

# Department of Neuropathology

## Outline and Research Objectives

The long-term goal of the research in our department is to determine the molecular events that lead to the development of Alzheimer's disease (AD). For this purpose, we are analyzing two proteins that have been implicated in the AD pathogenesis: amyloid  $\beta$ -protein (A $\beta$ ) and tau.

## Faculties and Students

Professor and Chair	Yasuo Ihara, M.D. (1991~)
Lecturer	Maho Morishima, Ph.D.
Associate	Satoru Funamoto, Ph.D. Kozo Motonaga, Ph.D.
Graduate Student.....	7
Secretary .....	1

## Past Research and Major Accomplishments

### Identification of posttranslational modifications of tau in paired helical filaments

Tau in paired helical filaments (PHF) is characterized by hyperphosphorylation and ubiquitination. The former causes the tau to migrate slower than dephosphorylated counterparts, and such particular tau makes three distinct bands on the Western blot, and called PHF-tau. We found that this phosphorylation somehow resemble that seen in fetal tau, because many phosphorylation-dependent monoclonal antibodies that react with PHF-tau recognize fetal tau. This indicates that fetal tau is phosphorylated to a greater extent than adult tau. We underwent the work to determine the exact sites for phosphorylation, using selective modification of phosphorylated Ser residues and their identification of amino acid sequencing and mass spec. As a result more than 20 phosphorylation sites in PHF-tau were determined: about half number of the sites were proline-directed, while the remaining were nonproline-directed. By our work the phosphorylation of PHF-tau was fully characterized.

PHF was found to be ubiquitinated in 1987, and since then we attempted to determine the ubiquitination sites in tau, but failed because of purification of ubiquitinated tau was very difficult and provided only very poor yield. From smeared tau (see below) we obtained a small amount of purified ubiquitinated tau, which was digested by lysyl endopeptidase and generated fragments were subjected to mass spec. Thus ubiquitinated sites were accurately determined,

and found in the microtubule-binding domain of tau.

One of the most remarkable characteristics of the tau in PHF is smearing on the blot from high molecular mass region down to 1-2 kD. The extent of smearing is well-correlated with the density of neurofibrillary tangles. We purified smeared tau using HPLC and analyzed protein chemically. Deamidation and isoaspartate formation were found, suggesting that smeared tau represents aging of NFT *in vivo*.

### (2) Determination of A $\beta$ 42 as the predominant species deposited in the brain

At that time, two A $\beta$  species were known, but their significance was unknown, although in the culture media A $\beta$ 40 that terminates in Val-40 is the major species. Using end-specific monoclonals that were developed by N. Suzuki, we found immunocytochemically that A $\beta$ 42 that terminates in Ala-42, a minor species among the secreted A $\beta$ , is predominant in the parenchyma of AD cortex. To learn about the temporal profile for A $\beta$  deposition in the human brain, we examined the brains from Down syndrome patients who invariably develop Alzheimer pathologies after the middle ages. In this way we found that the first species deposited in the brain is A $\beta$ 42.

### (3) Quantification of A $\beta$ accumulation in the human brain

With collaboration with N. Suzuki, we constructed two-site ELISA highly specific for A $\beta$ 40 or A $\beta$ 42. We first applied the ELISA for the quantification of A $\beta$ 40 and A $\beta$ 42 in the human brain. The biochemical alterations specific to AD may start much earlier than we previously thought. The work on CAA suggests four stages in the evolution: biochemically detectable stage, immunocytochemically detectable stage, histologically (Congo red) detectable stage, and clinically (multiple and recurrent hemorrhage in the aged) detectable stage. In other words, clinically overt patients are just a tip of the iceberg. The similar time course can be applicable to parenchymal A $\beta$  deposition; biochemical accumulation of A $\beta$  should start in the 40s (or even before). And AD patients are most

likely to represent a tip of the iceberg. A straightforward interpretation would be that AD is the consequence of the longstanding biochemical abnormalities.

Here, we focus on the nature of the initial biochemical abnormality, eventually leading to  $\beta$ -amyloidogenesis, rather than on why A $\beta$  accumulate in the brain in an exponential way. In particular, we are currently concerned about possible roles of low-density membrane domain (detergent-insoluble, glycolipid and cholesterol enriched domain; DIGs) in the A $\beta$  40 and A $\beta$ 42 metabolism, and in the earliest stage of  $\beta$ -amyloidogenesis.

As A $\beta$  accumulates, seemingly, A $\beta$ 42 shifts to higher density fractions, but A $\beta$ 40 tends to stay low. Possible explanations include: When a large amount of A $\beta$  accumulated, the low-density membrane domain may shift to higher density or A $\beta$ 42-bound low-density membrane domain may lose specific lipid composition (cholesterol and/or sphingolipid), resulting in shift to higher density. In either way, there may be a certain critical level of A $\beta$ 42 in the domain beyond which the membrane-bound A $\beta$ 42 cannot float.

#### **(4) Accelerated A $\beta$ accumulation in $\epsilon$ 4-bearing subjects**

We attempted to determine when A $\beta$  deposition starts in most human brains and how it is influenced by *apolipoprotein E* (*apoE*) allele  $\epsilon$ 4, a strong risk factor for late-onset AD. We successfully quantitated, using an improved extraction protocol and sensitive ELISA, the A $\beta$ 40 and A $\beta$ 42 levels in the insoluble fraction of the brains from many nondemented subjects aged 22 to 81 years. Both A $\beta$ 40 and A $\beta$ 42 were detectable in the insoluble fraction of the brains even from young subjects aged 20 to 30 years. The incidence of the subjects significantly accumulating A $\beta$  increased in an age-dependent manner; A $\beta$ 42 levels arose steeply in some subjects in their late 40s and this was accompanied by a much smaller rise in A $\beta$ 40 levels. *ApoE*  $\epsilon$ 4 was found to significantly enhance A $\beta$ 42 and, to a less extent, A $\beta$ 40 accumulation. These results strongly suggest that the presence of  $\epsilon$ 4 allele sets earlier in life the start of A $\beta$  accumulation in the brain.

#### **(5) Discovery of $\epsilon$ -cleavage using a cell-free system**

A $\beta$  is generated from  $\beta$ -amyloid precursor protein (APP) when  $\beta$ -secretase cleavage at the extracellular domain produces a 99-residue C-terminal fragment called CTF99 or CTF $\beta$ . Subsequent cleavage of CTF $\beta$  in the middle of the transmembrane domain by  $\gamma$ -secretase primarily produces either a 40-residue protein (A $\beta$ 40) or a 42-residue protein (A $\beta$ 42). Intramembrane

$\gamma$ -secretase cleavage of CTF $\beta$  should yield a 59- or 57-residue cytoplasmic C-terminal fragment (CTF $\gamma$ 41-99 or 43-99; APP712-770 or 714-770 according to the numbering of APP770 isoform) called CTF $\gamma$ . To learn more about the properties of  $\gamma$ -cleavage, we undertook to establish cell-free system for generation of A $\beta$  and CTF $\gamma$ , and characterize CTF $\gamma$ , the other product of this unusual cleavage. The generated CTF $\gamma$  consists largely of CTF $\gamma$ 50-99, and of CTF $\gamma$ 49-99, although the latter is several to ten-fold less than the former. Comparison of the cleavage sites of APP, APLP1, and APLP2 identifies new cleavage site: two to five residues inside the cytoplasmic membrane boundary. Therefore, this intramembrane cleavage is distinct from the A $\beta$ -generating  $\gamma$ -cleavage that occurs in the middle of the transmembrane domain (more than nine residues inside the membrane boundary).

It has been unclear why APP is cleaved near the middle of the transmembrane domain, whereas Notch 1 is cleaved immediately inside the cytoplasmic membrane boundary. Overall, our findings strongly suggest that the cleavage of Notch corresponds to this distinct type of cleavage rather than to  $\gamma$ -cleavage.

### **Current Research and Future Prospects**

#### **(1) Characterization of the substrate specificity of $\gamma$ -secretase**

1. Is  $\epsilon$ -cleavage a reality? Can we exclude the possibility that  $\gamma$ -cleavage is followed by swift, step-wise cleavages by a particular amino peptidase? Definite evidence requires demonstration of longer A $\beta$  (A $\beta$ 1-49 or 1-50) and/or the middle segment (A $\beta$ 41-49). Can one detect a longer A $\beta$  species in the membrane?
2. If it is reality, what is the meaning of  $\gamma$ -cleavage? Does the presence ragged C termini of A $\beta$  suggest participation of a particular carboxyl peptidase in the generation of A $\beta$ ?
3. If so, what is the relationship between  $\gamma$ - and  $\epsilon$ -cleavage? The two cleavages may not be in one to one stoichiometric relationship.
4. The general principle of type 1 membrane protein degradation. shedding, release of ectodomain, followed by intramembrane ( $\epsilon$ -) cleavage. How is the intramembrane segment left after  $\epsilon$ -cleavage degraded?
5. Is A $\beta$ 40 and A $\beta$ 42 production separable? How is A $\beta$ 40 or A $\beta$ 42 generation correlated to CTF $\gamma$ 49-99 or CTF $\gamma$ 50-99?

#### **(2) Mechanism of A $\beta$ accumulation in the brain**

1. At present the mechanism of FAD (Familial AD) can be understood by altered enzyme-substrate

relationship: Mutations in enzyme (presenilin) or substrate (APP) causes an (small) increase in the A $\beta$ 42 production, which eventually leads to AD. Patients with presenilin or APP mutants usually show higher levels of A $\beta$ 42 in the plasma. Presumably, whether this relationship is altered can be detected by increased levels of A $\beta$ 42 in the plasma. Another pathway to development of AD is being proposed: increased tendency to protofibril formation.

2. The role of ApoE4 is not to modify the enzyme-substrate relationship, but to mediate through other pathways, thereby leading to earlier build-up of senile plaques. Potential target would be in the exchange of A $\beta$  between cell membrane and HDL particles, in the transport of A $\beta$  through BBB or in the A $\beta$  fibrillization.
3. The thought into exponential accumulation curve may provide us with some insights into the mechanism. The accumulation curve may be simulated by the equation:  $A(t) = C(t-40)^n / 1 + (t-40)^n$ , where C: constant, t, age > 40; steepness in the accumulation depends on n. This equation represents cooperativity phenomena (phase transition), and may suggest that age-dependent A $\beta$  accumulation may be one of such examples in the biology. If so, the underlying mechanism can be explained by enhanced A $\beta$  aggregation force. A point is why the aggregation potential is enhanced. One possibility is a decrease in the degradation potential (decreased protease activity) during aging. However, it is a bit difficult to envision, because it has not so far been known that certain proteases are acutely inactivated during aging. The other possibility is altered environments induced by aging. Altered lipid composition and/or generation of a certain factor to promote A $\beta$  aggregation. Altogether, accumulation is a consequence of aggregation, and aggregation may be a consequence of an alteration in the environment, but may not be decreased protease activity. In this regard, it may be important to investigate the significance of A $\beta$  accumulation in the raft. First, to examine whether raft-accumulated A $\beta$  is younger than extracellularly deposited A $\beta$  by using pyroGlu-specific antibodies. Other markers for in vivo aging include isomerization and racemization of Asp residues and truncation of the A $\beta$  molecule.

### (3) Tau and neuronal cell death

What do we know about the significance of intracellular tau deposition, and what should we do in the future?

1. NFT itself is not so harmful to cell. NFT-bearing neurons are viable, and still attempt to maintain

dendritic arborization. NFT-bearing oligodendrocytes rather specific for FTDP-intron mutations still supply enough amounts of myelin. No demyelination is detected by a classical method.

2. But often, dendrites of NFT-bearing neurons are compromised, which reminds us of dying-back neuropathy. Presumably, curly fibers represent dying-back segments of basal dendrites isolated from neuronal cell bodies.
3. FTDP-17 is characterized by tauopathy and neuronal loss. P301L mutation is featured by extensive neuronal loss rather than NFT formation (see transgenic fly; Wittmann CW et al. *Science* 2001;293:711-4), while R406W mutation is featured by NFT formation rather than neuronal loss. Clinical course may depend on neuronal loss rather than NFT formation. This is consistent with the old view that the degree of dementia in AD is best correlated with the extent of neuronal loss.
4. Thus, the most important question about tau is why and how tau kills the neuron, rather than why and how it aggregates into NFT.
5. Significance of hyperphosphorylation of tau in PHF or PHF-like fibrils. Hyperphosphorylation of tau may be a consequence of degeneration of neuron, but not a cause of degeneration. Study on R406W brain showed that i) cytosolic R406W tau is less phosphorylated, which is consistent with an observation with CHO stable transfectant; ii) but the mutant tau composing PHF-like fibrils is hyperphosphorylated. A most likely interpretation is as follows. The mutant tau has a bulky residue close to its microtubule-binding domain, and when the mutant tau interact with tubulin or microtubules, a particular kinase cannot access to Ser-396 and 404, resulting in hypophosphorylation on these residues. Once the interaction with tubulin or microtubules is lost (by degeneration), the mutant tau takes a random structure as does wild-type tau, and these residues are freely accessed by kinase and become hyperphosphorylated.
6. To do: establishment of FTDP tau knock-in mice; attempt to increase the tau expression level by removing the neo gene by breeding with CAG Cre mice. Breeding with APP transgenic mice (for example, APP<sup>sw</sup> mice) may make the pathological phenotype to appear within lifespan, and P301L mice may develop NFT and neuronal loss much earlier than wild-type knock-in mice.
7. More data about tau in the cell biology may provide some keys to understanding of the pathogenesis of FTDP-17. For example, differential role of three-repeat and four-repeat tau should be more highlighted.

8. The data from transgenic fly suggest that expression of even wild-type tau may be somehow harmful to viability of fly (Wittmann CW et al. *Science*. 2001;293:711-4). This can be applied to synuclein also (Feany MB, Bender WW. *Nature* 2000;404:394-8). Presumably, the essential feature of FTDP-17 is neuronal death without overt NFT formation. But in many FTDP-17 cases, both neuronal loss and NFT formation coexist. This means that the initial step to neuronal death may be shared by the pathway to NFT formation. In this respect, recent observations may be significant (Zhukareva V et al. Loss of brain tau defines novel sporadic and familial tauopathies with frontotemporal dementia. *Ann Neurol*. 2001;49:165-75).
9. Currently there is no unifying hypothesis that can explain remarkable heterogeneity of FTDP-17. If it does exist, it must explain i) Why exonic mutation cause neuronal degeneration as well as ii) Why increased levels of four-repeat tau cause neuronal degeneration.

### Research Grants

1. Core Research for Evolutional Science and Technology from the Japan Science and Technology Corporation  
Year : 1996 ~ 2001  
¥483,000,000.-
2. Grant-in-Aid for Scientific Research on Priority Areas, Advanced Brain  
Science Project, from the Ministry of Education, Culture, Sports, Science and Technology, Japan  
Year : 2000 ~ 2002  
¥108,000,000.-
3. Research Grants for Longevity Sciences from the Ministry of Health and Welfare, Japan  
Year: 1999 ~ 2001  
¥ 6,000,000.-
4. Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology, Japan  
Year: 1999 ~ 2000  
¥ 3,700,000.-
5. Research Grants for Longevity Sciences from the Sasakawa Health Science Foundation, Japan  
Year: 1999  
¥ 2,900,000.-

### Select Publications

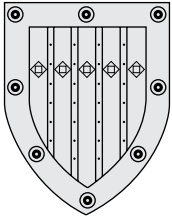
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12. Yoshida H, Ihara Y: t in paired helical filaments is functionally distinct from fetal t. — assembly incompetence of paired helical filament-t —. *J Neurochem* 61:1183-1186, 1993
13. Watanabe A, Hasegawa M, Suzuki M, Takio K, Morishima-Kawashima M, Titani K, Arai T, Kosik KS, Ihara Y: In vivo phosphorylation sites in fetal and adult rat tau. *J Biol Chem* 268:25712-25717, 1993
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# Department of Cognitive Neuroscience

## Outline and Research Objectives

The Department of Cognitive Neuroscience is one of the three departments composing the Speech and Cognitive Science Section. Since the establishment of this department in 1991, we have been working in the field of brain and cognition, especially from the point of view of language.

### Main research topics:

1. Research on the brain damaged patient: callosal syndrome, right temporal lobe syndrome, macular sparing, aphasia therapy
2. Functional magnetic resonance imaging (fMRI) during cognitive process: mental writing, writing, face recognition, reading comprehension, naming, reading aloud
3. Magnetoencephalographic (MEG) study of cognitive function: early component of the visual evoked magnetic field, motor imagery
4. Brain mechanism of Japanese kanji processing

### Teaching activities:

1. Graduate course: Speech science and language communication, Pathophysiology of speech and language Communication, Introduction to neuroscience
2. Master course: Cognitive neuroscience
3. Graduate course, Faculty of Literature: Experimental phonetics
4. Undergraduate course: Speech and language communication, Medical data processing
5. Undergraduate course, Faculty of Education: Fundamentals of speech science

### Clinical activities:

The assessments and therapies for aphasia, apraxia, agnosia, amnesia and dementia are conducted in collaboration with the Deptment of Neurology, Department of Neurosurgery and Department of Otorhinolaryngology

## Faculties and Students

Professor	Morihiro Sugishita Dr. Health Sci., Dr. Medical Sci. 1993~
Associate .....	2
Graduate Students .....	2.
Research Students.....	1
Guest Researchers.....	2

## Past Research and Major Accomplishments

### 1. Research on the Brain Damaged Patient

1-1) *Sugishita M, et al. Dichotic listening in patients with partial section of the corpus callosum. Brain 118, 417-427, 1995:* Despite the common assumption that damage to the posterior part of the trunk of the corpus callosum causes strong left-ear suppression, the results indicated that damage to the splenium causes strong left-ear suppression.

1-2) *Koike A, et al. Preserved musical abilities following right temporal lobectomy. Journal of Neurosurgery 85, 1000-1004, 1996:* No disturbances in the Seashore Measures were detected after temporal lobectomy on either side.

1-3) *Sugishita M, et al. Hemispheric representation of the central retina of commissurotomy subjects. Neuropsychologia 32, 399-415, 1994:* The region of the right (temporal) hemiretina represented by both hemispheres in letter processing, if it exists, was estimated as less than 0.6° from the foveal center.

### 2. Functional Magnetic Resonance Imaging (fMRI) During Cognitive Process

2-1) *Sugishita M, et al. Functional magnetic resonance imaging (fMRI) during mental writing with phonograms. NeuroReport 7, 1917-1921, 1996:* Four regions were activated during mental writing in all six subjects; the left intraparietal sulcus, the middle part of the left precentral sulcus and the posterior part of

the left superior frontal sulcus, the right intraparietal sulcus, and either or both of the left and right cingulate sulci. The left intraparietal region was usually the most extensively activated.

2-2) Katanoda K, et al. *A functional MRI study on the neural substrates for writing. Human Brain Mapping 13, 34-42, 2001*: During writing, activations were observed in the anterior part of the left superior parietal lobule, the posterior part of the left middle and superior frontal gyri, and the right cerebellum.

2-3) Katanoda K, et al. *Neural substrates for the recognition of newly learned faces: a functional MRI study. Neuropsychologia 38, 1616-1625, 2000*: The bilateral fusiform gyrus is involved, not only in face perception, but in a certain aspect of face recognition memory, and this aspect is related to the actual recognition of previously viewed faces rather than the processing of novel ones. The right parietal and frontal regions, in contrast, are differentially more associated with the detection of novel faces or retrieval effort.

### 3. Magnetoencephalographic (MEG) Study of Cognitive Function

3-1) Yoneda K, et al. *The early component of the visual evoked magnetic field. NeuroReport 6, 797-800, 1995*: The early component was observed in three of the nine subjects. The latency ranged from 40 to 45 ms in MEG and from 39 to 47 ms in EEG. The result of dipole localization analysis showed that its origin was cortical, and specifically, the striate cortex.

3-2) Ogiso T, et al. *The precuneus in motor imagery: a magnetoencephalographic study. NeuroReport 11, 1345-1349, 2000*: MEG was applied to subjects who imagined themselves hurdling in self-centered space. In three of six subjects all 300 trials in the motor imagery condition revealed the precuneus dipole. When we divided the 300 trials into four overlapping blocks, all six subjects showed precuneus activity.

### 4. Brain Mechanism of Japanese Kanji Processing

4-1) Sugishita M, Omura K. *Learning Chinese characters may improve visual recall. Perceptual and Motor Skills 93, 579-594, 2001*: From elementary through high school, Japanese children are required to memorize a large number of distinct visual forms, i.e., roughly 2,000 Chinese characters, and tremendous effort is expended in learning to read and write them. We hypothesized that early training in memorizing Chinese characters and the use of these characters in daily life shapes brain development and facilitates recall of visual forms in general. We administered the Wechsler Memory Scale-Revised (WMS-R) to a repre-

sentative sample of the normal Japanese population (316 persons, 100% Japanese) and compared their scores with data previously obtained from a representative sample of the normal U.S. population (316 persons, 82.5% Caucasian). Compared to the Americans, the Japanese group obtained significantly higher scores on these two visual recall subtests in all six age groups (16 to 74 years old).

### Current Research

Research on the brain damaged patients: callosal syndrome, right temporal lobe syndrome, macular sparing, aphasia therapy

1. fMRI during cognitive process: mental writing, writing, face recognition, reading comprehension, naming, reading aloud
2. fMRI study on speech dominance in split-brain patients
3. MEG study of cognitive function: visual evoked magnetic fields, motor imagery
4. Brain mechanism of Japanese kanji processing

### Future Prospect

1. Cerebral localization of neuropsychological deficits (aphasia, apraxia, agnosia and amnesia): The sites of lesions responsible for neuropsychological deficits will be more precisely localized with the MRI findings of the patient.
2. Brain functional imaging: Functional MRI study will be advanced with development of imaging techniques such as arterial spin labeling other than BOLD techniques and with refinement of experimental paradigm.

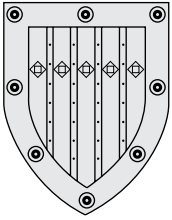
### Research Grants

1. JSPS Research for the Future Program (Higher Brain Functions) [FY1997~FY 2001] (\$1,095,000/ ¥131,000,000) "Neuroimaging contributions to the understanding of brain language mechanism"
2. JSPS Scientific Research (B) [FY1997~FY 1998] (\$79,170/ ¥9,500,000) "Neuroimaging contributions to the understanding music"
3. JSPS Exploratory Research [FY1997] (\$11,700/ ¥1,400,000) "Study on brain mechanism of spoken word discrimination by MEG"
4. MEXT COE Research (Neuroscience of Music) [FY1997-2001] (\$0/ ¥0) "Paradigm creation in behavioral neurology"
5. MEXT Scientific Research in Priority Areas (Mind Development) [FY1994-1998] (\$100,000/ ¥12,000,000) "Research on disorders of cognitive development by functional MRI"



## Select Publications

1. Takayama Y, Sugishita M, Akiguchi I, Kimura J. Isolated acalculia due to left parietal lesion. *Archives of Neurology*, 51, 286-291, 1994
2. Ishiai S, Sugishita M, Watabiki S, Nakayama T, Kotera M, Gono S. Improvement of left unilateral spatial neglect in a line extension task. *Neurology* 44, 294- 298, 1994
3. Sugishita M, Hamilton CR, Sakuma I, Henmi I. Hemispheric representation of the central retina of commissurotomized subjects. *Neuropsychologia* 32, 399-415, 1994
4. Takayama Y, Sugishita M, Kido T, Ogawa M, Fukuyama H, Akiguchi I. Impaired stereoacuity due to a lesion in the left pulvinar. *Journal of Neurology Neurosurgery and Psychiatry* 57, 652-654, 1994
5. Takayama Y, Kido T, Sugishita M, Ogawa M, Akiguchi I. A case of foreign accent syndrome without aphasia caused by a lesion of the left precentral gyrus. *Neurology* 44, 990, 1994
6. Takayama Y, Sugishita M. Astereopsis induced by repetitive magnetic stimulation of occipital cortex. *Journal of Neurology* 241, 522-525, 1994
7. Takayama Y, Sugishita M, Hirose S, Akiguchi I. Anosodiaphoria for dressing apraxia: contributory factor to dressing apraxia. *Clinical Neurology and Neurosurgery* 96, 254-256, 1994
8. Yoneda K, Sekimoto S, Yumoto M, Sugishita M. The early component of the visual evoked magnetic field. *Neuroreport* 6, 797-800, 1995
9. Sugishita M, Otomo K, Yamazaki K, Shimizu H, Yoshioka M, Shinohara A. Dichotic listening in patients with partial section of the corpus callosum. *Brain* 118, 417-427, 1995
10. Seki K, Yajima M, Sugishita M. The efficacy of kinesthetic reading treatment for pure alexia. *Neuropsychologia*, 33, 595-609, 1995
11. Sodeyama N, Tamaki M, Sugishita M. Persistent pure verbal amnesia and transient aphasia after left thalamic infarction. *Journal of Neurology* 242, 289-294, 1995
12. Takeda K, Sugishita M. Word length error types in Japanese left-sided neglect dyslexia. *Clinical Neurology and Neurosurgery* 97, 125-130, 1995
13. Nishiyama K, Momose T, Sugishita M, Sakuta M. Positron emission tomography of reversible intellectual impairment induced by long-term anticholinergic therapy. *Journal of the Neurological Sciences*, 132, 89-92, 1995
14. Takayama Y, Sugishita M, Fukuyama H, Akiguchi I. Localization in spatial vision. *Clinical Neurology and Neurosurgery* 97, 249-252, 1995
15. Endo K, Makishita H, Yanagisawa N, Sugishita M. Modality specific naming and gesture disturbances. A case with optic aphasia, bilateral tactile aphasia, optic apraxia and tactile apraxia. *Cortex* 32, 3-28, 1996
16. Sugishita M, Takayama Y, Shiono T, Yoshikawa K, Takahashi Y. Functional magnetic resonance imaging (fMRI) during mental writing with phonograms. *Neuroreport* 7, 1917-1921, 1996
17. Koike A, Simizu H, Suzuki I, Ishijima B, Sugishita M. Preserved musical abilities following right temporal lobectomy. *Journal of Neurosurgery* 85, 1000-1004, 1996
18. Nishiyama K., Sugishita M, Kurisaki H, Sakuta M. Reversible memory disturbance and intelligence impairment induced by long-term anticholinergic therapy. *Internal Medicine* 37, 514-518, 1998
19. Matsuda O, Saito M, Sugishita M. Cognitive deficits of mild dementia: a comparison between dementia of the Alzheimer's type and vascular dementia. *Psychiatry & Clinical Neurosciences* 52, 87-91, 1998
20. Kobayashi M, Takayama H, Mihara B, Sugishita M. Partial seizure with aphasic speech arrest caused by watching a popular animated TV program. *Epilepsia* 40, 652-654, 1999
21. Tanaka S, Kanzaki R, Yoshibayashi M, Kamiya T, Sugishita M. Dichotic listening in patients with situs inversus: brain asymmetry and situs asymmetry. *Neuropsychologia* 37, 869-874, 1999
22. Ogiso T, Kobayashi K, Sugishita M. The precuneus in motor imagery: a magnetoencephalographic study. *NeuroReport* 11, 1345-1349, 2000
23. Takayama H, Kobayashi M, Sugishita M, Mihara B. Diffusion-weighted imaging demonstrates transient cytotoxic edema involving the corpus callosum in a patient with diffuse brain injury. *Clinical Neurology and Neurosurgery* 102, 135-139, 2000
24. Katanoda K, Yoshikawa K, Sugishita M. Neural substrates for the recognition of newly learned faces: a functional MRI study. *Neuropsychologia* 38, 1616-1625, 2000
25. Katanoda K, Yoshikawa K, Sugishita M. A functional MRI study on the neural substrates for writing. *Human Brain Mapping* 13, 34-42, 2001
26. Sugishita M, Koike A, Shimizu H, Suzuki I, Ishijima B: Right temporal lobe function. *Journal of Neurosurgery* 95, 372, 2001
27. Sugishita M, Omura K. Learning Chinese characters may improve visual recall. *Perceptual and Motor Skills* 93, 579-594, 2001
28. Katanoda K, Matsuda Y, Sugishita M. A spatio-temporal regression model for the analysis of functional MRI data. *NeuroImage* 16, in press, 2002



# Department of Neuropsychiatry

## Outline and Research Objectives

This department of neuropsychiatry has the longest history among all that of Universities in Japan since 1886. In the past quarter century, unfortunately it had been in a unusual condition that two groups, "The Ward" and "The Clinic", had made independent clinical activities in this department and excluded each other. "The Ward" group was influenced by the movement "Anti-Psychiatry" in 1960. During this period the most part of budgets and personnel changes had been frozen. This unfortunate incident made the facilities of the ward and all laboratories of our department old-fashioned and obsolescent. In 1994, two groups had arrived a compromise with each other and started the care of patients in the psychiatric ward and clinic together, however more several years were needed for the regeneration of the department. Now it has already been "normalized", overwhelming the long and difficult period of past quarter century and we have regenerated the productive activities of neuropsychiatry.

Approximately 240 patients with various psychiatric disorders were admitted on last year (2001). Since 2002, new wards was established (34 beds in the secluded ward and 19 beds in the general ward) and this department became to be expected the more active roles on clinical fields in this year. Occupational therapy, art therapy and group therapy are performed in the ward. For the outpatients, we have two "Day Hospital Units" in this department; Day Hospital for young patients with psychotic feature based on cognitive behavioral approach and Day Care for autistic children using educational treatment models.

Research activities in this department are wide-ranged from social psychiatry to molecular biology. Main research activities in this department is as follows, 1) molecular biological studies of psychiatric disorders, 2) neuroscientific studies of stress, 3) psychopathological studies of early schizophrenia, 4) psychophysiological and neuropsychological studies of schizophrenia and epileptic psychosis, 5) community-based psychosocial treatment of chronic schizophrenia, 6) cognitive-developmental or pharmacological treatment of autism and Tourette syndrome, 7) neuroimaging studies of psychiatric disorders, and 8) neuropathological studies of dementias. Starting 2000, a nation-wide Project with the Grant of Japan Science and Technology Agency has organized under the management of this Department, entitled "Molecular mechanisms underlying stress-induced brain dysfunctions and development of diagnosis/treatment strategies on post-traumatic stress disorders (PTSD)".

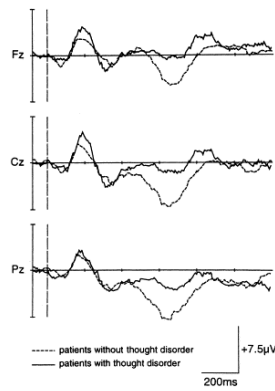
## Faculties and Students

Professor and Chair	Nobumasa Kato, M.D. (1998~)
Associate Professor	Nobuo Nakayasu, M.D. Akira Iwanami, M.D.
Lecturer	Koichi Tsunashima, M.D. Hitoshi Tsuda, M.D. Rinmei Fukuda, M.D.
Associate .....	10
Graduate Student.....	13
Secretary .....	4

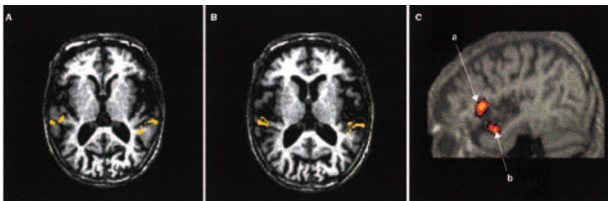
## Past Research and Major Accomplishments

Research activity in our department ranges over diverse fields in neuropsychiatry including biological

and social psychiatry. Professor Kato has been conducting and supervising the researches on the effect of stress on brain using animal models. Especially, various stress-induced changes in hippocampus have been elucidated. Dr Nakayasu had conducted intensive psychopathological research on early symptoms of schizophrenia and proposed the notion of "incipient schizophrenia" in 1990, which could be fundamentally different from its full-blown counterpart. At this early stage, he suggested that we could employ a combination of specific pharmacotherapy and psychotherapy to prevent the outbreak of this devastating illness. Dr Iwanami has been utilizing event-related potentials (ERPs) to investigate the neural correlates of cognitive dysfunction in schizophrenia, methamphetamine psychosis and other mental disorder.



Grand mean ERP waveforms in patients with and without thought disorder. (Iwanami et al. Schizophrenia Research, 2000)

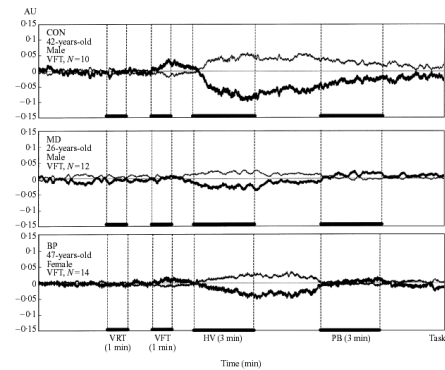


Spatiotemporal excitation patterns of N100m sources and SPECT imaging during musical hallucinations. (Kasai et al. Lancet, 1999)

ders. He found that specific parameters of ERPs correlates the severity of the illness. In the field of social psychiatry, our department was the first to introduce Day Hospital unit in 1980s and made extensive research on the efficacy of this treatment strategy. Our efforts had significantly contributed to the following nationwide introduction of this treatment approach in Japan.

### Current Research

The primary activity in our department is the government-supported study in the pathogenesis of stress-induced neuropsychiatric disorders, mainly, post-traumatic stress disorder (PTSD). The development of novel treatment strategy will also be expected. As one of the patient population, victims of the Tokyo subway sarin attack in 1995 kindly served themselves. Especially, we employ multimodal neuroimaging techniques (electroencephalography, event-related potentials, magnetoencephalography, structural magnetic resonance imaging, functional magnetic resonance imaging, magnetic resonance spectroscopy and near infrared spectroscopy) to elucidate the individual vulnerability to this condition and the neurobiological aftereffects of psychological trauma. Other research activities include 1) molecular genetics of psychiatric disorders, 2) basic neuroscience, 3) clinical and basic psychopharmacology, 4) the development of psychosocial treatment approach in autism, and 5) neuropathological studies of dementias.



Typical time courses of the NIRS variables during cognitive and physiological tasks. (Matsuo et al. Psychological Medicine, 2002)

### Future Prospects

The Last ten years were “The Decade of Brain”. Outstanding progress has been achieved in neuroscience in terms of scientific knowledge and technical innovation. In addition, the human genome project is almost done. Now we are jumping into the 21<sup>st</sup> century, “The Century of Mind”. Psychological terms should be translated into those of brain, which would provide the more solid basis for psychiatry as a branch of neuroscience. In this context, our basic and clinical research projects are in progress. For holistic understanding of patients suffering psychiatric disorders and their treatments, multi-disciplinary studies should be necessary under the collaboration with other neuroscience research groups.

As a university hospital, clinical practice is also important. We should be always keen to the needs of the public. To meet them, we opened several specialized outpatient clinics, such as PTSD or Child and Adolescent clinic, in addition to regular services. The teams for psychiatric emergency and palliative medicine are also being formed. In addition to clinical services, the public would expect the scientific outputs obtained through clinical studies. The system recruiting subjects or volunteers should be established immediately.

The curriculum for medical students and the training system for residents are now changing. Especially, the “super-rotation” system will begin in a few years. Our training system should be modified for residents to acquire the better clinical clerkship.

We will continue to make our best efforts to fulfill the three major missions of the university hospital, that is, research, clinical service and education.

### Research Grants

- 1999:Health Science Research Grants (Research on Brain Science) ¥18,000,000



2. 2000: Target Oriented Brain Science Promotion Program supported by the MEXT 2000-2002 ¥194,493,000
3. 2001: Japan-US Cooperative Reserch Project "Cooperative Brain Reserch 2001-2002 ¥2,700,000
4. 2002: Health Science Research Grants (Research on Health Service) ¥36,000,000

## Select Publications

### Basic

1. Jinde S, Matsui A, Morinobu A, Takahashi Y, Tsunashima K, Noda A, Yamada N, and Kato N. Elevated neuropeptide Y and corticotropine-releasing factor in the brain of a novel epileptic mutant rat: Noda epileptic rat. *Brain Research* 833, 286-290, 1999.
2. Shirayama Y, Hashimoto K, Matsuki H, Tsunashima K, Iyo M, Higuchi T, and Minabe Y. Increased expression of zif268 mRNA in rat retrosplenial cortex following administration of phencyclidine. *Brain Research* 839, 180-185, 1999.
3. Nagaki S, Fukumachi F, Sakamoto Y, Higuchi H, Miki N, Ono M, Sadamatsu M, Kato N, and Osawa M. Upregulation of brain somatostatin and neuropeptide Y following lidocaine-induced kindling in the rat. *Brain Research* 852, 470-474, 2000.
4. Kohda K, Wang Y, and Yuzaki M. Mutation of glutamate receptor motif reveals its role in gating and d2 receptor channel properties. *Nature Neuroscience* 3, 315-322, 2000.
5. Nishiyama M, Hong K, Mikoshiba K, Poo M-m, and Kato K. Polarity and input specificity of synaptic modification: critical role of Ca<sup>2+</sup> release from internal stores (underlined authors equally contributed). *Nature* 408, 584-588, 2000.
6. Watanabe K, Ashby CR Jr, Katsumori H, and Minabe Y. The effect of the acute administration of various selective 5-HT receptor antagonists on focal hippocampal seizures in freely-moving rats. *European Journal of Pharmacology* 398, 239-246, 2000.
7. Yasuda S, Ishida N, Higashiyama A, Morinobu S, and Kato N. Characterization of audiogenic-like seizures in naïve rats evoked by activation of AMPA and NMDA receptors in the inferior colliculus. *Experimental Neurology* 164, 396-406, 2000.
8. Minabe Y, Hashimoto K, Watanabe K, and Ashby CR Jr. Acute and repeated administration of the selective 5-HT(2A) receptor antagonist M100907 significantly alters the activity of midbrain dopamine neurons: an in vivo electrophysiological study. *Synapse* 40, 102-112, 2001.
9. Imai H, Nishimura T, Sadamatsu M, Liu Y, Kabuto M, and Kato N. Type II glucocorticoid receptors are involved in neuronal death and astrocyte activation induced by trimethyltin in the rat hippocampus. *Experimental Neurology* 171, 22-28, 2001.

10. Nishimura T, Schwarzer C, Furtinger S, Imai H, Kato N, and Sperk G. Changes in the GABAergic system induced by trimethyltin application in the rat. *Molecular Brain Research* 97, 1-6, 2001.
11. Takahashi J, Tanaka K, Morinobu S, Fujimaki K, Li ST, Kato K, Ohkawa M, Yamawaki S, and Kato N. Influence of restraint stress on the expression and the serine/threonine phosphatase activity of calcineurin in the rat brain. *Synapse* 40, 130-6, 2001.
12. Tsutsumi S, Akaike M, Arimitsu H, Imai H, Kato N. Circulating corticosterone alters the rate of neuropathological and behavioral changes induced by trimethyltin in rats. *Experimental Neurology* 73, 86-94, 2002.

### Clinical (genetics)

13. Kato T, Honda M, Kuwata S, Juji T, Kunugi H, Nanko S, Fukuda M, and Honda Y. A novel polymorphism in the promoter region of the tumor necrosis factor alpha gene: No association with narcolepsy. *American Journal of Medical Genetics* 88, 301-304, 1999.
14. Kunugi H, Hattori M, Nanko S, Fujii K, and Kato T. Dinucleotide repeat polymorphism in the neurotrophin-3 gene and hippocampal volume in psychoses. *Schizophrenia Research* 37, 271-273, 1999.
15. Kunugi H, Ishida S, Kato T, Sakai T, Tatsumi M, Hirose T, and Nanko S. No evidence for an association of polymorphism of the tryptophan hydroxylase gene with affective disorders or attempted suicide among Japanese patients. *American Journal of Psychiatry* 156, 774-776, 1999.
16. Kunugi H, Ishida S, Kato T, Tatsumi M, Sakai T, Hattori M, Hirose T, and Nanko S. A functional polymorphism in the promoter region of monoamine oxidase-A gene and mood disorders. *Mol Psychiatry* 4, 393-395, 1999.
17. Sadamatsu M, Masui A, Sakai T, Kunugi H, Nanko S, and Kato N. Familial paroxysmal kinesigenic choreoathetosis: an electrophysiologic and genotypic analysis. *Epilepsia* 40, 942-949, 1999.
18. Kato T, Kunugi H, Nanko S, and Kato N. Association of bipolar disorder with the 5178 polymorphism in mitochondrial DNA. *American Journal of Medical Genetics* 96, 182-186, 2000.
19. Narita K, Sasaki T, Akaho R, Okazaki Y, Kusumi I, Kato T, Hashimoto O, Fukuda R, Koyama T, Matsuo K, Okabe Y, Nanko S, Hohjoh H, and Tokunaga K. Human leukocyte antigen and season of birth in Japanese patients with schizophrenia. *American Journal of Psychiatry* 157, 1173-1175, 2000.
20. Tomita H, Nagamitsu S, Wakui K, Fukushima Y, Yamada K, Sadamatsu M, Masui A, Konishi T, Matsushita Y, Aihara M, Shimizu K, Hashimoto K, Mineta M, Matsushima M, Tsujita T, Saito M, Tanaka H, Tsuji S, Takagi T, Nakamura Y, Nakano S, Kato N, Nakane Y, and Niikawa N. Paroxysmal kinesigenic choreoathetosis locus map to chromosome 16p11.2-q12.1. *American Journal of Human Genetics* 65, 1688-1697, 2000.



21. Kato T, Kunugi H, Nanko S, and Kato N. Mitochondrial DNA polymorphisms in bipolar disorder. *Journal of Affective Disorders* 62, 151-164, 2001.
22. Tatsumi M, Sasaki T, Iwanami A, Kosuga A, Tanabe Y, Kamijima K. Season of birth in Japanese patients with schizophrenia. *Schizophrenia Research* 54, 213-8, 2002.

### **Clinical (pharmacology/neurobiology)**

23. Fukuda R, Sasaki T, Kunugi H, and Nanko S. No changes in paired viral antibody titers during the course of acute schizophrenia. *Neuropsychobiology* 40, 57-62, 1999.
24. Kato T and Kato N. Mitochondrial dysfunction in bipolar disorder. *Bipolar Disorder* 2, 180-190, 2000.
25. Fukuda R. Factors affecting serum haloperidol level assessed by longitudinal therapeutic monitoring. *Progress in Neuropsychopharmacology and Biological Psychiatry* 24, 1299-1318, 2000.
26. Morita S, Shimoda K, Someya T, Yoshimura Y, Kamijima K, and Kato N. Steady-state plasma levels of nortriptyline and its hydroxylated metabolites in Japanese: the impact of CYP2D6 genotype on the hydroxylation of nortriptyline. *Journal of Clinical Psychopharmacology* 20, 141-149, 2000.
27. Iwanami A, Okajima Y, Isono H, Shinoda J, Kasai K, Hata A, Fukuda M, Nakagome K, Kamijima K. Effects of risperidone on event-related potentials in schizophrenic patients. *Pharmacopsychiatry* 34, 73-79, 2001
28. Fujimaki K, Morinobu S, Takahashi J, Yamawaki S, Kato N, Kanno M, Okuyama N, Kawakatsu S, Otani K, Kusumi I, and Koyama T. Nucleotide sequence analysis of the binding site on the inositol 1,4,5-trisphosphate type-1 receptor in bipolar disorder-negative study. *Journal of Affective Disorders* 65, 139-143, 2001.

### **Clinical (neuroimaging)**

#### **Magnetic resonance spectroscopy**

29. Hamakawa H, Kato T, Shioiri T, Inubushi T, and Kato N. Quantitative proton magnetic resonance spectroscopy in the bilateral frontal lobes of patients with bipolar disorder. *Psychological Medicine* 29, 639-644, 1999.
30. Murashita J, Kato T, Shioiri T, Inubushi T, and Kato N. Age-dependent alteration of metabolic response to photic stimulation in the human brain measured by <sup>31</sup>P MR-spectroscopy. *Brain Research* 818, 72-76, 1999.
31. Kato T, Inubushi T, and Kato N. Prediction of lithium response by <sup>31</sup>P-MRS in bipolar disorder. *International Journal of Neuropsychopharmacology* 3, 83-85, 2000.
32. Murashita J, Kato T, Shioiri T, Inubushi T, and Kato N. Altered brain energy metabolism in lithium-resistant bipolar disorder detected by photic stimulated <sup>31</sup>P-MR spectroscopy. *Psychological Medicine* 30, 107-115, 2000.

#### **Near-infrared spectroscopy (NIRS)**

33. Matsuo K, Kato T, Fukuda M, and Kato N. Alteration of hemoglobin oxygenation in the frontal region in elderly depressed patients measured by near-infrared spectroscopy. *Journal of Neuropsychiatry and Clinical Neuroscience* 12, 465-471, 2000.
34. Matsuo K, Kato N, and Kato T. Decreased cerebral haemodynamic response to cognitive and physiological tasks in mood disorders as shown by near-infrared spectroscopy. *Psychological Medicine* 32, 1029-37, 2002.

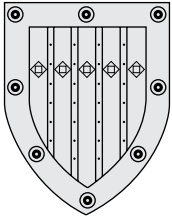
#### **Magnetic resonance imaging (MRI)**

35. Kasai K, Shenton ME, Salisbury DF, Hirayasu Y, Lee CU, Ciszewski A, Yurgelun-Todd D, Kikinis R, Jolesz FA, McCarley RW. Progressive decrease of left superior temporal gyrus gray matter volume in first-episode schizophrenia. *American Journal of Psychiatry*, in press.
36. Gilbertson MW, Shenton ME, Ciszewski A, Kasai K, Lasko NB, Orr SP, Pitman RK. Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nature Neuroscience*, in press.
37. Onitsuka T, Shenton ME, Kasai K, Nestor PG, Toner SK, Kikinis R, Jolesz FA, McCarley RW. Fusiform gyrus volume reduction and facial recognition in chronic schizophrenia. *Archives of General Psychiatry*, in press.
38. Kubicki M, Shenton ME, Salisbury DF, Hirayasu Y, Kasai K, Kikinis R, Jolesz FA, McCarley RW: Voxel-based morphometric analysis of gray matter in first-episode schizophrenia. *Neuroimage*, in press.
39. Anderson JE, Wible CG, McCarley RW, Jakab M, Kasai K, Shenton ME: An MRI study of temporal lobe abnormalities and negative symptoms in chronic schizophrenia. *Schizophrenia Research*, in press.
40. Lee CU, Shenton ME, Salisbury DF, Kasai K, Onitsuka T, Dickey CC, Yurgelun-Todd D, Kikinis R, Jolesz FA, McCarley RW: Fusiform gyrus volume reduction in first-episode schizophrenia: an MRI study. *Archives of General Psychiatry* 59, 775-781, 2002.

#### **Clinical (neurophysiology)**

41. Kasai K, Yamada H, Kamio S, Nakagome K, Iwanami A, Fukuda M, Yumoto M, Itoh K, Koshida I, Abe O, Kato N. Neuromagnetic correlates of impaired automatic categorical perception of speech sounds in schizophrenia. *Schizophrenia Research* 2002, in press.
42. Kasai K, Asada T, Yumoto M, Takeya J, Matsuda H: Evidence for functional abnormality in the right auditory cortex during musical hallucinations. *Lancet* 354: 1703-1704, 1999.
43. Kasai K, Okazawa K, Nakagome K, Hiramatsu K, Hata A, Fukuda M, Honda M, Miyauchi M, Matsushita M: Mismatch negativity and N2b attenuation as an indicator for dysfunction of the preattentive and controlled processing for deviance detection

- in schizophrenia: a topographic event-related potential study. *Schizophrenia Research* 35: 141-156, 1999.
44. Iwanami A, Okajima Y, Kuwakado D, Isono H, Kasai K, Hata A, Nakagome K, Fukuda M, Kamijima K. Event-related potentials and thought disorder in schizophrenia. *Schizophrenia Research* 42, 187-191, 2000.
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# Department of Neurology

## Outline and Research Objectives

The Department of Neurology as a division of clinical service in The University Hospital was established in 1964 separated from the third Department of Internal Medicine. The facilities for research in neurology were provided from the Faculty of Medicine as a division of clinical research in the Institute for Brain Research until March 1997. In April 1997, our department was reorganized as a Department in the Division of Neuroscience, Graduate School of Medicine.

Activities of our department include 1. clinical service in the out-patient clinic and the ward in the University Hospital, 2. undergraduate and postgraduate education of neurology in the Faculty of Medicine and Graduate School of Medicine, and 3. research in neuroscience in the Graduate School of Medicine. Besides a weekly ward round, a weekly clinical conference is held for discussion on cases with difficult problems. A weekly neuroimaging conference and journal club are also held. Moreover, a bimonthly "Neuroscience Club" with stimulating discussions is held where informal lectures are given by invited distinguished guests in the field of neuroscience from outside the University.

The Research Objectives of the Department of Neurology is to elucidate molecular mechanisms of neurological diseases and development of new therapeutic strategies based on our understanding of molecular mechanisms of neurological diseases.

## Faculties and Students

Professor and Chair	Shoji Tsuji, MD, PhD (2002)
Associate Professors	Shin Kwak, MD, PhD (1997)
Lecturer	Susumu Kusunoki, MD, PhD (1999)
Lecturer	Yoshikazu Ugawa, MD, PhD (1997)
Associate .....	4
Graduate student .....	18

## Past Research and Major Accomplishments

The research activities have been focused on elucidation of molecular mechanisms of neurodegenerative diseases based on molecular genetics approaches. We took a strategy for collecting as many as families with various hereditary neurological diseases. We have conducted a number of projects that include linkage mapping and eventual positional cloning of the causative genes. For the diseases in which we discovered the causative genes, analyses of molecular mechanisms of neurodegeneration have been conducted.

### 1. Identification of causative genes for neurodegenerative diseases

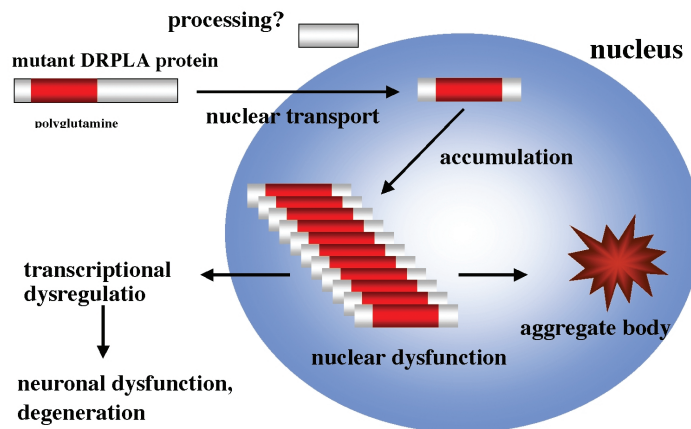
By linkage analysis, we have identified the loci for Machado-Joseph disease (Nature Genet. 4:300-304, 1993), hereditary progressive dystonia (Segawa disease) (Ann. Neurol. 37:405-408, 1995) and autosomal

recessive Parkinsonism (AR-JP) (Am. J. Hum. Genet. 60:588-596, 1997). Furthermore we have identified the causative gene for dentatorubral-pallidolusian atrophy (DRPLA) (Nature Genet. 6:9-13, 1994), hereditary progressive dystonia (Nature Genet. 8:236-242, 1994), spinocerebellar ataxia type 2 (SCA2), early-onset ataxia with ocular motor apraxia and hypoalbuminemia (EAOH) (Nature Genet. 29: 184-188, 2001), Charcot-Marie-Tooth disease type 2 (CMT2) (Cell 105:587-597, 2001) and SCA17 (Hum. Mol. Genet. 8:2047-2053, 1999, Hum. Mol. Genet. 10: 1441-1448, 2001).

### 2. Elucidation of molecular pathogenesis of neurodegenerative diseases

Based on identification of causative genes for the abovementioned neurodegenerative diseases, our laboratory further expanded our studies to elucidate the molecular mechanisms of neurodegeneration in these diseases. Our major effort has been focused on molecular mechanisms of neurodegeneration in polyglutamine diseases caused by expanded CAG repeats coding for polyglutamine stretches. We have created transgenic mice having a full-length human mutant DRPLA gene as a single copy, which carry various lengths of polyglutamine stretches (Q76, Q113 and Q129 mice at the same insertion site). The expression levels of the transgene were comparable to those of endogenous mouse DRPLA gene. Q129 mice showed severe neurological phenotype including ataxia,

**Figure. Molecular mechanisms of neuronal degeneration**



myoclonus and epilepsy with premature death by 16 weeks, while Q76 mice did not show any obvious phenotypes. Q113 mice showed milder phenotypes compared with those of Q129 mice. Given the fact that the insertion site of the transgene (human full-length mutant DRPLA gene) is identical in these Q129, Q113 and Q76 mice, these data strongly suggest that the variability in the phenotypes exclusively depends on the length of the polyglutamine stretches. Interestingly, neuronal loss was not evident in the brains of these mice, suggesting that neuronal dysfunction not but neuronal death underlies the basic pathophysiologic mechanisms of neurodegeneration. Detailed analysis of these mice demonstrated that the earliest neuropathological change was intranuclear accumulation of mutant proteins (diffuse nuclear staining) with preferential involvement of cerebellar nuclei, red nuclei, globus pallidus, subthalamic nuclei and cerebral cortex. Similar findings were also confirmed in human autopsied brains of DRPLA patients. These data strongly suggest that neuronal dysfunction is the basic molecular mechanisms of neurodegeneration in polyglutamine diseases.

Given the fact that nuclear dysfunction underlies the molecular mechanisms of neuronal degeneration in polyglutamine diseases, we investigated nuclear proteins that bind to expanded polyglutamine stretches. Employing yeast two-hybrid assays, we have found TAF130 (TATA-binding protein associated factor) binds to expanded polyglutamine stretches. The association was further confirmed by co-localization of TAF130 and expanded polyglutamine stretches not only in transient expression assays but also in human autopsied brains of DRPLA and MJD patients. Since TAF130 is involved in CREB-dependent transcriptional activation, association between TAF130 and expanded polyglutamine stretches raised the possibilities that suppression of CREB-dependent transcription, which has been demonstrated to be essential for neuronal survival and plasticity, leads to neuronal

dysfunctions. Suppression of CREB-dependent transcription by expanded polyglutamine stretches was confirmed by a reporter assay, and, furthermore, by monitoring endogenous cAMP responsive genes such as c-FOS. Expression profiling of the Q129 mouse brains further demonstrated down-regulation of many cAMP responsive genes including c-FOS and EGR-1.

To develop therapeutic approaches for polyglutamine disease, we have investigated possibilities for abrogating suppression of CREB-dependent transcriptional activation. We found that increase in intracellular cAMP levels abrogate the suppression of the CREB-dependent transcriptional activation. We have further demonstrated that histone deacetylase (HDAC) inhibitor has similar effects. Thus, stimulation of transcription by cAMP or HDAC inhibitors has potential roles in therapeutic measures for polyglutamine diseases.

## Current Research

### 1. Molecular pathogenesis of polyglutamine diseases

Current research is being focused to the following points. 1. Detailed analyses of transcriptional dysregulation in polyglutamine diseases. 2. Development of therapeutic measures based on the molecular pathogenesis of polyglutamine diseases.

For elucidating the mechanisms of transcriptional dysregulation, detailed expression profiling analyses of the Q129, Q113 and Q76 mice are being conducted. Similar expression profiling of human autopsied brains of patients with polyglutamine diseases is also being conducted. Abnormalities in CREB-dependent transcriptional activation in vivo are also being investigated by generating double transgenic mice for DRPLA and Cre-LacZ (Lac-Z gene was inserted under the promoter containing CRE.),

For development of therapeutic approaches, the



possibilities of increasing intracellular cAMP and administration of HDAC inhibitors are being investigated. To further establish a sensitive assay system, a sensitive assay system for CREB-dependent transcriptional activation using cultured cells is being developed.

## 2. Molecular pathogenesis of neurodegenerative diseases caused by deficiently in DNA repair

We have recently identified the causative gene, aprataxin, for an autosomal recessive spinocerebellar ataxia (early-onset ataxia associated with ocular motor apraxia and hypoalbuminemia). Preliminary studies suggest that aprataxin binds to XRCC-1, a key molecule involved in single strand DNA break repair. The hypothesis that XRCC-1 is involved in the single strand DNA break repair is further supported by the fact that aprataxin has a polynucleotide kinase-like domain.

Since a number of neurodegenerative diseases with mutations in the DNA repair systems have already been found, our finding further strengthens the functional role of DNA repair system in neurodegenerative diseases. Since the role of single strand DNA repair system in neurodegenerative diseases has not been well established, the future research will be focused on the roles of single strand DNA break repair in neurodegenerative diseases. The fact that hypoalbuminemia becomes evident only in adulthood further raises the possibility that transcription-coupled repair of highly actively transcribed genes such as albumin gene might be involved in this disease. This hypothesis is intriguing, since brain is the organ with highly active transcription activities.

## 3. Molecular pathogenesis of neurodegenerative diseases with complex trait

To elucidate molecular pathogenesis of sporadic neurodegenerative disease, we are organizing a consortium to establish a nation-wide collaborative network. To elucidate the molecular pathogenesis of such neurodegenerative diseases with complex trait, we need to establish large databases and to collect as many as samples. We are focusing on amyotrophic lateral sclerosis (sporadic ALS, and ALS in Kii Peninsula) and multiple system atrophy. Effort is being made to collect as many as multiples families for ALS and multiple system atrophy. We are performing both linkage analysis based on multiplex families and association studies (case-control studies). These two strategies will complement each other.

### Future Prospects

For future prospects, the following projects are being planned.

## 1. Development of therapeutic measures for polyglutamine diseases.

For accomplishing this goal, various approaches are being planned. Considering the cascade shown in the above Figure, the therapeutic approaches should be focused on 1. suppression of mutant gene expressions at the upstream cascade, and 2. enhancement of CREB-dependent transcriptional activation at the downstream cascade will be the two major targets.

## 2. Molecular pathogenesis of neurodegenerative diseases caused by deficiently in DNA repair

Detailed reconstruction studies will be conducted to elucidate the physiological functions of aprataxin. Knock-out mice for aprataxin gene are being generated, which should be essential not only for elucidation of molecular pathogenesis, but also development of therapeutic approached.

## 3. Molecular pathogenesis of neurodegenerative diseases with complex trait

To elucidate the molecular mechanisms of neurodegeneration of sporadic diseases, the following strategies are being planned. 1. large-scale collection of resources including database on clinical information and genomic DNAs based on a nation-wide consortium will be essential for future research. The collection of resources will be focused on a large scale case and controls, as well as, intensive collection of multiplex families. For example, we have recently identified 4 multiplex families with multiple system atrophy (MSA), suggesting detailed genome-wide analyses of these families may provide important findings as to the genetic component involved in these diseases. These two approaches (large scale case-control studies and intensive analyses of multiplex families) should complement, and allow us to identify the genes involved in the pathogenesis.

### Research Grants

Grant for the Research for the Future Program from the Japan Society for the Promotion of Science, a grant from the Research Committee for Ataxic Diseases, the Ministry of Health, Labor and Welfare,

"Identification of Genes Involved in Brain Diseases"

FY1996	94,998
FY1997	75,682
FY1998	78,658
FY1999	85,516
FY2000	76,286
FY2001	50,000
FY2002	47,000
	in thousands of yen

Grant for Scientific Research on Priority Areas (C) - Advanced Brain Science Project- from the Ministry of Education, Culture, Sports, and Science and Technology, Japan "Molecular mechanisms of neurodegeneration"

FY2001 66,600

FY2002/10/29 59,200

in thousands of yen

Grant for Scientific Research (A) from the Ministry of Education, Culture, Sports, and Science and Technology, Japan

FY2002 19,200

FY2003 11,300

in thousands of yen

Grant from the Ministry of Health, Labor and Welfare, Japan "Study for development of therapeutic measures for polyglutamine diseases!

FY1998 24,000

FY1999 24,000

FY2000 16,000

in thousands of yen

### Select Publications

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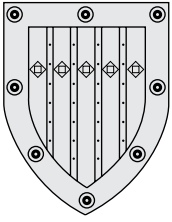


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# Department of Neurosurgery

## Outline and Research Objectives

The Department of Neurosurgery focuses its academic activities on patient care, research, and education. Services of the Neurosurgical Clinic are devoted to the care of patients who suffer from brain tumors, cerebrovascular disorders, head injuries, spinal diseases, malformations of the central nervous system, and functional disturbances of the brain. The research objectives of the department are based on these neurosurgical diseases. Clinical research has been performed by analyzing many neurosurgical cases. The attempts to develop new neurosurgical techniques are ongoing. Since the first Gamma-knife unit in this country was installed in 1991, many studies have been conducted which analyze the outcome of patients treated by this modality. To solve problems encountered in a clinical setting, cell biology and molecular biology of ischemic brain injury and brain tumors have been pursued as the two major research subjects. The research on ischemic brain damage has been carried out using rodent ischemia models. The objective of the research has been to determine the molecular mechanism underlying delayed neuronal death in the hippocampus following ischemia, but it has recently shifted toward the research on neuronal regeneration in the hippocampus by activation of endogenous progenitor cells. The research on brain tumors has mainly focused on the genetic analysis of tumor specimens obtained during neurosurgery. This research strategy has particularly employed the genetic analysis of malignant brain tumors.

## Faculty and Students

Professor and Chair	Takaaki Kirino, M.D., Ph.D. (1992~)
Associate Professor	Akio Morita, M.D, Ph.D.
Lecturer	Nobutaka Kawahara, M.D., Ph.D.
Lecturer	Tomoki Todo, M.D., Ph.D.
Associates .....	8
Graduate Students .....	7
Research Students.....	3
Secretaries .....	5
Research assistants .....	2
Residents.....	6

## Past Research and Major Accomplishments

The research performed by our department has been divided into three categories: clinical research, basic research on ischemic brain damage, and basic research on brain tumors.

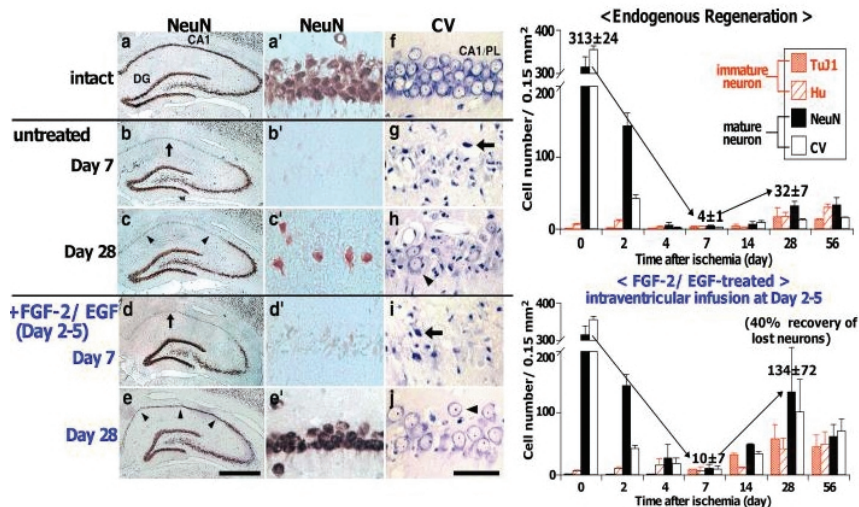
Research of clinical materials has been encouraged since it may be effective in improving current and future patient care. Most clinical publications by this department are case studies, such as the rupture of intracranial aneurysms (16, 17, 22, 25, 34, 41), arteriovenous or venous malformations (11, 32, 46), brain tumors (8, 15, 21), and other disorders (13). The results of Gamma-knife radiosurgery (6, 10, 12, 14, 19, 20, 33, 43) and the theoretical or technical aspect of neurosurgical treatment (18, 23, 38, 50) have also

been published. Thirty-two case reports have been published by this department in English (not listed in the references).

Our department has been continuously undertaking basic research as well. Currently, our basic research consists mainly of studies on ischemic brain damage and brain tumors. In our neurosurgical laboratory, one of the features is *in vivo* experiment using our microsurgical techniques on small animals. Another advantage of our laboratory is the availability of various specimens that are obtained during neurosurgical operations. In addition, we are rapidly expanding our research facility to take advantage of state-of-the-art techniques in cell and molecular biology. We collaborate with various laboratories of basic science and departments of engineering in an attempt to expand our future possibilities in clinical and research activities. Some of the major accomplishments in basic research in our department have been possible by mutual collaborations with such laboratories.

## Research on ischemic brain damage

When the brain is subjected to transient ischemia, the first detrimental event in the brain tissue is depletion of ATP and it is followed by anoxic depolarization of the neuronal membrane and a massive increase in the extracellular glutamate level. Glutamate is believed to be the major factor that



**Figure 1:** A small, but significantly higher number of mature neurons in the CA1 are seen at Day 28 (c, c', h). When FGF-2 and EGF are infused into the lateral ventricle of ischemic animals, CA1 pyramidal neurons degenerates extensively at Day 7 (d, d'), but the number of neurons later after ischemia markedly increases (e, e', j). (Cited and modified from Nakatomi et al. Cell 110:429-441,2002<sup>5</sup>)

injures the brain tissue during the early phase of cerebral ischemia. We attempted to examine the validity of this hypothesis by inducing focal cerebral ischemia in mice deficient in the  $\epsilon 1$  (NR2A) subunit of the NMDA receptor (39) in collaboration with Dr. Mishina of the Department of Molecular Neurobiology of this Graduate School. This experiment revealed that the brain deficient in glutamate neurotransmission is less vulnerable to ischemic insult.

The precise mechanism underlying ischemic brain injury that follows the initial extracellular glutamate surge and influx of  $Ca^{2+}$  remained unclear. Since a very brief ischemic insult can induce ischemic tolerance in the hippocampus (37), we postulated that the underlying mechanism is related to events induced by neuronal stress response. We found that significant changes in hsp70 and ubiquitin expression occur following brief transient ischemia (28, 49). The hsp70 message and protein levels increase in tolerance-induced brain tissue. Ubiquitin expression pattern is very similar to that of hsp70. When CA1 neurons die following ischemia, free ubiquitin is severely depleted, and at the same time, the level of conjugated ubiquitin increases. These findings led to the hypothesis that a disturbance in proteasomal function is the initial event that leads to neuronal apoptosis. We confirmed that under culture conditions proteasomal inhibitors induce cytochrome-c- and caspase-3-like protease-induced apoptosis (24). Then, we directly measured the proteasomal activity in the hippocampal CA1 region. This study demonstrated the selective proteasomal dysfunction in the CA1 sector after transient cerebral ischemia (1). This finding indicates that the protein-degrading system plays a major role in ischemic neuronal death following brief cerebral ischemia.

The decrease in the free ubiquitin level following brief cerebral ischemia may be attributed to protein degradation by activation of intracellular proteases, by direct protein denaturation, or by an increase in the level of nascent protein molecules as a result of disturbed protein synthesis. We have confirmed the disturbance of protein ubiquitination and, at the same time, inhibition of proteasomal function in CA1 neurons following brief cerebral ischemia. The mechanism of this dysfunction, however, is yet to be clarified. We hypothesized that activation of calcineurin, an intracellular protein phosphatase, is somehow related to proteasomal dysfunction since calcineurin inhibitor FK506 protects CA1 neurons following ischemia (47) and overexpression of calcineurin kills cultured glial cells and neurons (27). This hypothesis has not been fully tested.

Following brief ischemia, most of CA1 pyramidal cells die during 3-4 days following ischemia. The area becomes almost devoid of neurons and finally falls in classic gliosis. However, a close observation of this region for several weeks revealed a small but significant increase in the number of neurons in the CA1 subfield. Dr. Nakatomi (a graduate student of our department at that time) and Dr. Nakafuku (associate professor, Department of Neurobiology of this Graduate School) examined the possibility that the increased neurons are derived from endogenous neural progenitor cells (5). The result was positive. In addition, intraventricular infusion of FGF-2 and EGF resulted in a more than 40% increase in neuronal population in the CA1 region. The regenerated hippocampi by this procedure exhibited electrical activity when examined using slice preparations. The animals that had regenerated hippocampi performed better in the Morris water-maze task than the controls. We presented evidence that endogenous neural progenitors

can be induced *in situ* to replace the hippocampal neurons lost by ischemia. We further showed that regenerated neurons contribute to ameliorating ischemia-induced deficits in spatial cognitive functions, thus expanding the possibility of a novel neuronal replacement therapy for stroke. The neuronal changes in the CA1 sector are shown in Figure 1.

We have published other experimental results on cerebral ischemia (2, 26, 29) and on the cell biology of cerebral blood vessels (9, 31, 48).

### Research on brain tumors

Basic research on brain tumors conducted by our department has been based on specimens obtained during surgery and those donated by affiliated hospitals. A portion of these specimens were fixed in aldehyde fixatives (formalin) and embedded in paraffin. These materials were used for cellular analyses by MIB-1 staining and other immunostaining techniques. The remaining specimens were stored frozen until use for genetic analysis. We have examined many types of brain tumors such as meningiomas, oligodendrogliomas, and neurinomas in NF2 (neurofibromatosis type 2) patients.

Mutation of the NF2 genes was detected in 20-30% of sporadic meningiomas, whereas loss of heterozygosity (LOH) at chromosome 22q was found at a much higher frequency (35). To determine the correlation of merlin loss with NF2 genetic alteration, we performed a molecular genetic analysis of 50 sporadic meningiomas. Findings of this study strongly support the notion that NF2 is the sole target of 22q LOH in meningiomas and that the loss of merlin expression is always caused by alteration of NF2 gene, according to the classic "two hit" theory. Oligodendrogliomas frequently, but not always, show sensitivity to chemotherapy. Recent studies demonstrated that allelic loss of chromosome 1p is highly associated with this chemosensitivity. To clarify the molecular mechanism of this correlation, we examined comprehensive gene expression profiles of oligodendrogliomas with and without 1pLOH along with normal brain tissue using oligonucleotide microarray (GeneChip) (4). This study showed that biological differences between genetic subsets of oligodendroglioma are indeed reflected on the gene expression profile. We encountered a very rare case of malignant transformation of vestibular schwannoma following stereotactic radiosurgery. Genotyping of this tumor showed a TP53 mutation in the recurrent tumor that did not exist in the original tumor, suggesting that radiosurgery induced the malignant transformation (6). We performed molecular genetic analysis on non-selected gliomas and found that molecular genetic analysis of 1p/19q/10q/TP53 has significant diagnostic value, especially in detecting oligodendroglial tumors.

In addition, 1pLOH and TP53 mutations in gliomas may be markers of oligodendroglial and astrocytic pathways, respectively (7). We reported a case of endolymphatic sac tumor, a rare adenomatous tumor of the temporal bone, in a patient with von Hippel-Lindau (VHL) disease. Sequencing and microsatellite analysis of DNA samples indicated that VHL gene inactivation contributed to the oncogenesis of endolymphatic sac tumor (30).

We examined brain tumor specimens to analyze MEN1 gene mutation (36), telomerase activity (40), MIB-1 in central neurocytoma (42), and proliferation of chordoma (44). We also published experimental data on cultured glioma cells (3, 45).

### Current Research

We are continuously conducting clinical researches to improve neurosurgical technologies and future patient care in the neurological field. The department places strong emphasis on publishing clinical reports and particularly encourages young colleagues to do so. The objective of current clinical research in neurosurgery is gradually shifting to the improvement of less invasive neurosurgical procedures using microendoscope, navigation and robotic manipulators. This is now under way in collaboration with several departments in the Faculty of Engineering of our university. The department is involved in the nationwide epidemiological study of the incidentally-discovered unruptured cerebral aneurysms (Unruptured Cerebral Aneurysm Study of Japan, or UCAS Japan), and functions as the central office of this study.

We have been involved in experiments on the molecular mechanism underlying delayed neuronal death found in the hippocampal CA1 region following ischemia. Although we believe that this is a good model system for studying neuronal protection, experimental results obtained by us and other groups suggest that neuronal protection is extremely difficult and we are so far unable to propose clinically applicable methods of treatment. On the other hand, it turned out to be possible to induce practically meaningful regeneration of neurons in the hippocampal CA1 region by activating endogenous neural progenitor cells. Given these experimental results, we are now attempting to focus our experimental resources on experiments of regeneration of CA1 neurons.

We have been engaged in the genetic analysis of brain tumors. This strategy has been particularly effective for this department since comprehensive genetic analysis has been rapidly introduced and we can collect sufficient amounts of specimens during neurosurgical operations. This strategy is being continued but the focus on brain tumor experiments is now shifting toward research on treatment of malig-



nant brain tumors because Dr. Todo who has expertise in this field has just joined our department in January, 2003. He plans to start a clinical trial with genetically engineered retrovirus for the treatment of malignant brain tumors.

## Future Prospects

We will continue to carry out our three major research projects: i.e., clinical research, research on ischemic brain damage, and research on brain tumors. However, the main focus of each project may change. The future projects that we expect to conduct in the next 4-5 years are:

### 1. Clinical research

The aim will be the technical improvement of less invasive neurosurgical procedures using micro-endoscope, navigation, robotic manipulators as well as other devices. We will continue large-scale clinical studies such as UCAS Japan. Clinical studies on neurosurgical cases will be continuously encouraged.

### 2. Research on ischemic brain damage:

The main objective will be regeneration of hippocampal neurons following brief cerebral ischemia. We will focus on the feasibility of activation of endogenous neural progenitors under a clinical setting. The research on the mechanism underlying ischemic brain damage and ischemic tolerance will be continued.

### 3. Research on brain tumors:

We will continue genetic analysis of brain tumor specimens. Along with this investigation, we will start clinical research on the treatment of malignant brain tumors.

## Research Grants

1. Research grant from CREST (Core Research for Evolutional Science and Technology) by Japan Science and Technology Corporation "Molecular mechanism of delayed neuronal death", 1998-2002, ¥265,201,000
2. Research grant of 21st century medical development and promotion by the Ministry of Health, Labor and Welfare "Follow-up study of unruptured cerebral aneurysms diagnosed by brain check-up clinics", 1999-2001, ¥24,000,000
3. Research Grants in Natural Sciences by the Mitsubishi Foundation "Study of ischemic neuronal death and ischemic tolerance based on functional genomic/proteomic analysis", 2001, ¥7,000,000
4. Grant-in-Aid for Scientific Research (A) "Mechanism of ischemic cerebral damage by systematic expression analysis of genome and proteome", 2001-, ¥30,000,000

5. Research grant from SORST (Solution Oriented Research for Science and Technology) by Japan Science and Technology Corporation "Neuronal regeneration in the hippocampus following delayed neuronal death", 2002-, ¥75,000,000

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