



## Tripping on the Edge of Consciousness

By Jim Krueger

I've been lucky in my scientific life; I've always chosen the right order to shoot at scientific problems without knowing why. My colleagues and I have developed new ideas and produced many fine discoveries. I've always marveled at the commentaries about the scientific method and how science is supposed to progress; so formal, so structured, guaranteed not to go wrong. So logical, that human frailties could not influence scientific outcome. So comfortable, a safe secure way to earn a living. Or so I thought at the onset of my career. I have discovered that science is far more rewarding than I ever dreamed, an intense human endeavor, and a complete joy. Below I attempt to relate two experiences of discovery that contrast in many ways. What they hold in common is in the eye of the discoverer, the complete delight of sailing the deep blue uncharted waters of science.

### ***Down and Out in Boston and Harvard***

In the fall of 1974 I joined the esteemed laboratory of John Pappenheimer, a National Academy of Science member, as a postdoctoral fellow. He had recruited me to isolate Factor S, a substance that accumulated in CSF during wakefulness and that induced sleep. At the time neuropeptides were hot; it seemed new ones were being discovered almost every week. There was an unstated hope, based on the success of the endocrinologists, that there would be a "sleep hormone", a substance whose actions were specific to sleep and caused sleep. We knew that if we found that substance, it would be relatively easy to work out sleep mechanisms, give us insights into sleep function, and likely provide better sleep-inducing agents. These are big important issues so we worked hard.

At the time I joined the laboratory, John and his long-term collaborator, Manfred Karnovsky had shown that Factor S was a relatively low molecular weight peptide. They had determined its elution profile on gel filtration and had shown some of its biological actions, primarily the ability to enhance sleep. What they did not know was almost everything else, including the amounts it needed to induce sleep. My job, in theory, was simple. Just use chromatography, fractionate the sample, test each fraction for sleep-promoting activity, and then keep on repeating this cycle using different chromatographic techniques until the substance was pure. Once pure we would do an amino acid analysis to determine composition then determine amino acid sequence.

Six years passed. No structure. No publications. Much bad news. At one point a technician dropped a purified sample representing an entire year of work; she watched the frozen sample melt on the floor then wiped it up with a paper towel and threw it away! NIH cut off our funding in the middle of our grant, my salary was paid from that grant. John went to the Dean (his former student) to ask for my salary and was turned down. NIH had turned down a separate grant I had submitted; they had six people do a site visit to our lab.

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Without precise routines, detailed searching and meticulous recording, human ideas could never be validated nor replicated; but it is the human spirit of curiosity, the very human "need to know" that drives scientific progress. That element—the *human* conduit—provides the connection. It is to this humanness we owe all modern progress, and it is for those among us who believe that the creative human impulse basic to discovery is worth exploring that we dedicate this forum: *The Human Side of Science*.

*Thanks to Ken Campbell for giving creative birth to this series and to Jim Krueger for this second contribution. Volunteers are needed to continue this most entertaining and enlightening journey. Please let Ken or Jeanne Jensen know if you would willingly share your own human story.*

The visitors concluded we did not know what we were doing and that sleep was not regulated by chemicals but by neurons (the old soup and spark argument had resurfaced). I was within weeks of having no job and had little prospect of getting another in science. I had interviewed for other faculty positions; the response was always the same – get structure then come back. My stepfather had indicated to me that he would retire in a year's time and that I could take over his insurance business if I wanted. The thought of selling insurance rather than being at the forefront of science was rather depressing yet for a few weeks it seemed as if that might be my fate. The Office of Naval Research saved my position with only a week to go before my salary stopped. John had helped form ONR during WWII and many of his friends were still there; they gave us emergency funding for 6 months which paid my salary and thus kept food in the mouths of my two young children.

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Those 6 years were not wasted. We had made progress. We knew we were on the edge of figuring out what we were dealing with. We had scaled up and were extracting very large amounts of brain and urine. We had, with the help of Merck Co. and later Sterling Winthrop Co., extracted Factor S from 15,000 brains obtained from sleep-deprived rabbits and in a separate preparation 5000 liters of urine obtained mainly from medical students. We knew that Factor S was very potent; for instance after extracting the 15,000 brains we could not see anything in the test tube yet there was enough of it there to put about 40,000 rabbits to sleep! Every week we were making progress. Despite our financial woes, our samples were becoming clean enough that we could begin to discern amino acids upon analysis.

Finally, success. On a clear fall morning I climbed the two flights of stairs to where our amino acid analysis machine was located. There waiting for me were the results from hydrolyzed and unhydrolyzed samples we had loaded the evening before. I quickly made some ballpark estimates of the amounts of the amino acids that were present and realized we had good molar ratios. There was one peak present that was not a normal constituent of mammalian tissue; I had to consult the chromatography texts in the room to identify it as diaminopimelic acid. It was in equal molar ratio to the other amino acids indicating that it

was part of the molecule we were after. It was the next few minutes of my life that I will never forget; they lasted a very long time.

I knew what diaminopimelic acid was; it is the precursor to lysine. Humans do not have a D-amino acid decarboxylase and we can not thus make lysine from meso-diaminopimelic acid and that is why lysine is a dietary essential amino acid. I knew that dap was a component of bacterial cell walls; I knew that because Manfred's lab mainly studied macrophages and how they were stimulated by bacteria to produce superoxide. I had sat through many of his lab meetings, although often half asleep, I had learned of bacterial cell wall structures, my mind had thus been prepared.

After identifying diaminopimelic acid, I started a slow walk down the stairs back to my office. My mind began to race. My first thought was, maybe this is why one feels sleepy when sick. (I was unaware at the time that no one had ever measured sleep during an infectious disease; many years later Linda Toth and I would be the first to do so). Other implications of the finding began to sink in. Mammalian cells were using bacterial products to signal. Did our finding represent an example of endosymbiosis? (I was unaware of Lynn Margolis' hypotheses at the time so this thought was more troubling that it should have been). About halfway down the stairs I made the mental link to cytokines, which at the time were very new and only a few interferons had been cloned. I knew from Manfred's work that bacterial products were inducing production of cytokines in macrophages. No one had even suspected that they were produced in the brain let alone involved in sleep regulation (it would be several more years before we showed that). Yet I had the gut feeling at the time that this innate immune mechanism may also work in the brain but for another purpose. These thoughts were radical, out of the box. The implications I knew would be rejected by neurobiologists and especially by philosophers since it was hard to avoid the thought that bacteria were controlling, in part, levels of consciousness. What ever would happen to free will?

Many years have now passed. I can now see that that morning has led to many successful avenues of research and many successful careers in sleep research. Regardless, I remain melancholic because what I considered the most important part of those thoughts, the link between microbials and mind, has remained taboo. It is just too radical. Nevertheless, I am convinced that if anyone ever cites my work 100 years from now it will be for those thoughts which crystallized on that lovely fall morning.

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### Fat and Sassy in Memphis and Pullman

For the next 13 years of my life I was busy building my career, getting grants and job offers. My lab became very well funded. We published over 100 papers describing the biochemical regulation of sleep and the relationships between sleep and infectious disease. My most often cited paper, that showing the somnogenic properties of interleukin-1 was published in 1984 with Charles Dinarello, now a National Academy of Science member. I began to work with additional outstanding collaborators such as Drs. Ferenc Obal Jr. and Jeannine Majde. My students and postdocs were becoming successful. I had become part of the sleep establishment and had defended it to the hilt while on study section.

Yet, my radical thoughts from my scientific youth never left. They kept me thinking, I was ever on the outlook for their justification. I began, secretly at first, to question the dominant paradigm of sleep research. That being that sleep is a whole brain phenomena regulated by a sleep-specific set of circuits. I kept asking myself questions seldom addressed by sleep researchers, such as What is the minimal component of brain capable of sleeping? When did sleep start in evolution? Why do we sleep? Is there a connection between sleep mechanisms and sleep function? (There need not be; just think about circadian rhythms). Eventually, Ferenc and I entered into discussions about these issues and compiled a list of evidence that was eating away at the dominate paradigm in sleep research. For instance, Mukhametov had showed that dolphins sleep only on one side of the brain at a time. Countless lesion studies led to the simple conclusion that no matter what area of brain was lesioned, if the animal or person survived, it slept. This strongly indicated that no circuit in brain was necessary for sleep; a threatening concept to those studying those circuits. Slow stimulation of many areas in brain induced EEG signs of sleep. Isolated cortical islands waxed and waned through periods of EEG slow wave synchronization.

We also began to think hard about the theories of sleep function. None of them seemed sound to us (including my own that sleep served an immune function) because all of the proposed functions could be achieved without an animal losing consciousness. For example, it made no sense to us that sleep serves to remove one from "harms way" since to us it seemed to do the opposite. These discussions took place over a two year period; unlike my Fall 1980 experience, this was a slow, hard process. It required much more academic discipline because we knew we were heading for a new theory of brain organization as it applies to sleep and to sleep function and that it would challenge the powers that be. We came up with many ideas, several of them written, only to reject them or reformulate them because thought

experiments led to logic contradictions. I clearly remember a long luncheon conversation with Ferenc at a typical European sidewalk café during which we discussed the weaknesses of the theory. I was so engrossed in our discussion that I cannot remember today what city we were in. Finally, we agreed to publish a version of our theory. Its main tenets were that sleep was a property of small groups of highly interconnected neurons.

Thus, sleep and sleepiness were viewed as a statistical property of a population of neuronal groups in 2 or more states. A second major aspect of the theory was that sleep was targeted to areas in brain depending upon neuronal use during wakefulness. Third, we posited that sleep served to stabilize the brain's microcircuitry and

was thus critical for brain plasticity. These were radical ideas. These were threatening ideas to our friends and colleagues. These ideas provided an entirely new way to look a sleep mechanisms and function. At first the ostrich factor was strong; we were ignored. Within the year, however, Lee Kavanau of UCLA, came to similar conclusions using completely independent reasoning. Second, Alex Borbely, within whose lab in Zurich Ferenc has worked for a year, decided to test our theory. He showed we were right, sleep is targeted to areas of brain depending upon use during wakefulness.

Within the past few years our view of brain organization as it applies to sleep has become even more acceptable. Modern imaging studies have shown that there is differential activation of the brain during sleep that is dependent upon what part of the brain is used during wakefulness. Electrophysiological studies have shown that neurons asynchronously shift into a "sleep mode" as an animal is going to sleep. Our own biochemical studies have shown that sleep affects expression of molecules associated with synaptic plasticity differentially, depending upon the dynamics of the neuronal group's synaptic organization. Strong evidence that sleep affects the synaptic plasticity associated with memory formation has also provided support for our theory. Our view is thus beginning to replace the old paradigm. This is very rewarding and I guess I am even more of an establishment member now than ever.

But, you know, I can't get the radical thoughts of my youth out of my head. In fact, recently we (Abdur Rehman in my lab) described a peptidoglycan recognition protein in brain that varies with sleep. Stay tuned and hopefully you will see an aging revolutionary at it again.

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