M.A. RUDA
ORAL HISTORY INTERVIEW
April 6, 1999
May 18, 1999

Marcia Meldrum: Good morning. Today is the 6th of April. It's a Tuesday. We're sitting in Dr. M.A. Ruda's office at NIH, in Building 49. Would you say hello for the tape?

M.A. Ruda: Good morning.

MM: Yes, OK. So I think we're ready to go. Dr. Ruda, we'd like to start by having you talk a little bit about when you were growing up in Pennsylvania, and what important influences in your life led you into a career in biology. So, tell me a little bit. What kind of a background do you come from? Were there other scientists in it?

MAR: That's kind of starting at a very early point in time. I think I had always had an interest in science, just biology in general, and there wasn't any formal training or an experience or a relationship with anybody that was really responsible for it. I think I'm one of those people that just had an interest in biology all along, and it just developed as I went through school to getting a Ph.D. in neuroanatomy and doing the research that I've since followed up on.

MM: OK. That's really a shorthand version. You did biology in high school.

MAR: Yes, but nothing of any consequence. No. It was more a situation, I just had a natural interest in biology, animals and plants and just things of that nature, and so my first opportunity to really get some skills in this area other than – Well, I guess I did participate in high school in science fairs and won a couple of those. But it really was in college that I had my first opportunity to really get into more detailed and more interesting courses, and I did at the University of Pennsylvania and majored in biology there.

MM: And at that time were you interested in neurophysiology or any area of neural processing?

MAR: Well, actually, when I started out, I was probably more interested in marine biology than anything else. But by the time I was applying to graduate school, the area of neuroscience was really in its infancy and just taking off, and seemed very, very fascinating.

MM: That was like the early '70s.

MAR: Right, exactly. And I first applied to do a master's degree at Georgetown just in general biology because I wasn't a hundred percent sure what I was going to do with my career. So I went off to Georgetown. And even before I started there, I really had developed a strong interest in neuroscience and did some electron microscopy at Georgetown – actually, electron microscopy was also in its infancy – and had the good fortune of being accepted back at the University of Pennsylvania. They had just recently established the Institute of Neurological Sciences, which had funding for graduate students available, and very generous funding, I might say, especially by today's standards – it was a very nice program – and gave me the opportunity to go back and do a PhD there in neuroanatomy

with Peter Hand and Alan Rosenquist as the two key people that were my mentors at the time.

MM: And they were both neuroanatomists?

MAR: Yes. They were both neuroanatomists. They had very diverse interests, [more] than I did. Alan Rosenquist really is [interested in] the visual system and has always worked in that area. And Peter Hand's main area of interest was more in somatosensory cortex and thalamus areas. He did a lot of work with trying to identify the receptors and the areas of the brain that dealt with touch sensation of the vibrissae.

MM: The whiskers.

MAR: Right, right.

MM: OK. Now, forgive me because I know neurophysiology only from the standpoint of pain. What they were doing at this point, I mean, what you're talking about sounds like neuroanatomy is characterizing the ultrastructure or tracing the physiological pathways.

MAR: Well, I started out doing a little bit of both because the Institute of Neurological Sciences had the novel idea that if you were going to study the nervous system, you wouldn't be working in just one discipline. And so it was a very forward-thinking idea that the Institute had. And so I had opportunities to do both electrophysiology and neuroanatomy, and both areas are really just beginning at this time, and so anything you did was new and interesting. And I guess the reason I moved into the area of pain was more through somatosensory interests and the fact that when it came time to pick up a thesis project, I had done some reading on stimulation to produce analgesia and that whole idea, which seemed to be quite exciting. So I proposed that as a thesis project and they approved it, so I was able to go forward.

MM: So what kind of a project was it?

MAR: It was primarily neuroanatomy where I was able to identify the circuitry between the midbrain central gray, which was where the people were stimulated to produce the analgesia, and its brainstem or spinal or thalamic connections in order to try and identify what circuits might be responsible for the phenomena of stimulation to produce analgesia.

MM: Now I know in a lot of your work, you talk about circuitry, and it seems very interesting that – Let me see. It seems to me the point is frequently made that circuits are open to multiple functions, that each circuit can be open to integration of a variety of inputs, excitatory and inhibitory. Was this clearly understood at the time?

MAR: No. Ideas were very diverse in terms of how the nervous system really worked, because the research really had not been done, and so people hypothesized very simple circuits that were fixed and extremely rigid, and to a certain extent, that is a correct view of the nervous system. But the ideas have evolved over time to show that there's a lot of plasticity, a lot of change that can really happen. I think that that is something that just came about because of more advanced thinking of how the nervous system was put together. If you think about it in terms of body parts, for example, you have an arm, and

so you are not going to grow another one and you've got five fingers, etc., etc.; and so if your nervous system has a thalamus or brainstem, then it has neurons there, well, you know, that's what there is. It's a much more dynamic kind of situation than what was originally thought.

MM: Now, a lot of your work also has dealt with genetic expression. So at this time, had you done any particular training with molecular biology?

MAR: No, because molecular biology also is a field that maybe is only right now 10, 15 years old at the max, and molecular biology really started out in simpler systems than the nervous system, because if you go and look at it, it's a situation where homogeneity of the tissue is a key component of success in molecular biology. So you have PC-12 cells or something along those lines that you're really using as your system that you're studying, you have a very simple question as opposed to a nervous system where if you think it's just... For argument's sake, take a spinal cord and what you have there – you have motor neurons, you have sensory neurons, you have autonomic neurons, inner neurons, projection neurons, multiple neurotransmitters that are possibilities, etc. So each of the components is a very unique part of the system, and, as an individual part, represents only a small percentage of the whole.

MM: That's really interesting. My question was actually simpler, which is, had you done any thinking about genetics at this time? Had you done any formal training in it? I mean, molecular biology is a relatively new field, but there were people that were working on genetics then, on codons and genetic sequencing.

MAR: No. I really got into some of the molecular questions while I was here at NIH, and just using the tools of molecular biology as they would easily apply to the system we were studying, which was typically the spinal cord and the dorsal horn or the ganglia.

MM: OK. So, any other comments about working in Pennsylvania? It sounds like it was an exciting environment to do that.

MAR: It really was. I think that they had put together an excellent program, and if you think about the people that were there at the time, they are some of the original major players in neuroscience. Jim Sprague was chairman of the department. He had [Louis] Flexner there, he had Eliot Stellar. I could go on and on with names of people that, if you go into the history of neuroscience, are the key players. And so it was a very good opportunity to get very interesting training in an exciting environment.

MM: You were obviously aware of the work on stimulation-produced analgesia, and this was also a period when the IASP [International Association of Pain] was formed and the pain field became more formally organized. Did you participate in any of that? Were you aware of the journal Pain developing? Was this on your radar?

MAR: Yeah. I was aware of the organizations and all of that. I started out, I guess, in the Society for Neuroscience because that was just recently 25 years old, about four years ago, so it's 29 now, something like that. So it was before I started graduate school. I was just

finishing with college then. The Society for Neuroscience, with, I think, a hundred members, was getting in place, as opposed to the 20-some thousand that they have today. But I was aware of the American Pain Society and the IASP; and, as I said, at the Institute of Neurological Sciences, they had a very generous graduate student program. I had the opportunity of actually attending meetings, so it was a very good opportunity.

MM: So, what brought you here [to NIDCR] then? You graduated in '76, or you got your doctorate in '76.

MAR: Right.

MM: And so you were looking for postdocs.

MAR: I was looking for postdocs, and I started at NIH because I had the opportunity of doing electrophysiology and anatomy here, and both of those areas I was very interested in. So I started a postdoc and then was fairly successful in that and moved on to a tenure-track position and then was able to achieve tenure here at NIH and have pretty much my own lab and group of people that work together with me.

MM: And that's been over the last 20 years or so.

MAR: Yeah, about 20 years. No. Twenty-three, I think, by now. I hope nobody does the math to figure out how old I am.

MM: But you had choices. I mean, when you had your doctorate originally, you could have chosen an academic post, and you chose to come to NIH instead.

MAR: Right. I had.

MM: You decided to stay here.

MAR: Well, NIH has a lot of good opportunities that I was able to take advantage of, and so it's been a nice environment in terms of resources that are available to you and the fact that it is a real research environment. The requirements for teaching are nonexistent. And in some respects, it was good and it's bad. Today I would probably like to do a little bit of teaching, but there's no requirement of it for having a position here at NIH.

MM: And when you came, Ron Dubner hired you? Who was here then? I know Steve Gobel was here and you did some work with him.

MAR: Right. Ron Dubner was the new branch chief. It was not long after the branch was founded. And Steve Gobel was the other senior person that was here, and so I did work with both of them over time.

MM: Steve Gobel worked with the electron microscopy.

MAR: Right.

MM: And that's really fascinating to me, although it still mystifies me. I look at the pictures and the pictures look utterly beautiful, but – OK, simple question. What does it matter if a cell is a stalked cell or an islet cell? What does that mean?

MAR: They were used originally as identifying features so that you could imply function, and that was the only aspect of it. What you were searching for at that time were characteristics of neurons that could give you the opportunity to imply what their role in the nervous system might be. And so the islet cells, because of their structure, and then later on, the physiology that was done with them – it was possible to intracellularly fill them – were found or hypothesized to be inner neurons that were inhibitory. And so that was just a characteristic that was used. Whether or not it matters, not really, but the fact that the islet cells have a nice little territory that they receive their inputs from and then the stalked cells have the opportunity to send their dendrites down to different laminae so that they could get different input from primary afferents and other neurons implied certain levels of function. So that's why it was important at the time. Today, nobody talks much about islet cells and stalked cells. They've gone beyond that. You have a situation where you characterize the neuron as to what neurotransmitter it may have and other functional aspects of it, what kind of receptors it's expressing. But that's just an evolution of our knowledge about how the organization of the spinal cord is put together.

MM: So tell me, you came here and this is an interesting lab. You were in Building 30 then?

MAR: Yes. We were in the basement of Building 30.

MM: The basement? I guess you were safe from prying eyes.

MAR: You're being polite about what I would characterize the basement of Building 30 as.

MM: OK. So, tell me, how did you choose the problem that you wanted to work on, and how did you develop ways of working on that problem. Can you talk a little bit about that?

MAR: Yeah. Well, I think it's important in science to start out with a big-picture idea as to where a field is going and what the important questions in the future are really going to be, because science is a competitive business and you are not so unique that you're the only one to come up with an idea. Somebody else out there could have the exact same idea. So it's important to have the big picture as to where you think the field is going and then position yourself so that the studies that you're involved in are the ones that give important answers to questions that a lot of people are interested in. And so science is really about your ability to spot these and then do a good job in designing the study and analyzing the data.

MM: So can you be more precise?

MAR: Well, precise. How do you do this? How do you come up with a project? You can't only look in the area of pain research because that is what is already done. What I have found useful is to look in emerging areas of neuroscience to see what is being talked about, what is being researched, and then think if ideas or directions can be applied to problems that are important in pain research. And so that's the basis of how you come up with new ideas. If you have ongoing experiments, it's often easier to know what the next step is going to be, because in part it's a lot of times based on feasibility and techniques that are available as opposed to what you would like to do. Sure, you'd like to find a pill that would immediately relieve pain and have no side effects. I mean, I would love to do that. That would be a very

good service. However, realistically, that's not something that could be proposed as a research project. You need to come up with something a little bit more realistic.

MM: So, instead, what you were looking at, at least initially, was the release of various neurochemicals – serotonin, enkephalin.

MAR: Well, the first neurotransmitters that we were really able to work with were the amines. And, again, it was a matter of technique. You were able to visualize them originally because of Falck-Hillarp fluorescence, endogenous fluorescence. You were able to get [see] the monoamines. But then the next major technique that became available was really immunocytochemistry and the ability to make antibodies to things and then label them and figure out what role they had in the system you were working in. So, immunocytochemistry was a very major breakthrough. You had track tracing in the nervous system. Well, first of all, I guess you had Cajal, if you want to go way back, and visibility to identify neurons based on the staining patterns that he got in individual cells. But then track tracing, how things are put together, is a very important fundamental question. And so that gave a big boost to the whole field of neuroscience. Then the identification of a lot of novel neurotransmitters, neurochemicals that were the peptides, for example, again, gave you a good step. The next step would be identifying the receptors and your ability to really look at receptor distribution and receptor occupancy. All that was very important.

MM: Immunocytochemistry has been a very important part of your work.

MAR: Yes.

MM: And, as I understand it, what you do is, in looking for a specific neurotransmitter, you create an antibody?

MAR: Right.

MM: And then you label that with a radioactive substance.

MAR: No. It started out that way, but the techniques involved in using either a fluorescence tag or a permanent peroxidase tag.

MM: Yeah. Horseradish –

MAR: Horseradish peroxidase. Right, yes, mm-hmm. They were much more useful than the radioactivity.

MM: So, when you introduce those into the system and they react with wherever the neurochemical occurs, neurotransmitter occurs, they're going to react to it, and then you're going to get a fluorescence.

MAR: Right. You're able to visualize. You can visualize where the specific labeling is for whatever substance you have an antibody to.

MM: In all of your papers, you very carefully, however, say that what you're observing is not necessarily, say, enkephalin, but enkephalin-like immunoreactivity. So, we actually don't know what this is. We just think it looks like this.

MAR: Well, you end up being technically correct on these things because – especially the earlier papers using the technique had to use those qualifiers because remember, an antibody is not – You get into immunology, the whole concept of what an antibody is. It is made to a fairly small epitope of the molecule, and it is not necessarily true that that epitope doesn't exist in other molecules. And so you have a situation where something else can have that same epitope and therefore react to it. So, in the early days of immunocytochemistry, the "like" word was always used because people in immunology would make it very clear that that's what you needed to do to be accurate in terms of what your antibody was detecting.

MM: OK, next question. How, then, do you come to the finding that there – I mean, you identified some of these and you're able to observe them in different synaptic interactions. But there seems to be a continual process of observing new neurotransmitters and then neuropeptides, which seem to be a completely – well, not completely, but a different sort of animal. How do you identify new ones? I mean, you haven't even constructed any antibodies for them.

MAR: Well, a lot of what is done is if the new peptide is identified someplace else in the body, like most of the peptides were originally identified in the gut –

MM: Oh, really? I didn't know that.

MAR: Yes, exactly. So, it's a situation where they're not brain specific. And so if new peptides were identified, you had antibodies, you always tried "the nervous system" and see whether or not it would label anything specifically, because there's a whole new line of research that you could go into. You can also do studies where you try and target to identifying new, novel neurotransmitters, which are much more difficult to do. It's kind of like a fishing expedition, and sometimes you're lucky and sometimes you're not. So it depends on the strategy used, what fly you put on the end of the hook, if it's the one that that fish likes to eat.

MM: So, out of this work, what kinds of conclusions were you coming to?

MAR: What we were able to do is develop actual models of how the system was put together and potentially how it could interact with itself. We were looking in the spinal cord, and that's a very simple system with three major components. You have the intrinsic dorsal horn neurons, which either are local-circuit neurons or projection neurons; and then you have the primary afferents that carry in information from the periphery; and then you have the axons that descend from the brain and modulate these circuits. So if you start off with just this three-part system, you build on it from there by adding other characteristics such as the various neurochemicals that could come from each of these different sites, the various physiological responses. Are the neurons nociceptors, non nociceptors? Do the neurons respond exclusively to [one type of sensory input, such as touch, heat, pain, or are they multi-responsive, that is, are they] wide dynamic range [neurons? that give us clues] as to how the whole thing is put together.

MM: But the repeated comment I keep coming up with is functional diversity. I know I asked you about that a little bit at the beginning. Were you observing things that seemed complex, or was it clear that there were multiple interactions going on and you could identify each one? Or was it a pattern that looked – I mean, you could tell there was a lot, you could identify a

lot of different activities going on, and yet it was difficult to characterize them? I mean, what kind of a process is it to try to determine what specific neurochemical is involved in, say, an interaction between a primary afferent and a projection neuron? Do you understand what I'm saying? I know I'm not phrasing this very well.

MAR: Well, I think the question you're getting at is that, if you're looking at interactions and you have two things coming together, how much of an explanation does that really give you? Well, what started out that that's all we could do was two. Then multiple labels became available, and so every time you added another thing that you could identify in the system that you were working on, you increase the complexity of what you were looking at. But, by the same token, you also got more information about how its true function was going, what it was really doing, because the nervous system has a lot of diverse inputs that it gets [information from], and it's able to be modulated in many different ways. And so it's like coming up with pieces of a puzzle, and how are you going to put them together? And so, when you start to multiple-label things, you just get more and more pieces of the puzzle that help you out.

MM: Yeah. A puzzle is exactly the way it sounds. It sounds like a bunch of pieces and they all have all these little strangely shaped edges. It's a simplistic way of characterizing it.

MAR: Yeah.

MM: Now, in doing this, you were working primarily in cats, rats, mice? What kind of organism?

MAR: I started out in the very beginning, cats and monkeys, which were the main animals that we really use in neuroscience research, because there was an awful lot of behavior, and that was one of the first areas in neuroscience that people spent time on, so cats and monkeys were used an awful lot. It's now a situation where rodents are the key animals that you use. A lot of research is done in rats. And then, now with the ability of knockouts, transgenics, etc., mice, mice are becoming a major research animal in studying the nervous system.

MM: Well, I'm still thinking about the early '80s, and I know that Ron was working with the awake behaving monkeys at this point.

MAR: Yeah. I didn't really get involved in those projects. No.

MM: I just want to get an idea of the animal model we're working with. Is this an anesthetized animal where we're delivering different type of nociceptive stimuli to the periphery?

MAR: Well, the electrophysiology was all done that way, recording from the spinal cord of anesthetized animals and then seeing the response characteristics, looking at stimulation of various brain sites and seeing if it would alter the response characteristics or dribbling on various neurochemicals and seeing if that would change it. And so all those studies were done in anesthetized animals, and it moved on to – I guess intrathecal drug injections were the next area where you did have an awake animal that you could look at the behavioral changes.

MM: Okay. Because then, later, you start talking more about working with inflammation models, injury models. Am I getting ahead of the story?

MAR: No. It's a situation where, after the physiology in anesthetized animals had really played a major role in looking at the system, there was a very important need to develop models of persistent pain where the animals would be awake, moving around, and you would be able to look at how the nervous system is responding to these persistent pain stimuli. And so models of peripheral inflammation, hyperalgesia, were developed and really made an incredible leap forward in terms of our understanding how the nervous system encodes these pain messages, because the physiology that was done previously was really [with] an acute pain stimulus, and that is not the same thing.

MM: The hyperalgesia models were closer to chronic pain.

MAR: Yes, as if the pain persists over an extended period of time. And that's really where a lot of the ideas of neuronal plasticity developed because of the issue of the pain stimulus being almost like a learning situation. You could think about, well, how do you learn how to play the piano? How do you memorize something? You keep reading it and reading it and reading it, you work on it, you work on it, work on it, so you have this constant neuronal stimulation in order to learn the task. Well, if you have a painful part of your body, let's say, you know, gout, for example, or something that's relatively common, well, that thing is hurting all the time, and that message that I hurt, I hurt, I hurt, is constantly going into the nervous system and is being dealt with at certain levels. And so it requires the nervous system to deal with it, and so it's almost a learning episode for it.

MM: And over time it will lead to, it can lead to altered central processing.

MAR: Right, exactly, that you should change the way the nervous system processes its noxious stimuli.

MM: And that model has been very, very powerful in the last few years or so. But in your work, you've talked specifically about how that's affected genetic expression.

MAR: Right.

MM: Because when this model was first presented to me, what he said was – let's see, what did he say? It made sense. Persistent spontaneous discharge. I guess this was Gary [Bennett] who told me that, over time, persistent nociceptive inputs, like you said, will create a neuroma. OK? And then this will alter the structure of the nervous system. The spontaneous discharge will continue, and in response to that, there will be this altered central processing so that the brain will begin to interpret more and more signals as pain, the receptive fields of the nerves, of the neurons, will widen, and so, in other words, it was all described in a very – This is what the nerves are doing. It's very physical, no chemical. Just nerves. But when I read some of your papers and I started to think about it, I mean, this is really happening on a different level, because what we're essentially saying is not simply that nerves are doing different things. We're saying that there's a chemical process whereby new genes are being, or genes that were already present in the system are now being expressed in new ways or in multiple ways. Tell me what I'm talking about. So I have two questions here. I have probably three or four. Question one is, when did you begin to think about fitting this genetic component in, and sort of, how did you go about it? And then talk to me a little bit

about whether we can really understand this model without the genetic component. Or just go back to the first question. When did you start thinking about it?

MAR: I had to be reductionist. I mean, you know, my biggest criticism of molecular biologists is that they're too reductionistic. But you do get to a situation where changes in gene expression really are what drives the response, and so it's important to know what they are, be they at the level of neuropeptides or transcription factors or channels, receptors, you name it. They all end up being important components of what the response is all about. And so we started out right after the genes for the opioid peptides enkephalin and dynorphin had been identified, and so therefore you'd be able to make probes to find them in the spinal cord. And so we used two approaches, one, in situ hybridization in order to see where the cells would be and what the changes were.

MM: Now, when you say we, this was you and your colleagues. MAR: Just colleagues that I've had in the lab through the years. But it was an opportunity using in situ in order to look at individual neurons. Then RNA blots gave you the advantage of being able to quantify it in a very precise way as to how much of a response you would get. And one of the first studies we did was with the dynorphin gene, which is one of the more dramatic responses because it has practically undetectable constitutive expression, and then you give it a persistent pain stimulus and the gene just lights up. It has a very dynamic response to persistent pain. And so that was a fun one to work with from that perspective.

And we'd done that recently, rather than being so interested in dynorphin itself, but rather because of this dramatic induction that you can identify in persistent pain, I've almost used it as a way of seeing what manipulations can alter the response of various genes to painful stimuli. So it's almost become a marker in the laboratory for how the system responds to persistent pain. So it starts out being, this is an incredibly important opioid that is responding to painful stimuli, but then you can use it and its induction level as a measure.

We just completed a study looking at differences in male and female rats, female rats during different phases of the estrus cycle, and used dynorphin induction as a measure for changes. And you can identify a different response at the level of dynorphin mRNA expression to the same painful stimulus that correlates very nicely with the estrus cycle. So it's a tool now in that sense.

MM: So, again, with the persistent models, do you – How do I put this? We're talking now about a process which you could observe on many levels. You could observe it on the electrophysiological level in terms of continued, repeated firing of the nerves. You can observe it on the chemical level. You can observe it on the genetic level. And what we see is a plasticity. When you say plasticity, you essentially – you don't mean the nerves are necessarily growing and turning into new forms, although I suppose they could. What you mean is the system is changing. Can you describe – I know you described this in multiple papers. But can you describe sort of what happens in this model in terms of the NMDA receptors and so forth?

MAR: I won't touch the NMDA receptors. I'm sure you've heard about them ad nauseam.

MM: I'd love to hear about them.

MAR: NMDA receptors. I know. That was once thought to be the only receptor out there.

MM: Yes, that's right. Give me another one. MAR: Oh, gosh.

MM: I'm always interested in learning about new receptors.

MAR: Let me give you a different idea, and that is the concept of descending control, which is really what I started my pain research on as a graduate student. And we, a couple of years ago, did another study on descending control because it's coming back into vogue again as being an area that needs further attention. But what we essentially did was we went and did a very simple procedure where we cut the spinal cord. So what we did with that is we removed all the inputs from the brain so that what we had left was a nervous system circuit that was exclusively spinal cord and primary afferent. What you were able to identify is how the same level of primary afferent input would then alter how the neurons in the spinal cord responded. So you had a situation where this descending control, the net effect of it was this descending inhibitory control of the response of dynorphin neurons to painful stimuli. But we all know that descending control has excitatory inputs in addition to inhibitory inputs. But you just get rid of it, and the circuitry is such that it creates, especially, a net disinhibition of the response to pain.

So, you have a situation where now the neurons have an exaggerated level of activity. There's probably a lot more hyperexcitability potential, for a lot more excitotoxicity, since all the neurons are acting at more intensified levels, and so it gives a very nice indication of what the role of descending inputs might be. It also brings to one's attention the fact that you have spinal-cord-injury situations and oftentimes the issue of pain below the injury level is not paid attention to, but it's forgotten that these existing circuits in the spinal cord are still being activated. And they have the opportunity of involving the sympathetic nervous system and reflex activity with the motor neurons, etc., etc., so just because there's no conscious appreciation of pain does not mean that there's not a negative result of pain in these individuals

MM: The stress of it.

MAR: The stress of it, exactly. It's just knocking it out.

MM: That's really interesting.

MAR: Yeah. So [that was something I] recently finished, and it kind of comes almost full circle from descending control in graduate school and descending control at NIH.

MM: But descending control can be critically important, particularly in terms of behavioral responses.

MAR: Right, exactly. It's really where the conscious brain can exert control over what's happening in the first circuits that process pain messages.

MM: OK. So, let's talk a little bit, then, about gender and pain, switching gears a little bit. I know this is something that's you've been interested in for quite some time.

MAR: Right. Actually, there was the first task force on gender pain. I chaired that and we got off to a little bit of a start on those issues. It was a time when it wasn't talked about much or thought about in very much detail. But it's since become a very important area of research in general, which is gender differences or sex differences, depending on what one wants to use for descriptors, because gender always implies the cultural and social aspect of the behavior whereas sex does not do that. But then you get into sex and there's other baggage that comes along with using that term, so I think it's six of one, half a dozen of another at this point.

But the IASP has had an interest in this topic for a very long time, and actually worked to bring it to visibility at a number of lectures that were given, plenary lectures at the meetings that were on this topic. Right now there's a special interest group on gender and pain that is pursuing this area. We recently had a conference here at the NIH last spring on gender and pain that brought together people that are working in the area of sex differences and pain response, but in addition, people who were in areas of pain research where there was a very high proportion of women that experienced [the type of pain], and then we also had representatives from areas of neuroscience where they were really studying sex differences in nervous system organization and response. The three groups really came together very well in terms of exploring the topic.

MM: Yes. I think that was a really wonderful conference in terms of the diversity of the material, and yet how interestingly, how much it came to [that participants] interacted with each other.

MAR: Right. It really was very pleasing to see that it worked out that well and that there's been an awful lot of follow-up on it. We had more press coverage and interest in that topic than anything this Institute had ever seen. If you will, we were planning it and putting it together, I had a conversation – We put together a press briefing and all of that. And I thought, gee, if I can make the science section of the *New York Times*, I really made that. And my husband, who's not a scientist, says, "That's nothing. What about the *Wall Street Journal*?" And I can happily report that we did make the news summary for the *Wall Street Journal* on the *Gender and Pain Conference*; we were covered there, in addition to South America, Italy, several countries, so it didn't even stop in the United States. We had press coverage from many countries of the world, and so it was an important issue, brought a lot of visibility to the topic, and that, I think, is important to, number one, make people aware that there are these issues and they should be looked at clinically, and that maybe there are some very good research topics that should be highlighted. Actually, NIH, since that conference, has had an incredible increase in the number of grant [applications] on gender and pain; so, they're very pleased over that.

There's a follow-up conference on pharmacodynamics and sex differences that's going to be held in May, and I'm giving a talk at that on gender and pain issues. So, it's still ongoing and I think will ultimately lead to some very important observations on how there are these sex differences, because we have a situation where it's simplistic to think of us as the same. We're not. I mean, you could go back and forth. OK, there's the movie "My Fair Lady."

People know. People commonly say, "Why can't a woman be more like a man?" It's what we think. We're aware of it. But science, for some reason, has always treated males and females as incredibly similar entities.Well, yes, we are similar, but within that similarity, there are biological differences that are responsible for the fact that a man has male characteristics and a woman has female sex characteristics, and we're different, and this has to have an impact on the biology that we see. And it's not necessarily going to be a black-and-white difference. It's probably going to be more subtle differences because we're not totally different. We just have different elements of our biology that are contributing factors.

One of the ones that's easiest to think about is steroid hormones, because men and women have different circulating sex steroid hormones. And we know that even at the level of gene transcription, there are steroid-response elements on genes where these hormones can tag on and control actual gene transcription. So, it needs to be thought of; it needs to be kept in perspective. It's been anecdotally commented upon that certain chronic pain problems [affect] more women than men. Well, if that's the case, maybe it means something. Maybe that should be a component of the research. If somebody is investigating migraine headache, well, maybe it should have female rats as the experimental animal, considering migraines are typically a female kind of headache problem. And an important aspect of the migraine headaches might be missed if the research or the models that are being developed are used on male rats. So we need to clarify our thinking in that area.

We need to also clarify our thinking on how to design these experiments because you can't just pull a female rat out of the animal colony and do your study and say, "I have shown sex differences," because that's not acceptable, just to study a female. And to a lesser extent, that's true also for male animals. But for female animals, you have different levels of maturity, you have before puberty, you have childbearing years, you have menopause, you have beyond menopause.

MM: Do rats have menopause?

MAR: They probably do. They become less fertile. You get to a stage where their cycling changes, their estrus cycle, and so rat menopause – I don't know if people call it that, but old female rats probably don't cycle. So, you have a situation, even with hormone replacement therapy in a population of women, that they are all very different hormonal milieus that one has, and this can affect how various drug manipulations can solve a problem or exacerbate a problem. One area of research that is in the forefront in all this is really in cardiovascular issues, heart attacks and all, and they're actually finding that ion channels in the heart muscle have different response characteristics in males and females. And so if you're at that basic of a level of a difference, it can impact how the organism really responds to whatever medical treatment there's going to be. And so it's time now to see how much of the nervous system is impacted by these sex differences, and for that matter, even peripheral tissue events, arthritis, the development of that in men versus women.

MM: Arthritis is almost universal, and yet it affects the sexes differently.

MAR: Mm-hmm, yeah. And so, the bottom line is, whatever is identified will help both sexes. If one thing works better in a man or one thing works better in a woman, well, then, you give the treatment based on the sex of the individual. So, it has some very important implications that are not only for women, but also contribute to the health and well-being of men that develop these various problems. So it's an area of very important scientific research right now, and I hope that more and more people get interested in these questions in order to address them in more detail.

MM: And what interested me and what interests me in what you're saying – and this does sort of fit in with the rest of your work – is, I mean, a lot of this material I have been seeing in different works on pain anthropology and sort of cultural stuff on pain, going back many, many years, and ideas that women respond differently to pain, because women are – not in the negative sense – women are more emotional or women have more stamina or blah-blah-blah-blah. In any case, putting a lot of emphasis on women's socialization to act as women.

MAR: Sociocultural overlay that's on top of all the biology that we have. Yes.

MM: And I think most of the population thinks about this as almost as a kind of a truism. But what interested me in reading some of the abstracts from the Congress and sort of thinking about what you've been saying is, I mean, if we're really talking about processing, altered processing due to different chemistry and different endocrinological systems, which, you know, presumably are affected by descending inputs from the brain, which is presumably where culture is hidden in the body. But there's, you know, if that's not explored as an entire system, then we're really only looking at half of it. We're really only looking at a small part of the equation. We're really only looking at tip-of-the-iceberg stuff.

MAR: Right.

MM: And we never really learn why it is that more women have migraines or fibromyalgia or whatever it is.

MAR: Exactly. It's got to be one of the parameters as part of the research design so that you can get an accurate analysis of what the problem is all about. I was going to say that two of the major people in the IASP have always had an incredible interest in women and pain. One was John Bonica and his interest in the pain of childbirth, and he was really a very strong proponent of using some kind of pain medication during childbirth. A lot of very good science has shown that it does help the quality of the baby that's born, just because the pain of childbirth is an alerting kind of stimulus to acknowledge that it's going to happen. But if you think about it from the perspective of your bodily organs, the changes that happen then are very difficult. If you start moving male pelvises around to the extent that a female pelvis is going to move around, it's a lot of pain in there. It's not as if they were built differently. They still have all those nociceptors innervating that tissue. And so it's an important area that Dr. Bonica [identified].And then John Liebeskind. Some of the earliest papers looking at the basic science of gender difference in pain is really an area that John Liebeskind targeted. And so two of the really major players of the IASP and pain research were very much interested in the topic of gender and pain.

MM: I do see this as an area with key differences in people. I was particularly interested in Christine Makowski's paper on the kappa opioids.

MAR: Right, mm-hmm.

MM: Briefly, I guess what we're saying here is that with the kappa opioids, a particular class of analgesics, women seem to get better, more effective, and longer analgesia with them.

MAR: Right.

MM: What exactly are kappa opioids?

MAR: OK. There are different classes of opioids [that bind to different receptors in the brain.]

MM: Do we know why? Do we have any ideas about why these would be more effective?

MAR: It's not really known right now why it's more effective. But it is a situation where, with this class of drugs, if the research had only been done on males, it would have been tossed away as not a very good analgesic.

MM: Yeah. And, actually, I know some studies on, I think it's pentazocine from the early '60s, in which it essentially was. But go ahead.

MAR: Right. And so, you have a situation where you add this parameter of sex differences in your experimental group and analyze the data based on that and just go, well, yeah, the analgesic does seem to help women, and then, no, it doesn't seem to be that good of an analgesic in men. But it gives you another opportunity, another tool, to treat pain in women. So [kappa opioids] are one of those nice findings that hopefully will lead to further areas of research, because, remember, in order to test out all these new drugs, if they're only tested on males, you potentially are throwing away a lot of very good drugs.

MM: That's really interesting. OK. We're going to stop our interview now, it's 12:20 on April 6th, and resume it at a later time.

MM: OK. We're resuming recording, and it's 11:10 on the 18th of May. Hello again, Dr. Ruda.

MAR: Time flies!

MM: It certainly does.I wanted to ask you a couple of questions I guess mostly to help me sort of straighten things out in my mind. Can we talk a little bit about dynorphin? I mean, characterizing some of these peptides, and some of these neurotransmitters, dynorphin is widely distributed throughout the whole body. Right? Or throughout the entire nervous system.

MAR: Yes. It's found in many different areas. Interestingly, one place it is not found is in the dorsal ganglion, so there are no dynorphin neurons there. And it's not even induced, because we actually did all the negative experiments within the constriction injury model, the nerve transection. Examples where you typically would get novel gene induction, dynorphin still doesn't appear. There are a few, very, very few enkephalin -containing dorsal ganglion neurons, but no dynorphin.

MM: So, what exactly does that mean? Is that just sort of out of the loop of the excitability mechanism?

MAR: It essentially implies that the role for dynorphin at the spinal cord level is really going to be through intrinsic spinal cord neurons that are part of the circuitry, that it's not going to be primary afferent input that is the dynorphin component, but, rather, just the intrinsic spinal cord neurons.

MM: OK. And so this is why it's significant, I guess, that dynorphin synapses onto the thalamic projection neurons. Am I expressing that right?

MAR: Yes.

MM: That it's expressed at the synapse of the neurons which project from –

MAR: The spinal cord to the higher centers of the brain, yeah. They could also be neurons that project to the brainstem, because, remember, the ascending pathways, the projection neuron pathways, just don't go to the thalamus. There's a lot of research to say they make a couple of stops along the way with collaterals that end, you know, like the parabrachial area of the brainstem. And so it's a multi-tiered pathway where information is really processed.The other thing with the intrinsic dynorphin neurons in the spinal cord is that they only would not synapse on the projection neurons, but, rather, they also likely synapse on other inner neurons in the spinal cord. And so the circuitry that they're involved in is much more complex than just shutting off information that's on its way out.

MM: Right, right. It is somewhat complex, and it's subject to local inhibition. Am I right about this?

MAR: Yes, mm-hmm.

MM: And that what happens, then, in cases of chronic pain and persistent pain is the condition that's called excitotoxicity develops, is that there's a dysfunction of the inhibition. Am I right about all this? Am I expressing it correctly?

MAR: No. These are all –

MM: It's like a local circuit within the brainstem.

MAR: Within the spinal cord.

MM: Within the spinal cord, OK.

MAR: Right. The brainstem provides the descending modulation of the spinal circuits. So think of it as three parts. You've got primary afferents carrying messages in from the periphery.

MM: I've got that.

MAR: OK. Then you have the brain coming down to the spinal cord and synapsing on projection neurons and neurons, primary afferent terminals, pre-synaptic inhibition. And then you have

the intrinsic spinal cord neurons, which are projection neurons, and, in addition, local inner neurons that could be either excitatory or inhibitory inner neurons.

MM: Now, I think Mike said to me that originally there was some suggestion that dynorphin – It was clear that in the presence of nociception, and you said this to me too, that dynorphin was expressed a lot, that it was readily visible, whereas under non-pain conditions, it was harder to find it. You didn't see the dynorphin.

MAR: Right. It was a very low level of constitutive expression. And what that essentiallyis saying is that that particular peptide, the ultimate peptide that's going to be produced, it is being regulated at the level of mRNA in response to pain so that those very low levels of dynorphin mRNA that are expressed under normal conditions, and then when the pain stimulus comes in, there is this induction of the mRNA which theoretically then results in more dynorphin peptide and dynorphin beginning to play a more major role in the pain response.

Now, the other opioid in the spinal cord, which is enkephalin, is not regulated that way very much. There's a very high level of encephalin mRNA. There is some increase in response to pain, but it is not this massive induction that you get with the dynorphin. And so it gets down to the basic mechanisms of how the system is controlled. So, in addition to controlling it at a level of mRNA, you could control it at the level of transcription. So there's different places.

MM: And was there originally some sort of thought that maybe dynorphin acted as a modulator, as an analgesic, had an inhibitory effect on pain, and then later it became clear it was algesic?

MAR: I'm not sure anything is clear at this point. When it was first researched, since it was an opioid, the assumption was that it was going to inhibit pain.

MM: I'm sorry. When you say an opioid, is that because of its chemical composition?

MAR: Right, mm-hmm, yes, exactly, that the peptide is an opioid peptide in terms of what it’s made of.

MM: Opioid peptide. We thought it might be analgesic and we discovered it was algesic. OK. And you just said that you thought it, because it was an opioid peptide, it was thought it might have an inhibitory effect. OK. Go from there.

MAR: Right. So then, as its actual actions was being investigated further, it was more difficult to really say that it was acting as a suppression of pain pathways. And this was work that was done, a lot of pharmacological studies, studies using electrophysiology and all that, so---

MM: So it took a while to sort of figure this out. Or is it still not clear?

MAR: Well, it's still not a hundred percent clear what dynorphin is doing, because we tend to look at it in a very simplistic way, that it is dynorphin. Well, dynorphin is found in a large subpopulation of neurons, and so are we thinking of it in too simplistic a way to say they're all the same? I think that's not the case, that there is going to be different roles for different individual neurons [that express dynorphin]. You've got the very simple fact that they're found in different lamina, so there's a lot of them in the superficial laminae which can

directly be involved in nociceptive-specific kinds of processes; but it's also found in the neck of the dorsal horn, where the neurons that respond to pain are mainly wide-dynamic-range neurons, and so there's a lot of potential differences that just haven't been thoroughly investigated yet.

MM: And there's a statement in one of the papers that dynorphin works perhaps by increasing receptive fields, but that could have a lot of other applicability's besides pain, obviously.

MAR: Right, exactly.

MM: OK. You wrote a paper for *Science* in 1982 on the demonstration of encephalin synapses on projection neurons. So, again, we're talking about the same kind of thing. But you indicated that enkephalin was actually different. I mean, it synapses on the projection neurons in the thalamus, but it's not something which responds specifically to pain?

MAR: Well, if you look at the first clue that enkephalin is not only involved in pain pathways, is the fact that there are enkephalin-containing neurons in all laminae of the spinal cord except for the motor-neuron cell groups.

MM: OK.

MAR: And so it's outside of areas that are exclusively involved in pain processing, so encephalin is going to have multiple roles. Now, the enkephalin that occurs in the superficial laminae, which are intimately involved in pain processing, there it probably will have a major role. And since we demonstrated this direct synaptic input between enkephalin and projection neurons, it was an anatomical site where the enkephalin could interact with what messages were being sent out of the spinal cord.

MM: And the methods that we're using to characterize this, we talked about immunocytochemistry, and we talked about HRP [horseradish peroxidase] transport, and I've looked at some of the pictures. Okay. But essentially, a lot of this characterization has to be done by multiple methods. In the enkephalin paper, you labeled the ENK with – you're using immunocytochemical methods, creating an antibody to that. And at the same time, you had to trace the neurons back using HRP transport. Am I right about that?

MAR: Yes, mm-hmm.

MM: So it's really a case where one method alone is not sufficient to create a visual picture of all the actions that are going on?

MAR: That's correct. You really need multiple labels in order to identify interactions. And it's even a little more complicated than that. For example, if you take the peptide substance P, we commonly say that's the pain neurotransmitter from primary afferents. Well, the fact is that in spinal circuits, substance P originates from primary afferents, intrinsic spinal-cord neurons, some of which are projection neurons, and from axons that descend from the brainstem. So if you are looking at substance P in the spinal cord, other than the fact that it's substance P immunocytochemically stained, you don't know anything else about it unless you've gotten another marker or another way of investigating it.

MM: We do think that substance P, however, is analgesic. I mean, people refer to this as the pain peptide. And why exactly do we think that?

MAR: Well, substance P has been localized to small dorsal root ganglion neurons, and those are the ones that are classically the nociceptors that are found there. In addition, if you apply substance P to the spinal cord, the behavior that's elicited is that of a painful response.

MM: Okay. Another method is in situ hybridization. I need to understand this better. You create a probe of nucleic acid.

MAR: Right. With immunocytochemistry, what you're looking at is the end molecule, all right, for example, the peptide, the amino acid, whatever it is, that is the ultimate molecule that's going to stick to a receptor. All right? With in situ hybridization, what you're looking at is the gene that will encode the molecule that you're interested in. And so it's the same in that immunocytochemistry for substance P will identify the peptide-containing neurons, and in situ hybridization for substance P, which will be for the gene called preprotachykinin - protachykinin, will identify substance P-containing neurons. But you're identifying two different parts of the pathway to the end of what's called substance P.

MM: So why would you use one rather than the other? I mean, we know that the particular peptide that's present. We could reason backwards and assume there must be a gene there which is expressing it. Right?

MAR: Right.

MM: On the other hand, if the gene isn't being visibly expressed, as in the case of dynorphin, as we mentioned, having a low constitutive expression, then we can identify that it's still there sort of lying low.

MAR: Yes. There are conceptual and technical reasons for picking your technique, and the conceptual reasons would be, do you want to look at the end peptide product and its presence, or do you want to look at gene induction change in gene expression? So if that's what you want, you pick the in situ. The other aspect that you have to keep in mind is that with immunocytochemistry, you see axons and cell bodies, and sometimes [with] cell bodies, you need to pre-treat with something like colchicine in order to increase the level of peptide to get it to the point where the concentration is high enough for the technique to visualize it; whereas with in situ hybridization, that only identifies where the gene is, and the gene is in the cell body, so that's what you're going to be looking at, is the neuronal cell body, not the axons that use the molecule.

MM: Essentially what you do here is to create – What do I want to say? Are you trying to get the DNA to replicate? Is that what you're trying to do?

MAR: No. We're just trying to bind to it so that we can get a measure of how much of it there is. You could do it either with in situ hybridization or an RNA blot. They're identical conceptual methods. One is in a tissue section, the other one is after you've ground up the tissue section. So they have pluses and minuses. With the in situ, you could get laminar

specificity in terms of where the cells are. You could also get some indication of the amount of RNA that is present, but it's harder to quantify in situ than it is an RNA blot. But once you go to an RNA blot, you have a ground-up piece of the nervous system, and so you've lost the specificity in terms of individual neurons and individual locations where these neurons can be activated.

MM: OK. Here's a question for you. When I was talking to Ron Dubner, he seemed to say that you had introduced hybridization here in this lab. Would you say that was true?

MAR: The technique was really just beginning here at NIH, and Scott Young here at NIH was one of the first people to really develop the methodology. So the first study that we did was the collaboration with Scott in the very early, early days of in situ hybridization.

MM: OK. So, then, can we talk a little bit about your work with neonatal development, why you decided to look at this problem and what we think we've learned so far.

MAR: We began some developmental studies of pain probably about three years ago, and it grew out of our studies of changes in the nervous system's response to pain as one ages. We were actually doing aged-animal experiments and we found some very interesting things as to how the response to pain was actually increased in the aged animals as compared to young adult animals. And out of that grew an interest of, well, since it was an aging issue in very young animals, would it be similar or different? And when we began those experiments, we noticed that the behavioral response to the pain was very different in newborn rats than it was in an adult rat. It was an almost immediate shaking, vocalization, licking of the hind paw that we had just injected, and that doesn't happen in an adult rat. It usually takes a while before you get any response that's different from just injecting saline into the hind paw. And so we began to think about development of pain pathways, and there's been a lot of work in this area done by Maria Fitzgerald in University College of London and some really groundbreaking ideas in terms of pain in neonates. And one of the issues that came up was, from reading her papers a bit and also from things that I recalled way back from graduate school, was, what is the stimulus that's responsible for determining how the nervous system gets wired in pain pathways, because if you have all the sensory systems – vision, audition, olfactory, you name it – they all require –

MM: Learning.

MAR: I don't know if it's learning, but, rather, stimulation at a postnatal period in order for the pathway to properly develop. Well, pain, in our way of thinking, is not designed so that every five minutes after you've been born, you've got pain stimulation, a natural pain stimuli that's going in there and wiring you up. So, therefore, it had to be some other clue as to how the pathway came together. And, obviously, growth factors like nerve growth factors and all would play a major role in that, and if you look out in the periphery, growth factors are released in response to inflammatory stimuli, and so there was an opportunity there for some changes to really occur. So we could easily come up with an experiment to look at what were the critical determinants of how the pain pathways developed, other than spontaneous activity and the potential role of growth factors coming in there. So we decided to take the

other approach and ask the question, if during early neonatal times, we have activity caused by natural painful stimulation of the periphery, would this alter the development of pain circuits? And so what we did was we used neonatal animals [48 hours after birth], which roughly corresponds to about a 24-week human fetus through early postnatal times, and gave it a persistent painful stimuli, which was the injection of an irritant into the hind paw, and it became edematous and hyperalgesic and all of that. And then we waited until the animals grew up to be between at least eight weeks of age, which is a young adult rat, and we behaviorally tested these animals to find that they were hypoalgesic on that hind paw. But if they were then given a painful stimulus, an inflammatory stimulus, to that same hind paw, that they were hyperalgesic compared to the response that you would get to the untreated paw and the same inflammatory stimulus, so that their pain behavior was exaggerated. We've gone on to further characterize the circuitry that has developed in these animals and have found that there is, at the level of the spinal cord, a greater density of primary afferent, nociceptive-type primary afferent termination in the spinal cord segments, in laminae, that normally receive this innervation. In addition, there's also the presence of afferents at segments that are more caudal to what one would find under the normal situation. And so we've identified at the spinal level that there's alterations. This alteration persists up through the dorsal column nuclei, where there is also an increased level of terminal density. And we hypothesize that the changes that have occurred likely have gone to other higher centers of the brain that are involved in pain processing because of the presence of this natural noxious stimulation that occurred at a critical period of development.

Now, this could be some of the explanation for what is found in human situations. For example, the extremely low-birth-weight infants where they have found that they have exaggerated responses to painful stimuli as children, and so the experience that they have had early neonatally could also have altered how their nervous system that responds to pain has developed.

The importance and significance of this research is [that] we're drawing attention to the fact that the aggressive medical interventions that are currently available to treat the medical problems of neonates have to have a component of careful pain management both during the procedure and in the recovery time after it. It was somewhat surprising to me to learn that, until l987, it was unusual to have anesthesia for newborns because of the belief that the nervous system was immature and they didn't experience the pain. Well, we've gone on to realize that that's not true and that just simple observation of the behaviors of infants, newborns, to simple, common procedures such as heel stick or circumcision, that there are rather sophisticated responses that would suggest painful experiences.

MM: OK. We're continuing our interview with Dr. Mary Ann Ruda, and this is now tape two. You're familiar with Ron Melzack's work with restricted dogs all those years ago?

MAR: Actually, no.

MM: He raised some puppies in isolation from their littermates essentially, basically in isolation from contact. And I guess when they were still not nearly fully grown, about a year old, he

would then introduce them and they would meet other puppies and humans. And he found they had very little response to pain at all. You could hold up a lighted match and they would stick their nose right into the match and not draw back from it. And these observations were made in the late '40s, early '50s, and were, are usually cited as an early observation that pain must be a learned phenomenon. So I was wondering if you had done any work with, I guess, with animals who didn't seem to develop or have any ideas about animals not developing sensitivity to pain, in other words, being essentially hypoalgesic. Go ahead.

MAR: Well, we've not really done anything directly along those lines. If you remember, I said that if you give these rats that have experienced neonatal pain a pain stimulus, they are hypoalgesic. They become more hyperalgesic when you have an ongoing, persistent pain, and then you test them.

MM: So if you just like expose them to like a probe or something –

MAR: Heat probe, paw withdrawal, they're hypoalgesic.

MM: Right. They don't. OK. But then, in persistent pain, they show heightened response.

MAR: Right, [they are] more hyperalgesic because they show a greater response when you have the ongoing, persistent pain. But if it's just an acute pain stimulus, then they are hypoalgesic.

MM: What are you doing right now?

MAR: Well, we're in the process of further characterizing the changes that really have been created in the nervous system. We have here in the labs [been] doing some electrophysiology on spinal cord cells in these animals to see what differences [there are] in the response characteristics. We've also looked at Fos activity in the spinal cord to determine if there's more or less induction in neuronal activity that's really going on there. And we are also beginning some experiments to see what type of genes we might have further induced in these animals that experience the persistent pain during the neonatal time period, try and identify which ones could be major players in this response.

MM: You're describing essentially an inflammation model that we're using there, the injection of adjuvants.

MAR: Yeah, complete Freund’s adjuvant (CFA) is a very common one that’s used.

MM: And then you can compare cellular response.

MAR: Right.

MM: This is a useful model for you. Do you have any thoughts about different kinds of rat models? Gary Bennett developed the chronic constriction injury model, and it's obvious that has been very fertile in terms of developing some new pharmacological ideas. So do you have any comments about rat models which are easiest to work with and seem to be the most productive?

MAR: They all have their pluses and minuses in terms of what you're going to get out of them. And I think that, depending on the question you're asking, one or the other is the more appropriate one to pick. The constriction injury is really a neuropathic pain model, and CFA in a hind paw is more of an inflammatory kind of pain state. So you are getting a little bit different questions here.

You have issues such as the inflammatory state. One has to remember the immune system is playing a role there, and we know that there's a lot of cytokines and growth factors, etc., etc., that are likely being released into the tissue where the primary afferents could pick them up and transport them back to the cell body, and this could have an effect; whereas in a constriction injury model, you don't have this peripheral inflammatory component to the pain model. And so it's important to understand the specifics of these different models and what you're really looking at because they'll help, I think, in terms of the data that's being presented and the analysis, the validity of what you're trying to look at.

MM: You know what? Mike [Iadarola] showed me the other day some drawings which had been done by Jan Hylden, beautiful, huge, huge drawings that are impossible to reproduce.

MAR: You mean the neurons?

MM: Neurons, yes

MAR: That work actually was begun [by] Haruhide Hayashi. It was at a time where we were able to combine physiology and anatomy, and it was the intracellular HRP technique that was just extremely powerful and made a lot of very significant and important observations on the organization of pain pathways at the level of the spinal cord. Today, nobody would ever do it because it was so labor intensive. But it was at the time a very exciting way of looking at the nervous system. But you're right, the drawings are huge ones to get at the scale. You think of these tiny little neurons. But once you get to the point where you could actually draw them, it’s huge.

MM: They look like Chinese paintings. I was sure you might want to talk about some of the people you’ve worked with in your lab.

MAR: Ke Ren was great to have in the lab. He was just an excellent researcher and always did a very nice job and was a good supervisor and mentor of students too. He was very nice to have around, just a good person.

MM: Are there particular things that you would look for when selecting a fellow?

MAR: Enthusiasm, excitement, the ability to ask insightful questions so that you know that they're thinking. Technical skills, if they have them, great, but most times when people start, you're going to have to train them. For young people, you have to train them, so that's not a deciding issue to me. But it's really the enthusiasm and interest in the area, because if you have that, you're going to do a good job. If you're not interested in a certain research project, it's just, it becomes work, and those kinds of situations aren't that productive.

MM: I'm just wondering about the way this unit has developed. I mean, you've been here. You started working in neuroanatomy about 20-some years ago, and Mike [Iadarola] actually

came about 12 or 13 years ago, I think, so you've been working at NIMH earlier. And both of you have been working in molecular biology, and I sort of wonder – how do your projects complement each other or are they distinctly different or where do you collaborate and so forth?

MAR: Well, we've collaborated from time to time depending on what the project was and the skills that people bring to them. Mike's approach is more of a reductionist molecular approach than what we really do. We do do some molecular biology, but I'd say my research program is more systems neuroscience, and some of the techniques and tools we use have the molecular bent to them. So that's really where the major difference between our two approaches and experiments lie.

MM: What do you think? What's the most important paper you ever wrote, for you?

MAR: Probably the one I'm writing now.

MM: Previous to this.

MAR: Maybe it's this one. I don't know.

MM: What do you think is the most interesting or important work that you've done?

MAR: I don't think it's one paper. I think it's the compendium of pieces of the puzzle that we put together over the years, that it's like the body of the work that's more important than any one paper. The paper I'm working on right now, the developmental paper, probably will have the most lay interest because it really draws attention to an important area. And I guess the Gender and Pain Conference we ran last year I think also made a major impact on pain research, and, for that matter, even on gender-based biology, because it was noticed, and there's been a lot of follow-up, and so that is making a difference in the world.

MM: Do you want to talk about gender in your own work or in your own career? MAR: I think we covered that last time, didn't we?

MM: No. We talked about gender and pain a lot.

MAR: Oh. So you mean –

MM: Yes.

MAR: Sex differences in science?

MM: Yes. Well, let's see now. I mean, do you think that being a woman has changed the way your career was started, or has it – you have a fairly successful career. Have you felt that – I'm saying, would your career have been different if you'd been a man?

MAR: Parts of it probably. I think that, as in any profession, sex matters. You're blinding yourself if you don't appreciate that. It does make a difference. I don't go to the men's room. And by the same token, they're not coming to the ladies' room. And so there are things that are different in terms of our approaches and our accessibility to power and advancement. There is a difference there. And I think you just need to acknowledge it.

Men and women are different. My husband and I will tell you [when] we get into an argument. There's this book written by the woman at Georgetown. What's her name? *He Said, She Said?*

MM: Oh, yeah. I don't know. I don't remember her name.

MAR: But, anyway, we've read this book, and it's true. You could just really see the differences that are present. And when my husband and I argue, he'll tell me, you know, "Talk in boy," because I'm talking in girl. And I don't think I'm unique in that respect. We are somewhat different, and our approaches are different. And sometimes it's an asset and sometimes it's an impediment. And that's really true.

I try, when I have young people in the laboratory, we've gone from situations where it's been all girls to situations where it's been one guy and the rest girls, and now where I guess we're 50/50 right now. And we'll joke about it. For a while I had one male scientist, and they would call him my token. And we bring it up into the forefront and I make people appreciate, if I see a sex difference in their approach, that that's what's going on. I will comment to the women that work for me that a man wouldn't have done that, or "You shouldn't have let that happen. They shouldn't have gotten away with it. You should have stood up for what you deserve." It does matter, and you shouldn't just walk away and say, "Well, it really doesn't matter." That's girl, that's not boy. So it's there, and I think this whole concept of homogeneity is not true. Or, for that matter, this issue – we had a very interesting talk in this institute on diversity, and the analogy that was given was jars of jellybeans. And there was a jar that fit red jellybeans, a jar that fit green jellybeans, and a jar that fit blue jellybeans, and can we make a jar that fit red, green, and blue jellybeans? Well, I'm not sure. I think the red jellybeans like their jellybean jar. It fits them. They're comfortable, they're fine there. You have to have that jar that's going to have red, green, and blue jellybeans in it because that has to exist. But, by the same token, there also has to be the opportunity to go back to your red jar when you want to. You don't have to always be in this mixed environment.

MM: There's kind of a – not a truism, but [a frequent assertion] in some feminist studies of science that women scientists tend to be able to tolerate. Now, this is not from personal observation. This is what they tell me, that they will do the fine detailed work, the patient hours of observation that sometimes are required, very, very labor-intensive work, which many male scientists are simply not – they just won't do it, and they will try to make up for it by making swift intuitive leaps. Does that sound like a fair assessment?

MAR: Oh, for some people, yes. I think it just depends on the person, what their approach to science is all about. I'm not sure that it's only gender difference, because I think that I fight with my scientists all the time to get the big picture. You don't need to do one dinky little experiment, you know. What's the big goal here? What are you trying to get? And so, you know, the details are important, but you also have to have thinking that's broader in order to really do good science. You just don't need to do an experiment. It's not going to get you anywhere. But anyway, to throw it back to you, did you ask all the men that you

interviewed, the male scientists the same question? Or is it just because I happen to be a woman that I get the sex question?

MM: Well, I didn't ask the guys that. That's a perfectly, perfectly fair question. But you also do work on gender and pain.

MAR: Yeah. Well, I'll tell you the first – When I was in graduate school, there weren't a lot of women that really were in graduate school. And I was at the University of Pennsylvania and I was in the Institute of Neurological Sciences, which had a training grant from the NIH for a scientist, and it was a new program, etc., etc. And so they had set up a meeting with some of the graduate students in the program and these outside visitors that came into evaluate whether or not the money was being spent well. When I was in graduate school, I never felt sex differences or biases or anything like that. It just was Penn and the Institute and the people there, I was just one of the graduate students. And we went into this meeting, and I'll never forget it because this was one of the first times I've ever confronted it professionally, the fact that I was a woman, because we all sat around this big table and the fancy scientists that NIH had sent over there came in. And the chair of the thing looked around and says, "Well, I guess we'll get started here, and why don't we do ladies first," and I was it. And it was like, huh? What? Why me? Don't you have a better reason for beginning this conversation than the fact that you happen to see one woman sitting at the table? So –

MM: No, because I think it still is often true. It's almost something to take note of.

MAR: Unfortunately, the tables I sit at even still, today, [they start with] "ladies first" because there's only one at the table.

MM: Yes, I know that feeling. But have you noticed, though, some change over the last 20 years that you've been here? It seems to me there have always been quite a number of women scientists at NIH.

MAR: Yes, there have.

MM: More than in some areas of academe.

MAR: I think they try. But in my personal view – and that's all it is – the thing that gets in the way is the fact that men and women in general are different, and when it comes to evaluating performance, etc., etc., women and men are generally different, and so the same criteria, if they're applied, would, could be unfair.

MM: OK. Have you ever thought, in the last 20 years, of leaving and doing academia?

MAR: Yes. Actually, it would be kind of a fun thing to get more into a teaching environment and more student oriented, because here at NIH the research is very much your whole work, and a slightly different environment would be fun. Who knows, you know. Could leave tomorrow.

MM: Not until you get the paper finished.

MAR: Not until I get this paper finished. But after I get this paper finished, you know, there's no guarantees here.

MM: But can you imagine yourself being other than a scientist?

MAR: Yeah. I love art, I love music, I love a lot of the traditional female stereotype stuff, so, yeah, there are other things that would be fun for me to do. It's not as if science is the only thing that there is. And I'd pity the person that was so one-sided in terms of their approach to life.

MM: OK. Is there some, any aspect of your work – We talked about gender and pain quite a lot last week, or many weeks ago, actually. Is there any other aspect of your work that you'd like to talk about?

MAR: I think we pretty much covered it. You're very thorough.

MM: OK. I think we're going to conclude the interview, therefore, and it's now five after 12, and it's still May 18th. Thank you, Dr. Ruda.

MAR: Thanks.

End of Interview

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[1] The Institute of Neurological Sciences at the University of Pennsylvania was originally founded in 1953. It was renamed the Mahoney Institute for Neurosciences in 1985.

[1] Peter Hand as of 2015 is Emeritus Professor of Anatomy at the University of Pennsylvania and continues to help out during anatomy labs. Alan C. Rosenquist is Emeritus Professor of Neuroscience at Penn.

[1] The periaqueductal gray, or central, gray, in the midbrain is the primary cortical control center for pain modulation. Mayer, Liebeskind and colleagues reported on stimulation-produced endogenous analgesia in that area in 1971; see Mayer DJ, Wolfle TL, Akil H, Carder B and Liebeskind JC. Analgesia from electrical stimulation in the brainstem of the rat. Science 1971 Dec 24; 174: 1351-1354.

[1] PC-12 cells are a stem cell line derived from a medullary tumor in rats which can differentiate into neuron or neuron-like cells.

[1] Dr. James Mather Sprague (1928-2014), one of the pioneers of neuroanatomy and neurophysiology, was a member of the University of Pennsylvania Medical School faculty from 1950 until his retirement in 1983. He was one of the founders of the Institute of Neurological Sciences and served as Director from 1973 to 1980.

[1] Louis B. Flexner (1902-1996) joined the University of Pennsylvania School of Medicine as Chair of Anatomy in 1951, was the founding Director of the Institute of Neurological Sciences, and an important early researcher on the biochemistry of memory and learning.

[1] Eliot Stellar (1919-1993) was a physiological psychologist and pioneer in behavioral neuroscience. He joined the University of Pennsylvania faculty in 1960, served as Director of the Institute of Neurological Sciences 1965-73, and as provost 1973-78. He became Chair of the Department of Anatomy in 1990.

[1] The International Association of Pain, or IASP, was founded in 1973, following a seminal meeting of pain researchers in Issaquah, Washington, and the journal *Pain* began publication in 1975. The First IASP World Congress was held in Florence, Italy, that same year.

[1] The Society for Neuroscience was founded in 1969, by a subcommittee formed by the National Academy of Sciences; its first meeting, with 1300 attendees, was held in Washington, DC, in 1971.

Today SfN has nearly 40,000 members and some 30,000 attend its annual meetings. For more on the history of SfN, see: http://www.sfn.org/about/history-of-sfn .

[1] Founded in 1977.

[1] Ronald Dubner (1934 -) was Branch Chief of Neurobiology and Anesthesiology, later Pain and Neurosensory Mechanisms, from 1974 to 1996. He pioneered studies of pain in behaving animals and has been a leader in pain research throughout his career. See: http://history.nih.gov/exhibits/pain/docs/page_05.html. Since 1996, Dubner has been Professor of Pain and Neural Sciences at the University of Maryland School of Dentistry.

[1] Stephen Gobel, DDS, did extensive neuroanatomy work at NIDCR. He later became a Scientific Review Administrator at NIH.

[1] In 1960, Nils-Åke Hillarp (1915-1965) and Bengt Falck (1927 -), at the University of Lund in Sweden, developed a method of transforming the monoamines, specifically serotonin, dopamine, and norepinephrine, into substances that would fluoresce, enabling their detection with electron microscopy.

[1] Santiago Ramon y Cajal (1852-1934) was a Spanish pathologist and histologist whose microscopic studies of the brain and nervous system established that each neuron was a separate cell and laid the foundation of modern neuroscience. Cajal received the Nobel Prize for his work in 1906.

[1] The epitope is the part of the antigenic molecule recognized by the immune system.

[1] Neurons that respond to and transmit pain signals.

[1] That is mice with specific genetic alterations.

[1] For Dubner's awake behaving monkey studies, see http://history.nih.gov/exhibits/pain/docs/page_05.html .

[1] Intrathecal would be within the brain or spinal cord membrane.

[1] Gary J. Bennett, PhD, was a researcher in the Neurobiology and Anesthesiology Branch at NIDCR 1978-96; as of 2015, he was Canada Senior Research Chair in the Department of Anesthesia and Faculty of Dentistry at McGill University. He is perhaps best known for his paper with psychologist Richard Gracely on neurotoxicity, which is the subject in this paragraph. See: Gracely RH, Lynch SA and Bennett GJ. Painful neuropathy: altered central processing maintained dynamically by peripheral input. *Pain* 1992 Nov; 51: 175-194.

[1] A neuroma is a growth or swelling of nerve tissue.

[1] The N-methyl-D-aspartate, or NMDA, receptor is a highly specific glutamate receptor that plays a significant role in controlling memory and synaptic plasticity.

[1] "Gender and Pain: A Focus on How Pain Impacts Women Differently than Men," Bethesda, Maryland, April 7-8, 1998.

[1] The movie *My Fair Lady* (1964) is based on the Lerner and Loewe Broadway musical of 1956, which in turn is based on the play *Pygmalion* (1913) by George Bernard Shaw. One of the many hit songs in the movie, "Why Can't a Woman Be More Like a Man?" is sung by Rex Harrison as Professor Henry Higgins.

[1] John J. Bonica (1917-1994), widely recognized as the founder of the pain field, was Chair of Anesthesiology at the University of Washington for much of his career. He edited the first edition of *The Management of Pain* in 1953, founded a multidisciplinary pain clinic at UW and convened an International Pain Symposium in Issaquah, Washington, in 1973, which catalyzed the formation of the International Association for the Study of Pain.

[1] John C. Liebeskind (1935-1997), professor of physiological psychology at UCLA, was a founding member of the IASP and the APS. He is perhaps best known for his work on stress and stimulation-produced analgesia (see note 3), and also for his demonstration that persistent pain *is* harmful, in the stress it places on the immune system and other endogenous systems (see Liebeskind JC. Pain can kill. *Pain* 1991 Jan; 44(1): 3-4). He was also the founder of the Liebeskind History of Pain Collection at UCLA.

[1] Christine Miaskowski, RN, PHD, FAAN, was Professor of Physiological Nursing at the University of

California San Francisco as of 2015 and a highly respected pain researcher. For her work on the kappa opioids, see: Gear RW, Gordon NC, Heller PH, Paul S, Miaskowski C and Levine JD. Gender difference in analgesic response to the kappa-opioid pentazocine. *Neuroscience Letters* 1996 Mar 1; 205 (3): 207-209.

[1] Pentazocine is a synthetic opioid marketed by Sterling-Winthrop as Talwin for the treatment of moderate to severe pain. It was initially approved by the FDA in 1967 and is classified as a Schedule 4 drug.

[1] In situ hybridization is the use of a labeled strand of DNA or RNA to locate its complementary sequence within tissues or cells.

[1] W. Scott Young, MD, PhD, as of 2015 was Chief of the Section on Neural Gene Expression in the Laboratory of Cellular and Molecular Regulation at the National Institute of Mental Health. For the paper referred to, see: Ruda MA, Iadarola MJ, Cohen LV and Young WS 3rd. In situ hybridization histochemistry and immunocytochemistry reveal an increase in spinal dynorphin biosynthesis in a rat model of peripheral inflammation and hyperalgesia. *Proceedings of the National Academy of Sciences USA* 1988 Jan; 85 (2): 622-626.

[1] Maria Fitzgerald, PhD, was Professor of Developmental Neurobiology at University College London as of 2015. She trained with Patrick Wall and has been a leader in international pain research.

[1] See Hammond DL, Ruda MA. Developmental alterations in thermal nociceptive threshold and the distribution of immunoreactive calcitonin gene-related peptide and substance P after neonatal administration of capsaicin in the rat. *Neuroscience Letters* 1989 Feb 13; 97 (1-2): 57-62.

[1] Thinking on pain in neonates changed with the work of Kanwaljeet "Sunny" Anand, MD, PhD, who was a medical resident at Harvard in 1987. Dr. Anand now holds the St. Jude Chair for Critical Care Medicine at the University of Tennessee Health Science Center in Memphis. See Anand KJ, Hickey PR. Pain and its effects in the human neonate and fetus. *New England Journal of Medicine* 1987 Nov 19; 317 (21): 1321-1329.

[1] C-fos is an amino acid protein which plays a major role in many cellular functions and changes of gene expression.

[1] Freund's adjuvant, or CFA, is a commonly used mycobacterial solution that triggers an immune system response.

[1] See note 20.

[1] Michael J. Iadarola, PhD, was Chief of the Neurobiology and Pain Therapeutics Section at NIDCR as of 2015.

[1] Janice L. K. Hylden was a researcher at NIDCR in the 1980s and early 1990s. As of 2015, she was living and teaching in Washington State.

[1] Haruhide Hayashi was a researcher at NIDCR in the late 1970s and early 1980s. He later returned to Japan and as of 2011, was on the faculty of the Tohoku University Graduate School of Dentistry in Sendai.

[1] As of 2015, Ke Ren, MD, PhD, was Professor of Pain and Neural Sciences at the University of Maryland School of Dentistry, working with Ronald Dubner.

[1] See Ruda MA, Ling QD, Hohmann AG, Peng YB and Tachibana T. Altered nociceptive neuronal circuits after neonatal peripheral inflammation. *Science* 2000 Jul 28; 289 (5479): 628-631.

[1] The author was Deborah Tannen, who has written multiple books on this topic.