John Dowling Interview
Dr. Carl Kupfer Interviewee
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Dr. Kupfer: I am here at ARVO with Dr. John Dowling, whom I will be interviewing a second time. Mr. McManus interviewed Dr. Dowling in Boston the first time. John, one of the things that really surprised me in your first interview was your statement that many brain researchers and neurologists consider the retina as part of the peripheral system (chuckles). It was a shocker.

Dr. Dowling: Well, it is a shock for those of us who work in the retina. But it is astonishing how many meetings I've attended over the years where presenters talk about the retinal output as going to the brain. I point out that the retina is a part of the brain pushed out into the eye during development. Brain researchers tend to think of the retina as part of the peripheral nervous system, and that phenomena found in the retina don't apply to what's happening in the rest of the brain. So when I wrote my book in '87, I was very careful to make that point even in the title ("The Retina, an Approachable Part of the Brain"). And indeed Francis Crick in his book, "The Astonishing Hypothesis" picked up on that. But that was the only thing he said about my book in his book (laughter) - that John Dowling points out that the retina is a true part of the brain. Part of the reason for this misunderstanding is because the retina is not under the skull or in the spinal cord. It is the only part of the central nervous system not under the skull or in the spinal cord; thus, it is easy to think it is part of the peripheral nervous system. And, of course, we also have some unusual mechanisms in the retina. For example, in the outer retina we find neurons that respond to light only with sustained graded potentials and that don't generate action

potentials. I think many feel—"oh, that's not relevant to what's happening in the brain proper". But it is also the case that many of the mechanisms found first in the retina, are then found elsewhere in the brain. But then when the paper is written it suggests this is the first time this finding has been made in the central nervous system. I'll give you two examples.

Dr. Kupfer: That would be wonderful.

Dr. Dowling: The first is the discovery a number of years ago that dopamine modulates glutamate receptors. Andy Knapp and I showed that in retinal horizontal cells back in 1985, but then it was discovered again about 5 years later in another brain region with virtually no mention of what we had shown earlier. Another example is a recent paper in *Science* describing the neuromodulation of gap junctions in the brain. There was mention in the paper that this has been shown in horizontal cells, but the implication was that the retina is not the brain proper - they didn't put it quite that way, but the message was that the retina is not really part of the brain. These two examples relate to my own work, and there are others as well. There are also examples of things we've found in the retina that have not as yet been found elsewhere in the brain, but I am confident they will be found elsewhere in the brain down the road.

Dr. Kupfer: But one of the things slightly peripheral to this point is what Joram Piatigorsky said to me about 10 years ago. I had been telling him that I had a devil of a time talking to neurologists because I can't get their attention. When I'm talking about the retina and they're not interested.

Dr. Dowling: That's exactly right.

Dr. Kupfer: So what I had been doing was to preface my remarks by saying that the brain is an out-pocketing of the retina (laughter). So he said "Well I have something that may be of help here". He was working with some jelly fish at that time and a researcher by the name of Walter Gehring has done a lot of work with jelly fish. He found that what was a receptor to light with rhodopsin and a lens and everything else like that in these jelly fish, really didn't have much in the way of sending signals to "a brain" except for a few ganglia. So when do you call a few ganglia a brain and if it isn't then the eye precedes the development of the brain (chuckles). So I told Joram to enlarge on that and he's going to write something for me to put in, but that's the sort of thing we have to do to jog the thinking about these things.

Dr. Dowling: I see on page 6 of the transcript of the interview with Ed, I talk about the regulation of gap junctions by second-messenger systems.

Dr. Kupfer: Yes.

Dr. Dowling: And, also the regulation of a glutamate channels by second-messenger cascade.

Dr. Kupfer: Well, I just wanted to be sure that if I quote these they'll be complete and understandable.

Dr. Dowling: Yes.

Dr. Kupfer: That's good, okay. That takes care of page 6 and then on page 7 there's a very nice quote about Jeremy Nathans and, as a triumph of modern neuroscience, the elucidation of

visual transduction mechanisms. Now these would not be considered relevant to the central nervous system since the same situation doesn't exist there.

Dr. Dowling: That's true, but elucidating the genetics of the cone visual pigments was a tremendous achievement and paved the way for the work of Richard Axel and Linda Buck who uncovered the genetic basis of olfactory reception and for which they received the Nobel Prize a year or so ago. Indeed, our understanding of olfactory mechanisms has followed behind our understanding of visual system mechanisms in many ways. For example, a second messenger cascade is responsible for activating olfactory receptors. The second messenger is not cyclic GMP (as in photoreceptors) but cyclic AMP. But it is the same general principle that was found first in the retina. I am disappointed that there has not been a Nobel Prize for the elucidation of the visual transduction mechanisms because I believe this is one of the triumphs of modern neuroscience. Indeed rhodopsin is still the best model we have of the G- protein related receptor proteins. We know more about it than any of the other such proteins and this list includes the dopamine receptor and all of the other G-protein-related receptors as well as the olfactory receptors. The activation of the G-protein, transducin in the retina was pioneered to a great extent by Lubert Stryer, and the discovery of the cyclic nucleotide gated-channels came from photoreceptor studies. Working this out has led to an explosion of understanding of not only sensory systems, but also to our understanding of how neuromodulators work – that is, all of the monoamines including dopamine, nor-epinephrine, epinephrine, serotonin and all of the metabatropic receptors. A good deal of all of this has its origins in studies on the visual system.

Dr. Kupfer: Let me ask you a question. Is there a way of comparing the visual system with the somatic sensory system?

Dr. Dowling: Yes and no, but we don't know nearly as much about the somatosensory system as the visual system.

Dr. Kupfer: Right.

Dr. Dowling: In terms of activation mechanisms, we do know a fair amount about some of the touch receptors—the Pacinian corpuscle being a good example. It's a mechanoreceptor where deformations of the membrane activate channels directly. This depolarizes the cells to the point where they generate action potentials, which then carry the information into the brain. But how heat, cold and other temperature receptors work is still very much up in the air. We do now have evidence that the so-called Trp receptors are involved. Interestingly enough, it probably was the National Eye Institute that supported the work that led to the discovery of the Trp channels, which are becoming very important. Bill Pak was isolating mutations in fruitflies that affect the visual system and in one mutant he found that the ERG was transient rather than being sustained. So he called the mutation, transient receptor potential (Trp). When the protein was eventually isolated it turned out to be a channel and was named the Trp channel. This was the first of the Trp channels identified and the Trp channels have now been shown to be important in many somatosensory receptors including cold and heat receptors. It is also turning out to be a huge family of receptors. Again, it came from studies of the visual system, not the vertebrate vision system but the invertebrate visual system. I'll bet that if you looked to see who was supporting Bill Pak when he discovered the Trp mutant, it was the National Eye Institute.

Dr. Kupfer: I know the names, I know these people of course.

Dr. Dowling: So, there's another good example.

Dr. Kupfer: That's very good. On page 21 *(referring to the first interview conducted with Dr. Dowling on 11/5/2004 in Boston*), you said, "Certainly the work unraveling the photo transduction process in the photoreceptors was exceptional, with the big breakthrough coming in about the mid 1980's." And then you go on and make a prediction about gene therapy. I see your point, but gene therapy is fraught with all sorts of problems starting with making metal injections in the eye which the eye doesn't like (chuckles).

Dr. Dowling: Why I think gene therapy could work well in the eye is because between the retina and pigment epithelium is a collapsed ventricle. So you can inject there and get the vector to spread and infect the two critical cells—the photoreceptors and pigment epithelium. The eye is an encapsulated structure so you also don't have to worry about a virus vector spreading throughout the body, although there is the possibility of the vector going down the optic nerve. But nevertheless, I'll put money on it that gene therapy is going to work first in the eye.

Dr. Kupfer: Good. That certainly is encouraging.

Dr. Kupfer: On page 22 you talk about visual deprivation. When one thinks of visual deprivation one thinks first of amblyopia in patients, but I don't find that as exciting as what you've said here— that these findings are telling us more about brain development. I think that's really the pay-off.

Dr. Dowling: I should have made that a bit clearer. What I was talking about there were the findings of David Hubel and TorstenWiesel. If you take a visually inexperienced monkey and record from area V1 of the cortex, the neurons are amazingly adult-like. They may not respond quite as vigorously as they will in a month or so, but you find all the types of cortical cells - simple cells, complex cells, specialized complex cells and so on and so forth. The circuitry is pretty much all there in the newborn, visually inexperienced monkey. They then went on to show that this circuitry is initially extremely labile. It can be modified for a period – during the so-called critical or sensitive period. These results have really shaped our understanding of brain development. We've come to realize that by intrinsic-genetic-mechanisms much of the brain circuitry is put together *in utero,* but then in the young animal it's very modifiable; that's where environment comes in. Until the experiments of David and Torsten, which have held up amazingly well, the guess was that circuits formed as a result mainly of experience or environment.

Dr. Kupfer: Right.

Dr. Dowling: But now I think we've come to realize that the circuitry is there to begin with, but it's early experience that shapes it and can alter it. I'm not sure it can be enhanced by early experience, but it certainly can be modified in some very interesting ways. Some experiments that have come from other systems (perhaps not supported by the Eye Institute) have provided us models and ways of thinking about brain development that would never have come about if it were not for those classic early studies of David and Torsten.

Dr. Kupfer: So other systems have followed—in other words if you go into the somatosensory system, the connections are already in place.

Dr. Dowling: As far as I know, yes and this is true for even more sophisticated parts of the central nervous system. So for example, some have said the Hubel-Wiesel results may be the case for area V1, but what about higher cortical areas? The best evidence here comes from the work of Charlie Gross who looked at very young monkeys and the face recognition area in the inferior temporal cortex. He found that early on the neurons respond remarkably like they do in the adult, though there are some if's, and's, and but's. What I shall do, Carl, is to send you my book that was published a year or so ago by the Joseph Henry Press of the National Academy of Sciences. It talks about the development of the brain, the adult brain and the aging brain. I use many examples from the visual system including a fair discussion of David and Torsten's work and the work of Charlie Gross which I would guess is supported by the Eye Institute. But some of the work that has derived from this approach has been stunning. I'll give you one example, but if I'm getting off the track, stop me.

Dr. Kupfer: No, not at all.

Dr. Dowling: Some of this work is important not only for an understanding of brain development, but also for how we should be teaching our youngsters. Let me tell you about the research of Eric Knudsen. He works on barn owls. You've probably seen movies of these owls; they can rotate their heads almost 360 degrees. They cannot move their eyes in the socket, so when they look at something they must move their heads. They also have very acute hearing, so if they hear something off to the side, they move their head to bring what they are hearing into congruence with their visual field. Following up on the kind of experiments that David and Torsten did – Eric showed a number of years ago that if prisms are put on young owls that distort where the animal is looking – in other words,

they look too far to the right or too far to the left, the young animal quickly compensates for that. They can adjust how far the head moves, but adults can't. There is a critical period for making the adjustment. Then he went on to show that when animals are making the adjustment, certain neurons sprout new processes and the presumption is that they make new synapses accounting for the adjustment. Next, he did a terrific experiment that I think is extremely important. He took a young owl, and put on the prisms until they compensated. While still in the critical period, he took the prisms off the owls and they re-compensated back to what would have been the case if they never had prisms on. Then he let the animals grow up and put the prisms back on. If you do that experiment in the normal adult animal they would never adjust, but these animals did. This suggests strongly that the young animals made new synapses while they were making the initial adjustment, and that these synapses then persisted and could be reactivated later. This is saying something that's familiar to all of us. As a youngster you learn to ride a bike and even if you don't ride a bike for 20 years, and get on a bike again you can ride it. You don't need to relearn to ride all over again; the circuitry underlying bike riding has persisted. But as an adult, try to learn to ride a bike, or play golf, or tennis and it's extremely difficult. These are the ideas that we presently have.

Dr. Kupfer: Can you give me the reference to that book?

Dr. Dowling: Yes, let me give you the book. I'll send it to you.

Dr. Kupfer: Good. That will be perfect.

Dr. Dowling: In the book, I also talk about neural degenerative diseases and how studies on retinitis pigmentosa have led the way in understanding these diseases.

Dr. Kupfer: That's another area that I would like to talk about On page 29, you talked about the vitamin A and E story. Vitamin A seems to be beneficial in RP but vitamin E probably interferes some way with vitamin A being mobilized. And yet we still have ophthalmologists who give vitamin E to their RP patients, John Heckinlively being the major person who I just don't understand at all. Is this something that you've come across? Is this an isolated event? Have you heard of other people who don't believe these results?

Dr. Dowling: Let me make some comments about clinical trials and why people may not appreciate the results. If you take a group of people that have all forms of RP, as Eliot did in that trial, and you give them a potential therapy – in this case vitamin A, it was the case that for some people it worked, but for the majority of people it didn't work. When you put the data together, the effect is so small on average that it doesn't seem significant. And because there are risks to taking large amounts of vitamin A, many physicians felt it was not worthwhile prescribing it. Indeed, why put people under risk by asking them to take such high doses of vitamin A if the effect is so small? But what Eliot and one of his students have since done is to show that certain forms of RP are much more responsive to vitamin A than other forms. Where a mutation resides in a gene can be critical. For example, in those cases of RP in which the mutation is close to the amino end of the rhodopsin molecule, there is a substantial beneficial effect of vitamin A. On the other hand, if you have a mutation at the other end of the molecule – even though it's coded by the same gene, vitamin A doesn't work. The same goes for light deprivation. It's been shown also to have a beneficial effect if the mutation is at one end of the rhodopsin molecule but not at the other end. What this is saying, of course, is something that is now very familiar – that retinitis pigmentosa isn't one disease – it is 200 diseases. And if

you're going to start to treat somebody you've got to know what particular genetic form you're dealing with. Further, there will be no therapy for any disease that's going to work for everybody. And yet today we still talk about Alzheimer's as one disease, Parkinson's as one disease, Schizophrenia as one disease. Two weeks ago, I was reading in the *New York Times* a story about Parkinson's disease and a researcher was quoted as saying that drug therapy for Parkinson's disease is disappointing in that it only helps 50% of the people; thus we need to find a way to help everybody with the disease. What she was proposing is that they do deep brain stimulation on all patients. But, if you have any therapy that helps 50% of a population, you're doing marvelously! What is needed is to understand the genetic profile of a group of patients, to figure out who will be responsive and those who are not going to be responsive to a particular therapy. Why do some people with the same mutation come down with RP at age 20 and others don't show any signs of it until age 60? And that's got to be due to small variations in the genome, as well perhaps, to environmental effects and so on. Just think if Merck could have predicted the 5% of the patients who reacted adversely to Vioxx, they wouldn't be in the deep water that they're in now.

Dr. Kupfer: That's right.

Dr. Dowling: Another example is the drug that Biogen marketed for multiple sclerosis that they withdrew about a year ago because of adverse side effects in a small percentage of the people who were taking it. For many people with multiple sclerosis; it seemed to be a miracle drug. You know this is the conundrum. We need to understand the genetic profile of patients. We've been moving too slowly in my view in trying to get at that. If we had a drug that cured 50% of the RP patients wouldn't we be thinking we're doing beautifully?

Dr. Kupfer: Yes, we would. Is there's anything further that you wanted to comment on in this regard?

Dr. Dowling: No.

Dr. Kupfer: Otherwise it's pretty straight forward and real enjoyable. Now the only other thing is—this is unbelievable to me (chuckles) of course. Here is the 100th Council meeting of the NEI, and a program was put together to sort of review what happened in the first meeting and who was there and what they said to each other, and then what has happened throughout those hundred Council meetings and what the future is going to be. It was a day long meeting with Jack McLaughlin, Paul Sieving, and a list of others. And they have a list of Research Highlights; here's the list, I sent it to you. So I called Jack and said, "Gee Jack, I'd really like to know who put this list together. What list? So I told him for the Council's 100th Anniversary. He said 100th Anniversary? I don't know anything about that.

Dr. Dowling: When was this?

Dr. Kupfer: Here it is, February 14, 2002. So I found myself in a time-warp because Jack didn't know anything about it and Paul couldn't remember anything about it. And then I had this document, which I happened to find on the Web—on the Internet, and I just pulled it up and no one seems to know anything about it. So I figured I'd go to you for clarification on the story of unraveling the visual transduction cascade.

Dr. Dowling: Yes. That is an interesting story. To start at the beginning, it was realized very early on that there had to be some way for the visual signal to get from the disc membrane, where

we knew the bulk of the rhodopsin was, to the plasma membrane where it was believed the channels were that caused the photoreceptor potential. The story begins, then, with the first recordings from the photoreceptors and horizontal cells by Svaetichin and Tomita, as well as the electron microscopy of photoreceptors which showed that the discs were separate from the plasma membrane and that there had to be a diffusible signal going from disc to plasma membrane. Bill Hagins first showed convincingly that there was a dark current which is shut down in the light. That finding, along with Tomita's recordings showing a low resting potential of photoreceptors and that photoreceptors hyperpolarize in the light with a resistance increase, indicated there had to be a substance that kept channels in the outer segment membrane open in the dark. But what was the substance? First was the calcium hypothesis which came from Bill Hagins who showed that he could change the response of photoreceptors substantially by changing Ca^{2+} levels. Then Mark Bitensky showed cyclic nucleotides were in photoreceptors, and thus they could also play such a role. This was very early in the story of the cyclic nucleotides. Then a whole host of people – our own lab was involved as was Deric Bownds, Lubert Stryer and a host of others. All of the experiments suggested that cyclic GMP was involved, but calcium also seemed to be important and so it was a puzzle with people coming down on one side or the other. And so the cyclic GMP vs Ca^{2+} wars went on about 10 years.

The breakthrough was made by Fesenko, a Russian who did an experiment that if any researcher in the United States proposed to a study section, would have been laughed out of the room. What he did was to take a patch electrode, put it on an outer segment membrane and pull off that patch of membrane. Then he applied a cyclic nucleotide, cyclic GMP, directly to the membrane and showed that it opened channels. At that point in time, it was thought by everyone that the way the cyclic nucleotides acted was not

directly on proteins or on channels, but via kinases, so that if you were to do this experiment you would never add the cyclic nucleotide directly to the membrane; you would add a kinase along with the cyclic nucleotide. But Fesenko (and I'd love to know from him why he did the experiment) put the cyclic nucleotides directly on the membrane and the channels opened. That was the breakthrough because it showed that cyclic GMP directly gated channels in the outer segment membrane. Fesenko sent a paper to *Nature*, they sent it around for review, and although the details are still a little murky, they apparently sat on it for six months. In the meantime, King-Wai Yau did the same experiments. Now whether he did it after Fesenko – I think he had heard about Fesenko's experiment when he was at a Dahlem Conference in Berlin – or independently of him is unclear. But he did the same experiments and wrote a paper saying that it worked and then *Nature* published both papers.

Dr. Kupfer: At the same time?

Dr. Dowling: No, Fesenko's was first and then Yau's a little bit later.

Dr. Kupfer: Where did Fesenko work? What laboratory?

Dr. Dowling: He had his own laboratory. I'm pretty sure it was in Moscow. I think it was the only really important experiment he ever did.

Dr. Kupfer: I don't think this is the history of the NEI (chuckles).

Dr. Dowling: The reason I mention this is that the lore at that time was that the cyclic nucleotides only act via kinases and they, themselves, couldn't open channels. To continue the story, the

role of calcium was soon uncovered as playing an important role in photoreceptor adaptation. That work was done pretty much in this country by a variety of people, including Denis Baylor who played a particularly important role in advancing our understanding of photoreceptor physiology. When put all together, it is an impressive piece of biology.

Dr. Kupfer: Okay, we have that well covered. How about the other discoveries on the list. For example, the first viral therapy that was first discovered by Herb Kaufman

Dr. Dowling: Herb Kaufman—right.

Dr. Kupfer: How about the first human gene to be cloned? Was that an eye gene?

Dr. Dowling: I don't know.

Dr. Kupfer: Well it may not be. No one wants to take credit for it.

Dr. Kupfer: Who was responsible for uncovering the genetic basis of retinoblastoma?

Dr. Dowling: Retinoblastoma? Ted Dryja was involved.

Dr. Kupfer: Ted Dryja, that's who I was trying to think of, yes.

Dr. Dowling: With Bob Weinberg.

Dr. Kupfer: Exactly.

Dr. Dowling: But that work also derived in large part from the work of Al Knudson.

Dr. Kupfer: Oh yes the double hit.

Dr. Dowling: There is another advance that was very important, the crystal structure of human rhodopsin. Paul Hargrave was involved here, I believe.

Dr. Kupfer: Hargrave, yes.

Dr. Kupfer: Do you have any final comments?

Dr. Dowling: Yes, one. I think it's fair to say that the retina is today still the best understood piece of the brain in terms of its circuitry, physiology and what it does and how it does it. Some of the papers I've heard at ARVO this year have just confirmed my conviction of this, and, of course, the Eye Institute with its support of basic researchers, the ARVO meeting can take much credit for the enormous progress we have made.

Dr. Kupfer: I'm going to turn this off.

End of Interview