Small Animal Gamma-Ray Imaging

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Abstract

This document is a report on my research in the area of Small Animal Gamma Ray Imaging, with an emphasis on Single Photon Emission Computed Tomography, or SPECT. Our project team imaged the uptake of radioactive ligands through the blood stream of a live mouse. We use radioactive Iodine-125 (^{125}I) as our isotope. The ligand used is RTI-55, a cocaine analog, and it is commercially tagged with ^{125}I. Our imaging apparati are two 125 mm diameter cylindrical position-sensitive photo-multiplier tubes (PSPMT), which detect the position of emission of the gamma rays. [^{125}I]RTI-55 was injected into the tail vein of a mouse, and view the time-dependent uptake with our PSPMT's.

To this date, this group has obtained many two-dimensional images of the uptake. My research was aimed at extending our capabilities to the production of three-dimensional images. Single Photon Emission Computed Tomography (SPECT) is the computer algorithm that we use to produce 3-D images. We have imaged static, inanimate objects (phantoms) in 3-D, but we face problems when trying to extend this to imaging a live mouse. One problem is that the [^{125}I]RTI-55 travels through the mouse at a certain rate. This puts a time constraint on the collection of data for the SPECT program, since one is trying to get snapshots at different stages of the uptake.

Introduction

This project is a collaboration of the Physics and Biology departments at the College of William and Mary and the detector group at Jefferson Lab. Thus, my research has many biological and physics-related applications and implications.

Our group has previously had much success in producing 2-D images of the uptake of [^{125}I]RTI-55. These images are well documented in previous reports[1,6]. Figure 1 shows a set of images showing the uptake of [^{125}I]RTI-55 over a time period of six hours.

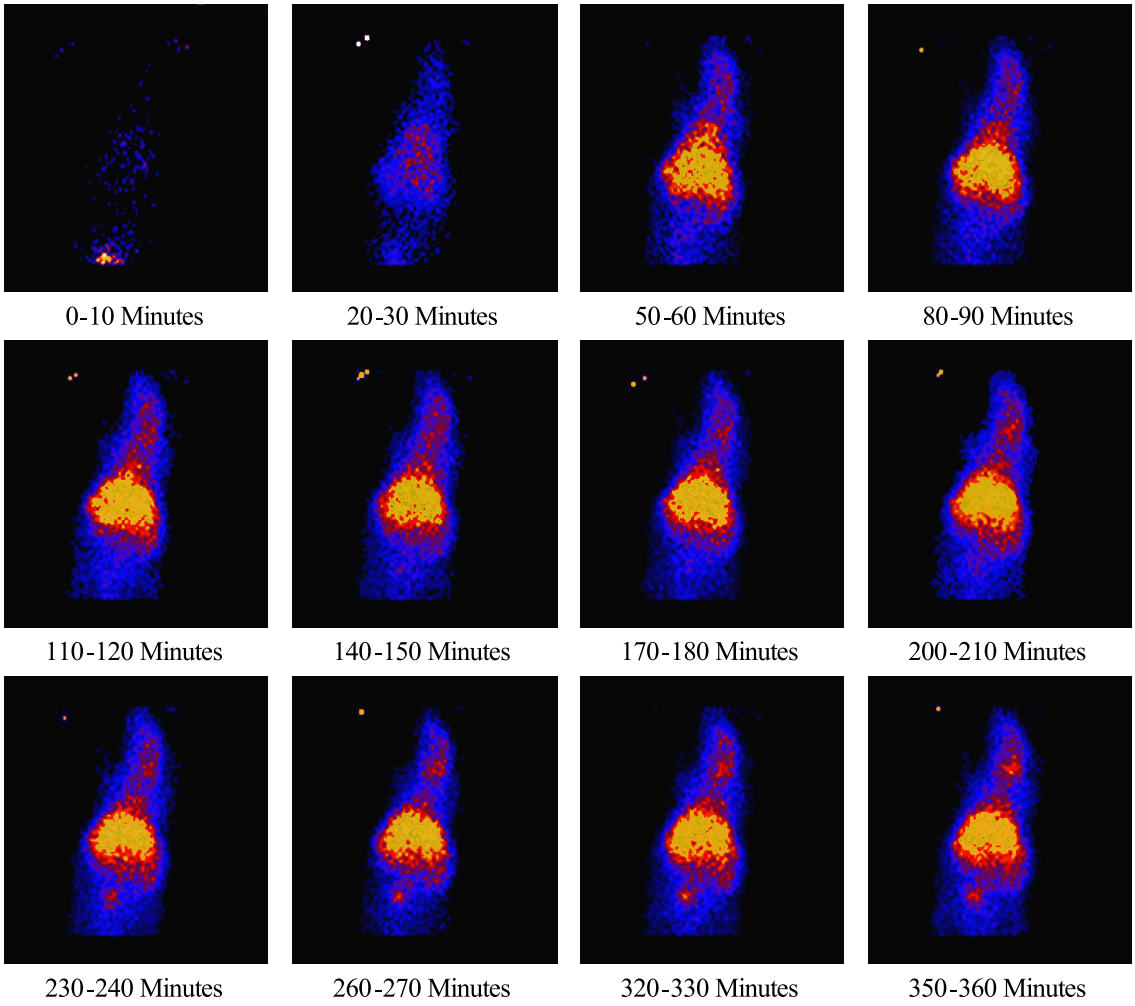

Figure 1 The uptake of [^{125}I]RTI-55 in a mouse is shown as a function of increasing time—from left to right, top to bottom. The minute interval to which each image applies is given below the image. The tail of the mouse is at the bottom in each image, and the head is at the top.

As one notices, after injection into the tail vein of the mouse, the [^{125}I]RTI-55 travels through the blood stream to various regions in the mouse's body.

The uptake of RTI-55 is of interest to medical fields, because it is able to pass through the blood-brain barrier. In Figure 1, one can notice the collection of the ligand (RTI-55) in the head region.

The intention of this research was to enable us to produce 3-D images similar to those in Figure 1. A SPECT computer program allows us to reconstruct a 3-D volume from any angle. By taking the imaging data at a variety of angle and using the SPECT computer program, it is possible to produce such 3-D images. Such images can be of considerable biological and medical interest.

As mentioned above, we have as yet only been able to successfully produce 3-D images of an inanimate phantom. We call these objects "phantoms", which is a general term for any object or material that approximates the properties of the actual object of interest (mouse). Phantoms are usually cheaper to acquire and to work with, and easier to manage. In our case, mice are more expensive, they have to be anaesthetized before imaging, and require a biologist to be present at all times to monitor the mouse's status. The phantoms we employ are clear Lucite cylinders, two inches long and one inch in diameter. We bore holes of 1 to 2 mm in diameter into the top and sides of the cylinder, and inject ^{125}I into these holes. The holes are then sealed with small metal screws and glue, and the static distribution of the radioactive Iodine can be imaged.

As with any phantom there are limitations and properties that do not exactly match the actual object of interest. Our phantoms approximate the size and distribution properties of a mouse, but they do not incorporate flow of the ^{125}I. This fact does not

raise problems when taking data for 2-D image production. However, when attempting to produce SPECT, the flow through a mouse introduces a time constraint on the collection of data, which cannot be approximated or tested with the use of a static phantom. Since the ^{125}I does not move in the phantom, data acquisition for SPECT in the phantom can take as much time as needed for adequate data (usually about 3 hours). However, if we are to extend SPECT to the case of an actual mouse, this time constraint must be taken into account (time limit = ~30 minutes). This is the main problem we face in the production of 3-D images of the uptake in a mouse. Solutions are proposed later in this paper, when SPECT is fully explained and the limitations can be better understood.

Concepts of RTI-55

Safely inside the confines of a fume hood, we inject [^{125}I]RTI-55 into the tail vein of an anaesthetized mouse. We then image the bio-distribution of this radioactive-tagged ligand as it is taken up through the bloodstream. RTI-55 binds to certain proteins, called receptors, on the outside of cells. Thus, the bio-distribution of the ligand may be used to tell us the distribution of the gene expression that is responsible for the presence of such receptors.

In our experiment, RTI-55(Beta-CIT), also known as tracer methyl 3 Beta-(4-iodophyl) tropane-2 Beta-carboxic acid methyl ester, is labeled with ^{125}I, and its dynamic bio-distribution is imaged over an interval of time. RTI-55 is a cocaine analog, and it binds to receptors called “transporters” that are involved in the movement of dopamine and seratonin across cell membranes in the brain. The molecules of RTI-55, as with certain other drugs, mimic the shape of the seratonin molecule, which biologists say is

responsible for making humans feel “good” and “happy”. RTI-55 successfully passes through the blood-brain barrier and is taken in by these receptors.

RTI-55, however, is also recognized as a toxin by the mouse’s body, and it is taken through the liver via the blood stream. The liver acts to dispose of such toxins and thus in our images a large concentration of ^{125}I is seen in the mouse’s liver.

Regardless of where the radioactive ligand ends up, its position, upon emission of 32 keV gamma rays via the decay of ^{125}I, is recorded by a position sensitive photo-multiplier tube, which is linked to KMAX data acquisition software.

Experimental Setup

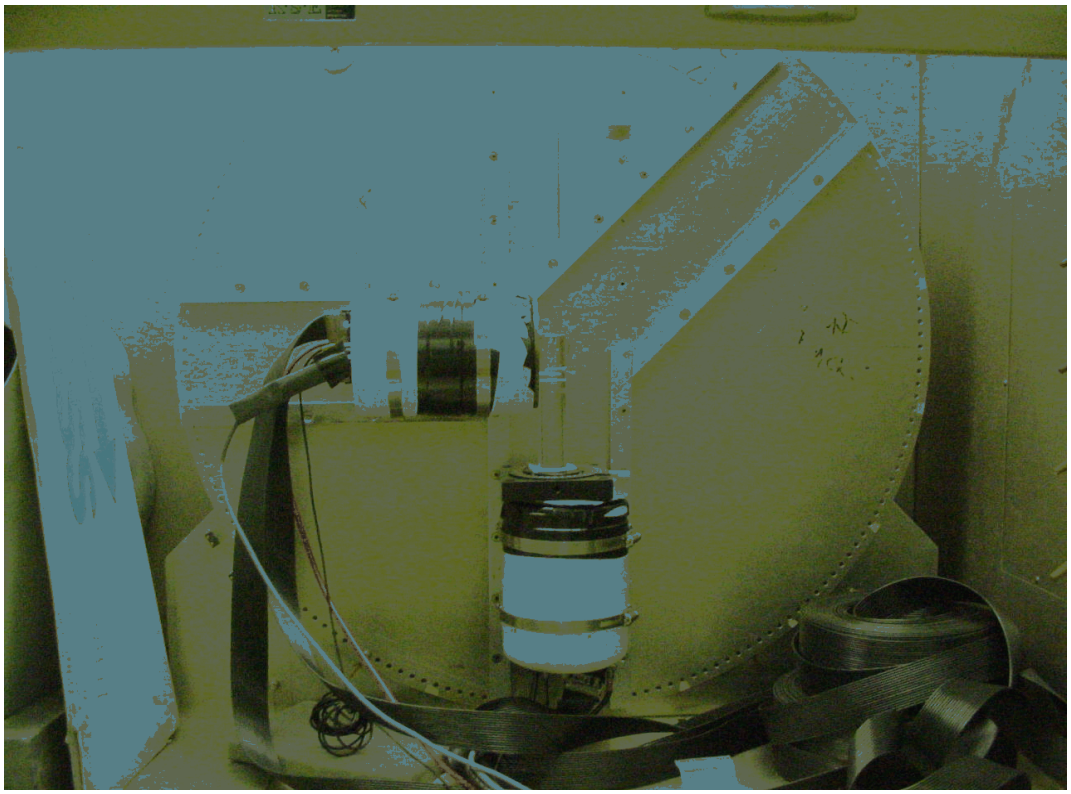

Figure 2 Above is the experimental setup for a SPECT run. This image shows our PSPMT's, and the gantry on which they are currently mounted. The clear, cylindrical object positioned vertically on top of the bottom detector is a phantom. The phantom is on an additional five inches of clear, cylindrical Lucite, for positioning purposes.

The present apparatus includes two Hamamatsu R3292 125-mm and (more recently) one 25-mm position sensitive photo-multiplier tubes (PSPMT's). In these tubes we currently employ parallel-hole collimators. Research is currently under way to test different types of collimators, including pinhole collimators, for their resolution and field of view. (For a full description of PSPMT's and those used in our experiment, please see

Weisenberger [1], pp. 8-17, 45-52, 84-86.) From the PSPMT's we achieve position-sensitive images of the distribution of ^{125}I inside a living mouse.

^{125}I has many distinct characteristics from other radioactive elements that make it advantageous in the imaging of mice. It emits low energy gamma rays of about 35 keV, which have enough energy to exit the body of the mouse (average path length of about 1 cm through live tissue [1]) yet is low enough in energy so as not to pose any great threat to lab workers. The half-life of ^{125}I is 60.2 days, and thus it can be stored much longer than the isotope ^{131}I, which has a half-life of only 8 days. A further problem with ^{131}I is that emits higher energy gamma rays of 364 and 637 keV. For a full discussion of radioactive isotopes of biological interest, see Weisenberger [1], p.3 and pp. 42-45. He delineates the aspects of many isotopes, and gives a detailed description of the properties of ^{125}I.

The collimator used in gamma ray imaging is very important and specific to the type of radioactive isotope used. The low energy photons emitted by ^{125}I allow us to use a copper-beryllium parallel hole collimator, which is a lower Z material than the commonly used lead and tungsten. The advantage of using CuBe collimators is that the many holes can be fashioned by etching very thin individual layers of the collimator one at a time, then the layers can be glued on top of each other until the desired thickness of the collimator is reached. We currently use collimators with thicknesses of 5 mm and 7 mm. The thickness of a collimator and the hole size and wall thickness determine its resolution and sensitivity. With parallel hole collimators we have achieved a spatial resolution of about 2 mm in a phantom, and about 3 mm in a mouse. One loses resolution in the mouse experiments owing to the fact that the mouse is farther away from

the face of the detector during imaging and more scattering occurs in the mouse than in the phantom. Distance from the collimator is a major determinant of resolution. Research is now being done by a member of our project team to determine the possible advantages of using pinhole collimators. A pinhole collimator has only one hole in its center, instead of the hundreds in the parallel hole collimator. Weisenberger [1] discusses the mathematical relations that approximate the resolution of parallel-hole collimators and pinhole collimators on pages 10-16 of his thesis. Converging and diverging-hole collimators are also discussed in these.

Ultimately, parallel-hole collimators were employed in this experiment because they usually offer higher sensitivity than competing designs, without sacrificing spatial resolution. Also it is known that when imaging a wide field of view, as we do with a mouse, the pinhole collimator loses resolution far from its center axis. If one were to use it only for imaging the brain of a mouse, however, this would not be of consequence.

Figure 3 shows a cross sectional view of the 125 mm diameter R3292 position sensitive photo-multiplier tube coupled to the CsI(Na) crystal array scintillator:

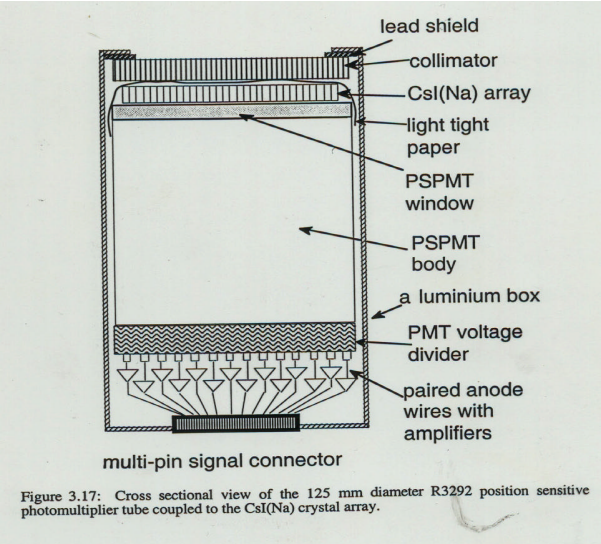

Figure 3.17: Cross sectional view of the 125 mm diameter R3292 position sensitive photomultiplier tube coupled to the CsI(Na) crystal array.

Figure 3 A cross sectional view of the 125 mm diameter R3292 position sensitive photomultiplier tube coupled to the CsI(Na) crystal array [1].

The characteristics and function of the scintillator and photo-multiplier tubes are described be detail by Weisenberger [1], pp. 16-17, and 71-74.

Single Photon Emission Computed Tomography—SPECT

In this project we seek to produce a three-dimensional image of the bio-distribution of ^{125}I-labeled RTI-55. In essence, tomography is the production of a 3-D image built by a computer code from multiple 2-D images. The object that contains a distribution of the radioactive substance is imaged by the gamma cameras for three minutes (adjustable) every six degrees (adjustable) for a full 360-degree rotation. The exact length of time that data are taken for each image and the degree interval can be adjusted in order to produce better images, or to produce more recorded gamma ray events, as necessity demands.

An experimental run of collecting data for SPECT, as we have done it, includes the following. We position one gamma camera (more than one can be used to gather images, but thus far we have only used one) so that it is horizontal. The phantom (we have yet to collect data for SPECT using a mouse) is then placed on a rotating stage within a few millimeters of the face of the detector (see Figure 2). Holes have been bored into the phantom, ^{125}I placed into the holes, and the holes have been sealed. The rotating stage is calibrated by tick marks every degree, so that the angular position of the phantom can be set precisely. We then begin taking data with the phantom oriented at the 0^{th} degree. The KMAX data acquisition program records the x and y coordinate of each event over the allotted time interval (usually 3 minutes). Once the first three minutes are up, data acquisition automatically stops and the image is saved from the 0^{th} degree position. The stage is then rotated six degrees, and data are acquired for another three minutes. This image is saved for the 6^{th} degree position, and so on.

Once all the data have been taken, the images are then passed as arguments to the SPECT program. This tomography computer program, written in IDL by Dr. Steve Meikle [7], then reconstructs a three-dimensional image of the object. It takes horizontal slices of the images and stacks them vertically. The volume can then be freely rotated in the IDL program to be viewed from any angle—side to side, above and below.

Below is a particular orientation of a 3-D image produced using this SPECT program. The axes are labeled to display orientation. A representation of the phantom is on the right. The object imaged was a clear Lucite cylinder. Inside the bored holes is the radioactive ^{125}I.

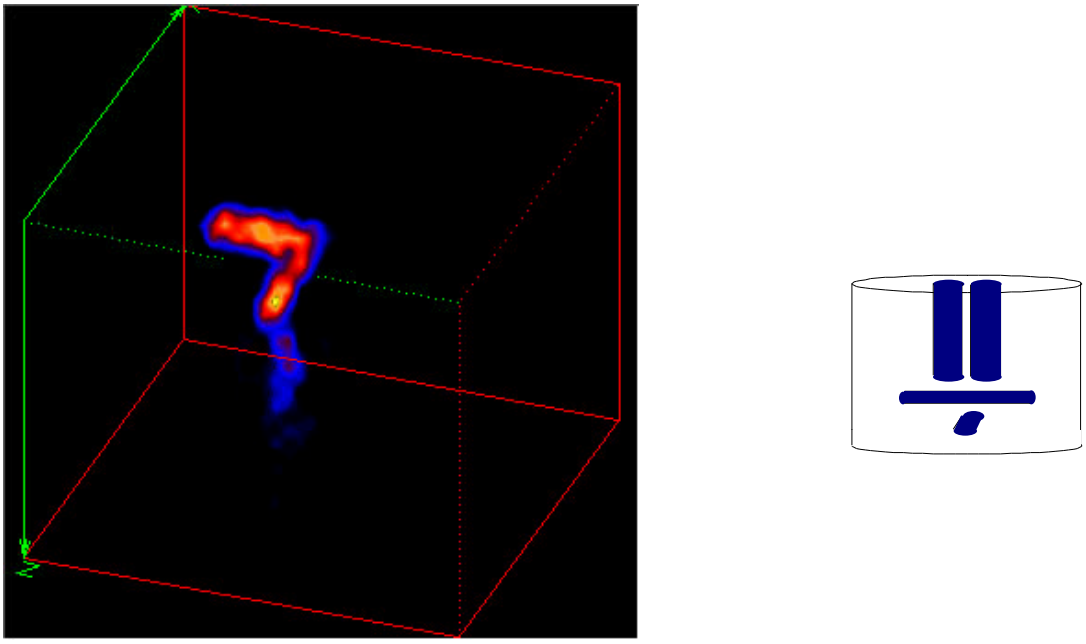

Figure 4 Left is the 3-D image produced by SPECT. Right is a pictorial representation of the phantom used in the experiment.

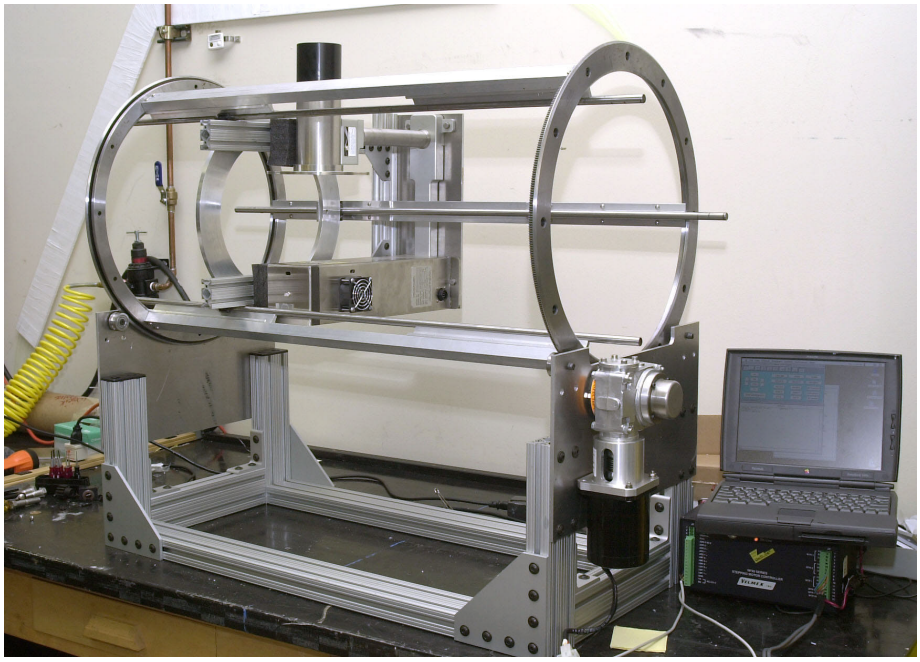

Figure 5 The new rotating gantry, which, at the time of this paper, has yet to be put to use.

Future Plans

We have recently designed a cylindrical gantry, which has been fabricated by the William and Mary Physics Shop (see Figure 5).

Using the new rotating gantry along with three 1-inch PSPMT's, we hope to be able to efficiently and quickly produce SPECT images of a phantom or mouse.

The rotation of the gantry will be operated by a computer-controlled VELMEX stepping motor. This will reduce the human error and inefficiency of the process of rotating the stage, which was done previously by hand. (Notice the Lixi X-ray machine shown in Figure 5 mounted on the gantry.) The three one inch detectors will be placed 120 degrees apart, equidistant from the center of rotation of the gantry. The mouse will

be mounted on a stage that will move axially down the center of the gantry. This stage will be driven by a stepper motor, as well.

The stage will pause in line with the Lixi x-ray machine, while an x-ray image is produced. It will then continue its travels through the axis of the gantry, where it will again pause, aligned with the detectors. Once here, it will remain for a number of hours, while data are taken for the production of tomography. The stepping motor and the data acquisition software will be coordinated, as data are taken for a few minutes every six degrees. As there will be three detectors, only a total angle of 120 degrees will be swept out by the gantry's rotation.

The timeline for one full data set for tomography will need to be no longer than 20-40 minutes, when another data set will begin to be acquired. This time limit poses a problem for taking SPECT data. Under our current modus operandi, it would take about one hour to acquire each data set, which would be long if we are to get the desired snapshot effect of the uptake of the ligand. Research is currently being done to determine the minimal acquisition time for a single SPECT data set. Data have been taken, and the results, when compared with multiple other data runs, will guide us to the solution to this time limit problem.

After the mouse has been imaged there for one or more hours, several snapshots will be had (each taking 20 minutes), and from each one a three dimensional reconstruction of the biodistribution of the ^{125}I will be produced. Thus, we will be able to view up to fifteen different stages of the 3-D uptake of the ^{125}I, as well as the many different 2-D images that could also be of interest.

After such a period of time, the mouse is returned to its cage to sleep off the anesthesia.

An added modality

During the summer of 2000 we added a Lixi X-ray machine (shown in Figure 5) to our experimental setup, giving us the ability to produce dual modality imaging. We are able to overlay a 2-D gamma ray image on the x-ray image. This makes it easy to locate the source of gamma rays with reference to bone structure in the mouse. When we are able to produce 3-D SPECT images of a mouse, the x-ray pictures will allow for precise referencing of the gamma ray events.

Findings Concerning Bio-distribution

We have repeatedly seen evidence that the radioactive ligand gathers in the liver region, thyroid region, and the head region.

A computer program called "Region of Interest" (ROI), written by Rob Saunders [4], allows us to plot the number of events as a function of time in any region of the body. Once data have been taken, we can create time cuts in any desired time interval (usually 10 minutes), and plot the number of events taken over each interval throughout the whole experimental run.

As expected, the radioactive pharmacokinetic ^{125}I-labeled RTI-55 is found to pass through the blood brain barrier and be taken in by the transporter receptors. The Region of Interest plot allows us to know exactly what percentage of the drug makes it to the brain, and potentially what areas of the brain it is in. In the future we will use our new

one-inch detectors to focus more closely on the brain uptake. Using these will increase our resolution, and, using SPECT, we will be able to determine, more specifically, which areas of the brain are taking in the ligand, and how much. Figure 6 shows the layout of the ROI program, and the results of two different ROI plots of the head and liver of the mouse for the uptake of RTI-55 over six hours.

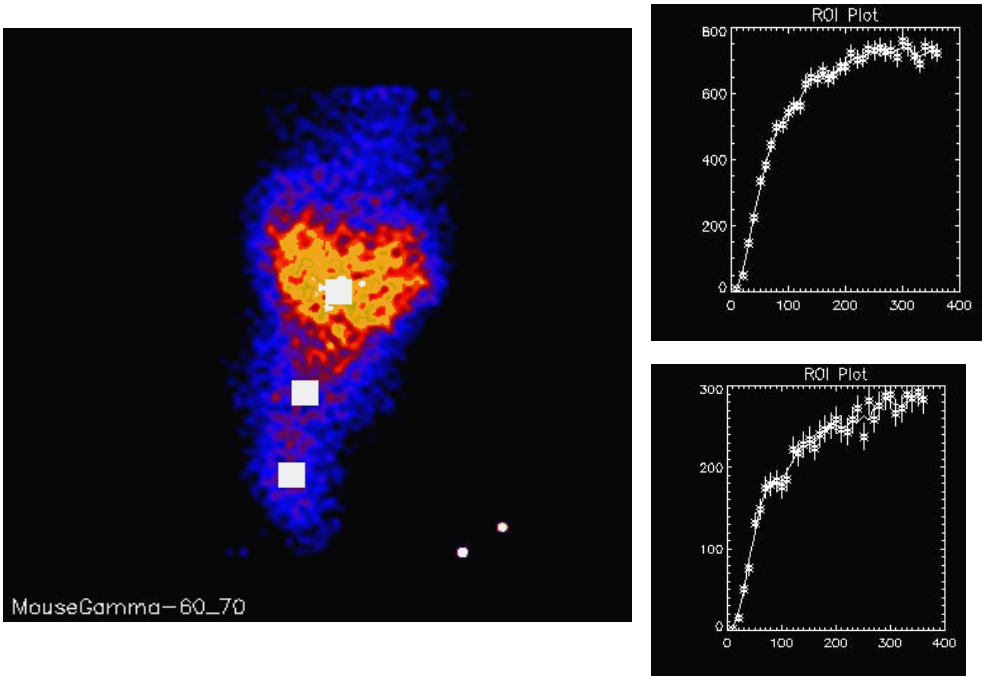

Figure 6 Left: Image of mouse, with ROI squares shown for clarity. Head is down, tail is up. The liver region is in the abdomen (middle of the picture). Upper Right: The ROI plot of the brain—number of gamma ray counts vs. time. Upper Left: ROI plot of liver—same axes as brain.

The Author's Areas of Specialization

My contributions to this project have been varied. Thus, it has been possible for me to get a good overview of all of the different facets of the project. I have been studying the IDL programming language, and have learned how to apply it. This has also helped me in understanding the limitations of the hardware and software that is used here.

This has proved important when trying to produce SPECT images. The code must be fully understood in order for resolution and efficiency to be maximized when trying to take SPECT data on a mouse.

I have also participated in the construction and design of the experimental setup. I designed and built the x-ray mount. I also participated in the biological research and oversaw the construction of the rotating gantry.

My main area of interest has been in the maximization of resolution. Initially, I read articles and did research on collimators. All collimators have an intrinsic resolution limitation. Determining what this is, and trying to maximize resolution without sacrificing sensitivity has proved an interesting goal. Weisenberger discusses this comparison, and the intrinsic resolution in his thesis ([1] pages 8-16 and pages 84-86). (see also, [2] and [5])

Further research is being done on collimator resolution by Luke Ng, an undergraduate physics student and member of this project team. Pinhole collimators are being built now, to be tested in the near future, to determine whether these will have any advantages over the parallel-hole collimators that we are currently using. This will allow us to put theory and these pinhole collimators to the test.

Finally, I have designed several Lucite phantoms with bored holes to be used for phantom experiments. Since mice are costly and time consuming to deal with, we have done many runs with these plastic cylinders to determine our resolution and to experiment with different methods of data acquisition.

Conclusions

Many plans have been made future improvements to this project. In the past year, while I have worked on this project, there have been many changes and improvements to the experimental setup and the images produced. Our next goal is improvement in resolution, and the first production of a SPECT image of a mouse. These two things will be more readily achieved when the new rotating gantry is put to use. The use of this gantry alone will increase efficiency and capabilities of image production.

Three sets of data have been taken in order to research the most efficient ways of collecting SPECT data. To decrease the acquisition time to something on the order of 30 minutes or less, we need to determine if it is possible to increase the angular interval between images, without great reduction in resolution. We are also trying to determine the minimal time interval that can be employed, again, without losing resolution. Maximizing the angular interval and minimizing the time needed for each angle can decrease the total time of acquisition, and would enable one to get a 20-30 minute, static snapshot of the uptake of the radioactive ligand in a live mouse.

When these improvements are realized, the usefulness of these results to the biomedical field will be substantially enhanced.

Bibliography

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