Serial Measurements of Positron-Emitting Isotope Activity in Rat Brain

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SUMMARY This report describes the construction and performance characteristics of a device capable of making rapid serial measurements of rat brain radioactivity following the administration of a positron-emitting isotope. Two bismuth germanate oxide (BGO) scintillation detectors in a coincidence detection circuit are used to achieve the necessary degree of collimation. Data from the device can be used to characterize cerebral biochemical and physiological processes using computer-assisted mathematical models.

Recent technical developments have made it possible to construct positron-emission tomographs (PET), that are capable of producing quantitative data describing the 3-dimensional distribution of tracer compounds in human organs. These data can be used to solve appropriate mathematical models of biochemical and physiological processes and to make deductions concerning the behavior of the labeled compound. This report describes the construction and operational characteristics of a device that can be used to make serial measurements of whole brain radioisotope content in a small animal following the administration of a positron emitting nuclide. This device will be used to study cerebral metabolic processes as a prelude to human PET studies or under conditions that would be expected to cause permanent alterations in brain morphology or biochemistry (e.g. glucose metabolism during severe hypoxia, and ammonia metabolism following the administration of the glutamine synthetase inhibitor L-methionine S-R sulfoximine). It is also relevant where direct tomographic studies of humans or primates with large brains are precluded by ethical and financial considerations.

Theoretical Considerations

The photons produced by the annihilation of positrons travel in almost exactly opposite directions. This colinearity makes it possible to use electronic circuits that require coincident detection of events in opposing crystals to achieve a high degree of collimation. A combination of electronic and physical collimation is used in this device. Figure 1 shows the relative positions of the animal, lead shielding and bismuth germanate oxide (BGO) crystals. Points A, determined by the edges of the lead collimators, determine the boundaries within which one of the pair of gamma photons produced by positron annihilation can be detected; points B, determined by the plane of the upper or lower surfaces of the lead collimators, determine the boundaries within which both of the annihilation photons can be detected, and point C is the position where the probability of detecting both photons from the annihilation pair is greatest. A stereotaxic rat-head holder is used to ensure constancy of head position from study to study, thus eliminating variations in counting geometry.

The shape and dimensions of the lead collimators were chosen based on theoretical and empirical considerations and are shown in relationship to a midsaggital section of the rat brain in figure 2. Ideally, the device should register all of the events that occur within the brain and none of those that occur outside of the brain (no extracerebral contamination).

In a preliminary series of experiments, rats were anesthetized with phenobarbital (150 mg/kg, i.p.) and were given an intravenous injection of 14N-ammonia after removal of the scalp, masseter, and occipital muscles. The shape and dimensions of the collimators were varied until the ratio of coincidence counts, measured with the brain in situ, to the coincidence counts after brain removal and replacement with a radiation absorber was highest. The highest ratio (10:1) was obtained with an inverted trapezoidal collimator as shown in figure 2. The lower plane passes through the interaural line and is parallel to the base of the skull. The exact dimensions are as shown in the figure. Note that the 10:1 ratio will vary with the chemical form of the isotope, increasing as the brain:extracerebral tissue ratio rises. Positrons that originate outside of the coincidence zone and travel into the zone before annihilation also affect this ratio: thus the performance characteristics will also vary with positron energy.

Description of System

A standard stereotaxic head holder (David Kopf, Model 900, Tujunga, CA) equipped with 3.5 cm offset earbars and a riser block was selected to provide a rugged means for securing each animal. The offset earbars raise the animal above the structural elements of the frame and simplify the placement of the collimators. Cylindrical BGO crystals measuring 2.5 cm in height and diameter, equipped with RCA 4885 photomultiplier tubes and voltage dividers, were obtained from the Harshaw Chemical Co. (Solon, OH). The crystals were shielded with lead (3.7 cm minimum) on all sides. A Power Designers model 1570 power supply (Westbury, NY) was used. Photomultiplier amplifiers (model 612 AM), discriminators (model 620 BL) and coincidence logic units (model 622) were purchased from LeCroy Research Systems.
FIGURE 1. Scale drawing of rat brain radioactivity monitoring device. The head of the rat is held in place with a stereotaxic head holder with offset ear-bars. The BGO crystals are collimated and shielded with lead (see also fig. 2). Assuming complete absorption of annihilation radiation by lead, the intersecting lines at points A limit the zone from which single photons will be detected, lines B limit the zone of coincidence detection and C is the position where coincidence detection probability is greatest.

FIGURE 2. The coincidence collimation zone. The inverted trapezoidal zone of coincidence collimation is shown superimposed on a mid-sagittal section of the rat brain. The collimator was constructed from lead that is 50 mm thick, the bases are 16.0 and 18.0 mm and the height is 12.0 mm. The inter-aural line lies on the one base. Reference points are from the atlas of König and Klippel.

System Performance

The effectiveness of the collimators in excluding coincidence events outside of the collimated zone is shown in figure 4. In this study EG&G Ortec (Oak Ridge, TN) photomultiplier tube bases with pre-amplifiers (model 276), delay line amplifiers (model 460), timing single-channel analyzers (model 420A) and a fast coincidence circuit (model 414A) were substituted for the LeCroy electronics. The discriminator was set to accept all photons with an energy of at least 50 keV. A line source (0.058 cm diameter) was filled with $^{14}$C-carbonate and surrounded by 0.5 cm of birch wood on each side. The source was moved through the plane equidistant from the detectors (passing through points A in fig. 1) and the true coincidence count rate was determined. For this experiment the trapezoidal collimators were replaced by a collimator with a rectangular hole 1.6 X 1.2 cm cut through 5.0 cm of lead.

The results of this study are shown in figure 4, and indicate the very high rejection rate for events that occur outside of the collimated zone. When the experiment was repeated with the single channel analyzer...
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Figure 4. System response to line source. A 0.058 cm tube was filled with 14C and surrounded by 0.5 cm of wood, to simulate positron absorption by tissues, and moved vertically through a plane equidistant from both BGO crystals. The boundaries of the lead collimators and the effectiveness of the system in excluding events originating outside of the collimated zone is shown. Events from outside of the zone are a function of the range of positrons in the system (distance from origin of positron and site of annihilation). See text for dimensions of collimator and energy discriminator settings.

Figure 5. The effect of increasing the coincidence resolving time (2t) on the true coincidence detection efficiency. Due to "jitter" in the system, the true coincidence count rate rises as the resolving time is increased to about 40 nsec. Observations made with 15N and include appropriate corrections for radioactive decay and random coincidences.

Figure 6. 15N-ammonia extraction by the brain. 15N-ammonia (0.10 ml in 50 mM Ringer HEPES, pH 6.8) was injected into the carotid artery via a catheter. These data demonstrate the ability of the system to make rapid serial measurements of isotope activity.

adjusted to the 360–660 keV photopeak for 511 keV radiation detected by BGO crystals, there was no change in the fraction of the coincidence counts measured outside the collimator boundaries, indicating that coincidence counts due to scattered radiation are an unimportant source of error in this system.

Within the nanosecond time frame of the detection system, the pulses for true coincidence events, the result of the annihilation of a single positron, do not arrive at the coincidence logic circuit at exactly the same time. A coincidence resolving time must be selected that includes as many true coincidence events as possible yet minimizes the random coincidence rate. Figure 5 shows the effect of increasing the coincidence resolving time on the detection of true coincidence events. Since this system allows the user to calculate the random coincidence contribution for each measurement, a relatively long resolving time can be used. The coincidence resolving time can be adjusted by varying the width of the pulses produced by the discriminator and measured by placing an 90Sr source in the coincidence zone. 90Sr produces single 513 keV gamma photons, thus all measured coincidence events are random, and the resolving time can be calculated from the coincidence and singles counts recorded by the system.

The linearity of this system at high count rates was confirmed by placing an 14C-carbonate source in the coincidence zone and making serial measurements of the true coincidence count rate and the single photon count rate for each crystal. These rates remained linear at 0.5 million single counts per sec and 50,000 coincidence counts per sec. Circuit elements that produce pulses with a short duration must be used to avoid pulse pile-up at high counting rates. These linearity determinations were made with 20 nsec pulses from the LeCroy discriminators. The 500 nsec
pulses from the Ortec model 420A timing single-channel analyzers make them unsatisfactory for coincidence counting at rates over 1000 counts per sec.

System Applications

This system is capable of measuring radioactivity in the rat brain rapidly and sequentially. Figure 6 shows the data obtained by the system following an intracarotid injection of $^{13}$N-ammonia. A 300 g male Wistar rat (Lab Supply, Indianapolis, IN) was anesthetized with diethyl ether, curarized, and ventilated with 70% nitrous oxide and 30% oxygen. The external carotid artery was catheterized with PE-10 tubing (2 cm) mated to PE-50 tubing. The catheter tip was advanced to the bifurcation of the carotid artery, and 0.10 ml of $^{13}$N-ammonia in 50 mM Ringer-HEPES, pH 6.80, 10 mCi/ml was injected and the cerebral radioactivity was monitored at 0.2 second intervals. The ammonia extraction fraction was measured by the method of Raichle et al. The extraction fraction was 18% and was constant with injection volumes of 0.05, 0.10 and 0.20 ml. This value is similar to that obtained by Lockwood et al. using the Oldendorf internal standard method.

This example demonstrates the utility of this system for applications that require rapid serial measurements. Other measurements, where the rate of change of cerebral radioactivity is low, such as those that might be encountered in $^{13}$C-glucose, or $^{18}$F-fluorodeoxyglucose metabolic studies, are also practical. Stable geometry and calibration with phantoms should also permit this device to be used for making absolute measurements of brain radioactivity.

This system is well suited to small animal studies that use positron-emitting isotopes. Present applications include its use in studies that precede human investigations using positron-emission tomography, and in studies requiring sequential measurements of cerebral radioactivity, especially those that are likely to alter cerebral morphology, e.g. hypoxia, where the use of larger, more expensive animals such as rhesus monkeys, is precluded.

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References

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