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Neurotransmitter Receptor Imaging in Living Human Brain with Positron Emission Tomography

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New neuroimaging technology can label specific receptors in living human brain with ligands tagged with radio-isotopes, and then anatomically localize and quantitate them with positron emission tomography (PET). This approach requires a multidisciplinary team, including radiochemists who prepare tagged ligands, pharmacologists who develop *in vitro* and *ex vivo* techniques for quantitating receptors in experimental animals, and PET experts who adapt these techniques for *in vivo* study of human subjects. This article outlines the principles of quantitative PET analysis by examining the numerous biochemical, kinetic and anatomical methods available for neurotransmitter receptor quantitation in experimental animals, as well as their applications to the study of neurotransmitter receptors in living human brain with PET.

Modern conceptualization of the brain includes chemical neurotransmission via numerous neurotransmitters and neuromodulators, acting upon a multiplicity of receptors organized in an array of unique neuronal pathways. Modern conceptualization of disorders of the brain, as well as the treatment of these disorders with pharmacological agents, focuses upon the process of chemical neurotransmission as well. A new technique, positron emission tomography (PET), has emerged from the collaboration of high technology computer hardware and software with biotechnology advances in the understanding of neurotransmitter receptors in the living brain (see Sedvall, Farde, Persson, and Wiesel, 1986 and Stahl, Leenders, and Bowery, 1986 for reviews). In the last few years, this new research discipline has been applied to clinical neuroscience and is currently making rapid technological strides: high

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technology electronics has produced sophisticated cameras which can simultaneously image many sections of the brain in short periods of time and reconstruct these images with the aid of computer algorithms; radio chemistry has advanced sufficiently so that new drugs which label neurotransmitter receptors can be tagged with positron emitting isotopes so as to generate imaging ligands.

All of these advances are merely setting the stage for what should become the most valuable applications of this technology, now developed to a sufficiently high degree for serious clinical neuroscientific study. Neuroimaging studies in humans can now begin to map neuroreceptors in the human central nervous system (CNS), search for new diagnostic criteria for neurologic and psychiatric disorders, monitor treatments with neuropsychopharmacologic agents, and even assist in the discovery of new drugs and receptors in the human CNS.

The present article will review the background of quantitative PET analysis of neurotransmitter receptors in living human brain by outlining chemical neurotransmission in the CNS, as well as examining the numerous biochemical, kinetic, and anatomical analyses available for neurotransmitter receptor quantitation in experimental animals. Recent studies will be reviewed showing how these preclinical quantitative techniques can be applied to the study of neurotransmitter receptors in living human brain with PET, using specific examples from the dopamine neurotransmitter system. Suggestions as to the future developments of this potentially powerful technique in the clinical neurosciences will be discussed.

Chemical Neurotransmission in the CNS

Messengers, Modulators and Receptors

Contemporary understanding of chemical neurotransmission in the CNS has advanced considerably since Dale (1954) and his co-workers first conceptualized its existence. The original proposal of an electrical stimulus in a nerve causing the release of a chemical neurotransmitter at a synaptic connection between a neurone and its effector has evolved into a much more complex model. Chemical signalling, between neurones, is now known to be mediated by fast signals or slow signals. The amino acids GABA and glutamate are hypothesized to represent the principal fast chemical signalling agents for the "off" and "on" responses, respectively, of the CNS (Iversen and Goodman, 1986). Almost all other brain neurotransmitters can be thought of as slow-acting modulators of the two main fast transmitting agents. Thus, the monoamines and the neuropeptides may serve principally to modify the excitability of nerve cell groups.

A fascinating new concept in chemical neurotransmission is the idea that transfer of information between neurones can occur not only via the classical

"anatomically addressed" system, but also by a "chemically addressed" system (Iversen, 1986). The "anatomically addressed" system corresponds to the conventional view of the nervous system, where a synapse between two nerves allows for the transfer of information from the first nerve terminal to the second nerve only. However, with the development of the concept of neuromodulation as a common mode of chemical signalling, it seems that precise one-on-one anatomical connections between presynaptic terminals and effector cells may not always be needed, or even desirable. Thus, if the function of neurotransmission, in some cases, is to modify groups of neurones rather than individual cells, the chemical transmitter may be released in a diffuse manner, as has been suggested by Iversen (1986). Neurones release different neurotransmitters, but if only some target cells possess receptors for these substances, then a specific transfer of information is still possible at sites considerably remote from the neurone releasing the neurotransmitter. Therefore, the old concept of hardwired neurocircuits with fast "on" and "off" chemical signals has been replaced largely by the concept of a chemical soup, whereby the subtlety of chemical neurotransmission is perhaps far greater than we had hitherto imagined. Chemical neurotransmission at each effector site may be mediated via biological mixtures of agonists or of neurotransmitters and neuromodulators. Recognizing that the number of chemical messengers in the central nervous system is large, and that individual neurones may release mixtures of these substances, makes the idea of "chemically addressed" information transfer particularly relevant to the potential understanding of CNS disorders as well as to CNS drug therapies. If one assumes that there are 50 different chemical messenger substances in the human brain (Snyder, 1984), and that all permutations among these are possible, then there are no less than 1,225 different combinations of messengers which neurones could contain and release, assuming they were only to contain coexisting pairs of substances.

Neuroimaging techniques are poised in a powerful position to be able to visualize the human brain not only as a series of hardwired pathways and exhaustive chemical maps, but also by the much more dynamic concept of the chemically addressed nervous system.

Biochemical Analysis of Neuroreceptors

In Vitro Kinetics

Neuroreceptors occur in such small concentrations that it makes direct chemical analysis difficult if not impossible. The discovery and quantitation of individual neurotransmitter receptors has been rendered possible, however, by using specific chemical ligands, labeled with long-lived isotopes (^{3}H, ^{14}C, ^{125}I), and coupling these chemical tools with the kinetic methodology of ligand binding methods. The receptor binding assay is performed by adding a tissue

containing the receptor binding membranes with a radiolabeled selective ligand for the receptor of interest. When incubated together, receptor binding membranes bind the radiolabeled ligand both specifically (at the receptor) as well as non-specifically (adsorption to non-receptor sites). At the end of the incubation, the tissue is centrifuged, or filtered, to produce a pellet, and the excess unbound radioligand is discarded. The radioactivity in this tissue pellet is counted and comprises both specifically bound ligand as well as non-specifically bound ligand.

Simultaneously, receptor binding membranes are incubated with radioactive ligand, plus an excess of non-labeled ligand. This maneuvre results in all of the specific binding sites of interest being occupied by unlabeled ligand, whereas the labeled compound only binds to the non-specific adsorption sites. At the end of an incubation period, this tissue is centrifuged or filtered, and its radioactivity counted, and quantitated as non-specifically bound ligand.

To determine the amount of specific binding to the neurotransmitter receptor of interest, subtract the radioactivity counts in non-specific binding from those in total binding. Plotting the results over a wide range of concentrations of ligand added, will result in a specific binding plot known as a Scatchard plot, whose slope and intercept are important kinetic constants. The x intercept, B_{max}, represents maximum binding and is a quantitative measure of the total number of specific receptors in the membrane study. The slope represents $^{-1}/K_D$. K_D, known as the equilibrium dissociation constant, is the concentration of radioligand at which half of maximum binding occurs.

Anatomical Analysis of Post-Mortem Neuroreceptors

In Vitro Autoradiography

Neurotransmitter receptors can be accurately mapped in post-mortem tissue from animals or humans by a technique known as *in vitro* autoradiography. This is also a radioligand technique, normally using a ^{3}H-labeled ligand which is incubated with a slice of intact tissue. Sections of frozen brain tissue are dried in an incubator with radioactive ligand in a concentration that results in specific binding, and with other drugs to ensure selective binding only to the receptor of interest. Once conditions have been optimized to result in preferential binding of the ligand to the receptor of interest, the tissue is washed, dried, and placed against a dry photographic emulsion. The radiation from the radiolabeled ligand then exposes the film in proportion to the tissue concentration of the ligand, and therefore in proportion to the number of neurotransmitter receptor binding sites. This technique enables receptor binding to be visualized in relation to the tissue anatomy. While regional binding can be assessed from membranes prepared from specific brain areas,

the resolution afforded by autoradiography is much greater. This is illustrated in Figure 1, using the dopamine-2 receptor (D_2) ligand, ^{3}H-spiperone. Four

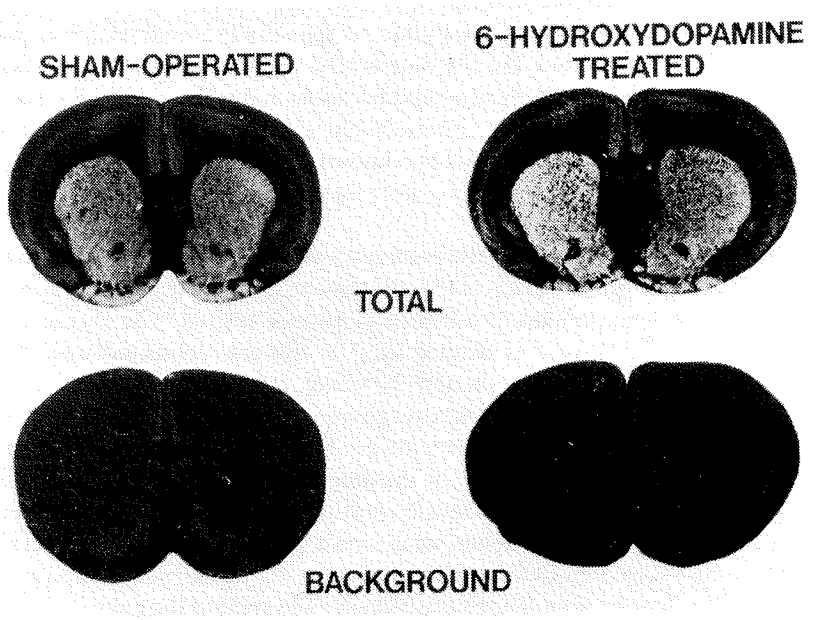
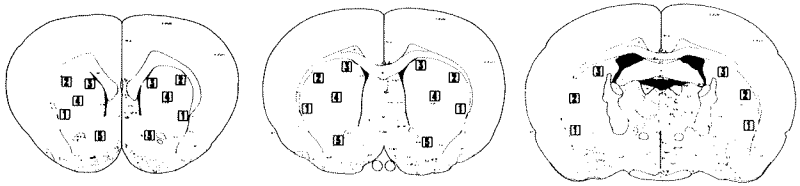


Figure 1: Comparison of dopamine D_2 receptor binding in normal rat striatum and after unilateral denervation of the substantia nigra. Two male Sprague-Dawley rats (160g) were anaesthetized with equithesin and placed in a stereotoxic frame with the toothbar positioned at +5mm. A Hamilton syringe was inserted into the brain with the needle tip positioned 3.8mm behind bregma, 1.4mm laterally and 7.7mm below the dura. One rat was injected with 8μg 6-hydroxydopamine HCl (free base equivalent) in 4μl saline containing 0.1% ascorbic acid. The other rat was injected with saline alone. After 15 days the animals were tested for turning behaviour with apomorphine (1mg/kg s.c.). A positive lesion was taken when 30 turns occurred within 5–15 min after injection. Thirty days after the 6-hydroxydopamine or saline injections the animals were perfused fixed by intracardiac perfusion with 0.1% paraformaldehyde in 0.01M phosphate buffered saline. 10μm cryostat sections were prepared and stored at –20°C overnight. The sections were then thawed, rinsed and incubated for 45 min at 20°C in 50mM tris-HCl buffer (pH 7.4) containing NaCl 120mM, KCl 5mM, $CaCl_2$ 2mM, $MgCl_2$ 1mM and 0.8 nM ^{3}H-spiperone (26.5 Ci/m mole NEN). The sections were then rinsed twice in ice-cold buffer for 2 min followed by a dip in ice-cold water for 2 seconds. The dried sections were juxtaposed to ^{3}H-sensitive LKB Ultrofilm for 5 weeks. The images were then developed. The upper two images show total binding in a sham-operated and lesioned brain. The injection in both cases was into the substantia nigra innervating the striatum shown on the left of each picture. The whiter regions indicate the presence of radiolabel. Note the high density in the lesioned striatum. The lower two images show the background levels obtained by incubation of adjacent sections in the additional presence of unlabeled haloperidol (10μM).

images are shown which are derived from two rat brains. In Figure 1 the binding to normal (i.e., sham operated) rat brain sections is shown both with total binding and background or non-specific binding. Background binding was obtained by concomitant incubation of the section with excess (100μM) unlabeled haloperidol. Clearly the amount of specifically bound ^{3}H-spiperone is high within the striatum, but the distribution within this region is uneven. The subtle differences in density across the striatum could never be observed in membrane binding studies. Interestingly, the distribution of D_2 sites observed in Figure 1 accords with the known distribution of dopaminergic fibres which innervate the more rostral regions of the striatum (Doucet, Descarries, and Garcia, 1986).

The sections in Figure 1 were also prepared from a rat 30 days after unilateral (left) 6-hydroxydopamine lesion of the substantia nigra. The denervated side is on the left of the image labeled "6-hydroxydopamine treated." Clearly there is an increase in the density of binding sites on this side compared with the control striatum in the same section. However, the extent of the increase varies across the striatum with the greatest change occurring in the dorsolateral regions and nucleus accumbens. Doucet, Descarries, and Garcia (1986) indicate that the density of dopaminergic nerve terminals in the dorsolateral regions is twice that in the ventromedial areas. Thus, it might be expected that dopaminergic denervation would give rise to a greater increase in the former region as observed. Quantitation of this change is shown in Figure 2. The density of binding sites within defined areas of the striatum have



ANTERIOR **MEDIAL** **POSTERIOR**

Location	ANTERIOR Receptor density Left	Right	Difference	MEDIAL Receptor density Left	Right	Difference	POSTERIOR Receptor density Left	Right	Difference
1	2.44±.08	2.14±.07	0.30*	3.14±.05	2.67±.07	0.47**	3.40±.06	2.74±.04	0.66**
2	2.75±.09	2.46±.06	0.29*	2.97±.04	2.65±.04	0.32**	2.64±.03	2.57±.06	0.07
3	2.26±.04	1.78±.09	0.48**	2.55±.05	2.09±.05	0.46**	1.72±.04	1.70±.05	0.02
4	2.42±.04	2.20±.11	0.21	2.29±.07	2.10±.05	0.19*			
5	2.43±.07	2.20±.04	0.41**	2.20±.03	1.86±.04	0.16*			

Data obtained from three sections at each position from 2 rats.

6-OH-dopamine injected into the substantia nigra on the side corresponding to the striatum in the left of each diagram.

Values are in μCi/g wet tissue.

*p<.05 and **p<.001: binding site density was significantly greater on lesioned side comared to control side

Figure 2: Regional changes in D_2 receptor binding after unilateral lesion of the substantia nigra with 6-hydroxydopamine. The method was as described in the legend to Figure 4. Binding densities in regions indicated by the squares on the line drawings were determined by image analysis using a Quantimet 970 analyser. Reference to brain paste tritium standards placed on the same films facilitated the quantitation.

been determined sequentially in 10μ sections. Figure 2 illustrates the results obtained in sections from anterior, medial and posterior regions of the striatum. The data were obtained 30 days after unilateral denervation and the denervated side is on the left in all cases.

Emulation of PET-Scans

Ex Vivo Autoradiography

Although the above description of *in vitro* autoradiography is useful in quantitating post-mortem neuroreceptors, it does not give the same information as a PET-scan, because the latter employs radioligands administered systemically to live patients. Fortunately, the technique of *ex vivo* autoradiography has evolved to help neuroscientists quantitate visual images in tissue slices, yet emulate the circumstances of a human PET-scan by giving the radioligand isotope systemically to experimental animals.

Ex vivo autoradiography employs a trace amount of radioactive ligand injected intravenously into live animals. After the ligand binds to receptors, the animal is sacrificed, and frozen sections of brain are rapidly dried and placed against a photographic emulsion, much as described above for the technique of *in vitro* autoradiography. The density of photographic grains in *ex vivo* autoradiograms is again in direct proportion to the concentration of radiolabeled ligand present. The advantage of the *ex vivo* technique is that it can emulate the PET-scan by creating the same experimental conditions whereby the drug is systemically administered, must pass the blood-brain barrier to gain access to specific receptors within the central nervous system, and is exposed to potential systemic metabolism of the radioligand, yet it retains all the quantitative power of *in vitro* autoradiography. Disadvantages of the *ex vivo* technique are that many radioligands which can measure neurotransmitter receptors in *in vitro* incubations cannot penetrate the brain, and thus are not available for measurement of neurotransmitters in the *ex vivo* technique. Also, it is possible that the binding of radioligand to receptor is labile, and the washing procedures, and tissue preparation procedures may distort the image on the autoradiograph such that it does not represent any longer what was actually occurring *in vivo*. Despite these limitations, the emulation is extremely powerful as it allows the neuroscientist to approximate what is occurring in PET-scans of living subjects in a manner not possible in *in vitro* autoradiography.

An example of the use of *ex vivo* autoradiography is shown for the dopamine system in Figure 3. This figure is taken from an article by Kuhar, Murrin, Malouf, and Klemm (1978) in which they describe the distribution of ^{3}H-spiperone binding sites in rat brain sections two hours after intravenous administration. The autoradiograms were obtained by juxtaposing the sections

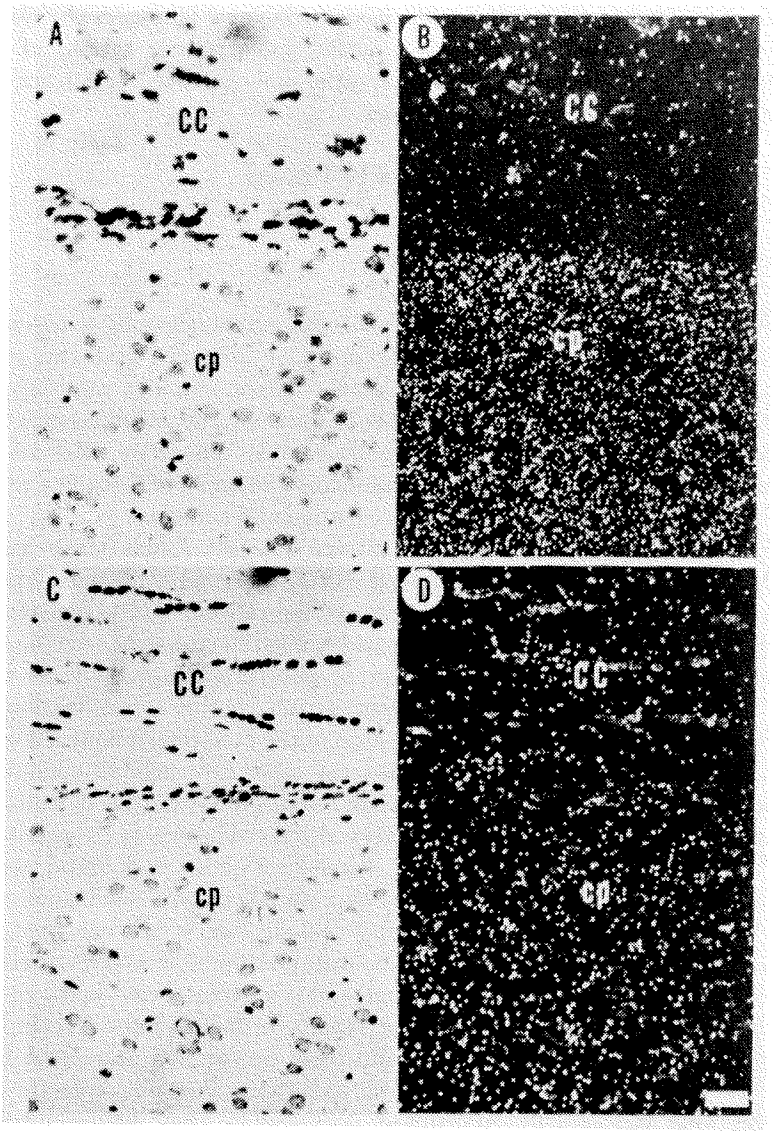


Figure 3: Autoradiographic localization of ^{3}H-spiroperidol binding sites in the caudate putamen (cp) and corpus callosum (CC). A (bright field) and B (dark field) show an autoradiogram from an animal injected with only ^{3}H-spiroperidol. Frames C and D show a similar area from an animal injected with ^{3}H-spiroperidol and (+)butaclamol. Note the striking localization of grains to the caudate putamen in A and B and the dramatic reduction of binding due to the (+)butaclamol in C and D. Bar = 30μ. From Kuhar et al., 1978, "Dopamine receptor binding in vivo: the feasibility of autoradiographic studies", *Life Sciences*, *22*, 203–210. Reprinted with permission from Pergamon Journals Ltd. © 1978.

to dry coverslips coated with photographic emulsion. After developing, the image appears as silver grains deposited on the coverslip above the section. Unlike the film method, this coverslip technique allows the grains, which correspond to radiolabeled ligand, to be viewed in relation to the tissue morphology. In Figure 3 the morphology is shown under transmitted light on the left, while the same area viewed under dark field optics on the right shows the photographic grains as white dots. Note in Figure 3 that a large number of grains is present over the caudate putamen with much lower levels over the corpus callosum. When the dopamine ligand (+)butaclamol (5mg/kg) was injected prior to ^{3}H-spiperone (Figure 3) the grain density in the striatum was reduced from 276 $\pm$ 21 to 64 $\pm$ 10 per 2500 μ^2. No change was observed in the corpus callosum and (–)butaclamol, the inactive isomer, produced no change in either region.

Visualization of Living Neuroreceptors

Qualitative PET

Long-lived isotopes such as ^{3}H and ^{125}I, which are used for *in vitro* studies and for animal investigations, are not safe, and therefore, are not appropriate for human investigations. Fortunately, a number of isotopes are available which are short-lived and therefore safe for human investigations. These include ^{11}C, ^{18}F, ^{13}N, and ^{15}O (Table 1). In practice, however, the short half-lives of these isotopes (i.e. 2–110 min) limits their applicability, somewhat in proportion to the shortness of their half-life. As it is necessary to prepare the isotope in a cyclotron and then to incorporate it into a neuroreceptor ligand of interest,

Table 1

Properties of Ideal Ligands for PET-Scanning

1. Ease of rapid labeling with positron emitting isotope.
2. Adequate preclinical data from biochemical, kinetic, and anatomic quantitative methods to characterize the pharmacology of the ligand.
3. K_D values less than 10nM.
4. High selectivity.
5. Stereoselectivity.
6. Rapid penetration of blood brain barrier.
7. Little or no metabolism to active ligands.
8. Low non-specific binding.
9. Regional selectivity of specific binding.
10. Saturability of binding.
11. Acceptable biological activity at therapeutic doses.

the time delay between production of the isotope and injection of a labeled drug for scanning of a human subject means that in practice ^{18}F (half-life 110 min) is the preferred isotope, although ^{11}C isotopes (half-life 20 min) can be used in many circumstances where rapid chemical syntheses can be achieved.

The principles of double photon positron emission imaging are shown in Figure 4. Firstly, a positron emitting isotope such as ^{11}C or ^{18}F undergoes decay

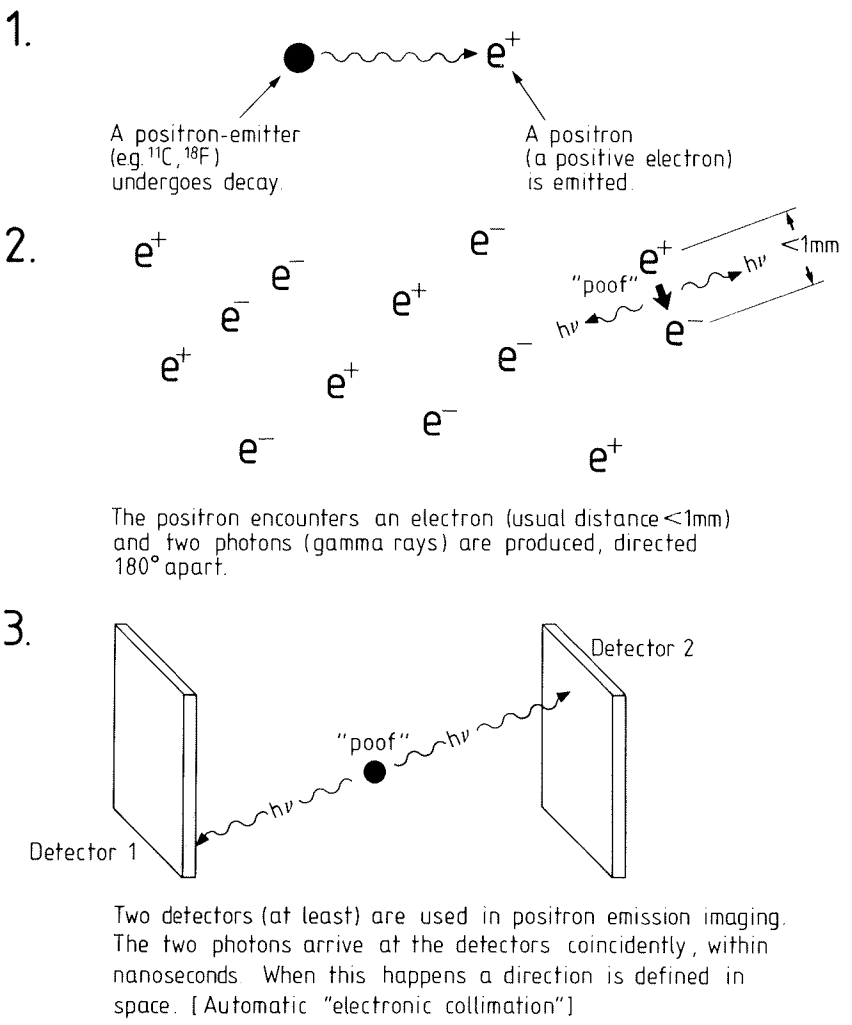


Figure 4: Principles of Double Photon Positron Emission Tomography Imaging.

and emits a positron, which is a positive electron. Secondly, the positron encounters a nearby electron and a matter/antimatter annihilation occurs ("poof"). Since the positron must travel up to a millimeter away from an electron for a reaction to occur, the spatial resolution of the image is affected by how far a distance is traversed. Thus, the visualizing of a positron will occur in a radius of 1 mm from the actual receptor where it originated. Finally, the products of the annihilation mentioned above are two photons (gamma rays) which are released in opposite directions.

If the positron emission reaction has occurred within an imaging device (Figure 5) the two photons will arrive at the detectors coincidentally within nanoseconds and be detected, quantitated, and oriented in space by a process known as automatic electronic columnation. The summation of all this data ultimately produces an image which can be viewed on the computer screen (e.g., see Figure 6).

Much of the success of neurotransmitter receptor imaging rests upon the specificity and the selectivity of the ligand of choice. Ligands in general are neurotransmitters or drugs which have been labeled with a radioactive tag. A successful ligand must achieve penetrability to the brain, not be metabolized to co-ligands which compete for binding, and achieve a high degree of receptor specific binding and a low degree of non-specific or background binding in the brain. In practice, this usually requires K_D values of 10nM or less. Under such conditions, ratios of specific to non-specific binding as high as 10, and at least as high as 2, must be achieved within a few hours after injection so as to render practical the use of these ligands for PET imaging.

A high receptor affinity appears to be an important property of the ligand if prolonged *in vivo* receptor binding is to be achieved. Other useful criteria for selection of ligands for human PET-scanning are shown in Table 1. The requirements shown in Table 1 are often not met by drugs which are otherwise useful for *in vitro* experimentation. Unfortunately, this has limited the number of isotopes available for human PET-scan imaging of receptors.

A partial list of labeled drugs which have been successfully utilized for imaging neuroreceptors by PET in either animals or humans are shown in Table 2. This is by no means a comprehensive list, as the number of drugs labeling receptors is multiplying rapidly as well as the ability to radiolabel them.

If the requirements in Table 1 are met, injection of a ligand can actualize a visual image of neurotransmitter receptors in the brain (see Figure 6). A good deal of debate exists as to whether useful information can be derived from such qualitative visual images rather than the quantitation of these images discussed below. One must remember, however, that traditional clinical neurodiagnostic techniques have relied entirely upon qualitative interpretation of images in order to gain an insight into human pathology. Thus, if neuroreceptors in humans can be labeled under standard conditions, and brain images empirically correlated with brain pathology, it is nevertheless a useful diagnostic tool.

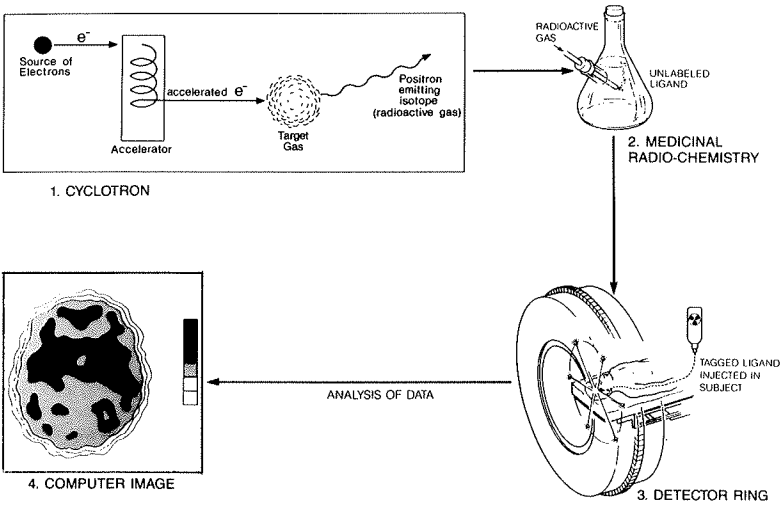


Figure 5: 1. Low energy cyclotron produces positron emitting isotopes (e.g., ^{11}C and ^{18}F). These isotopes are formed in their gaseous state.

2. The radioactive gas is used to tag the ligand which will be injected into the subject. The PET-scan shows how the brain cells consume this tagged ligand.

3. Each time a positron is emitted, and it encounters an electron, two photons (approximately 511-kiloelectron volts) are released 180° apart. Typically, after an intravenous injection of a low dose of ligand, 1 million of these photons are detected by the scintillation crystals mounted in the detector rings surrounding the head of the subject. The detector is linked to a computer where information about the time and location of photon emission is stored. The computer then uses time coincidence to determine the line of position along which the positron emitter decayed. In addition, the small differential in time of arrival of the two photons can be used to estimate the position of the source along this line of flight. A time difference in arrival at two detectors of 300 picoseconds corresponds to a 9cm travel difference. The computer then assimilates all the information about the position of the many photons released, and translates this data into an image.

4. Although photons will be emitted every time a positron meets an electron, more photons will be released from the sites of specific binding since the radioactive positron emitters are tagged to the specific binding ligand. The image displayed by the computer will show how the tagged ligand is distributed in the brain by ascribing a different colour to each level of binding.

Unfortunately, such an approach would not be based upon any quantitative understanding of what the images themselves represent in terms of classic kinetic parameters. Nevertheless, it does have the benefit of clinical judgement, and is one approach to the use of PET-scanning in humans at the present time. It is especially used by clinical neuroradiologists who are pragmatically oriented.

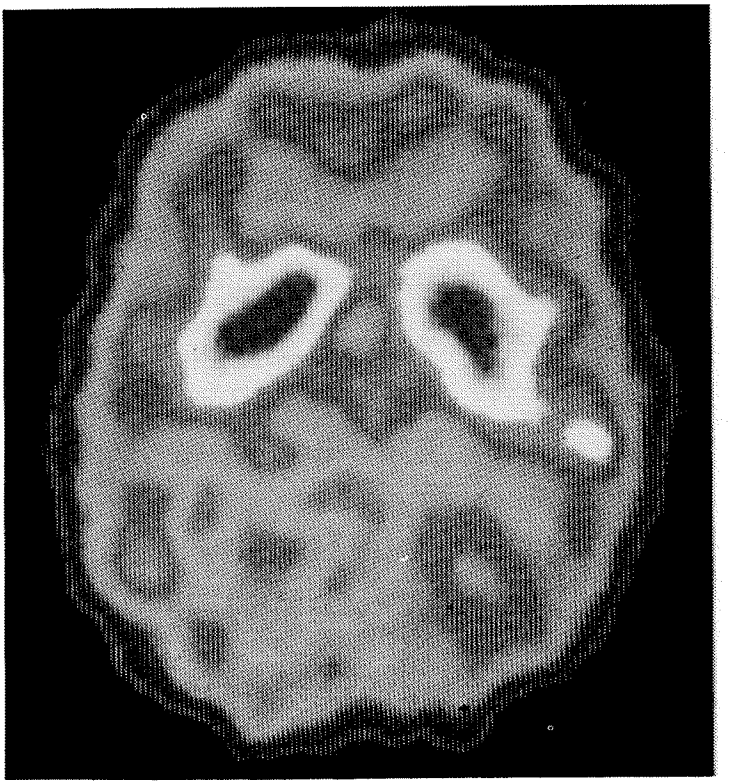


Figure 6: Axial cross section through the brain of a healthy control subject who was scanned over time for one hour after the injection of 2-(^{11}C)-methylspiperone. Although the initial distribution has a pattern similar to blood flow, gradually the specific binding of the ligand to the striatum dominates the image.

Neuroscientists and neuropharmacologists familiar with the quantitative biochemical, kinetic, and anatomical analyses of receptors *in vitro* are very enthusiastic about the possibility that such analytical tools might be applied to PET techniques to take the visual images a step further beyond mere qualitative pictures of the human brain. This pursuit is under intense current investigation.

Kinetic Analysis of Living Neuroreceptors

Quantitative PET

After experiencing the thrill of achieving the qualitative visual labeling neurotransmitter receptors in the brain, it became a goal of serious investigators in this new discipline to visualize regional receptor binding so that

Table 2

Partial List of Ligands Available for Imaging Neuroreceptors by PET

Neuroreceptor	PET Ligand
Dopamine 1	^{11}C-SCH-23390
	^{76}Br-bromo-SCH-23390
Dopamine 2	^{11}C-raclopride
	^{11}-C-piquindone
	^{11}-C-N-methyl-spiperone
	^{76}Br-bromospiperone
	^{18}F-methylspiperone
Serotonin 1A	^{11}C-buspirone
1B	^{125}I-pindolol
Serotonin 2	^{11}C-N-methyl-spiperone
	^{76}Br-spiperone
	^{18}F-methylspiperone
	^{11}C-ketanserin
	^{11}C-methylketanserin
	^{11}C-ritanserin
	^{11}C-methylbromo-LSD
Serotonin reuptake sites	^{11}C-imipramine
	^{11}C-paroxetine
	^{11}C-citalopram
Benzodiazepine	^{11}C-R015-1788
	^{11}C-suriclone
Opiates	^{11}C-carfentanil
	^{11}C-etorphine
	^{11}C-diprenorphine
Alpha-1 adrenergic	^{11}C-prazocin
Beta-adrenergic	^{125}I-pindolol
Cholinergic muscarenic	^{11}C-dexetimide
	^{11}C-QNB

at least a rough estimate, if not a quantitatively exact estimate, of neurotransmitter receptor distributions in various regions of the brain could be made (see Sedvall, Farde, Persson, and Wiesel, 1986, and Stahl, Leenders, and Bowery, 1986 for review). As mentioned earlier, only a few of the potential ligands used in *in vitro* studies are ideal for quantitative studies of PET in humans. For those acceptable ligands under current investigation, a number of mathematical models (using different compartments), as well as kinetics

(applied to either tracer doses or to Scatchard-type analysis), have been devised for PET-scans in living humans. At least four models are currently under investigation for the quantitation of PET-scan images. The first model (Gjedde, 1981; Patlak, Blasberg, and Fenstermachen, 1983) calculates an influx constant, K_i by solving equations for the ratio of brain radioactivity in different compartments of the brain to plasma radioactivity at various times of sampling. According to this method, determining the K_i value is useful because in those instances where it represents a unidirectional transfer process, it is proportional to the concentration of the substrate being transported. Several investigators have utilized this model for quantitating the uptake of fluorodopa by dopaminergic neurones. As a result, fluorodopamine synthesis from fluorodopa, is now assumed to be unidirectionally transported into synaptic vesicles. This model may not be suitable for other neurotransmitters since release and metabolism of the neurotransmitter, during the time of the PET-scan, is not accounted for by this model. It can therefore only be useful in those circumstances where such processes are assumed to be negligible. This model also does not account for metabolism of the radiolabeled ligand in the periphery. Errors are introduced if the plasma radioactivity does not represent pure unmetabolized radioligand. Nevertheless, K_i has been proposed to be a reasonable quantitative measure of synaptic vesicle dopamine concentrations and/or transport, which in turn may be a reasonable quantitative measure of how many presynaptic dopamine nerve terminals are present in the tissue slice being imaged.

Another model is the three-compartment model (Mintun et al., 1984). It categorizes radioactivity into three components: the input function from plasma, which is estimated by determining arterial radioactivity; specific plus non-specific binding which is estimated by scanning over brain region containing the receptors of interest; and finally, specific binding which can be estimated by mathematical calculations. A mathematical transformation has been proposed to quantitate receptor binding not by determining B_{max} or K_D, but by determining a parameter known as the "binding potential," which is the product of B_{max} and K_D. The binding potential may reflect the capacity of a given tissue for ligand binding site interaction.

A third model is a different three-component model (Wong et al., 1984), but also measures specific plus non-specific binding by scanning over a brain region containing the receptors of interest. However, this model measures non-specific binding by scanning over brain that does not contain receptors of interest. This model attempts to quantitate the receptor of interest by determining the ratios between two scanned areas over time. It assumes that the slope of the rising ratio over time is proportional to specific receptor binding.

Both of these three-compartment models have been applied to the neuroleptic spiperone, which probably labels mainly dopamine-2 receptors in

the striatum and mainly serotonin-2 receptors in the cortex. In neither of the models, though, is it clear that one can assume non-specific binding in brain areas that have receptors of interest to be identical with non-specific binding in areas of brain that do not have the receptors of interest. Furthermore, the kinetic assumptions for Scatchard plotting are not fulfilled. This is because the total amount of ligand (labeled plus unlabeled) is not necessarily present at an *in vivo* concentration near K_D. Whether the binding potential parameter of the three-compartment model represents a biologically meaningful measurement is also unproven at this time.

Some of the limitations of the three-compartment models mentioned above could be overcome if it is possible to do a full Scatchard plot as proposed by Sedvall and co-workers (1986). This model literally attempts to perform a Scatchard plot in the living human brain by deriving data from four to five PET-scans performed at multiple concentrations of a receptor ligand. This model also requires that the ligand be given in doses such that the total ligand concentration (i.e., labeled plus unlabeled) is present at the receptors at several concentrations at or near K_D. This in turn implies that the human subject may experience a biological effect from the drug ligand administration, which may be undesirable (for example, dystonia). The latter model not only requires that the labeled ligand have no active metabolites but also it should have the many features mentioned in Table 1. As mentioned earlier, few radioligands are suitable candidates for this technique and the labor-intensive, relatively invasive use of multiple PET-scans, with varying doses of radioligand is not always clinically feasible. Finally, estimation of the free pool of ligand from Scatchard plots in this model can be very problematic when a large amount of non-specific binding occurs. Nevertheless, whenever the technique of Scatchard plotting is available, it is a powerful quantitative methodology which can be applied towards the analysis of neuroreceptors in living human brains.

Current Applications and Future Developments

Much of the time and effort of current investigators in this field has been occupied with attempts to upgrade the utility of the high technology and biotechnology expertise so as to make PET-scanning in humans feasible and quantifiable. Now that this seems to be the case, several applications of these techniques can begin. One of the most obvious applications is to scan the brain and to develop neurotransmitter receptor maps of the human central nervous system in an attempt to create an atlas of the "anatomically addressed" human CNS. Another application would be to quantify the kinetic characteristics of neuroreceptors in normal human CNS so as to compare with the kinetics of these same receptors in various neurologic and psychiatric disorders. The possibility that receptor abnormalities exist in various neurologic or psychiatric

disorders could lead to the discovery of new diagnostic tools for such disorders.

PET-scanning also has a powerful role in assessing potentially useful new drugs. For example, the technique can be employed in order to determine whether a peripherally administered drug is getting into the central nervous system, whether it is working on the postulated receptors of interest, and what the acute effects of the new drug are on the binding at receptors. Also, many chronically administered neuropharmacological agents have important effects upon the number of neurotransmitter receptors, either up-regulating them, as in the case of chronic dopamine receptor antagonist administration, or in down-regulating them as in the case of chronic antidepressant administration. The ability to quantitate neurotransmitter receptors will allow clinical neuroscientists to determine whether the chronic administration of CNS drug therapies have important quantitative effects on specific neurotransmitter receptors. This would also be a potential manner of monitoring psychopharmacologic treatment in patients who either do or do not respond to a drug.

One exciting potential dimension of the PET-scan, which has not yet been demonstrated, is the possibility that one may use it to explore chemical neurotransmission in health and disease in a dynamic manner. That is, it may be possible that some neurotransmitter receptor ligands, such as ion channel binding agents, produce different images when the ion channel is open as compared to when it is closed. Thus, a region of the brain in which a great deal of neurotransmission is occurring, such as during a seizure or during a degenerative episode of neurones, may have a different state-dependent neuroreceptor binding profile than normal brain. This provocative but unproven possibility remains an exciting one for monitoring the status of receptors during the stages of illness of the brain. Finally, the PET-scan offers exciting possibilities for the discovery of new drugs and new receptors in brain as radionuclides become increasingly available as new chemical entities from the pharmaceutical industry.

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for studies requiring high spatial resolution, regional quantification, or rapid sequential imaging.

The rotating gamma camera approach is preferable for routine clinical imaging, because of its availability and because it can be used for other types of tomographic and non-tomographic imaging. The major constraint on rotating gamma camera tomography is sensitivity. The low sensitivity for each tomographic slice is compensated by the fact that the gamma camera collects volumetric information as opposed to the single slice information obtained with the multidetector system. Improvements in collimator design (the slant-hole, long-bore, and fan-beam collimators, for example) and in reconstruction algorithms have substantially improved the quality of SPECT perfusion images using the Anger-type gamma camera. Satisfactory tomographic imaging has been achieved with the rotating gamma camera using all of the perfusion agents described above.

Quantification of Cerebral Blood Flow

The initial distribution of I-123 IMP is directly proportional to regional cerebral blood flow. Quantification of flow after intravenous injection is not an easy matter, however. There are both biologic constraints imposed by the mathematical model, and physical contraints imposed by the instrumentation.

A variation of the indicator fractionation technique of Saperstein has been proposed to measure regional blood flow using the tracer I-123 IMP (Kuhl et al., 1982). This method assumes a diffusible tracer and requires that: (1) the tracer is delivered continuously to the brain, (2) the tracer is freely diffusible and completely removed on a single pass through the brain, (3) the tracer back diffuses slowly from the brain to the blood, and (4) the tracer can be distinguished from its polar metabolites.

These techniques depend upon accurate measurement of the absolute tracer activity within regions of the brain. Currently, only special purpose imaging devices come close to meeting the specifications for such a task. Photon attenuation and partial volume effects add to the difficulty.

It is probably more realistic and certainly simpler to limit quantitative schemes to the measurement of relative activities within brain regions. The ratio of activities between left and right regions can be used to evaluate asymmetries in focal diseases such as cerebral infarction while the ratio of activities between affected and unaffected regions can be used in degenerative disease such as Alzheimer's disease. The major biological constraint on relative quantitation of tracer uptake is that, while initial uptake is proportional to blood flow, tracer distribution becomes a function of the partition coefficient with efflux. Therefore, imaging must be carried out as soon after injection and as rapidly as possible and tracers should be used with as little efflux from the brain as possible. While relative quantification of tracer uptake will not

of positron emission tomography (PET) facilities have suggested that the abnormalities in flow and metabolism are focal and most extensive in the posterior temporoparietal area.

Those techniques which have been developed to study regional brain perfusion and metabolism in Alzheimer's disease have used either positron emitting tracers or radioactive gases. Both methodologies require special purpose instrumentation and are technically difficult to perform with precision and accuracy. PET is an exceedingly costly technology and is inappropriate for patient screening and for evaluating neurologic diseases in any setting other than a tertiary medical center. PET should be restricted to helping us better understand the physiology of disease processes and to developing potential applications which will lead to the development of appropriate commercially available single photon radiotracers.

A family of amines have been developed which accumulate in the brain proportional to cerebral blood flow. I-123 IMP (isopropyl iodoamphetamine) and its related radiocompounds have an initial distribution related to regional cerebral blood flow. The tracers remain within the brain without significant clearance for sufficiently long intervals so that tomography can be performed either using special purpose instrumentation or the more generally available rotating gamma camera. With I-123 IMP or the more recently introduced Tc-99m labelled cerebral perfusion agents, we can begin to explore the potential clinical role of cerebral profusion imaging in the dementias.

Radiopharmaceuticals

IMP

N-isopropyl I-123 p-iodoamphetamine (IMP) is highly lipophilic, moving across the blood:brain barrier with almost complete extraction during a single passage through the cerebral circulation (Hill et al., 1982; Winchell, Horst, Braun, Oldendorf, Hattner, Parker, 1980). The initial intracerebral distribution of IMP follows regional cerebral blood flow (Kuhl et al., 1982). Once inside the brain, it binds to nonspecific sites forming a lipophobic complex. As a result, IMP does not redistribute significantly within the brain for at least an hour after intravenous injection.

In the human, brain uptake of IMP is rapid, reaching 45 percent of its maximum in two minutes (Holman, Lee, Hill, Lovett, Lister-James, 1984). While there is clearance of the tracer from the brain, probably due to the production of lipophilic metabolites, this washout is counter-balanced by slow release of IMP from the lungs back into the circulation. Thus, brain activity remains constant from 20 minutes to at least 60 minutes after injection. Although the gray/white matter activity ratio remains constant during the immediate postinjection period, intracerebral redistribution of the tracer does

occur and can be demonstrated by one hour or less by autoradiography (Moretti et al., 1983). By 24 hours, the gray/white matter activity ratio has reversed and activity is higher in the white matter (Holman et al., 1983).

The initial uptake of IMP correlates with cerebral blood flow over a wide range of flows (Kuhl et al., 1982), but may be altered in some diseases independent of changes in cerebral blood flow. While most tumors do not concentrate IMP, cerebral metastases from malignant melinomas and oat cell tumors take up the tracer avidly, particularly if they are actively metabolizing monoamine amphetamine precursors (Holman, Wick et al., 1984). These observations have important ramifications if cerebral blood flow is to be measured using IMP and point to the need for a thorough understanding of the effects of drugs and diseases on the biodistribution of these tracers before they are used routinely to assess cerebral perfusion.

HIPDM

Kung and his colleagues have investigated a group of iodophenolic diamines which, like IMP, exhibit high uptake and long retention times in the brain (Kung, Tramposch, and Blau, 1983). Their most promising agent, N,N,N′-trimethyl-N′-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine (HIPDM), shows rapid brain uptake in the monkey reaching four to five percent of the injected dose within one hour after injection.

HIPDM is taken up more rapidly by the brain than IMP, reaching 75 percent of maximum brain levels by two minutes (Holman, Lee et al., 1984). The peak brain activity is significantly lower for HIPDM than for IMP, however. The lung clearance is slower for HIPDM than for IMP and the amount of tracer reaching the liver is less. Despite its limitations relative to I-123 IMP, I-123 HIPDM has been used successfully in numerous clinical studies of regional cerebral perfusion.

T-201 DDC

Thallium-201 diethyldithiocarbamate (DDC) has recently been suggested as an alternative to I-123 IMP (deBruine, van Royen, Vyth, deJong, van der Schoot, 1985). The development of this imaging agent is based on the observation that exposure of thallium intoxicated patients to large doses of DDC causes increased neurotoxicity. Tomographic images obtained after the intravenous injection of the tracer show high gray matter uptake and look similar to images obtained with I-123 IMP. The perfusion map obtained with T1-201 DDC is very similar to that obtained with I-123 IMP in terms of both quality and biodistribution. There is almost complete extraction of the tracer after carotid injection. Unlike I-123 IMP, T1-201 DDC washes out of the brain very slowly with no evidence of washout 60 minutes after carotid injection.

Can perfusion SPECT imaging play a role in the assessment of patients suspected of having Alzheimer's disease? To answer this question properly, we must divide the issue into manageable parts. The first and easiest question to answer is: Can patients with a clinical diagnosis of Alzheimer's disease be distinguished from age-matched controls? In such patients, marked decreases in blood flow are seen particularly in the parietal area, suggesting that there may be a perfusion signature which could be helpful in the identification of the disease (Figure 2). We studied a group of patients who met NINCDS-ADROA criteria for Probable Alzheimer's disease and compared them to age matched controls (Johnson, Mueller, Walshe, English, and Holman, 1985). We compared the I-123 IMP activities in the parietal, frontal, temporal and striate cortex to the activity in the cerebellum. We normalized the tracer activity to the cerebellum because the cerebellar activity is the same in Alzheimer's disease as it is in the age-matched controls, and because the cerebellum does not demonstrate pathologic changes when examined at post-mortem.

In our study, the cortical:cerebellar ratios were significantly lower for most regions of the cortex in patients with Alzheimer's disease than in the control subjects. Relative I-123 IMP uptake was decreased in the posterior parietal (73% of control), frontal (77%), and lateral (76%), medial (82%) and posterior temporal (83%) cortex. Smaller reductions were seen in the striate cortex (91%). The posterior parietal cortex was the most sensitive marker of

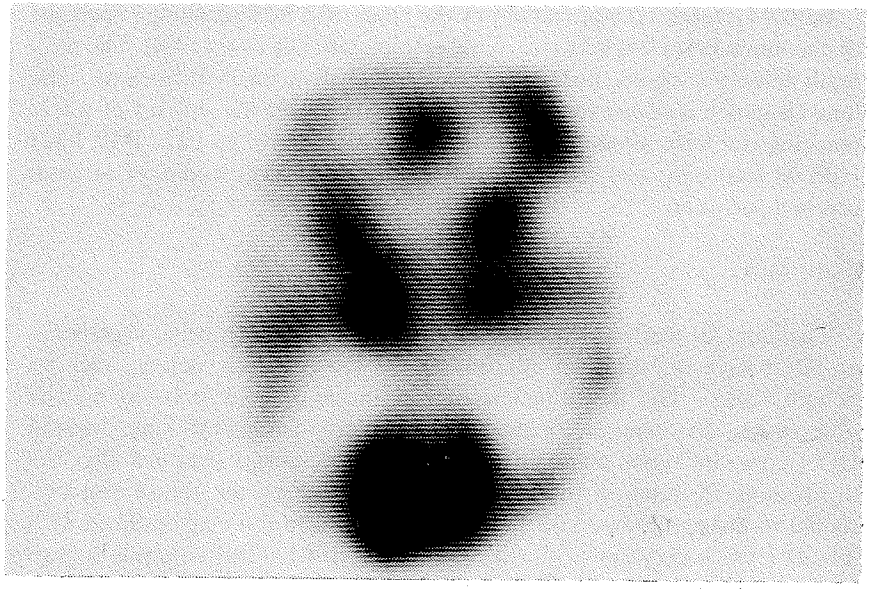

Figure 2: SPECT in a patient with Alzheimer's disease. Note decreased IMP uptake, particularly in the associative cortex.