FINAL REPORT

06/01/1997 to 05/30/2000

Development of Dopamine Receptor Radiopharmaceuticals For The Study of Neurological and Psychiatric Disorders

DE-FG02-ER62540

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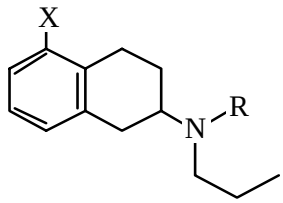
Our goals in this grant application are directed towards the development of radiotracers that may allow the study of the high-affinity state (functional state) of the dopamine receptors. There have been numerous reports on the presence of two inter-convertible states of these (G-protein coupled) receptors *in vitro*. However, there is no report that establishes the presence of these separate affinity states *in vivo*. We have made efforts in this direction in order to provide such direct *in vivo* evidence about the presence of the high affinity state. This understanding of the functional state of the receptors is of critical significance in our overall diagnosis and treatment of diseases that implicate the G-protein coupled receptors. Four specific aims have been listed in the grant application: (1). Design and syntheses of agonists (2). Radiosyntheses of agonists (3). *In vitro* pharmacology of agonists (4). *In vivo* distribution and pharmacology of labeled derivatives. We have accomplished the syntheses and radiosyntheses of three agonist radiotracers labeled with carbon-11. *In vitro* and *in vivo* pharmacological experiments have been accomplished in rats and preliminary PET studies in non-human primates have been carried out. Various accomplishments during the funded years, briefly outlined in this document, have been disseminated by several publications in various journals and presentations in national and international meetings (Society of Nuclear Medicine, Society for Neuroscience and International Symposium on Radiopharmaceutical Chemistry).

1. Design and Syntheses of Agonists.

Our basic approach towards the development of tracers that may allow the study of high affinity states arises from the following facts: Critical amino acid residues in the D-2 receptor serve two principal purposes: 1) bind dopamine and 2) trigger a response subsequent to binding of dopamine. Structural requirements of dopamine in order to attain the two separate goals are distinct but have some overlap. Our approach has been two-fold: One is the development of "high affinity agonists", such as ZYY-339 and its analogs; and second is the development of "silent agonists".

Silent Agonists

A number of agonists for the dopamine D-2 receptor have been reported. A significant amount of evidence has accumulated using dopamine D-2 receptor agonists which show the importance of interaction of the nitrogen as well as the hydroxyl groups. Extensive site-directed mutagenesis studies in adrenergic receptors have inferred similar findings on the importance of the nitrogen in binding to the receptor active site and the role of the catecholic hydroxyl groups in interacting with the serine residues (Mansour et al., 1990; Cox et al., 1992). Interaction of the hydroxyl groups with the serine residues results in signal transduction via coupling to G-proteins.



Silent Agonists

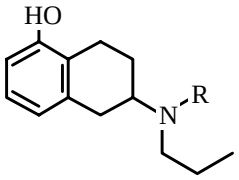
X = H, F, Cl; R = Alkyl, Cycloalkyl, Fluorocycloalkyl

Figure-1. Chemical structures of silent agonists.

We had postulated that by removing the hydroxyl group and substituting it with a hydrogen, chlorine etc (Figure-1) would provide "silent agonists" with slower dissociation rate constants. This would then make the development of imaging agents based on agonists somewhat easier. However, our binding affinity data and some reported data indicates the necessity of the hydroxyl group at the 5-position in order to maintain high affinity for the D-2 receptors. It may be inferred from this preliminary data that the hydroxyl group may play a more significant role at the 5-position. Thus in summary, silent agonists without the 5-hydroxyl group exhibit low affinity and may not thus be able to serve as good PET imaging agents.

High Affinity Agonists

Using structure-activity information on the various agonists for dopamine receptors, such as 2-(*N*-phenethyl-*N*-propyl)-amino-5-hydroxytetralin (PPHT), we have synthesized high affinity agonists which may be developed as potential *in vivo* radiotracers in order to exclusively study the high-affinity state. We have prepared various analogs of PPHT which contain a alkyl, substituted aryl and cyclohexyl group instead of a phenyl group. Incorporation of the cyclohexyl group as in 2-(*N*-cyclohexylethyl-*N*-propyl)amino-5-hydroxytetralin (ZYY-339) significantly enhances the affinity of these compounds. In order to radiolabel the agonists with carbon-11, three compounds which differed in their affinities for the D-2 receptor were chosen for in vivo studies (Figure-2).



Compound	R	Affinity (nM) [a]	Log P [b]
1.	$CH_3CH_2CH_2$ (5OH-DPAT)	2.5±0.40	1.37±0.03
2.	$C_6H_5CH_2CH_2$ (PPHT)	0.65±0.10	3.034±0.003
3.	$C_6H_{11}CH_2CH_2$ (ZYY-339)	0.01±0.005	3.089±0.006

[a] Affinity, IC_{50} values using ^{3}H-spiperone; [b] Log P were measured by partitioning the compounds between *n*-octanol and phosphate buffer at pH 7.4.

Figure-2: In Vitro Affinities and Lipophilicity of Selected Agonists

2. Radiosyntheses of agonists.

In order to evaluate the ability of the high affinity agonists in vivo, we carried out carbon-11 radiosynthesis of three derivatives (a general reaction scheme is shown for ^{11}C-ZYY-339 in Figure-3). Radiosynthesis of 2-(*N*-cyclohexylethyl-*N*-1'-^{11}C-propyl)amino-5-hydroxytetralin (^{11}C-ZYY-339), 2-(*N*-phenethyl-*N*-1'-^{11}C-propyl)amino-5-hydroxytetralin (^{11}C-PPHT) and 2-(*N*-propyl-*N*-1'-^{11}C-propyl)amino-5-hydroxytetralin (^{11}C-5OH-DPAT) has been carried out. Radiosynthesis of ^{11}C-propionyl chloride and subsequent coupling to form an amide of the respective precursors followed by reduction provides the radiotracers in 5 to 10% radiochemical yield in specific activities of 500 to 1000 Ci/mmol after reverse-phase HPLC purification.

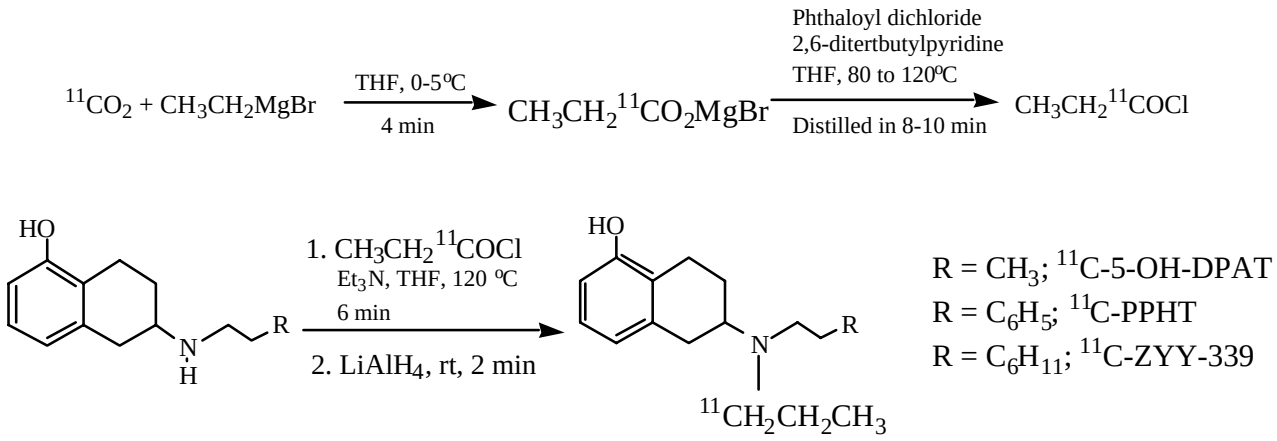


Figure-3: General Scheme for Carbon-11 Radiolabeling of the Agonists

In vitro autoradiographic studies have been completed with all the three carbon-11 derivatives and a manuscript describing the radiosyntheses and in vitro autoradiographic data has been published (Shi et al., 1999).

3. *In vitro* pharmacology of agonists.

In ^{3}H-spiperone assays using rat striata, ZYY-339 exhibited subnanomolar affinity for D-2 receptor high-affinity sites (IC_{50} = 0.010 nM), while 2-(*N*-phenethyl-*N*-propyl)amino-5-hydroxytetralin (PPHT) and 2-(*N*, *N*-dipropyl)amino-5-hydroxytetralin (5OH-DPAT) exhibited an affinity of 0.65 nM and 2.5 nM, respectively. Removal of the phenolic hydroxyl however, decreased the affinity dramatically. We also analyzed the effect of 5'-guanylylimidodiphosphate (Gpp(NH)p, 150 microM), the non-hydrolysable GTP analog on ZYY-339 using ^{3}H-spiperone assays. Binding of ZYY-339 was reduced by more than an order of magnitude at the high affinity site, indicative of an agonist behavior. Further pharmacological studies of other derivatives (agonists and silent agonists) are in progress and a manuscript describing these findings is under preparation (Yang et al., 1999).

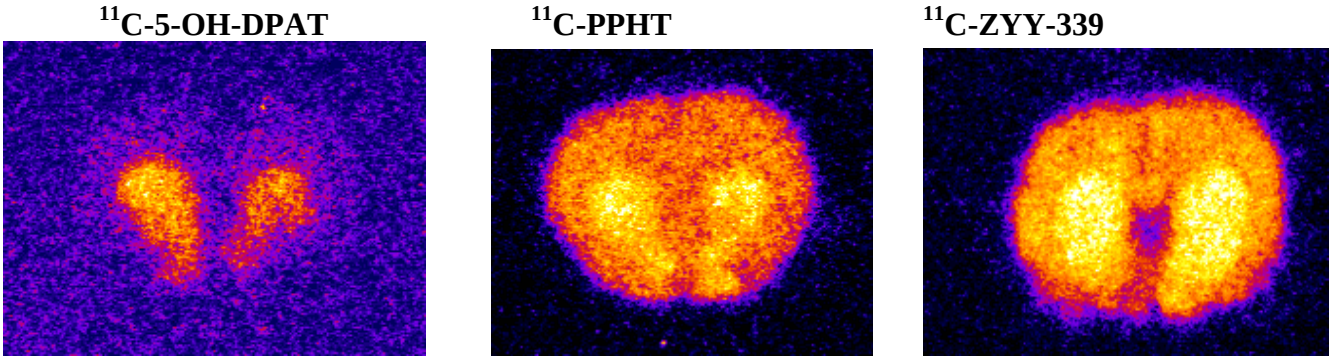


Figure-4 *In Vitro* Rat Brain Sections showing binding of ^{11}C-DPAT, ^{11}C-PPHT and ^{11}C-ZYY-339

Coronal tissue sections (20 µm) were placed in 50 mM Tris HCl buffer (pH 7.4, 25 ºC) containing 120 mM NaCl and 5 mM KCl and were preincubated for 15 minutes. The slices were then incubated with ^{11}C-5-OH-DPAT, ^{11}C-PPHT and ^{11}C-ZYY-339 at concentrations of approx. 0.1 to 1 nM) for 30 minutes at 25 ºC. Non-specific binding was defined as the binding remaining in the presence of 10 µM (*S*)-sulpiride. Following incubation, tissue sections were briefly washed twice for 0.5 min period each with cold 50 mM Tris HCl buffer, pH 7.4, followed by a quick rinse in cold deionised water. The slices were apposed to phosphor screens and read by the Cyclone (Figure-4).

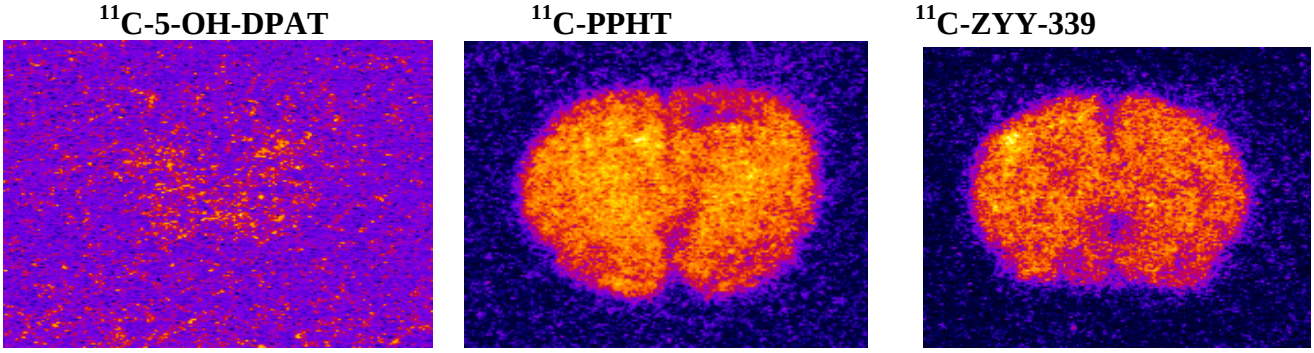


Figure-5. *In Vitro* Rat Brain Sections Showing Effects of Gpp(NH)p on the Binding of ^{11}C-DPAT, ^{11}C-PPHT and ^{11}C-ZYY-339

In the case of experiments with 5'-guanylylimidophosphate (Gpp(NH)p), which is known to convert the HA-affinity sites to LA-affinity sites (Nobrega and Seeman, 1994), brain slices were preincubated for 15 mins at 25 ^{o}C with the above mentioned buffer containing 150 µM of (Gpp(NH)p). The slices were incubated with ^{11}C-5-OH-DPAT, ^{11}C-PPHT and ^{11}C-ZYY-339 at concentrations of approx. 0.1 to 1 nM) for 30 minutes at 25 ^{o}C. Non-specific binding was defined as the binding remaining in the presence of 10 µM (*S*)-sulpiride. Specific binding with all the three radiotracers was significantly reduced suggestive of high-affinity state binding (Figure-5).

4. *In vivo* distribution and pharmacology of labeled derivatives.

In vivo studies with the radiotracers were carried out by intracardiac administration of the respective radiotracers in groups of Sprague-Dawley rats (controls, prereserpinized with 5 mg/kg and haloperidol, 1 mg/kg, pretreated). Male Sprague-Dawley rats (approx. 200 g) were administered 100 µCi of ^{11}C-5-OH-DPAT by intracardiac injection in a volume of 200 µl. A group of rats were prereserpinized (5 mg/kg, ip) 24 hrs prior to the experiment. Haldol (1 mg/kg) was injected 15 min. prior to the injection of the radiotracer. Animals were sacrificed at 15 mins pi. Figure-6 shows the selective binding in the striata which was reduced in the presence of haloperidol. It must be noted that all these experiments are with the racemic mixture and thus a significant amount of non-specific binding may be attributed to the less-active isomer.

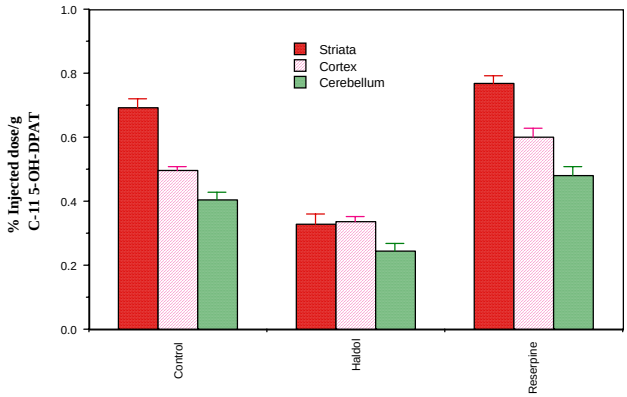


Figure-6. ^{11}C-5-OH-DPAT in Rat Brain Regions

Both ^{11}C-ZYY-339 and ^{11}C-PPHT indicated preferential localization in the striata compared to cerebellum (S/C = 1.94 and 1.22 respectively; Figure-7). Pretreatment with reserpine improved the binding in the striata for both radiotracers as seen in the striata/cerebellum ratios (2.17 and 1.44, respectively), whereas haldol reduced this selective binding. A high degree of cortical binding was observed with both the radiotracers. Improvements of specific activities are also underway in order to improve the extent of specific

binding. Further experiments are underway in order to demonstrate the nature of this binding i.e., to the high and/or low affinity state of the D-2 receptor using autoradiographic methods.

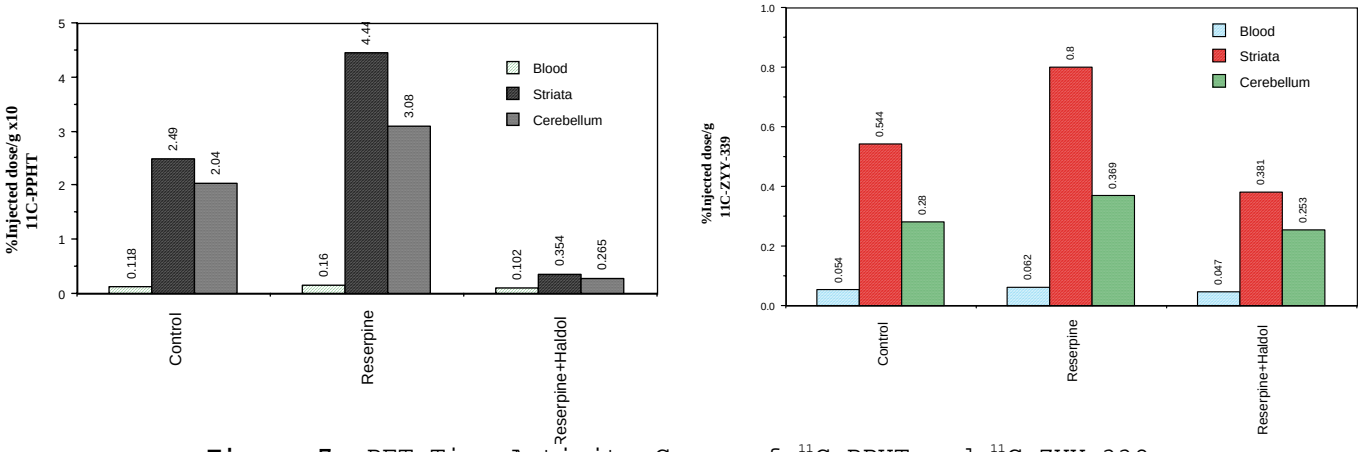


Figure-7. PET Time-Activity Curve of ^{11}C-PPHT and ^{11}C-ZYY-339 in Rat Brain Regions

5. PET Studies of labeled derivatives in Monkeys.

Rhesus monkeys were used to carry out PET studies with the agonists. The head of the animal was placed in the gantry of a Siemen's CTI HR+ scanner and positioned in place with the use of adhesive tape. Image slices of the whole brain parallel to the canthomeatal plane were acquired in 3-D mode. A 2.4 mm thick brain slice from a PET study of a rhesus monkey post-injection of ^{11}C-5-OH-DPAT (3.43 mCi). Localization of ^{11}C-5-OH-DPAT in the striata is seen clearly, while some cortical binding is also seen (Figure-8). The image is a sum of dynamic frames from 11 to 57 minutes.

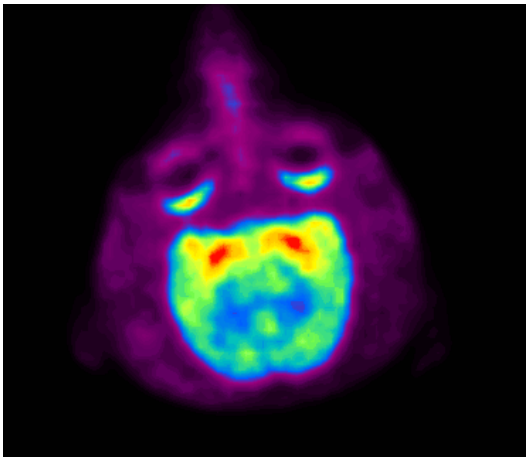


Figure-8. PET Time-Activity Curve of ^{11}C-5-OH-DPAT in Monkey Brain Regions

Image slices of the whole brain parallel to the canthomeatal plane were acquired in 3-D mode. A 2.4 mm thick brain slice from a PET study of a rhesus monkey post-injection of ^{11}C-PPHT (2.5 mCi). Localization of ^{11}C-PPHT in the striata is seen clearly, with a larger amount of cortical binding compared to that of 5-OH-DPAT is seen (Figure-9). The image is at 57 minutes.

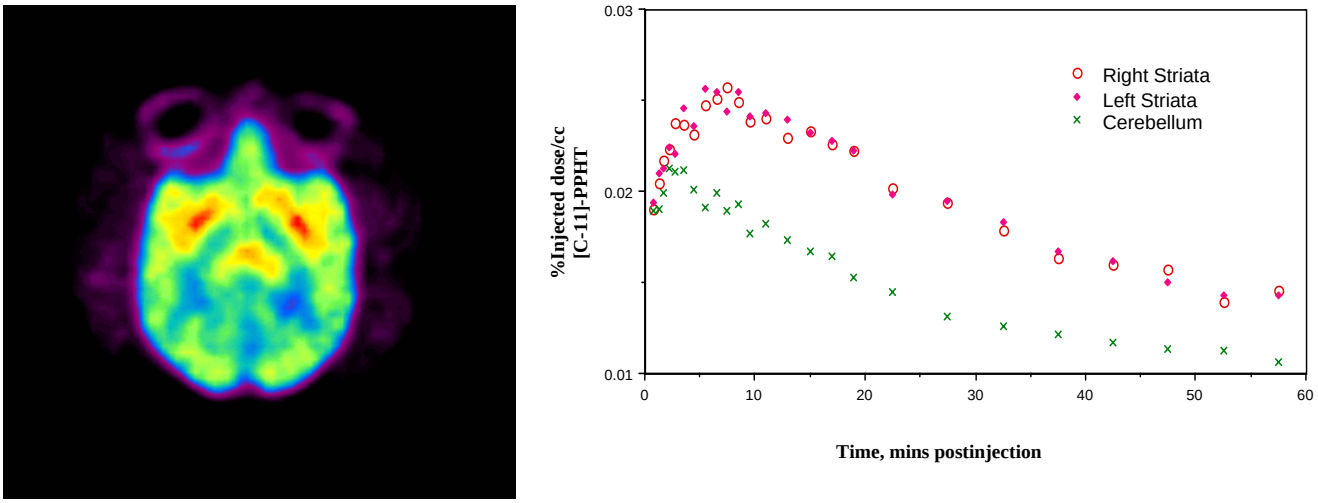


Figure-9. PET Time-Activity Curve of ^{11}C-PPHT in Monkey Brain Regions

Image slices of the whole brain parallel to the canthomeatal plane were acquired in 3-D mode. A 2.4 mm thick brain slice from a PET study of a rhesus monkey post-injection of ^{11}C-ZYY-339 (0.75 mCi). Localization of ^{11}C-ZYY-339 in the striata is seen clearly, while a significant amount of cortical binding is also seen (Figure-10).

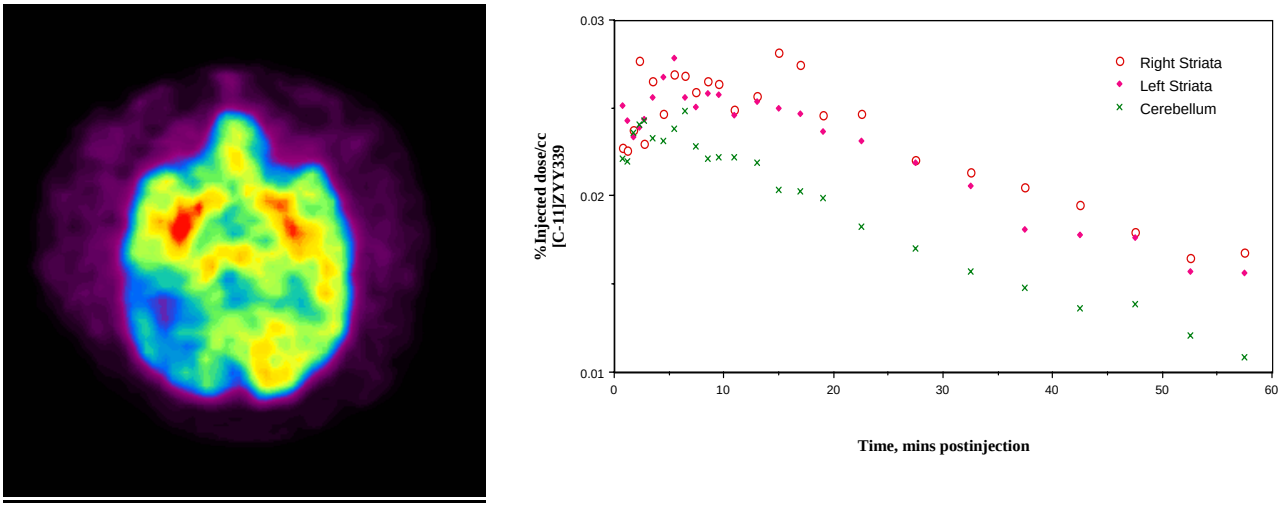


Figure-10. PET Time-Activity Curve of ^{11}C-ZYY-339 in Monkey Brain Regions

Image slices of the whole brain parallel to the canthomeatal plane were acquired in 3-D mode. A 2.4 mm thick brain slice from a PET study of a rhesus monkey post-injection of ^{11}C-ZYY-339 (0.7 mCi).

Localization of ^{11}C-ZYY-339 in the striata (red) is seen clearly, while some cortical binding is also seen (yellow-green). The image is a sum of dynamic frames from 42 to 57 minutes.

Summary

- We have prepared the high affinity dopamine D-2 receptor agonist derivatives ZYY-339 and its various analogs. Guanine nucleotide analog, Gpp(NH)p reduces its affinity *in vitro*.
- Attempts to develop analogs without the phenolic hydroxy group as potential PET radiotracers were not successful due to the marked reduction in the D-2 receptor affinity.

- Carbon-11 analogs, ^{11}C-5-OH-DPAT, ^{11}C-PPHT and ^{11}C-ZYY-339 have been succesfully prepared in moderate radiochemical yields.
- The three agonists, ^{11}C-5-OH-DPAT, ^{11}C-PPHT and ^{11}C-ZYY-339 indicated preferential localization in the striata compared to the cerebellum. Pretreatment with reserpine improved the binding in the striata for both radiotracers as seen in the striata/cerebellum ratio plots below, whereas haldol reduced this selective binding.
- Autoradiographic experiments, *in vitro*, indicate the binding of the three agonists to the high-affinity sites. Efforts will be made to demonstrate the high-affinity state binding *in vivo*.
- Preliminary PET studies with the three agonists indicate selective localization in the striata. A striata to cerebellum ratio of approximately 2 was obtained in the case of the three radiotracers. It must be noted that all experiments have been carried out with the racemic (*R,S*)- ^{11}C-5-OH-DPAT. It is known that the (*S*)-isomer of 5-OH-DPAT has higher affinity than the (*R*)-isomer (Malmberg et al., 1994). It is therefore likely that the radiolabeling of the pure (*S*)-isomer of 5-OH-DPAT may result in a higher target to nontarget ratios. Furthermore, due to the somewhat complex radiosynthetic procedures, specific activities of ^{11}C-5-OH-DPAT are approximately 500 to 1000 Ci/mmol and are considered to be moderate (Shi et al., manuscript under review). It is likely that improvements in specific activities will further improve the quality of the PET data. Studies are currently underway in order to identify one of the agonists for further investigations.

Publications During this Grant Period

Journal Articles

1. **Mukherjee, J**., Yang, Z.Y., Lew, R., Brown, T., Kronmal, S., Cooper, M. and Seiden, L.S.: Evaluation of *d*-amphetamine effects on the binding of dopamine D-2 receptor radioligand, [F-18]fallypride in non-human primates using positron emission tomography. *Synapse*, **27**: 1-13, 1997.
2. Kao, C-M., Yap, J.T., **Mukherjee, J**. and Wernick, M.N.: Image reconstruction for dynamic PET based on low-order approximation and restoration of the sinogram. *IEEE Transactions in Medical Imaging*, **16**: 738-749, 1997.
3. **Mukherjee, J**. and Yang, Z-Y.: Evaluation of monoamine oxidase B inhibition by fluoxetine (Prozac): An in vitro and in vivo study. *Eur. J. Pharmacol*., **337**: 111-114, 1997.
4. **Mukherjee, J**., Das, M.K., Yang, Z-Y. and Lew, R.: Evaluation of the binding of the radiolabeled antidepressant drug, ^{18}F-fluoxetine (^{18}F-Prozac) in the rodent brain: An *in vitro* and *in vivo* study. *Nucl. Med. Biol.*, **25**: 605-610, 1998.
5. **Mukherjee, J**. and Yang, Z-Y.: Monoamine oxidase A inhibition by fluoxetine (Prozac): An in vitro and in vivo study. *Synapse*, **31**: 285-289, 1999.
6. **Mukherjee, J**., Yang, Z.Y. and Lew, R.: *N*-(6-[F-18]Fluorohexyl)-*N*-methylpropargylamine: A fluorine-18 labeled monoamine oxidase B inhibitor for potential use in PET studies. *Nucl. Med. Biol*., **26**: 111-116, 1999.
7. **Mukherjee, J**. and Yang, Z.Y.: Development of *N*-[3-(2',4'-dichlorophenoxy)-2-^{18}F-fluoropropyl]-*N*-methylpropargylamine (^{18}F-fluoroclorgyline) as a potential PET raditracer for monoamine oxidase. *Nucl. Med. Biol*., **26** (6): 619-625, 1999.
8. Yang, Z-Y. and **Mukherjee, J**.: *N*-[1-Cyclopropylmethyl-2-pyrrolidinyl)methyl] substituted benzamides: Synthesis and dopamine D-2 and D-3 receptor binding affinities. *Med. Chem. Res*., **9**: 1-8, 1999.
9. **Mukherjee, J**., Yang, Z.Y., Brown, T., Wernick, M., Yasillo, N.J., Ouyang, X., Chen, C-T., Mintzer, R. and Cooper, M.: Preliminary assessment of extrastriatal dopamine D-2 receptor binding in the rodent and non-human primate brains using the high affinity radioligand, [F-18]fallypride. *Nucl. Med. Biol.*, **26** (5): 519-527, 1999.

10. Shi, B., Narayanan, T.K., Yang, Z.Y., Christian, B.T. and **Mukherjee, J**.: Radiosynthesis and in vitro evaluation of 2-(*N*-alkyl-*N*-1-^{11}C-propyl)-aminotetralin analogs as high affinity dopamine D-2 receptor agonists for use as potential PET radiotracers. *Nucl. Med. Biol.*, **26** (7): 725-735, 1999.
11. **Mukherjee, J**., Narayanan, T.K., Christian, B.T., Shi, B., Dunigan, K. and Mantil, J.: *In vitro* and *in vivo* evaluation of the binding of the dopamine D-2 receptor agonist, ^{11}C-(*R,S*)-5-hydroxy-2-(di-*n*-propylamino)tetralin in rodents and non-human primate. *Synapse,* **37**: 64-70, 2000.

Presentations

1. Pan, X., La Riviere, P.J., Ye, J., **Mukherjee, J**. and Chen, C.T.: Efficient sinogram smoothing for dynamic neuroreceptor PET imaging. Presented at the *SPIE Medical Imaging Symposium*, Newport Beach, California, February 22-28, 1997.
2. **Mukherjee, J**., Das, M., Yang, Z-Y., Brown, T., Lew, R., Cook, E. Jr. and Cooper, M.: Is monoamine oxidase inhibition involved in the therapeutic effects of Prozac? Presented at The Society of Nuclear Medicine 44th Annual Meeting, June 2-5, 1997, San Antonio, Texas (*J. Nucl. Med*., **38**: 284P, 1997).
3. **Mukherjee, J**. and Yang, Z-Y.: Evaluation of monoamine oxidase B inhibition by Prozac. Presented at the Society for Neuroscience 27th Annual Meeting, New Orleans, LA., October 25-30, 1997 (*Soc. Neurosci. Abstr*., **23**: 1997; abstract #435.4).
4. **Mukherjee, J**. and Yang, Z-Y.: Monoamine oxidase inhibition in vivo by fluoxetine. Presented at The Society of Nuclear Medicine 45th Annual Meeting, June 7-11, 1998, Toronto, Canada (*J. Nucl. Med*., **39**: 41P, 1998).
5. **Mukherjee, J**. and Yang, Z-Y.: *N*-(6-[F-18]fluorohexyl)-N-methylpropargylamine: A PET radiotracer for monoamine oxidase B. Presented at The Society of Nuclear Medicine 45th Annual Meeting, June 7-11, 1998, Toronto, Canada (*J. Nucl. Med*., **39**: 231P, 1998).
6. Christian, B., **Mukherjee, J**., Cooper, M., Adityanjee, Morris, E., Satter, M. and Mantil, J.: Specific binding of [F-18]fallypride and [F-18]desmethoxyfallypride in Macaque monkeys. Presented at The Society of Nuclear Medicine 45th Annual Meeting, June 7-11, 1998, Toronto, Canada (*J. Nucl. Med*., **39**: 207P, 1998).
7. **Mukherjee, J**.: In vivo competition of dopamine with antagonists: A hypothesis for fallypride and raclopride. Presented at "*The Second International Symposium on Functional Neuroreceptor Mapping of Living Brain*", Ann Arbor, Michigan, June 12-14, 1998 (*Neuroimage*, **7**(4):A9, 1998).
8. **Mukherjee, J**., Shi, B. and Yang, Z-Y.: Development of dopamine D-2 receptor agonists as potential in vivo imaging agents for PET. Presented at the Society for Neuroscience 28th Annual Meeting, Los Angeles, CA., November 7-12, 1998 (Session 340.2).
9. Roberts, A.D., DeJesus, O.T., Schneider, M.L., Schueller, M.J., Shelton, S.E., **Mukherjee, J**. and Nickles, R.J.: Dopamine system characterization of rhesus monkeys exposed in utero to moderate dose of alcohol. To be presented at The Society of Nuclear Medicine 46th Annual Meeting, June 6-10, 1999, Los Angeles, California (*J. Nucl.Med*., **40**: 108P, 1999).
10. **Mukherjee, J**., Shi, B. and Narayanan, T.K.: Evaluation of dopamine D-2 receptor agonists, ^{11}C-ZYY-339 and ^{11}C-PPHT as potential in vivo imaging agents. Presented at The Society of Nuclear Medicine 46th Annual Meeting, June 6-10, 1999, Los Angeles, California (*J. Nucl.Med*., **40**: 304P, 1999).
11. Christian, B.T., **Mukherjee, J**., Shi, B. and Narayanan, T.K.: Using parametric images of [F-18]fallypride to measure striatal and nonstriatal binding. Presented at The Society of Nuclear Medicine 46th Annual Meeting, June 6-10, 1999, Los Angeles, California (*J. Nucl.Med*., **40**: 287P, 1999).
12. **Mukherjee, J**., Shi, B., Narayanan, T.K. and Yang, Z.Y.: "Radiopharmaceuticals for Imaging the Brain". To be Presented at the symposium on "*Future of Nuclear Medicine Physics and Instrumentation*", Chicago, IL., March 19-21, 1999.

13. Shi, B., Narayanan, T.K., Yang, Z.Y. and **Mukherjee, J**.: Radiosynthesis and in vitro evaluation of 2-(*N*-alkyl-*N*-1-^{11}C-propyl)-aminotetralin analogs as high affinity agonists for dopamine D-2 receptors. Presented at the *XIIIth International Symposium on Radiopharmaceutical Chemistry*, St. Louis, Canada, MO., June 27 to July 1, 1999 (*J. Label. Compds. Radiopharm*., **42**:S384-S386, 1999).
14. **Mukherjee, J**., Narayanan, T.K., Shi, B. and Christian, B.T.: Evaluation of dopamine D-2 receptor occupancy in vitro by clozapine in rodents using ^{18}F-fallypride. Presented at the Society for Neuroscience 29th Annual Meeting, Miami Beach, Florida, October 23-28, 1999 (Session 630.11).