receptors were first identified and isolated in the seventies of last century. Up to that time they were more or less plausible concepts, no molecular realities. After a time of tedious protein identification and purification, the field exploded with the advent of cloning technologies.

Diversity through multiplicty: Classical neurochemistry had discovered a rather limited number of neurotransmitters so far, small molecules transmitting the electrical signal from the presynaptic side of a synapse over the synaptic cleft to the postsynaptic side where they trigger an electrical signal again (or a signaling cascade of molecules within the target cell, respectively). The paucity of neurotransmitters seemed to be in contrast to the vast multiplicity of functions in the brain. One would expect that novel properties of many molecules interacting as 'systems' emerge from complexity. How can this happen if only one dozen or two transmitters and their respective receptors are available? The solution (at least in part) was found in the multiplicity of receptors: Most of the transmitter receptors are hetero oligomeric proteins. They occur in various combinations of similar but distinctively different polypeptide chains. The prototype inhibitory $GABA_A$ Receptor e. g. is a pentamer composed of four (resp. 5) types of polypeptide chain coming in a number of variants. Theoretically one can easily construct more than thousand different hetero pentamers with them. Of course not all of them occur *in vivo*. But it opens not only the possibility of quite a variety of tissue specific expression and function of the various subunits and by that of different receptors. It also offers a multitude of small molecule-binding sites, which are often located at the interfaces between subunits. The recently published high resolution 3D structure of a $GABA_A$ Receptor elucidated by electron cryo-microscopy with the receptor protein heterologously expressed in HEK cells and functionally reconstituted in lipid nanodiscs is proof of this principle. This receptor is an inhibitory ligand gated chloride channel. Its specificity for benzodiazepine binding requires the presence of the ß2 subunit. The transmitter GABA binds to the αß interface while benzodiazepines bind (non competitively) to the αγ interface, the high resolution images of which give optimal information for rational drug design. The molecular basis of receptor multiplicity and functional diversity of drug targets is hoped to lead to more specific drugs with fewer side effects. But on the other hand it introduces a level of complexity which makes research more challenging. This may be a reason why we observe that many of the major drug companies gave up CNS research alltogether. They are not willing to risk the time and money it takes to bring a drug candidate from the "bench to the bedside". This observation is in favor of investing more into government supported basic research at universities and public institutions.

Toxins as tools in Neurochemistry: The best molecular neuropharmacologist is still 'Mother Nature': Many animals use neurotoxins as a means for defense and for hunting prey. Most of them interact in minute amounts with maximal specificity with key functional sites in the nervous system of enemies resp. prey. By this they supply science with efficient tools to identify and analyse such sites: Snake venom toxins *(e.g. α-Bungarotoxin)*, Black widow spider toxins *(α-Latrotoxin)*, Scorpion toxins, Tetrodotoxin, but also sea anemone toxins and toxins from plants and bacteria are a few examples of the long list of this potent tool kit which served basic science to isolate receptors, ion channels, synaptic components, and other signaling molecules.

This leads me to a more political aspect of my activity in the years starting in the early eighties of last century: my cooperations and friendship with colleagues in the former Soviet Union. Traditionally life scientists were oriented westwards. Accepting a position in Berlin (then called "*Westberlin*") in 1979 made me turn around and look for links to the East, hoping to pierce tiny holes into the *Iron Curtain*. Of course official support of these activities on the Russian side was stimulated by non-scientific afterthoughts circling around the deadly neurotoxins (Leitenberg and Zilinskas 2012), but the positive 'side effect' was a vivid exchange of contacts, symposia and summer schools. These contacts resulted in fruitful cooperations with the best Russian neurochemists, culminating in a 1 1/2 year visit in my laboratory by Victor Tsetlin, who was awarded a Humboldt professorship.

Trends of the past, present, and future

Neurochemistry came a long way, from medicine, chemistry, biochemistry, to its present day state. In other words: it proceeded from biological *systems*, through *reductionism* focussing on simple models and functional components, and it arrived again at *systems* biology. The basic questions, the starting point of this essay, remain unanswered. The common answer to both questions is: *ignoramus*, but they will be answered, if not by this or the next generation by a generation to come: with new ideas and new methods.

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Bionotes

Prof. a. D. Dr. rer. Nat. Ferdinand Hucho
Institut für Chemie und Biochemie
Freie Universität Berlin
Thielallee 63
14195 Berlin
Tel. +49 30 3248693
e-mail: hucho@chemie.fu-berlin.de

Ferdinand Hucho, born 1939 in Berlin, studied Chemistry in Freiburg/Breisgau. He obtained his PhD in1968 with work on the catalytic mechanism of a bacterial enzyme under the supervision of Kurt Wallenfels, He did his first postdoc 1969–70 with Lester Reed at the University of Texas at Austin working on the regulation of multi enzyme complexes. His second postdoc he did in the lab of Horst Sund at the University of Konstanz/Germany. There he got his Habilitation in 1973 with work on the regulation of pyruvate dehydrogenase by a protein kinase. Short visits to Cold Spring Harbor and to Paris allowed him to switch to neurochemistry. In 1979 he became a professor (with tenure) at the University of Konstanz. The same year he moved to the Freie Universität Berlin. His main field of interest were the structure and functional mechanism of neurotransmitter receptors. In 1997 he was elected as a member to the Berlin-Brandenburg Academy of Sciences and Humanities. There he initiated a long term monitoring project observing the development of Gene Technology in Germany.