March 3, 2005 Interview with Dr. Robert Innis, NIMH, Molecular Imaging Branch

"I'M A TOOLMAKER!"
DEVELOPING RADIOACTIVE TRACERS FOR PET STUDIES

Robert Innis is a Senior Investigator at NIMH, and Chief of the Molecular Imaging Branch, laboratory with an emphasis on brain imaging using PET (positron emission tomography). The overall goals of Dr. Innis's laboratory are to develop new radiotracers that image molecular targets in the brain, to evaluate these tracers in animals and healthy human subjects, and then to extend their use to patients with neuropsychiatric disorders.

CW: Dr. Innis, in 2001 you became chief of the Molecular Imaging Branch, which was then new laboratory at NIMH in the Section on PET imaging. Could you begin with telling me what brought you here?

Robert Innis: I do brain imaging particularly with PET, Positron Emission Tomography. The things that attracted me to the NIH were largely the resources that were available to do this state-of-the-art brain imaging because it's a very expensive methodology requiring extensive infrastructure like cyclotrons and cameras and a multi-disciplinary research team.

CW: Can you give some more details about the state of the art facilities that you have available here?

RI: For PET, Positron Emission Tomography, you must first be able to make a radioactive atom or a radionuclide and that's done in a cyclotron. And at NIH, in the Clinical Center they have three cyclotrons, which is three times as many as most places have. Then, after the cyclotron makes this radioactive atom -- it's a Carbon-11 CO2, carbon 11 labeled radioactive Carbon Dioxide -- that radioactive carbon has to be quickly incorporated synthetically with chemistry into some radio labeled probe that will go to the brain and label some specific protein in the brain or someplace in the body as a whole. In addition to having this cyclotron there has to be an extensive radiochemistry facility in order to make the radio labeled probe that is subsequently injected into an animal or human subject. The National Institute of Mental Health in particular was very interested in this methodology because it allows a way to look at proteins that may be involved in normal brain function as well as what goes wrong in the brains of people with neuro-psychiatric disorders. So if you want to try to measure these proteins, this is one of the major ways, if not the only way, in order to be able to do that in a living human brain.

Because NIMH was interested, they were willing to make a large investment in a new radiochemistry laboratory here. Then after you make the radio labeled probe, the radio-tracer, the radio-ligand which binds to a specific protein in the brain, then you have

to inject it into an animal or human and do imaging. The imaging facilities here for PET are really extensive. They currently they have two cameras just for imaging in rats, they have four to five cameras for imaging in monkeys and humans. So it's really extensive facilities and also the experience -- personnel who are multi-disciplinary and skilled in many different areas.

CW: Your focus is on imaging proteins. Is PET the most promising approach to brain imaging now?

RI: For measuring specific proteins in the living human brain it may be the only one right now really, for the vast majority. And it's an issue of sensitivity. Many of the proteins that we're interested in, that we think may be involved in psychiatric illnesses or neurological illnesses, many of those proteins are present in very low concentration and in order to be able to measure them we need a very highly sensitive method to be able to do so. PET is currently unique in the imaging methodologies for doing that. If you want some specific numbers, I would say that if you compare MRI methods, magnetic resonance imaging as a whole, their sensitivity: They can measure lets say the targets that are maybe present at 10^{-4} molar. But PET can probably go down 10^{-12} molar. So it's just many, many orders of magnitude higher sensitivity, and many of the proteins that we're interested in measuring are present at 10^{-9}, 10^{-10} molar. So it is out of bounds for being possible to measure, at least currently, with MRI techniques and we need some other higher sensitivity method. The downside is that you have to use radioactivity, but the advantage is you have much greater sensitivity.

Because of the unique sensitivity of the PET, and also I might say because of the specificity, you can make drugs that will bind to just one specific protein. So because you can measure specific proteins at low concentrations in vivo, this seems like a very valuable technique that could be. So NIMH put in a lot of money in order to make this radio-chemistry lab; and the purpose of this lab is to develop many new ligands, because it has great potential, but there has been relatively little payoff so far. As we develop the new ligands, the idea is to make the knowledge about them available to other PET centers throughout the United States. We are currently working on perhaps twenty different targets at various stages of work, in terms of cold chemistry to radio labeling to imagining in animals going on to humans. And to some patient disorders. It's a broad number, we're working on about twenty different target proteins right now.

CW: So you say the development of radio-labled ligands has a great potential but little payoff so far. What makes it so difficult?

RI: This is developing a radio-labeled drug. It's called a radio-pharmaceutical. Many people know that pharmaceutical development is very risky. Less than one out of a hundred compounds turn out to work. Well it's a similar success rate, maybe it's a little better success rate than that, but it is a low success rate because you have to make a radio-pharmaceutical that is going to work and the characteristics of that are different

from a therapeutic agent. Therapeutic agents, many of those don't work out. So it's an inherently risky business and difficult in order to make it. The other difficulty is -- I mean there has been some successes -- I would say the difficulties are the high failure rate in developing pharmaceuticals, including radio-pharmaceuticals, and also the technically complex nature of it. Few places have cyclotrons available and radio-chemistry facilities, but that is expanding.

"MONEY DRIVES THE CAPITALIST MEDICAL SYSTEM IN THE UNITED STATES"

I think one of the reasons why this is a good time to get into this kind of work is that PET is now beginning to expand in a manner that is very similar to MRI in, let's say, the 1980s. In the past few years insurance agents, including the government's *Medicare*, has started to reimburse for selected PET scans, and these in particular are PET scans to measure metabolism with *FDG*, a glucose analog. And many tumors and their metastases will have increased glucose uptake so that you can localize the tumor and its metastases with this PET tracer. So it's been re-approved, it's been approved by Medicare for reimbursement and money drives the capitalist medical system in the United States. Not only that, but the charge for the FDG scan is pretty high, so that you can make a profit, so that cash starved hospitals say, "Profit? Well, we'll set up a PET too." In fact, some companies have established mobile PET cameras on trucks that travel from hospital to hospital, like they have for mammograms. As medial centers themselves get PET cameras and get set up to do it, then they have a significant component of the infrastructure there in order to be able to do the research studies. And the same thing happened with MRI, where it was a limited utility, and when there was reimbursement for it, every hospital gets an MRI. While the MRI is there, you have the infrastructure and you can work on research, and functional MRI is sort of invented, and then the infrastructure was there and you could do it. So it seems like PET could expand now.

CW: So your lab works on twenty different tracers, you say, which are designed to help scientists and later physicians using PET to target specific molecules in the brain that are associated with disease or brain function. Can you say which ones are the most promising so far?

RI: These are twenty different targets, and for any target we might have several ligands. There are many targets that may have potential clinical utility. I can give you an example of one that I've worked on in the past. When I was previously at Yale University, I helped to make a probe for a particular target that's a marker in *Parkinson's disease*. That is now used in the European Union to aid in the diagnosis of Parkinson's disease. Specifically we radio labeled a chemical analog of cocaine, and it binds to the *cocaine receptor* and it just so happens that patients with Parkinson's disease lack the cocaine receptor. With this radio labeled probe, or with the absence of that, you can aid in the diagnosis of Parkinson's disease. There has been a lot of work now done on a probe for Amyloid, a protein, which builds up in *Alzheimer's disease*. Two other

research groups have developed probes that could measure this protein and may be able to diagnose Alzheimer's disease before the onset of symptoms.

CW: When you chose these tracers, is your research always driven by a specific disease, or what is it that makes you go into a specific direction?

RI: If we're fortunate enough to know a very clear connection to a disease then that can be one of the reasons for going into it. So in the case of Alzheimer's disease, we really do need to be able to measure the amount of Amyloid in the brain, this protein which builds up and kills the nerves. In the case of Parkinson's disease, we really do need to know the *dopamine neurons* that are gone. So we are trying to make a new and improved amyloid probe and we have other animal and human studies looking at Parkinson's disease. But unfortunately in the majority of cases we don't have a clear connection like that. Because it isn't like, let's say for schizophrenia, that we have one target that we know that if we could measure that, would be sure to get valuable information for the diagnosis or treatment of schizophrenia. That's not known. The vast majorityof radiolabeled PET probes would not be "diagnostic agents", like in Parkinson's or Alzheimer's, but really research tools to be able to explore pathophysiology. So these are more like tools to be used to understand what may be going wrong, to look at the time course, to look at the effect of treatment, etcetera. However, we work on a tracer only if we think it would extend to humans, and we would only do it if there is some reasonable evidence that that target could be involved in an important way in physiology or pathopyhsiology.

CW: Next to the dopamine receptor you focus on the nicotinic receptor, is that correct?

RI: Well maybe we could take an example of the *nicotinic receptor*. This is the receptor in the brain that nicotine binds to. It's thought to be the receptor that mediates the addictive proprieties of smoking. There could be reasons to look at that in terms of addiction, let's say quite clearly with regard to nicotine addiction, but there could be other utility too in looking at cognitive function and agents which enhance nicotine receptor function may enhance cognition and that could be helpful in schizophrenia or Alzheimer's disease, and in Parkinson's disease. There are some drugs that act at this nicotine receptor now, which enhance cognition. We worked to develop a probe of the nicotine receptor, and then we've looked at it recently in Parkinson's disease and there is a substantial loss in Parkinson's disease. We might want to follow that up now to look at Parkinson's disease with or without dementia. So it's a research tool to study pathophysiology. We have a good probe for that receptor now and there'd be questions on its use as a clinical research tool. For that, we always collaborate with a clinical group that is, a specialist in Parkinson's disease, or cognitive function in Parkinson's disease.

CW: Since much of your work involves animal studies, maybe you can say a little bit about what you're doing at the NIH right now?

RI: Okay, I'll give you an example. We're collaborating with Ron McKay, in the NINDS, who studies embryonic stem cell transplantation. Stem cells might be a useful treatment, they say, of Parkinson's, Alzheimer's, and diabetes. One of the issues that will come up is that if some stem cells are developed for the treatment, we'll have to have some way of monitoring whether the stem cells have survived and whether they're growing and doing what they're supposed to do. So, in a project in collaboration with Ron McKay we did the imaging and Ron McKay and his group did all the molecular biology and interesting stuff. We're sort of the toolmaker, we are the maker of the biomarker. But he has shown that you can poison a rat and give it Parkinson's disease in one half of its brain so it is hemi-Parkinsonian and it will have abnormalities. Then he treats the animals with embryonic stem cells, which can be genetically modified, and a lot of them turn into the dopamine neurons, the neurons that are destroyed in Parkinson's disease, and when he does this, the animals get better. So this is a great animal model of Parkinson's disease and it could be a good one, if it were ever extended to human subjects, to try to develop a marker to see if you can measure the survival and growth of that transplant, and we've done that. That is, we can image a protein marker on the dopamine neuron and thereby monitor the survival and growth of the cells, and that is correlated with behavioral improvement in those animals. So that would be an example of an animal study in which we're demonstrating the feasibility of this as a biomarker.

CW: Are there other examples?

RI: We're doing a lot of different studies. We have done a study in collaboration with the NIAAA to look at monkeys that have had maternal deprivation. The monkeys that have maternal deprivation that is, they're raised by peers, they're not raised by their mother, and when they grow up they have abnormalities; they don't socialize, they're very shy, they don't explore, and when exposed to alcohol they will have bouts of alcoholism. So, the analogy of all this to the human situation is very clear and it could well be that maternal deprivation is doing something to the brain which then continues into adulthood to lead to these behaviors. That could be responsible for alcoholism, let's say shyness and withdrawal, and the investigators, Drs. Dee Higley and Steve Suomi had evidence that there's a decreased amount of one particular transmitter in the brain, *serotonin*, if they measured in cerebral spinal fluid, the fluid around the brain.

So we did a study in these animals and looked at regularly reared animals and maternally deprived, and sure enough we had a marker for the serotonin neurons; it was actually the *serotonin transporter*. It's the target site where Prozac works, so it's the "Prozac receptor" and it was markedly depleted in many areas of the brain. So one could think about some interventions, which are being thought about now, to enhance serotonin transmission and would reverse the animals' problems. Maybe that could be a model for disorders in humans. It's also thought to be a potential model in alcoholism, so we are now doing a study of the same "Prozac Receptor" in alcoholic subjects. So there's a direct connection to look and see if we see similar things in the human disorder of alcoholism.

By the way, our results are coming back exactly the opposite. It's really quite strange. The alcoholics are markedly increased in their level of the serotonin transporters whereas these maternally deprived monkeys are in the other direction, so [laughs] we are left with more questions than answers.

CW: So you were really surprised by your results!

RI: Yeah, yeah.

CW: That's fascinating. Do you have an idea why that would be?

RI: No. It's going to take some more work, but what's nice is that you could think about additional studies that could be done in both animals and humans to try to explore that. For example, in those maternally deprived monkeys they're also very aggressive. They're shy but aggressive. So, we're going to look now at alcoholics, at those alcoholics with aggression, some have markedly increased aggression compared to others, and we'll look at some other parameters. That may be one aspect of it that we would have to look at, and that could be refined or done in both humans and animals. So that's another great, great advantage about the work here, beyond any technical facility resources, the capability of doing bench-to-bedside work with very, very outstanding researchers in those areas to examine these questions in a broad way, it's a big positive at NIH.

CW: You have mentioned Parkinson's, Alzheimer's, schizophrenia, addiction. Is there another field, another study, that would be worth mentioning?

RI: Anxiety disorders and depression are certainly active areas of research here. And again, some of those cases -- in the case of anxiety disorders and depression we do not know like a single protein that we definitely want to measure in order to know that we're going to have diagnostic information. It isn't like measuring amyloid in Alzheimer's disease, or measuring dopamine neurons in Parkinson's disease. So it is developing tools that we think could be useful in understanding the pathophysiology or the treatment of these disorders, and we have several markers in development or early studies on that.

I guess one that I might mention here is there's strong evidence in the case of depression that the mechanism of action of many antidepressants, from drugs to electroconvulsive therapy, that they all work by a common mechanism increasing a *second messenger system* in the brain, by increasing the *cyclic AMP* messenger system in the brain. So we have implemented a probe here that will measure cyclic AMP phosphodiesterase, which will measure a particular aspect of this second messenger system. So in addition to measuring receptors that are located on the cell membranes we are trying to move beyond that to second messenger systems. We have implemented that tracer here, we have developed methods to appropriately quantitate it in rats, and we hope

this year to start studies in patients with depression. But again, it would be a tool, we develop it because we think that it would be involved in either the pathophysiology or the treatment of depression.

CW: **Can we go back to the first example you gave, dopamine. What makes it so difficult to measure dopamine in the living human brain?**

RI: Well, there are different aspects of it. What you want to be able to do is to measure dopamine transmission. Dopamine is a chemical neurotransmitter in the brain. It is released from one neuron, diffuses, travels across a gap, and then interacts with a post-synaptic receptor, like a D2-receptor, and then after that there might be a second messenger system or cyclic AMP dependent systems. So you'd want to measure the synthesis of dopamine, you'd want to measure the release of dopamine, you're going to measure its interaction with the receptor, and you'd want to measure what effects binding to that receptor has. So you have to develop in vivo, just like it has been developed in vitro, probes all along the way in order to be able to do that.

There are probes already developed to measure synthesis, and there are probes available to measure some of the receptors like the D1 and the D2-receptor. There are some novel ways of trying to measure the amount of dopamine released, which I might talk about more -- you might be interested -- and then the dopamine signal is terminated, is ended by the dopamine being taken back up into the first neuron, and that's done via the dopamine transporter, which is also the cocaine receptor. So you have markers on the first neuron pre-synaptically, and on the second neuron post-synaptically. And all of these aspects could be of interest or be abnormal in one area or another of the brain.

An example of the dopamine released can be valuable here. Take *amphetamine*, or speed, the way speed works is to release dopamine --it causes dopamine to be released into the synapse and that release of dopamine causes the euphoria and the high and probably is related to the addictive properties of amphetamine and some other stimulants like that. So what we, and others, have done is to label the D2 receptor that is post-synaptic that is, on the second neuron, when dopamine comes out it binds to that receptor. We put in a radio-tracer that also binds there so we can measure this D2 receptor before and after we give amphetamine. We've done a series of studies in monkeys and in humans and in patients. And when the amphetamine causes the dopamine to be released it displaces the radio-ligand, it knocks it off the receptor, so therefore the binding goes down, and the amount that the binding goes down is proportional to how much dopamine is released.

Well one of the leading hypotheses of *schizophrenia* is that the psychotic symptoms -- hallucinations, delusions -- are caused by increased dopamine release in the brain. So in order to test this we did this D2 receptor imaging before and after giving amphetamine and we're able to show that patients with schizophrenia had a much greater release of dopamine than healthy subjects. In addition, in healthy subjects the amount of

dopamine released was related to the high. They got higher if they had more dopamine. – Even though they had the same dose of amphetamine, they had different amounts of dopamine released and different effects. The higher they got by certain measures or activation, the more dopamine that was released. In the schizophrenic patients there can be a transient -- short lasting, one hour, two hours increase in psychotic symptoms, paranoid symptoms typically, and the transient increase in psychotic symptoms was related to the amount of dopamine released. So that was really some of the first evidence clearly linking dopamine release in the brain to psychotic symptoms in patients. So that was able to help explore the pathophysiology.

Now, in addition, I mean probably some of the greatest disability that patients with schizophrenia have is not just the psychotic symptoms but other symptoms called negative symptoms of withdrawal -- social apathy, lack of initiative, lack of emotional responsiveness, and also cognitive impairment, sometimes full-blown dementia -- and that these cognitive deficits may impair overall life even greater than the psychotic symptoms. So there's a lot of interest in looking at why is there this cognitive decline in patients with schizophrenia, and one of the leading causes has to do with dopamine transmission in the cortex. So we and others are trying to develop probes to measure this in the cortex, not just in the inner portion of the brain called the striatum, and we have some studies to look at that now, but it's difficult to do in general because there's much less dopamine transmission in the cortex quantitatively, let's say, than in the striatum, so you have very low density sites that you are measuring and it becomes important to develop probes to be able to measure dopamine transmission in the cortex as well as the sub cortical areas, and that's a need for a new tool. So, I'm a toolmaker!

[laughter]

And toolmakers have their roles.

CW: Yeah. [laughs] That's great. Okay. So these technologies that are available to look at the brain, so far have they already helped to get a different understanding of emotion and cognition or is this still something that's in the future?

RI: I think vis-à-vis -- I think there's much more in the future than there's been in the past, at least I hope so. It's just the tip of the iceberg. And, there are other imaging methodologies that may be better for some of the questions that you have with regard to cognition and emotion like functional Magnetic Resonance Imaging, fMRI, where you can do things more quickly and you have no radiation exposure and you have higher resolution, and you can do these studies repeatedly so that fMRI has been far more valuable so far, really, than PET, in studying emotion and cognition. But in terms of trying to look at a specific protein, I think there you're at with PET, and insofar as the drugs that are likely to be developed for neuropsychiatric disorders will be targeting specific proteins, there can be extra utility for this sort of measure for the therapeutic development.

The drug companies recognize that and most of the big drug companies have either set up PET within their own facilities or are collaborating with other sites. We have several collaborations ourselves with Big Pharma because they want to develop the radio labeled compounds that will help them -- when they make the therapeutic agent it helps them in the therapeutic drug development -- and we work with them to make the radio labeled probe because it can be a useful research tool to study pathopysiology. So that so-called public/private partnership is strongly encouraged and has been very beneficial to us.