

## 6. Brain Imaging Project



Tetsuya Suhara, M.D., Ph.D.  
 Director, Department of  
 Molecular Neuroimaging,  
 Molecular Imaging Center, NIRS

### ***Outline of Research Career:***

Dr. Suhara received the Ph.D. from Jikei University School of Medicine in 1991 for his study of dopamine receptor binding *in vivo*. He began to work at NIRS in 1989. In 1992-1993, he studied in the PET group of the Department of Clinical Neuroscience, Karolinska Hospital, Sweden. He has researched on brain functional imaging for a long period. He serves as a visiting professor in the Department of Neuropsychiatry, Nippon Medical School from 2004, and in the Graduate School of Medicine, Yokohama City University from 2006.

**Contact point:** [suhara@nirs.go.jp](mailto:suhara@nirs.go.jp)

### ***Objectives:***

#### ***1. Neuroimaging Research***

- 1) Clinical research: The mechanism of mental disorders

Abnormal inter-regional connectivity has been reported in neurophysiological studies of schizophrenia. Dopamine systems interact with several other neurotransmitter systems with direct or indirect synaptic connections, and aberrant interactions among different neural networks could lead to an unusual inter-regional dopaminergic tone.

- 2) Neuropsychopharmacological research

Studies on imaging of neurotransmission were conducted in search of molecular interactions that critically modulate the release of dopamine in the striatum.

- 3) Generation of animal models of neuropsychiatric disorders

Our aim in establishment of *in vivo* systems modeling neurological and psychiatric conditions was to construct an experimental paradigm for comprehensive investigations of neurodegenerative disorders including Parkinson's disease, on pharmacological, behavioral and histopathological bases.

- 4) Development of novel PET tracer

Exploitation of imaging agents included radiosynthesis and characterization of [<sup>18</sup>F]SPA-RQ, a newly produced radioligand for visualizing neurokinin 1 receptors, which are major binding sites for substance P neuropeptide in the brain. The primary focus of the present assessment was elucidation of diversity in the pharmacokinetics and pharmacodynamics of the radiotracer among species ranging from small rodents to non-human

primates. Additionally, evaluation of radiolabeled agonist for dopamine D2 receptor, was also planned to be performed by using animal PET.

#### ***2. Neurogenetic Research***

A genetic screen for mutations, which were induced by ENU (N-nitroso-N-ethylurea) or X-rays in the medaka (*Oryzias latipes*), has identified many mutants that exhibit deformities in the brain morphogenesis and abnormalities in behaviors. These mutants are thought to have mutations in the genes that play important roles in the brain formation and brain functions.

#### ***3. Brain Toxicological Research***

The studies have been performed to elucidate the mechanisms of irradiation-induced brain damage and to find protective methods (compounds) against this damage.

#### ***4. Imaging Research of Gene Expression***

We have studied the methodology to detect iNOS gene expression in cellular and individual animal levels using an expression vector containing the iNOS promoter sequence and the dopamine D2 receptor gene.

## **Progress of Research:**

### **1. Neuroimaging Research**

#### 1) Clinical research

##### *Connectivity of regional D2R in schizophrenia*

A number of morphological and neurochemical abnormalities have been found to characterize schizophrenia. In particular, the dopamine D2 receptor has been investigated extensively, since the chronic use of amphetamine can cause psychotic symptoms and dopamine D2 receptor antagonists are the most widely used drugs for the treatment of schizophrenia. On the other hand, abnormal inter-regional connectivity has been reported in neurophysiological studies of schizophrenia. Dopamine systems interact with several other neurotransmitter systems with direct or indirect synaptic connections, and aberrant interactions among different neural networks could lead to an unusual inter-regional dopaminergic tone. In this study, we investigated the connectivity of regional dopamine D2 receptor binding in PET data from 10 drug-naive patients with schizophrenia and 19 healthy controls. We applied a structural equation method to evaluate the connectivity of regional D2 receptor binding in schizophrenia patients and normal controls. By this method, the inter-regional correlations of D2 receptor binding were decomposed to assign numerical weights (path coefficients) to the anatomical connections. This computational method allows for the assessment of changes in the inter-regional associations of entire systems. The results indicated that the network models of the patients and normal subjects were significantly different. As to the individual path coefficients, (a) connectivity between cortical regions was different between groups; (b) connectivity from the prefrontal cortex, parietal cortex, and thalamus to the anterior cingulate differed from that in controls; and (c) connectivity from the prefrontal cortex to the anterior cingulate and thalamus via the hippocampus was observed in normal subjects but not in patients. These results suggest that a systems-level change reflected in the connectivity of D2 receptor binding is present in schizophrenia.

##### *Appropriate dosage setting of antipsychotics by PET research*

Conventional antipsychotics tend to elicit extrapyramidal symptoms at clinical doses, but dose optimization could reduce the risk of such side-effects. *In-vivo* receptor-binding studies have suggested that 70-80% of dopamine D2 receptor occupancy provides the desired antipsychotic effects without extrapyramidal symptoms (EPS). In terms of dose optimization based on the occupancy, there has

not been enough supporting data regarding the clinical doses of the respective antipsychotics.

Although two conventional benzamide antipsychotics, sulpiride and sultopride, are prescribed at similar doses (300-1200 mg), and their clinical potency is considered to be equivalent, sultopride has been reported to induce more EPS than sulpiride.

In this study, we measured extrastriatal dopamine D2 receptor occupancy of sulpiride and sultopride using PET, to investigate the rationale of their clinical dose.

Subjects were 21 male healthy volunteers. PET scans were performed with [<sup>11</sup>C]FLB 457 before and after a single administration of several doses of sulpiride or sultopride. Quantification of dopamine D2 receptor binding potentials was done with the simplified reference tissue model. Dopamine D2 receptor occupancies were calculated using binding potential. Although dopamine D2 receptor occupancy increased as the dose increased for both drugs, the doses required to obtain similar receptor occupancy (70-80%) were quite different: 1010-1730 mg for sulpiride but 20-35 mg for sultopride. In terms of dose, sultopride has about 50 times greater potency than sulpiride based on dopamine D2 receptor occupancy. The calculated optimal dose range for sulpiride overlapped with the upper range of the registered clinical doses. On the other hand, the registered clinical doses of sultopride were about 10 times higher than the calculated optimal doses. The present results suggest that a much lower dose of sultopride would be sufficient to treat psychotic symptoms.

Appropriate dosage setting of conventional antipsychotics based on dopamine D2 receptor occupancy would be helpful for rational antipsychotic therapy.

#### 2) Neuropsychopharmacological research

##### *More reliable measurement of endogenous striatal dopamine by novel D2 agonistic PET tracer*

Radiolabeled agonist for dopamine D2 receptor was employed to quantitatively monitor alterations in the release of endogenous dopamine subsequent to administration of potential direct and indirect modulators of dopaminergic neurotransmission. Measurements were done by performing PET scans for awake monkeys and rats, and then estimating specific binding of the radiotracer to the striatal D2 receptors.

In addition, changes in the radioligand binding in these animals after administration of methamphetamine, a potent modifier of the dopamine release, were detected in a reproducible

manner. Hence, the new agonistic tracer was conceived to facilitate reliable estimation of endogenous dopamine present in the synaptic cleft.

### 3) Generation of animal models of neuropsychiatric disorders

#### *Prediction of dopaminergic dysfunction in the animal model of Parkinson's disease with PET*

A new experimental system for quantifying presynaptic and postsynaptic dopaminergic neurotransmission in nigrostriatal neurodegeneration recapitulating Parkinson's disease was constructed by carrying out PET scans with [<sup>11</sup>C]raclopride (antagonistic radioligand for dopamine D2 receptor) and [<sup>11</sup>C]PE2I (radioligand for dopamine transporter) in conjunction with behavioral tests for the same rat in the time course following unilateral injection of 6-OHDA into the medial forebrain bundle. Alterations of dopamine transporter and D2 receptor, as assessed by the binding of the radiotracers, were tightly correlated with the dose of 6-OHDA administered to each rat, while the dopamine transporter was more vulnerable to lesioning than was the D2 receptor, producing a partial discrepancy between presynaptic and postsynaptic changes in pathophysiological circumstances. Methamphetamine-induced rotational behaviors, which reflects laterality of dopaminergic functions, was observed in the rats injected with 6-OHDA at relatively high doses, and was thus indicated to emerge as a consequence of severe impairments in the presynaptic elements of dopaminergic neurotransmission. These revelations provide a mechanistic basis for motor deteriorations in Parkinson's disease, and clarify the utility of PET studies on multiple components of neurotransmission combined with behavioral assays.

### 4) Development of novel PET tracers

#### *Establishment of two novel tracers*

Protocols to robustly synthesize [<sup>18</sup>F]SPA-RQ and an agonistic radioligand for dopamine D2 receptor were established, and pharmacological evaluation of these tracers by applying them to PET and autoradiographic measurements for brains of rodents and non-human primates is currently going on. Radioligand for noradrenalin transporter was also successfully produced in a series of pilot experiments, and several remaining technical issues, which are relatively minor, are being figured out.

## **2. Neurogenetic Research**

We have started positional cloning of several medaka mutants in order to identify these genes and to understand the gene functions. The selected mutants were who (white out), tac (tacobo), act

(albino with cloudy tectum), and gac (growing act). All of the mutations were genetically mapped at each chromosome, and several candidate genes were isolated. We found that who mutant, which shows a hypochromic anemia and an unusual swimming behavior at later stages, was caused by a mutation in the gene for  $\delta$ -aminolevulinic acid dehydratase (ALAD). This is the first successful positional cloning of the medaka induced mutants. who mutants represent a model for the human disease, ALAD-deficiency porphyria. Another mutant, tac, which exhibits a short body length and smaller brain subdivisions, was caused by a mutation in the gene lam  $\gamma$ 1, which plays a role in laminin formation. The mutants, act and gac were genetically mapped at linkage groups (LG), LG 15 and LG 14, respectively.

## **3. Brain toxicological Research**

The protective effects of a free radical scavenger on brain tissue injury induced by heavy-ion irradiation were investigated. The left cerebral hemispheres of Sprague-Dawley rats were irradiated at a dose of 100Gy with charged carbon particles (290 MeV/nucleon, 5mm spread-out Bragg peak). Some rats were administered with a free radical scavenger, MC-PROXYL, just before irradiation. After irradiation, a conventional behavioral test and histological examination of the brain were done. The results showed that behavioral changes in walking patterns and rotation when suspended by their tail were observed at 8 week after irradiation, and the distinctive histological changes like necrosis, vascular dilation and tissue swelling at the irradiated region were also induced around 8 weeks after irradiation. Irradiation-induced histological and behavioral changes were mitigated in most rats that were injected with the free radical scavenger.

## **4. Imaging Research of Gene Expression**

Two types of expression vector were prepared: iNOS promoter + D2 receptor and iNOS promoter + D2 receptor + EGFP. They were transfected to rat glioma cells (C6) and macrophage cells (Raw264.7) and the clones responding to LPS and INF- $\gamma$  were selected. The EGFP expression in Raw264.7 cells measured by fluorescent microscopy and flow cytometry showed that the fluorescence intensity increased 2-5 times after stimulation with LPS and INF- $\gamma$ . In contrast, expression of D2R mRNA and protein in C6 cells measured by RT-PCR and Western blotting, respectively, was not significantly affected by the stimulation. These findings suggest that C6 cells transfected with the construct containing D2R receptor sequence are not suitable

for *in vivo* PET detection of iNOS expression in rats.

**Major Publications:**

- 1) Inaji M., Okauchi T., Ando K., Maeda J., Nagai Y., Yoshizaki T., Okano H., Nariai T., Ohno K., Obayashi S., Higuchi M., Suhara T. Correlation between quantitative imaging and behavior in unilaterally 6-OHDA-lesioned rats. *Brain Res*, **1064**:136-145, 2005.
- 2) Obayashi S., Matsumoto R., Suhara T., Nagai Y., Iriki A., Maeda J. Functional organization of monkey brain for abstract operation. *Cortex*, in press.
- 3) Takano A., Suhara T., Yasuno F., Suzuki K., Takahashi H., Morimoto T., Lee Y.J., Kusuhara H., Sugiyama Y., Okubo Y. The antipsychotic sultopride is overdosed - a PET study of drug-induced receptor occupancy in comparison with sulpiride. *Int J Neuropsychopharmacol*, **9**:1-7, 2005.
- 4) Yasuno F, Suhara T, Okubo Y, Ichimiya T, Takano A, Sudo Y, Inoue M. Abnormal effective connectivity of dopamine D2 receptor binding in schizophrenia. *Psychiatry Res*, **138**:197-207, 2005.
- 5) Yamamoto N., Ishikawa Y., Yoshimoto M., Xu H-G, Bahaxar N., Sawai N., Yang C-Y, Ozawa H., Itou H. A new interpretation on the homology of the teleostean telencephalon based on hodology and a new eversion model. *Brain Behav Evol*, in press.