Brain PET imaging in awake mice and its application to human disease models

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RIKEN Center for Biosystems Dynamics Research

RIKEN BDR RIKEN Center for Biosystems Dynamics Research

Outline

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- ✓Overview of our research group
- ✓Brain PET imaging in awake mouse
- ✓Other PET study using small animals

Facility of Molecular Imaging at RIKEN

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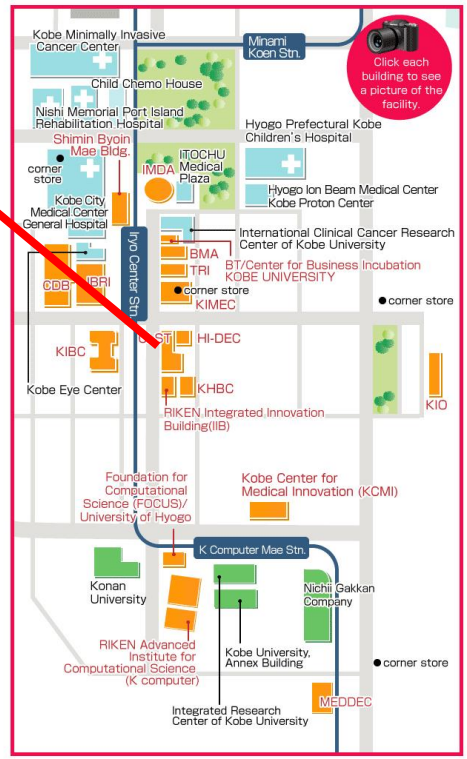
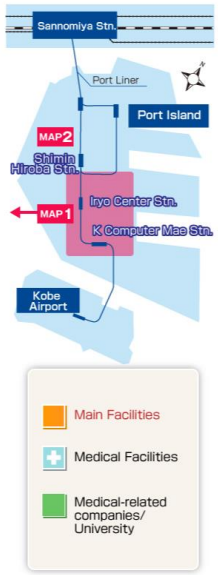
4-storied
Lot area: 6,000m²
Total floor area: 8,600m²

- 4F: Laboratory animal breeding area
- 3F: Office, Optical imaging, MALDI-TOF-MS, etc.
- 2F: Office, Synthetic organic chemical labs, etc.
- 1F: RI laboratory area, PET, MRI, etc.

KOBE Biomedical Innovation Cluster
（神戸医療産業都市）





https://www.fbri-kobe.org/kbic/

Radioisotope laboratory area

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Major instrumentation

- 2 Cyclotrons
- 14 Automated synthetic robotics
- 2 PET/CT scanners for animals
- 1 CT scanners for small animals
- 8 Hot labs (including one GMP-based)
- 2 PET scanners for small animals
- 1 Optical imaging scanners for animals
- 1 MRI (3.0T) for animals and humans

PET scanner

PET/CT scanner

Cyclotron

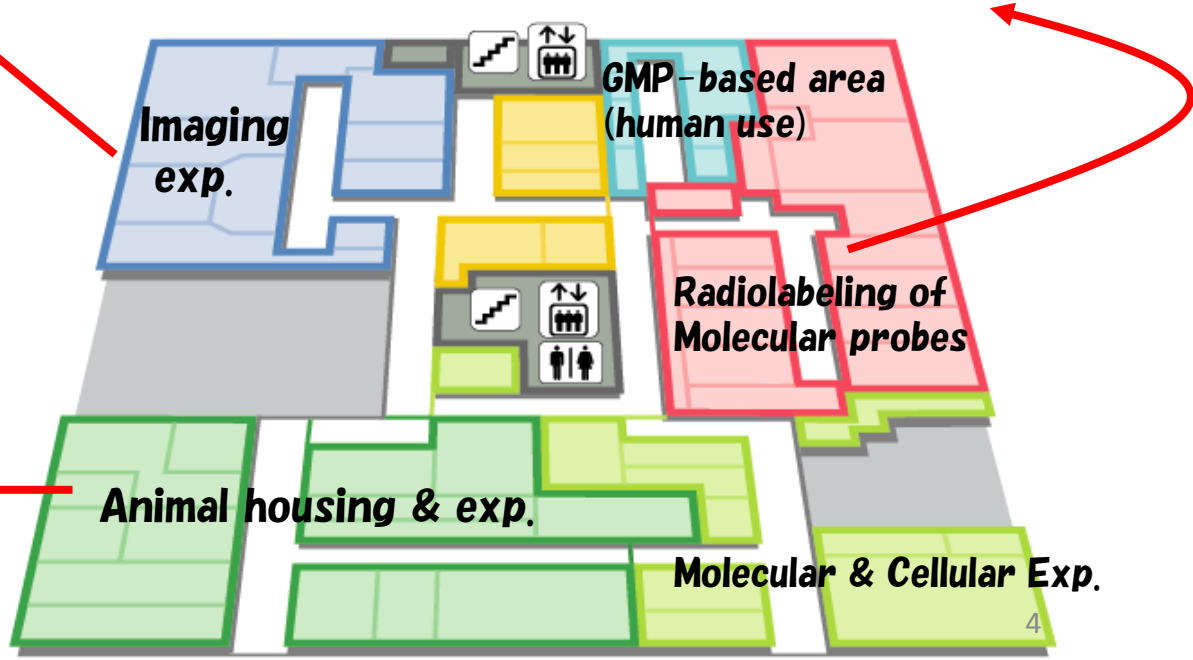
Hot Cell

Automated synthesizers

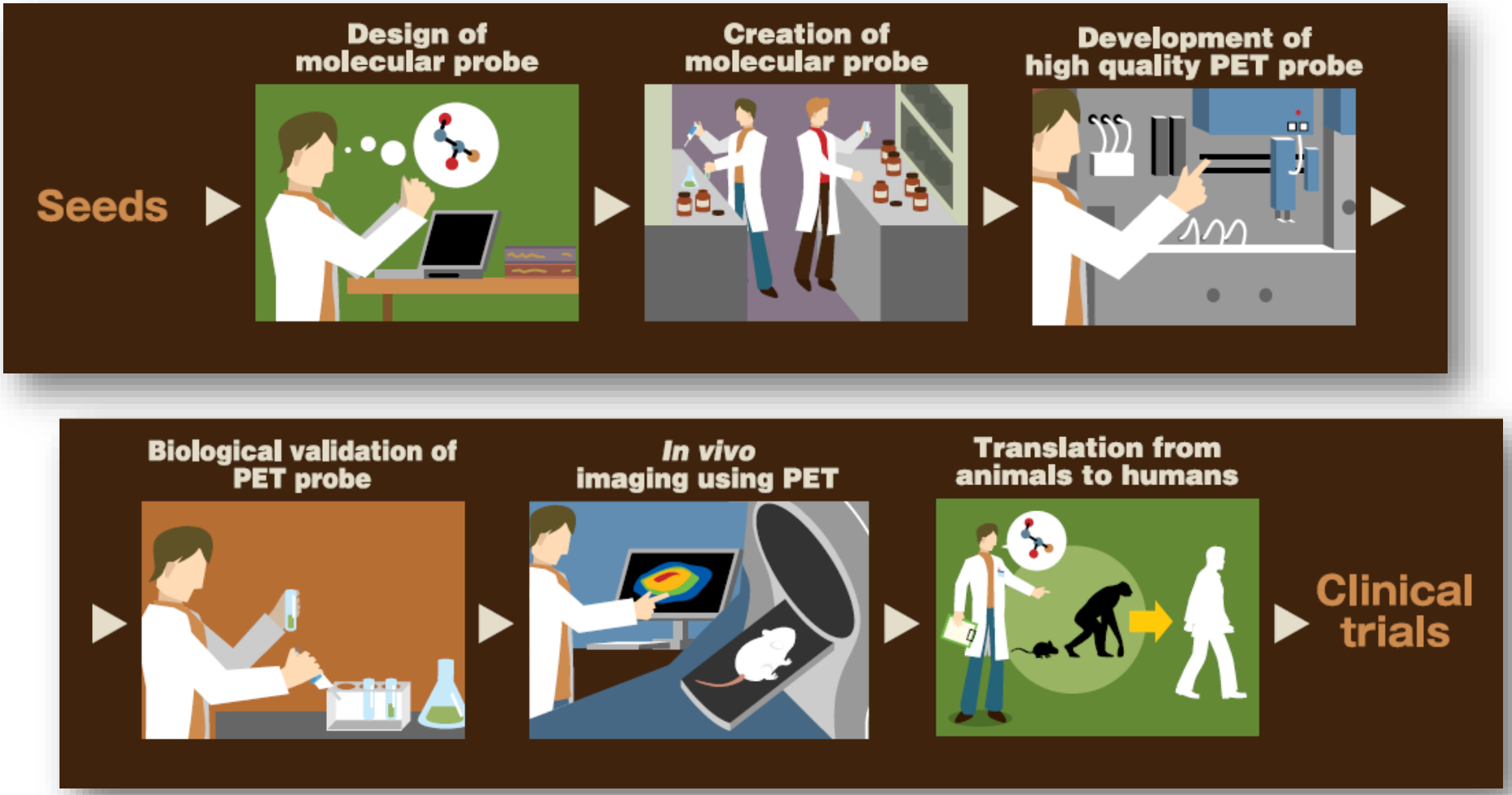
CT scanner

Optical imaging scanner

3T MRI



Workflow of Molecular Imaging



Workflow of Molecular Imaging

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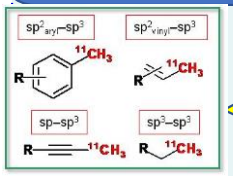
Leading Chemistry

Creation of >212 Original Molecular Imaging Probes!!

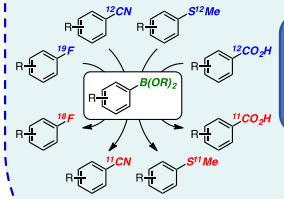
Design & Elaborate Synthesis of Imaging Probes

Labelling technologies on optimized position

Labelling technologies w/o loss of activities



4 Types of *C*-[^{11}C] Methylation Reaction



Molecular Renovation

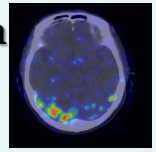
Metabolic Stability of Imaging Probes *in vitro & in vivo*

Molecular Imaging for Bio-functions & Pathophysiology

Novel Molecular Imaging Technologies

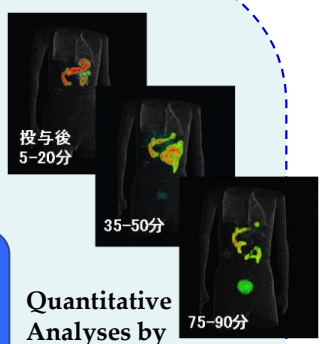
Proof of Concept

Seamless Approach From Animals to Human!!



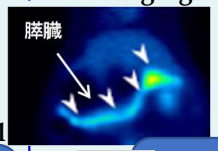
Mab Imaging for Cancer Metastasis

Clinical Studies



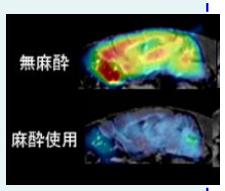
Quantitative Analyses by Oral Administration in Humans

Pancreatic β-cell mass imaging



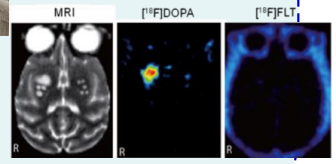
Pancreas β-cell

Molecular Imaging for Pharmacokinetics & DDS



Un-anesthetized & Anesthetized Animals

Molecular Imaging for Efficacy of Drugs & Therapies



Monitoring of iPS Cell Therapy with Human Dopaminergic Neurons on Monkey PD model

PET

Why is PET central for application to human and functioning animals?

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1. **Ultra-high sensitivity (thus microdose)**

   ultra-high specific radioactivity (short half-life):

   ***i.e.*, injection mass = 0.1-10 nmol (30-3,000 ng, if MW=300)**

1′. **Little perturbation to intrinsic system**

2. **Broad spectrum of target molecules**

   A variety of positron emitters for the radiolabel

   ^{11}C, ^{18}F, ^{13}N, ^{15}O; *"Physiological!"*, ^{64}Cu, ^{68}Ga, ^{76}Br, ^{89}Zr, ^{124}I

   principally, any organic compounds can be introduced

3. **Highly quantitative even in depth of the body**

   coincidence detection of annihilation photons

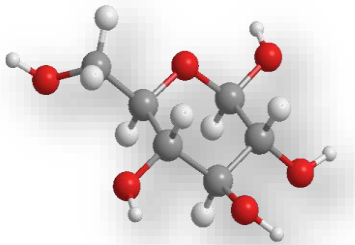
   accurate attenuation correction

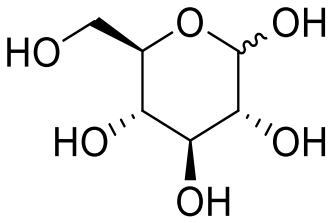
Positron emitters

Radionuclides	Half-life	Nuclear reaction	Common precursor
^{11}C	20.4 min	$^{14}N(p, \alpha)^{11}C$	$^{11}CO_2$, ^{11}CO, $^{11}CH_4$
^{13}N	9.96 min	$^{16}O(p, \alpha)^{13}N$	$^{13}NO_3^-$, $^{13}NO_2^-$, $^{13}NH_3$
^{15}O	2.03 min	$^{14}N(d, n)^{15}O$ $^{15}N(p, n)^{15}O$	$^{15}O_2$, $C^{15}O_2$, $H_2{}^{15}O$
^{18}F	110 min	$^{18}O(p, n)^{18}F$	$^{18}F^-$, $^{18}F_2$
^{68}Ga	68 min	$^{68}Ge(p, n)^{68}Ga$	^{68}Ga
^{64}Cu	12.7 hrs	$^{64}Ni(p, n)\ ^{64}Cu$	^{64}Cu
^{76}Br	16.2 hrs	$^{76}Se(p, n)^{76}Br$	^{76}Br
^{89}Zr	3.26 days	$^{89}Y(p, n)^{89}Zr$	^{89}Zr
^{124}I	4.18 days	$^{124}Te(p, n)^{124}I$	^{124}I

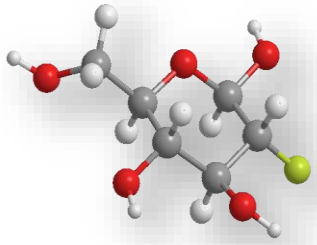
[^{18}F]FDG-PET imaging

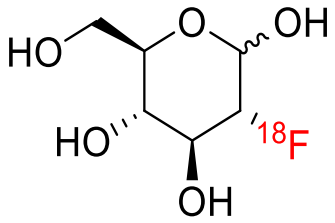
D-glucose
（ブドウ糖）



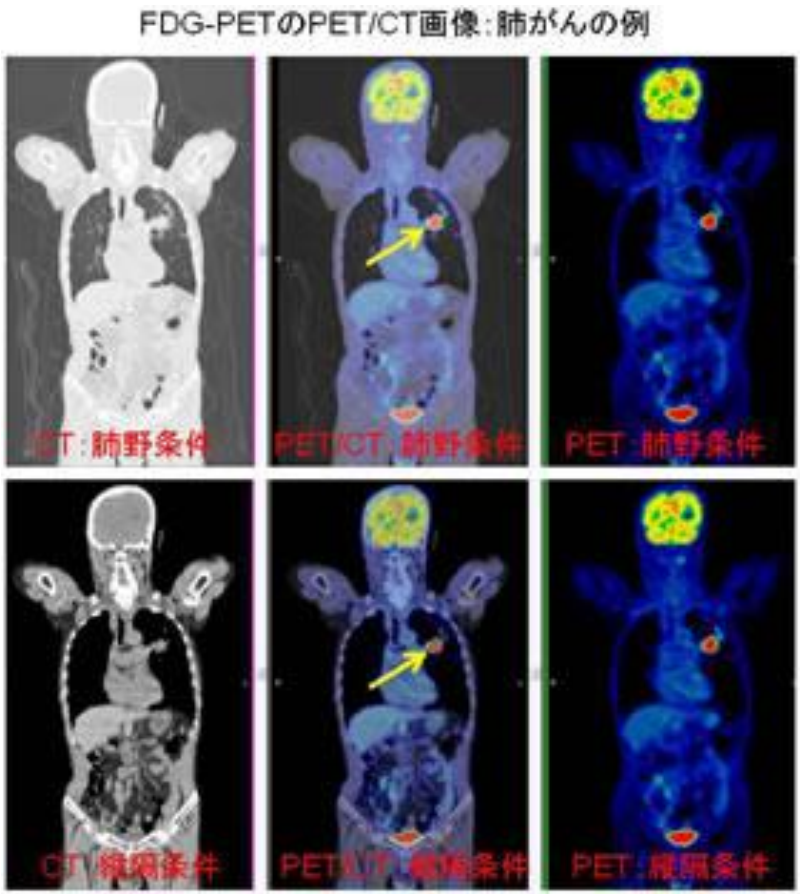


2-fluoro-2-deoxy-D-glucose (FDG)





Human PET images with [^{18}F]FDG



滋賀県立総合病院研究所HPより
http://www.shigamed.jp/pettest.html

Laboratory animals in our studies

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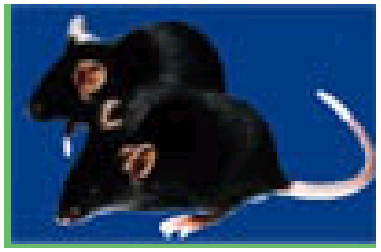
Rodents
（げっ歯類）

Mouse



Rat

- Homogeneity
- Short life span
- Inexpensive
- Large number
- Easy animal handling
- Gene modification (mouse)

Non-human primates
（非ヒト霊長類）

Marmoset

Monkey

- Resemble to human
- Longitudinal course
- Expensive
- Small number
- Difficult animal handling
- Higher brain function

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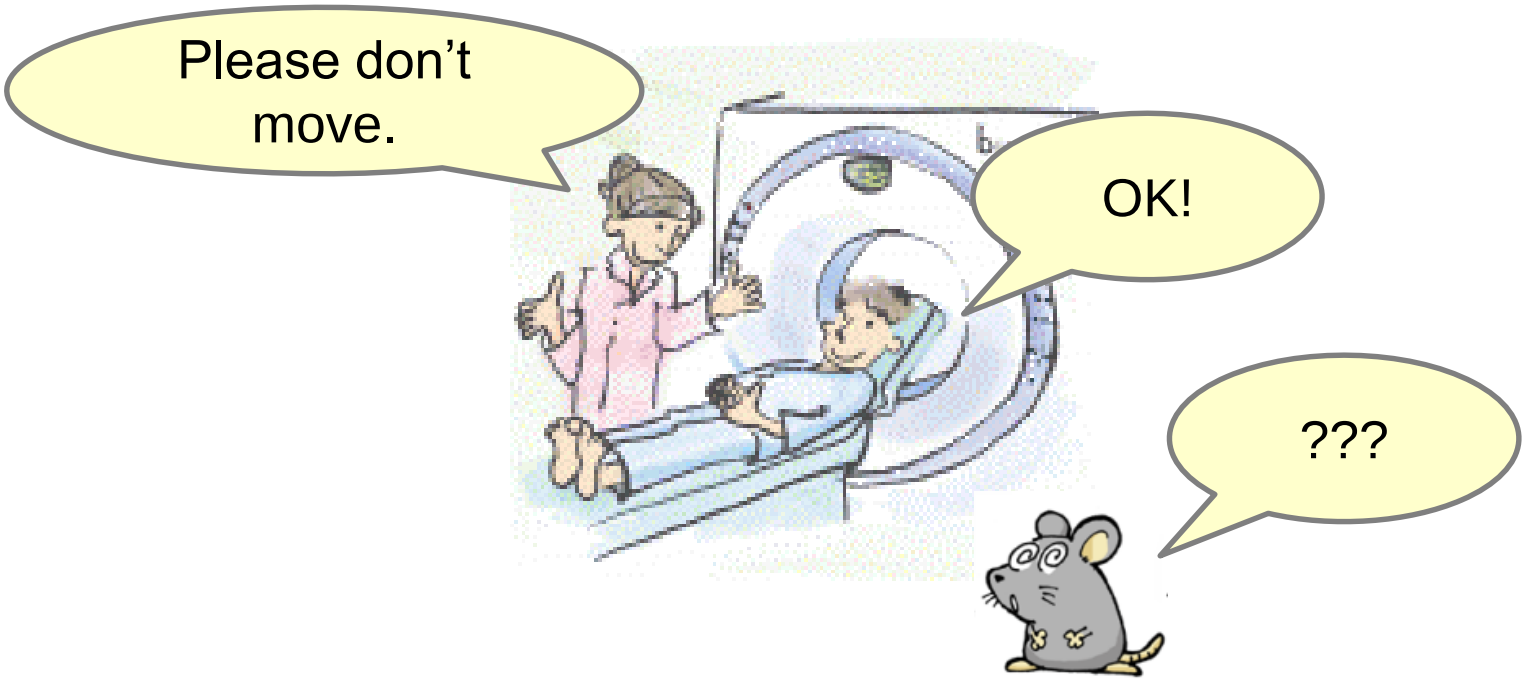
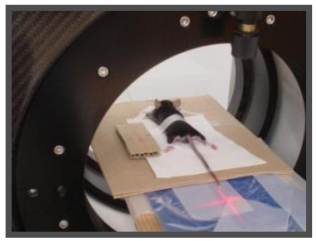
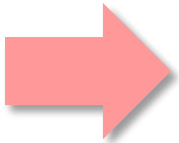
Why is anesthetics needed?



Under anesthetic condition…

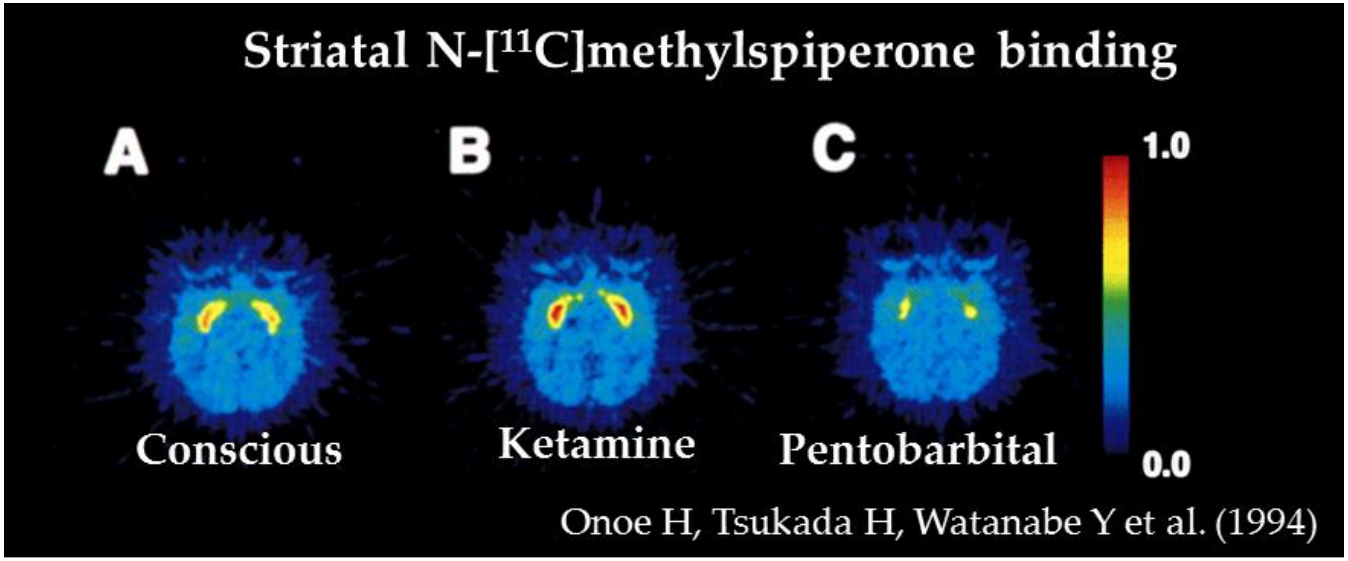




Body temp.
Heart rate
Respiration
Neural activity

Why is anesthetics needed?

A PET study using Monkey



A method for brain PET imaging in awake mouse

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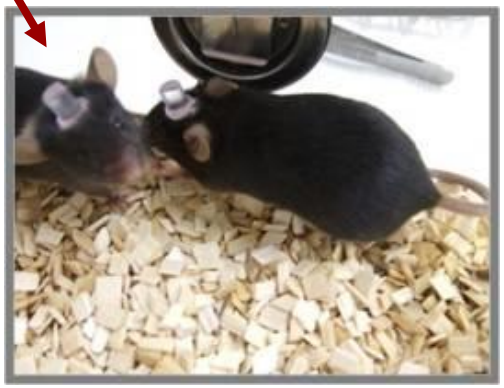
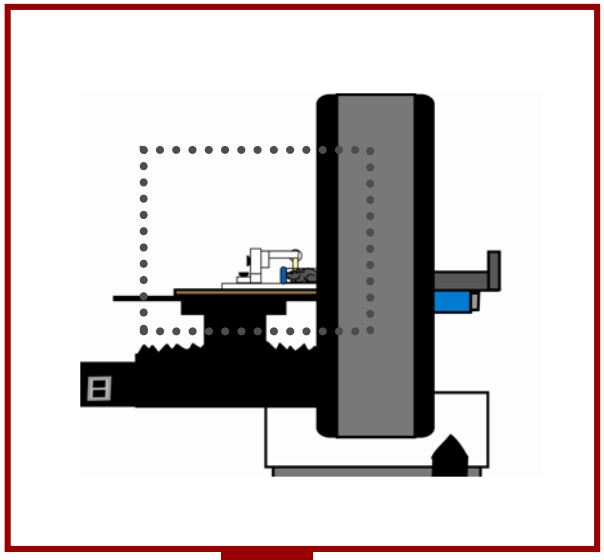
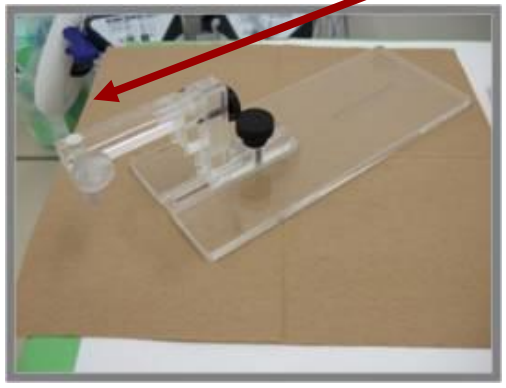
Overview of the apparatus for immobilization

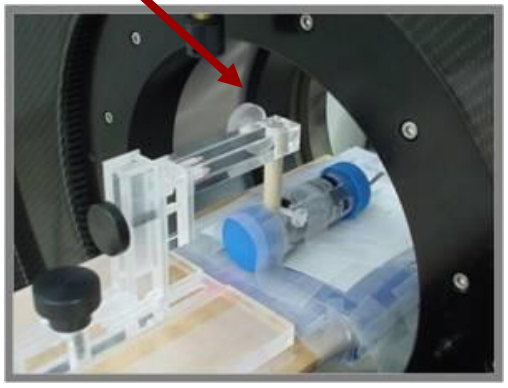
The head-holder

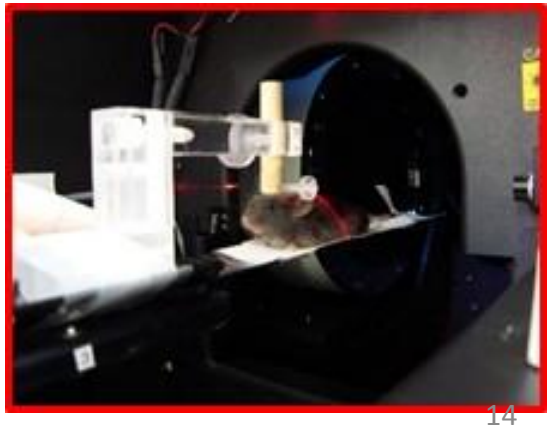




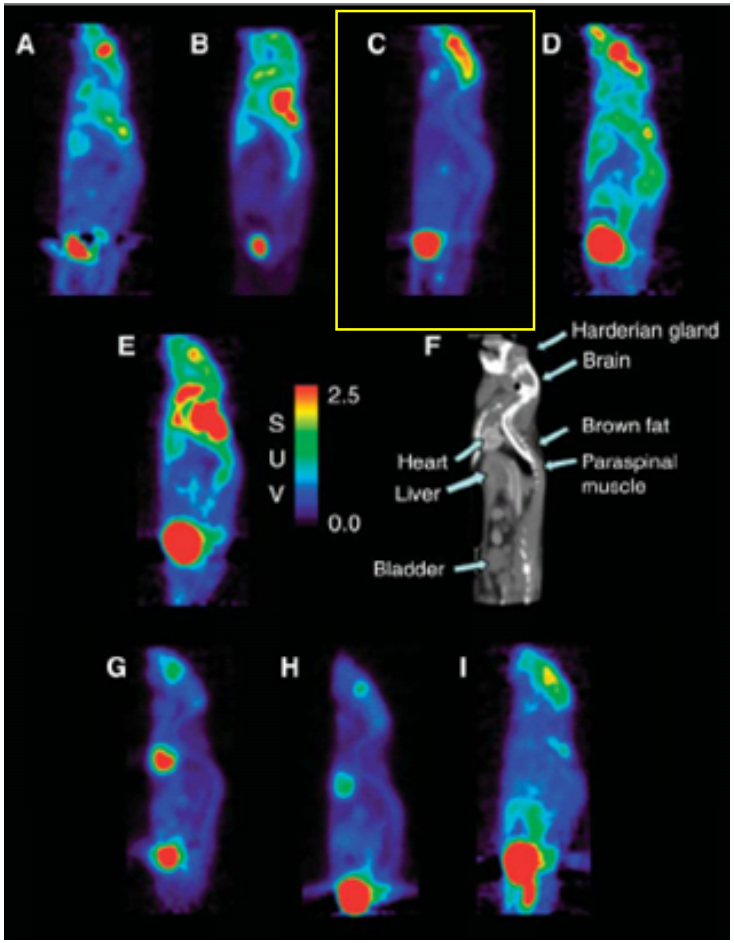
The apparatus for the head-hold







Influence on [^{18}F]FDG biodistribution under various conditions

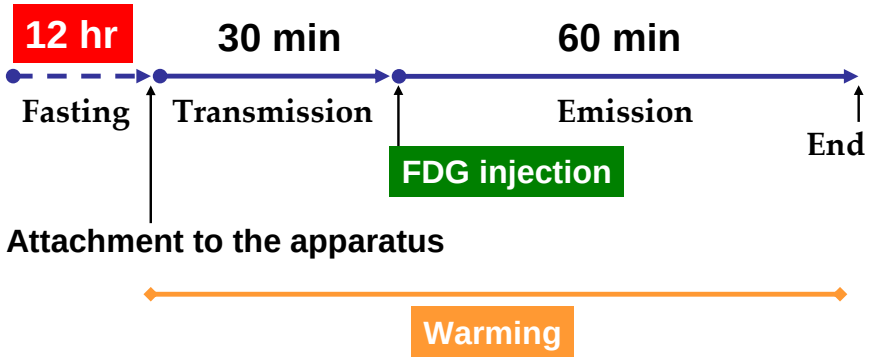


Fueger et al. (2006)

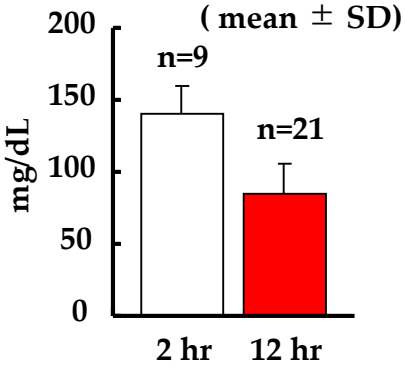
	Fasting	Warming	Anesthesia
A	No	Yes	No
B	Yes	No	No
C	Yes	Yes	No
D	Yes	Yes	No
E	No	No	No @ inj. FDG Isoflurane during PET scan
F	microCT sagittal view		
G	No	Yes	Isoflurane
H	Yes	Yes	Isoflurane
I	Yes	Yes	Ketamine

Improvement of brain [^{18}F]FDG uptake in awake mice

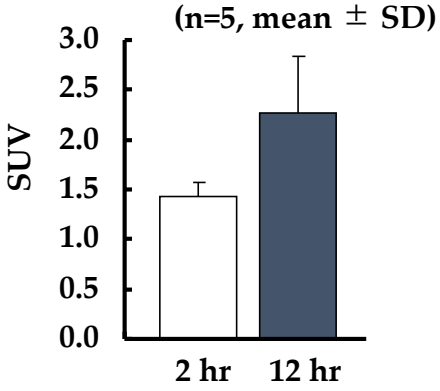
Experimental procedure



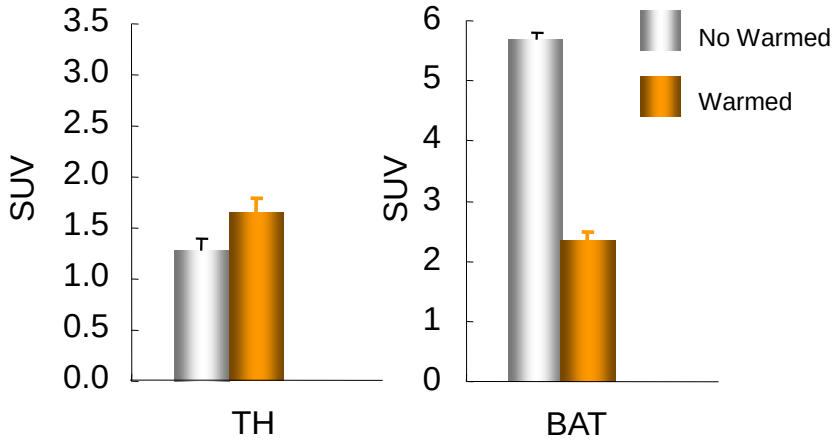
Blood glucose



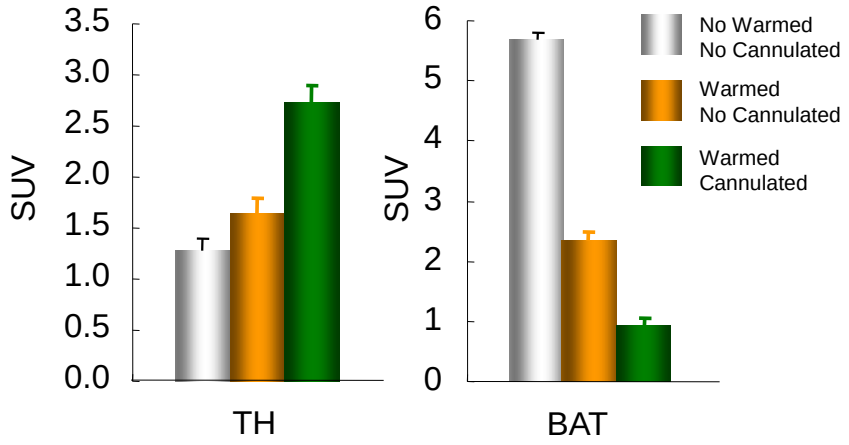
Thalamic [^{18}F]FDG uptake



Warming



FDG injection



Establishment of brain PET imaging method in awake mouse

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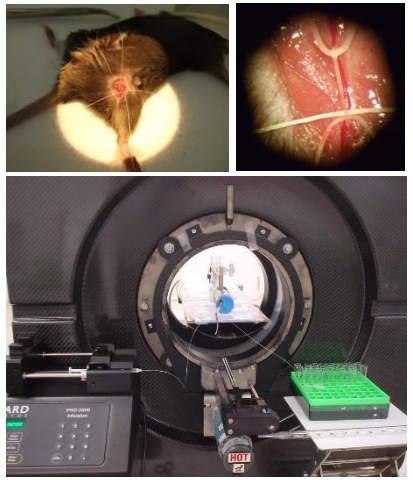
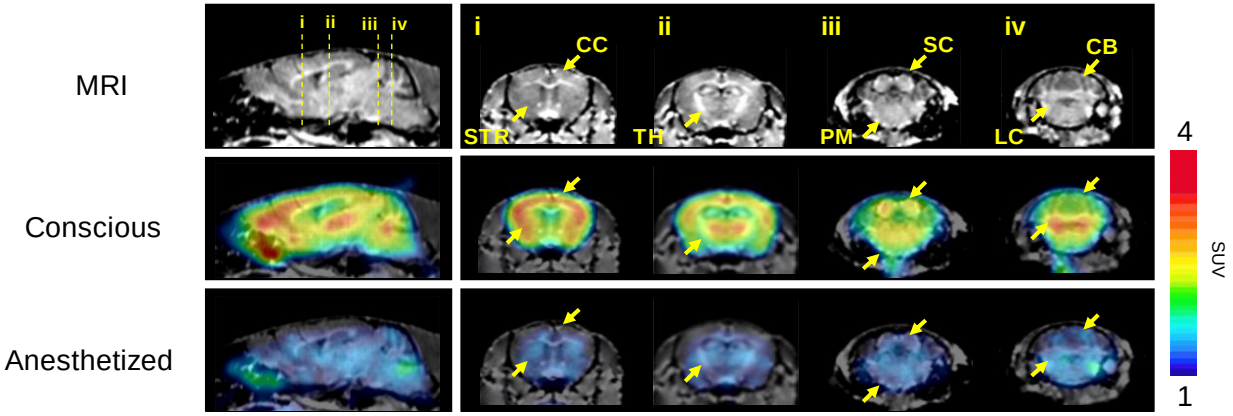
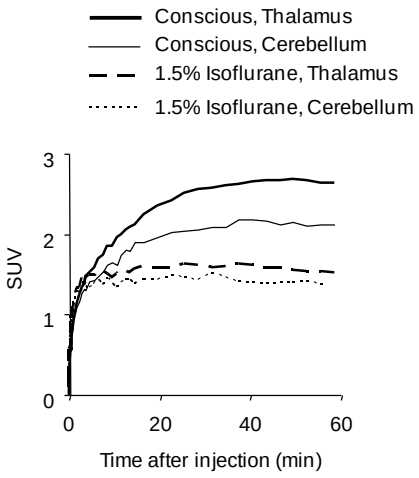
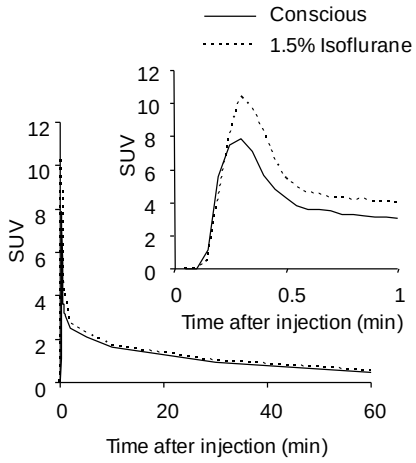
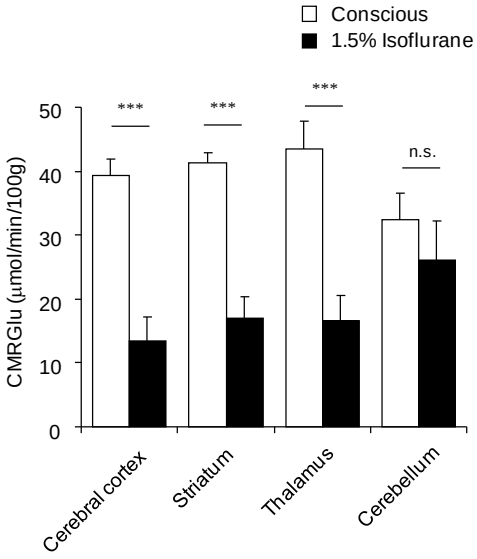
Arterial Blood Sampling











Mizuma et al., *J Nucl Med (2010)*

Tauopathy

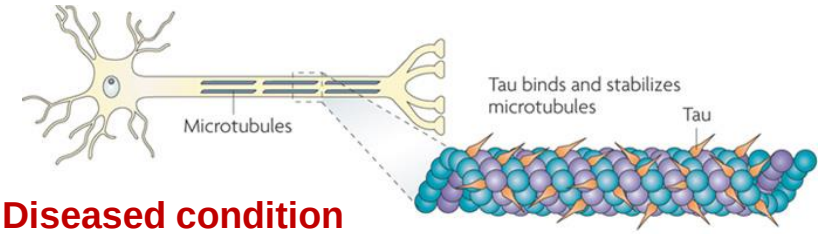
Tauopathy is a class of neurodegenerative diseases, caused by the pathological aggregation of hyper-phosphorylated tau proteins.

Related disease

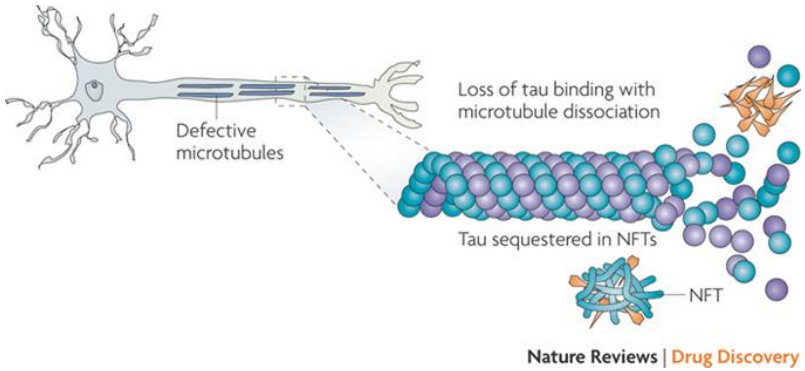
- Alzheimer's disease (AD)
- Frontotemporal dementia (FTD)
- Corticobasal degeneration (CBD)
- Frontotemporal lobar degeneration (FTLD)

Structures of microtubule

Healthy condition



Diseased condition



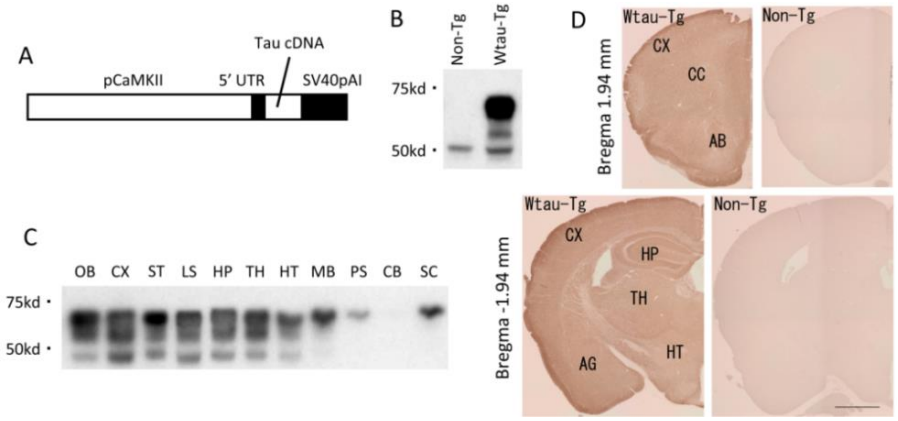
Brunden et al., *Nat Rev Drug Discover* (2009)

Comparison of other mouse models of tauopathy

Mutation/construct	Promoter	Cognition	Motor	Pathology
4R/2N isoform	Thy 1	Impairment	Deficit	Hyperphosphorylated PHFs
P301L	Thy 1.2	Impairment	Deficit	Tangle pathology detectable at 2.5 M
rTg4510 (P301L)	Prion protein	Impairment	Deficit	Tangle pathology detectable at 3 M
P301S	Prion protein	Impairment	Deficit	Synapse loss, glial activation
Tg601 (4R/2N)	**CaMK II**	**Impairment**	**Normal**	**?**

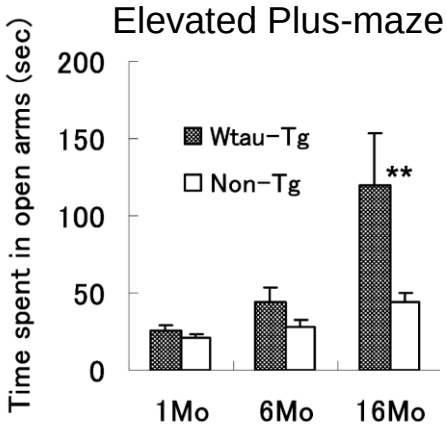
Tg601 mouse

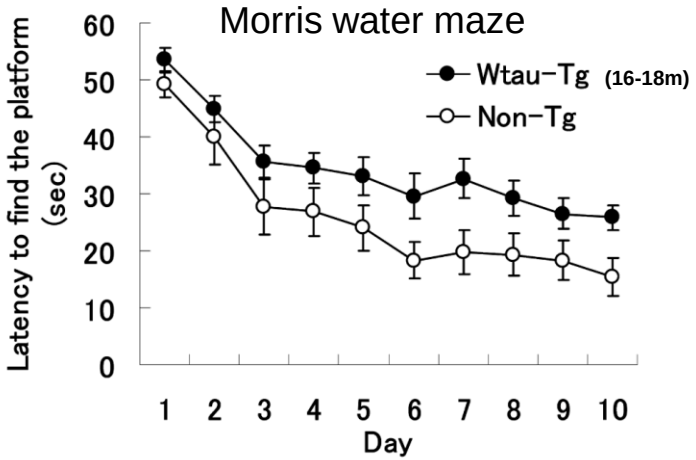
Exogenous tau protein level



Tg601 mouse was uniquely developed as a model of tauopathy, which overexpressed the wild-type human tau protein (4R2N type) under the control of CaMK-II promoter.

Behavioral characterization

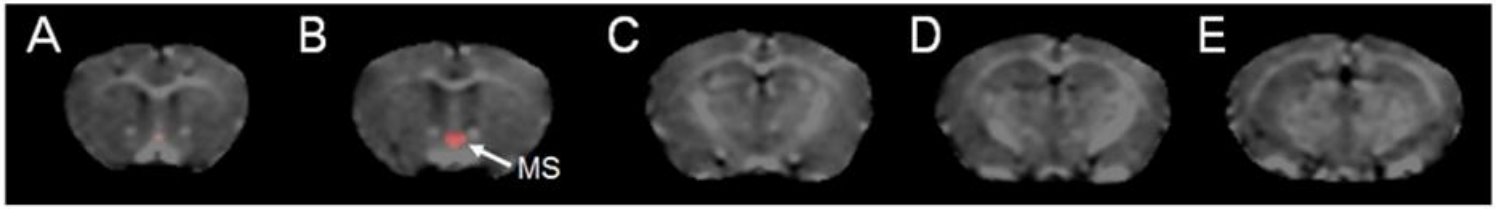




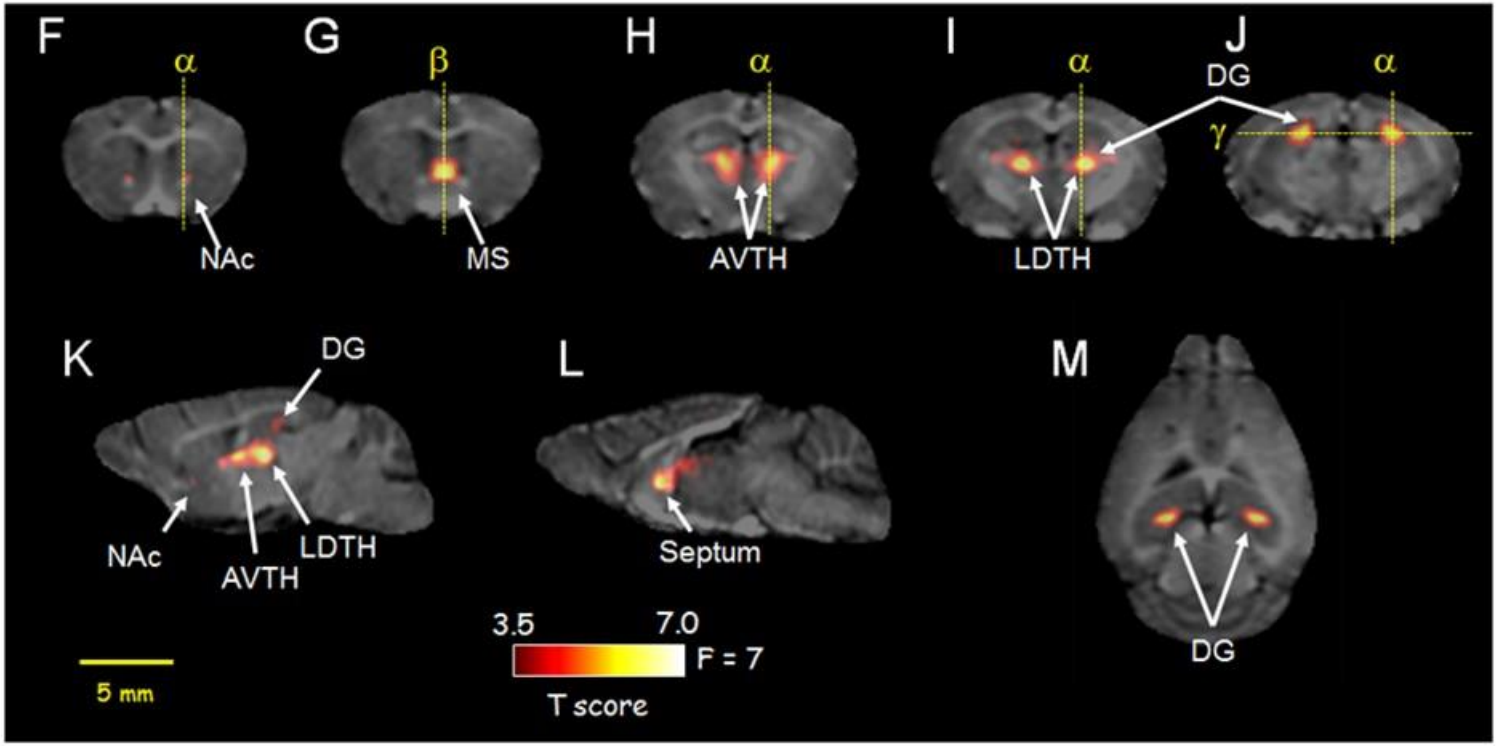
Kambe et al., *Neurobiol Dis* (2011)

Decrease in [^{18}F]FDG uptake in Tg601 mice

Adult (6 months)



Old (18-months)



Hara et al., *Curr Alzheimer Res*, in press

Low septo-hippocampal connectivity in old tg601 mice measured by *ex vivo* DT tractography

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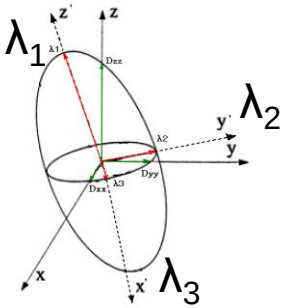
DT tractography

For visualization of neuroanatomical tracts

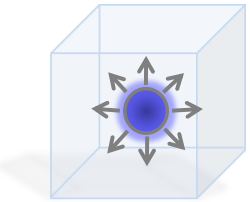
e.g. disease progression, brain development

Fractional anisotropy (FA)



Basser et al., *Biophys J,* (1994)

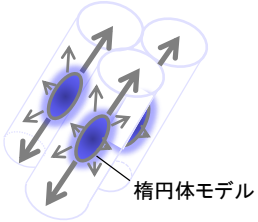
等方性拡散



$\lambda_1 = \lambda_2 = \lambda_3$

FA = 0

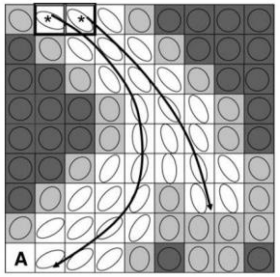
異方性拡散



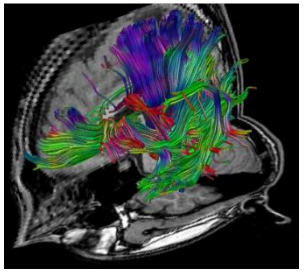
$\lambda_1 \gg \lambda_2 = \lambda_3$

FA≒1

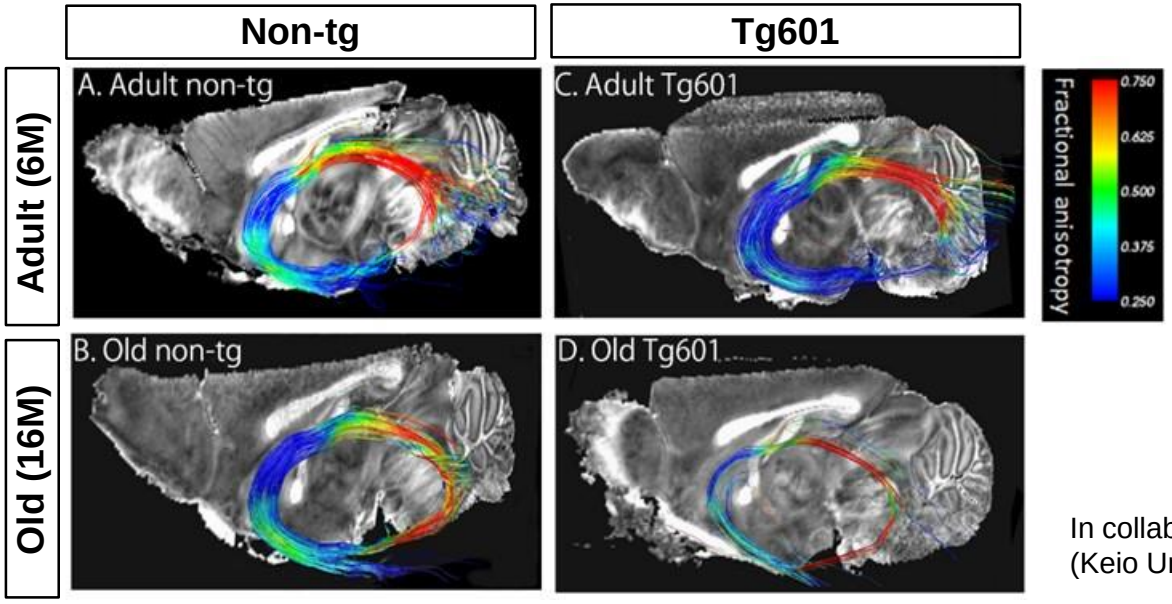
追跡方法



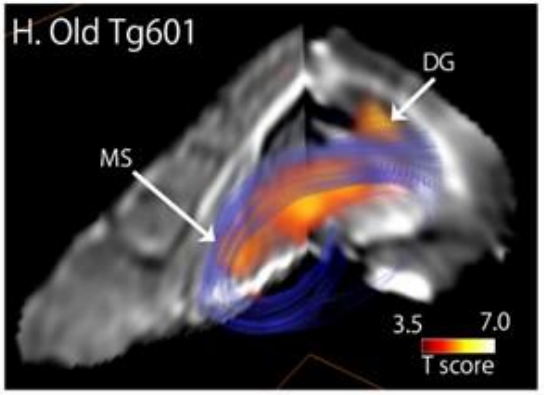
トラクトグラフィ



Mori & Zhang, *Neuron* (2006)



With [^{18}F]FDG T-map



In collaboration with Drs. Keiko Hikishima & Hideyuki Okano (Keio Univ. & Cent. Inst. Exp. Animals)

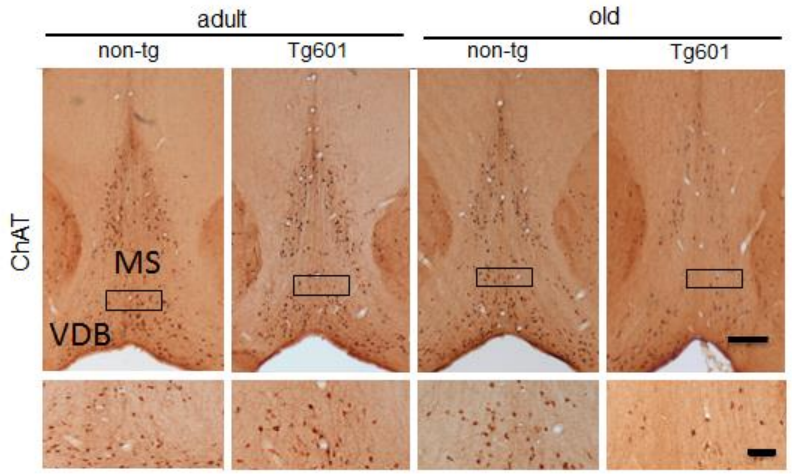
Hara et al., *Curr Alzheimer Res*, in press

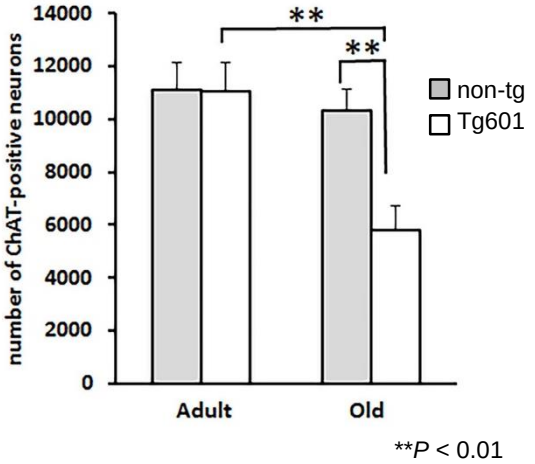
The number of choline acetyltransferase (chat)-positive neurons decreased in the medial septum in old tg601 mice

Immunohistochemistry

ChAT

Cholinergic neuron

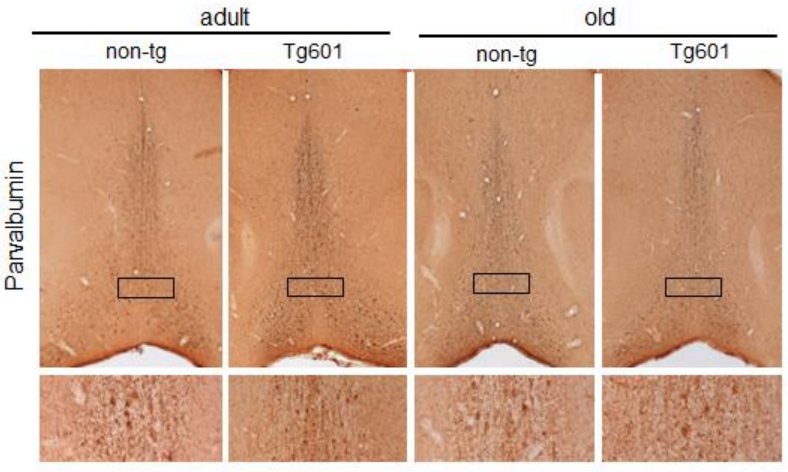


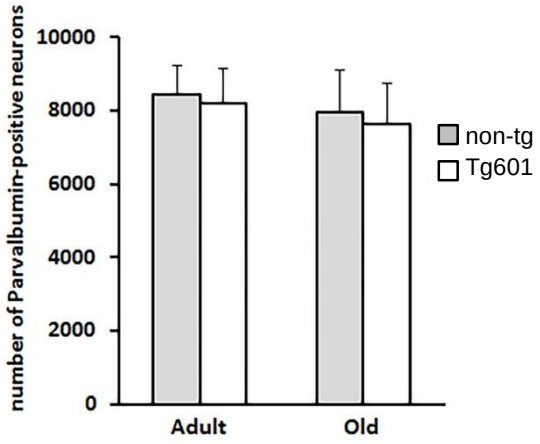


$^{**}P < 0.01$

Parvalbumin

GABAergic neuron





Hara et al., *Curr Alzheimer Res*, in press

Autistic Spectrum Disorder (ASD, 自閉症)

RIKEN BDR RIKEN Center for Biosystems Dynamics Research

Diagnostic criteria

Diagnostic and Statistical Manual, Fifth Edition, DSM-5 (2013)

A. Persistent deficits in social communication and social interaction

A-1. Failure to initiate or respond to social interactions
A-2. A total lack of facial expressions and nonverbal communication
A-3. Absence of interest in peers.

B. Restricted, repetitive patterns of behavior, interests, or activities

B-1. Stereotyped or repetitive motor movements, use of objects, or speech
B-2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns or verbal or nonverbal behavior
B-3. Highly restricted, fixated interests that are abnormal in intensity or focus
B-4. Hyper- or hyporeactivity to sensory input or unusual interest in sensory aspects of the environment

C. Symptoms must be present in the early developmental period

D. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.

E. These disturbances are not better explained by intellectual disability or global developmental delay

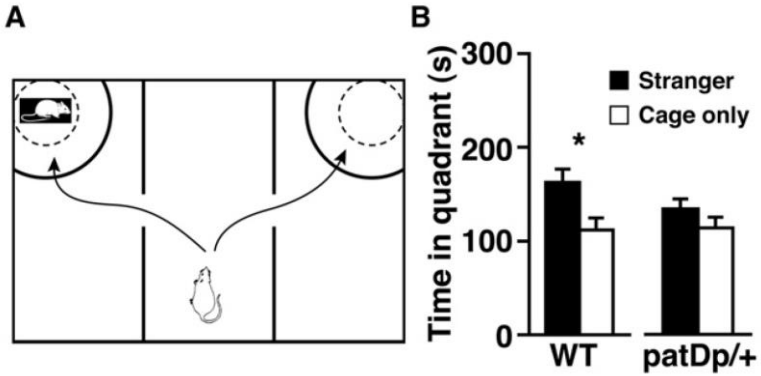
Prevalence About 1 in 68 children has been identified (about 4.5 times more boys than girls)

Treatment There are no medications that can cure ASD or treat the core symptoms.

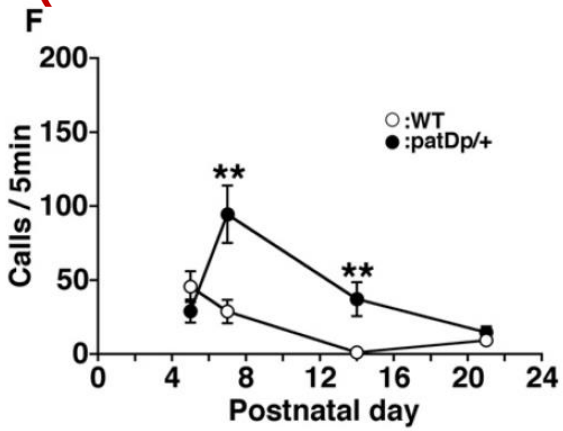
CDC homepage: https://www.cdc.gov/ncbddd/autism/index.html

ASD-like symptoms in patDp/+ mouse

Social interaction (three-chamber test)

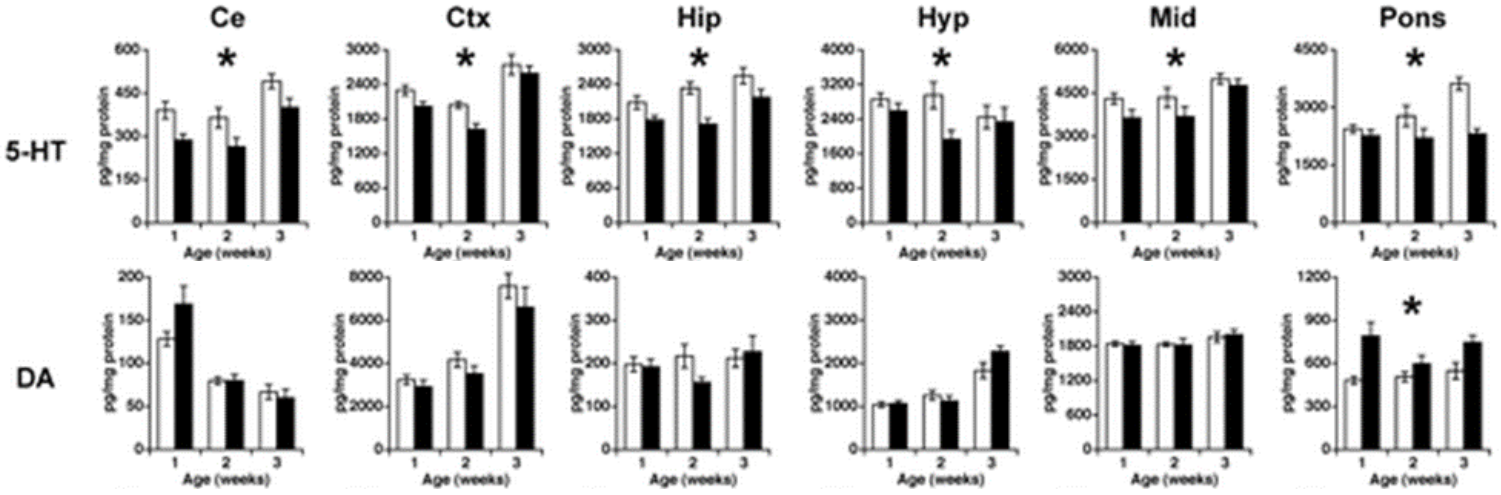


Communicative behavior (ultrasonic vocalization)



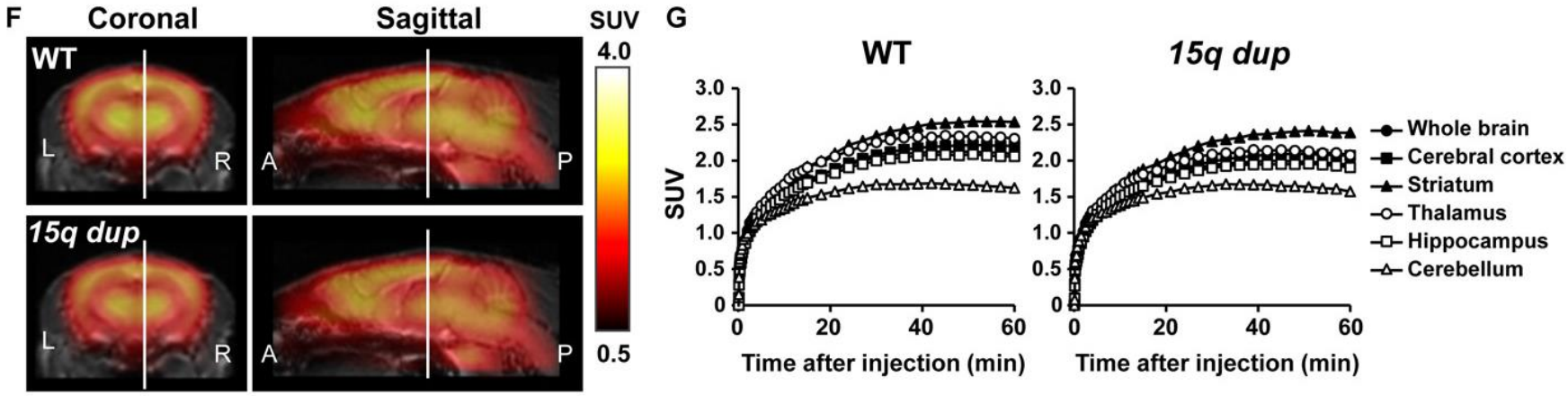
Nakatani et al., *Cell* (2009)

Brain monoamine levels



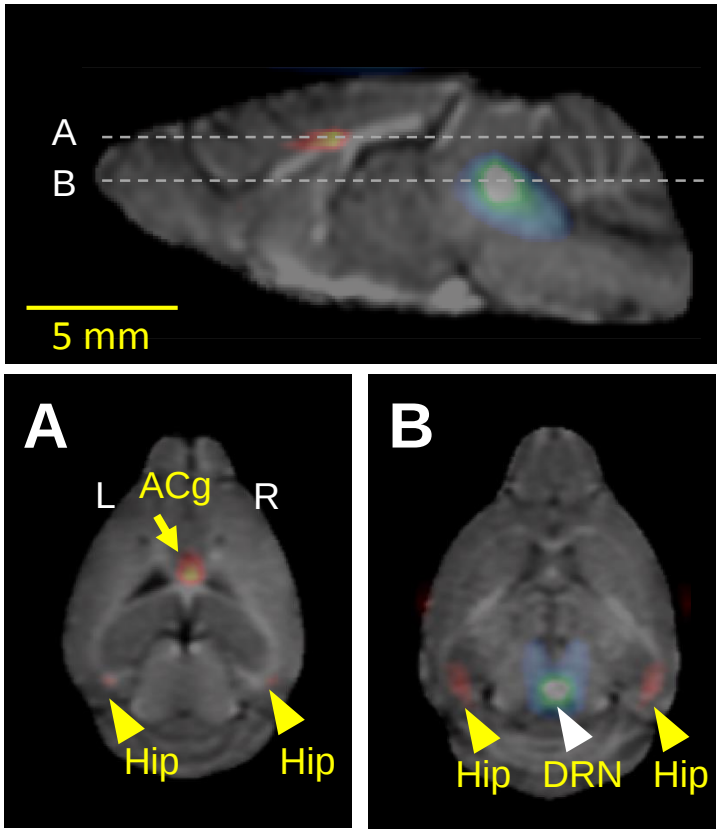
Tamada et al., *PLoSONE* (2010)

PET images with [^{18}F]FDG

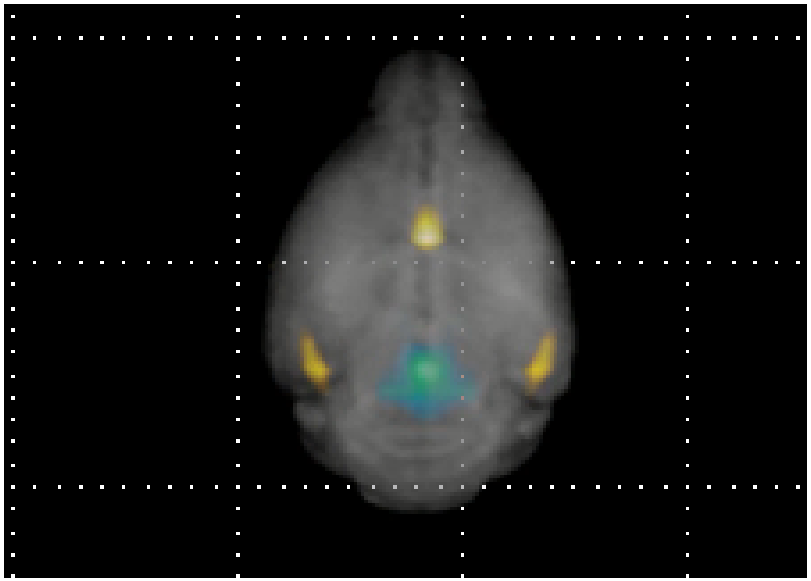


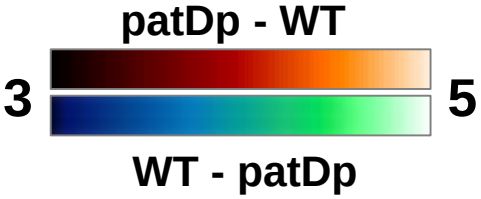
Nakai et al., *Sci Adv* (2017)

A voxel-based statistical analysis



3D image

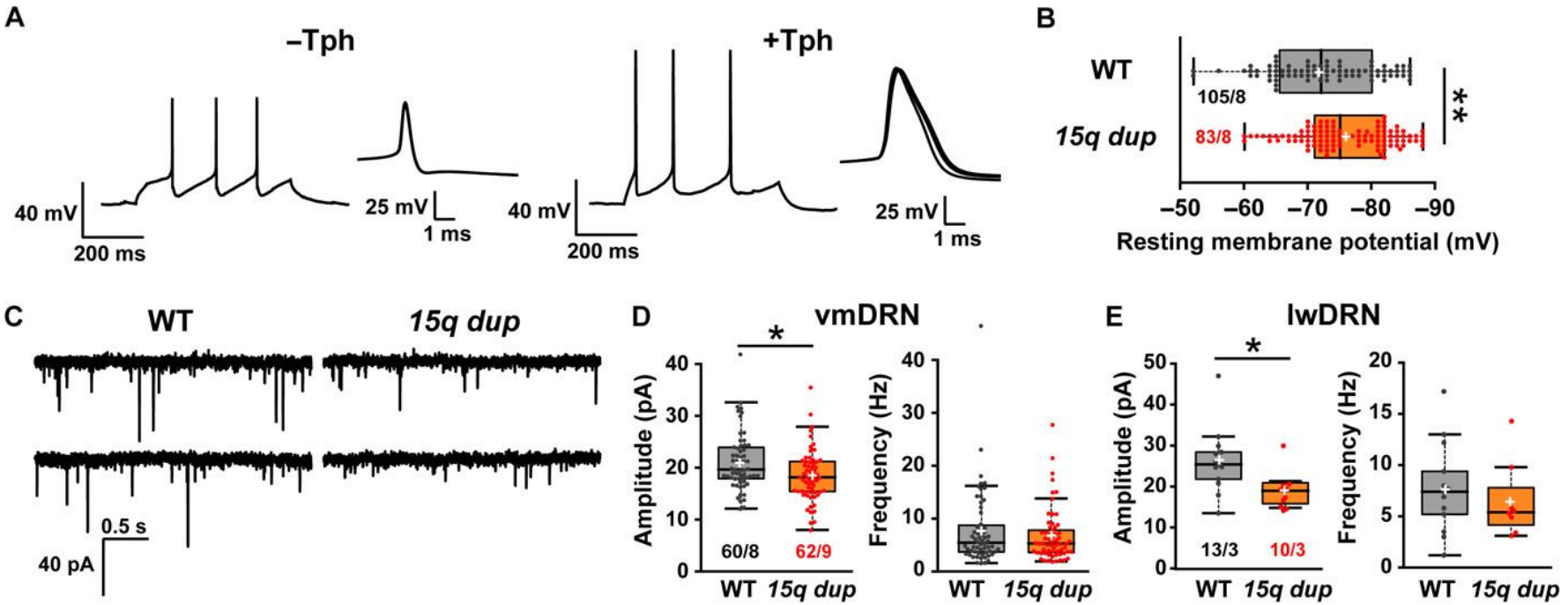




uncorrected

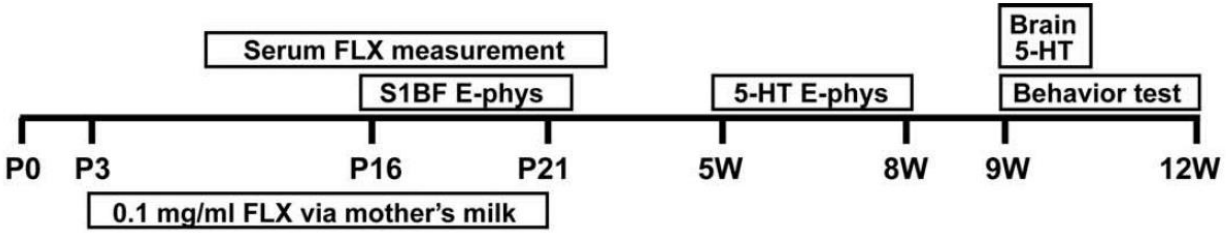
Nakai et al., *Sci Adv* (2017)

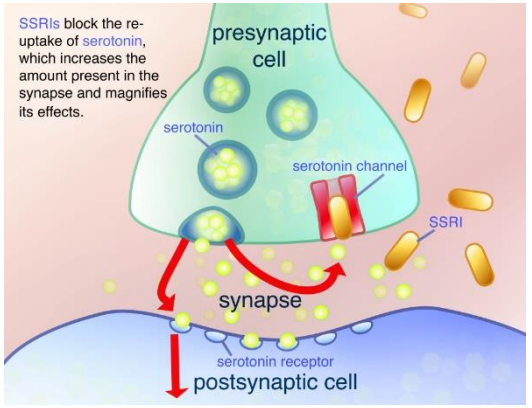
In vitro electrophysiological study

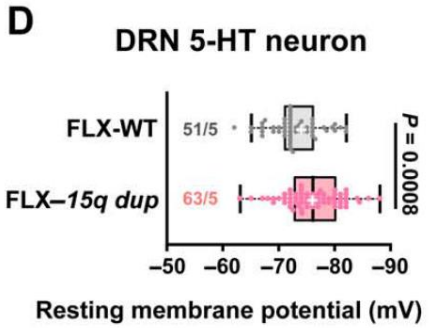


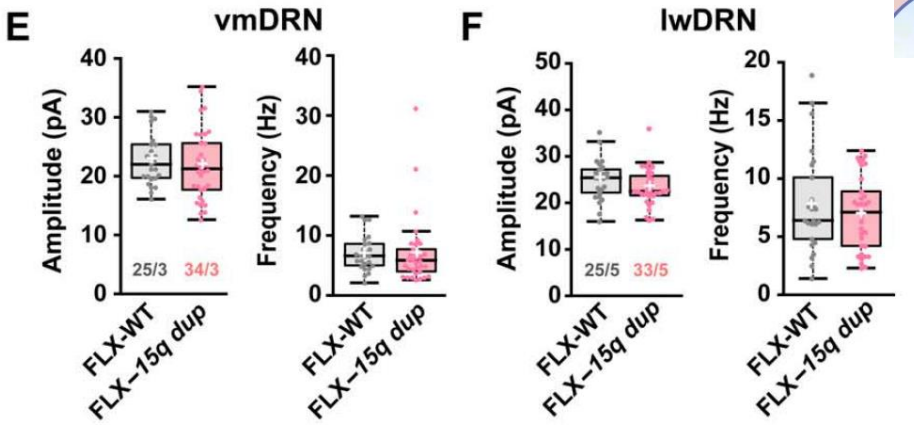
Nakai et al., *Sci Adv* (2017)

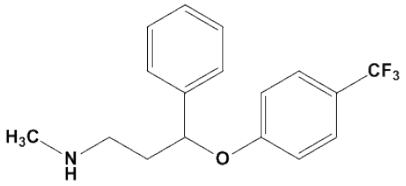
Restoration by SSRI treatment



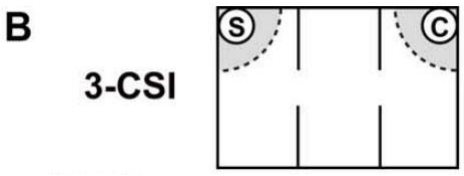


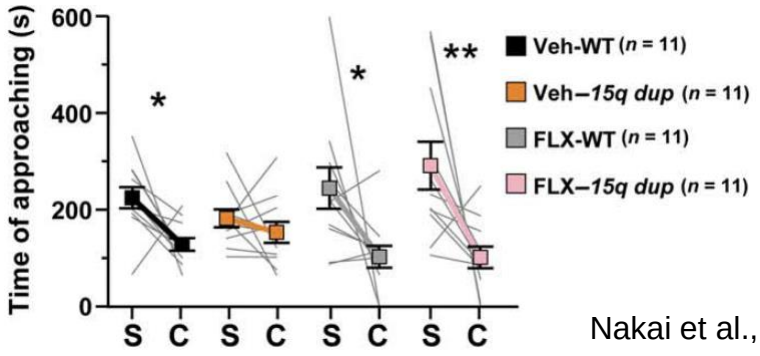






Fluoxetin (Prozac®)





Nakai et al., *Sci Adv* (2017)

Summary

1. *Ex vivo* DT MRI tractography and *in vivo* voxel-based statistical analysis of [^{18}F]FDG-PET revealed the selective involvement of the septo-hippocampal pathway in a wild-type tau overexpressing Tg601 mouse model.
2. Histological analysis identified decreases in the number of ChAT-positive neurons in the septum.

1. A voxel-based statistical analysis of [^{18}F]FDG-PET revealed that glucose metabolism was decreased in the DRN in patDp/+ mice
2. In vitro electrophysiological study identified the abnormalities of 5-HT neurons in DRN.
3. The abnormalities of the model mouse were improved by SSRI treatment

Our PET imaging method would provide for detection of regional brain abnormalities and for efficient biological evaluation method in the process of drug developments.

Outline

RIKEN BDR RIKEN Center for Biosystems Dynamics Research

✓Overview of our research group

✓Brain PET imaging in awake mouse

✓Other PET study using small animals

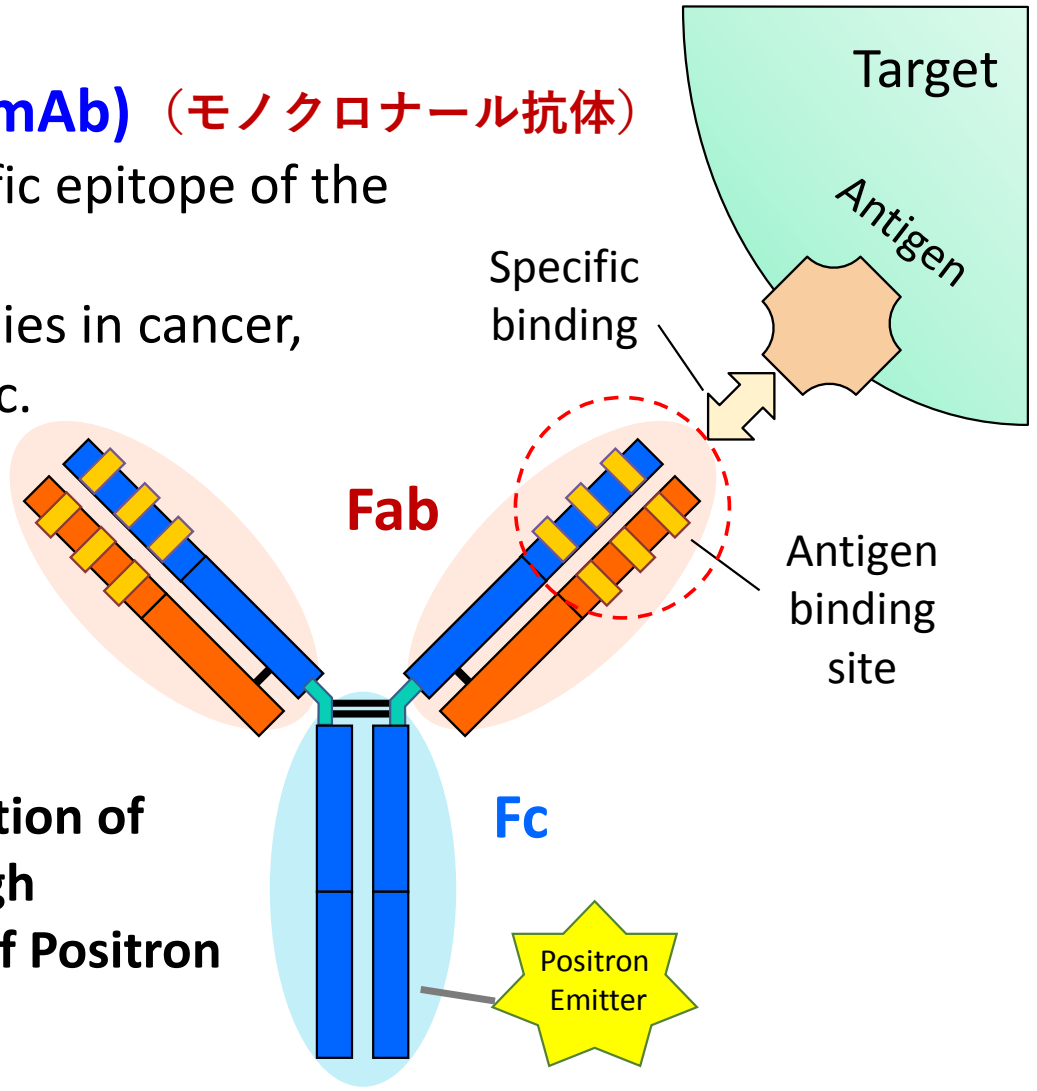
Overview: Immuno-PET

Dr. Kanayama

Monoclonal antibody (mAb)（モノクローナール抗体）

- selectively bind to specific epitope of the antigen
- used for targeted therapies in cancer, autoimmune disease, etc.

Antibody preparation
（抗体医薬）



Immuno-PET:

the tracking and quantification of radiolabeled mAbs with high resolution and sensitivity of Positron Emission Tomography

PET Imaging of ^{64}Cu-Labeled Trastuzumab

Dr. Kanayama

RIKEN BDR RIKEN Center for Biosystems Dynamics Research

- ^{64}Cu production

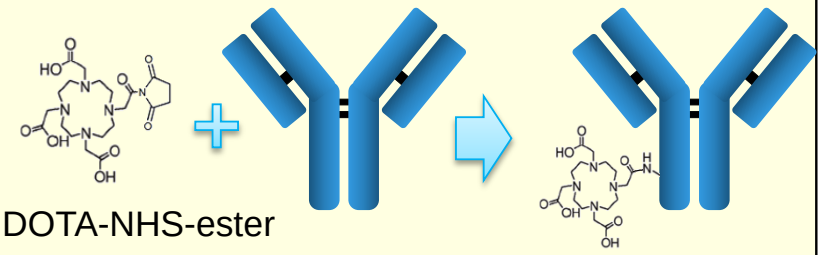
^{64}Ni (p, n) ^{64}Cu

^{64}Cu

Small Cyclotron

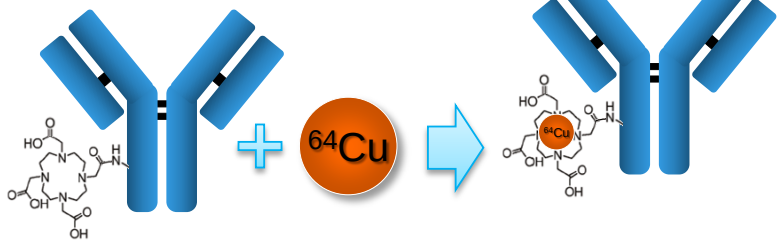
99%~ enriched ^{64}Ni plated target

- Chelator conjugation



DOTA-NHS-ester

- ^{64}Cu labeling



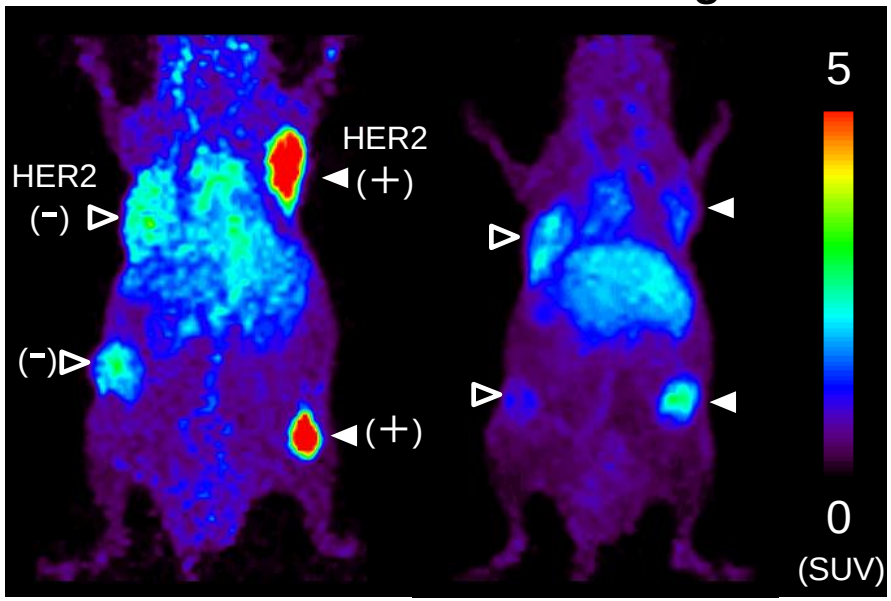
- **PET imaging of tumor-bearing mouse**

mAbs: **Trastuzumab (anti-HER2)**

Mouse IgG1 isotype control

Imaging: 30 min of data acquisition was performed at 57 h after injection.

^{64}Cu-DOTA-Trastuzumab	**^{64}Cu-DOTA-control IgG**



PET images are shown as MIP image.

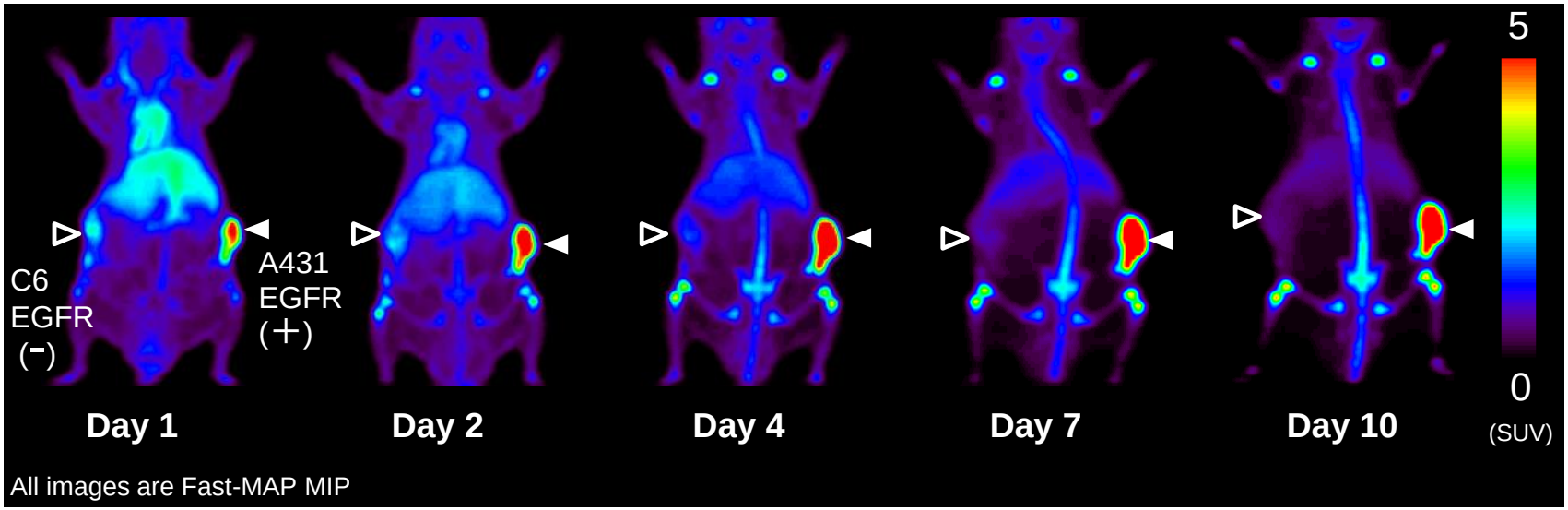
Long-Time Observation of ^{89}Zr-Cetuximab

Dr. Kanayama

RIKEN BDR RIKEN Center for Biosystems Dynamics Research

We also performed the longitudinal tracking of ^{89}Zr-labeled cetuximab using EGFR-positive and negative tumor-bearing mice.

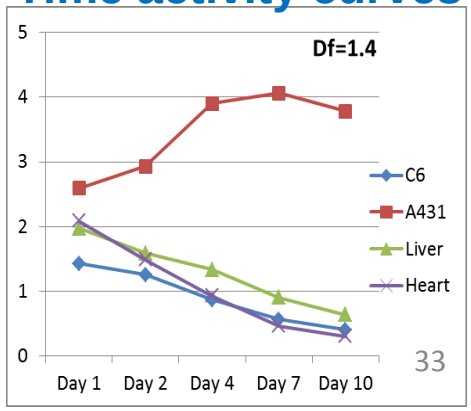
Zirconium-89 (half-life, 78.9 h)



High activity observed in the EGFR-positive tumor region for 10 days.
However, the bone accumulation was observed in spine and several joints. The accumulation seemed to increased with time.

Once internalized, ^{89}Zr is considered to remain in the cell.
So ^{89}Zr dissociation might occur during circulation.

Time activity curves



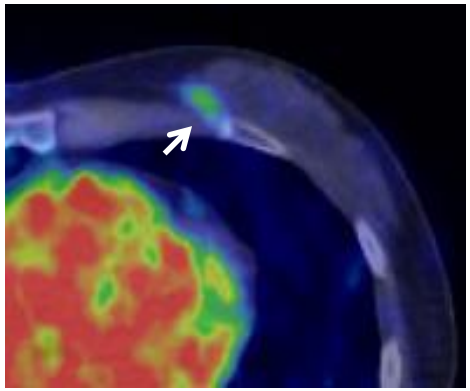
Clinical PET trial: ^{64}Cu-DOTA-Trastuzumab

Dr. Kanayama

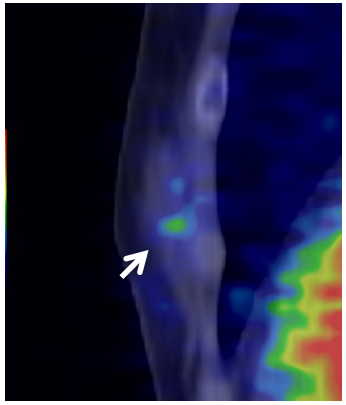
Primary cancer lesion (Breast)

Case 1

Axia

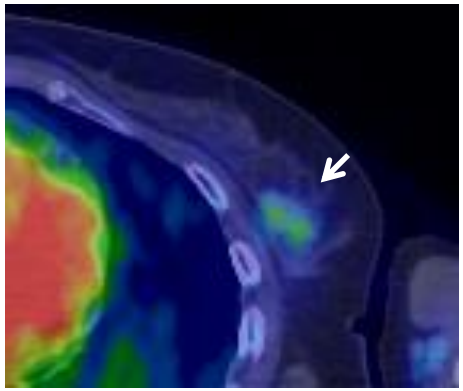


Sagittal

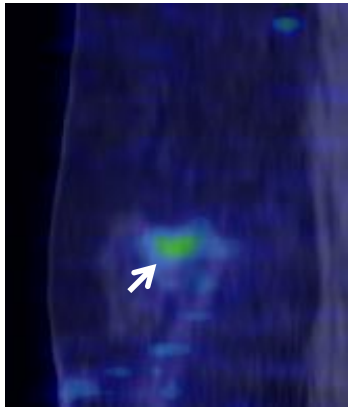


Case 2

Axia

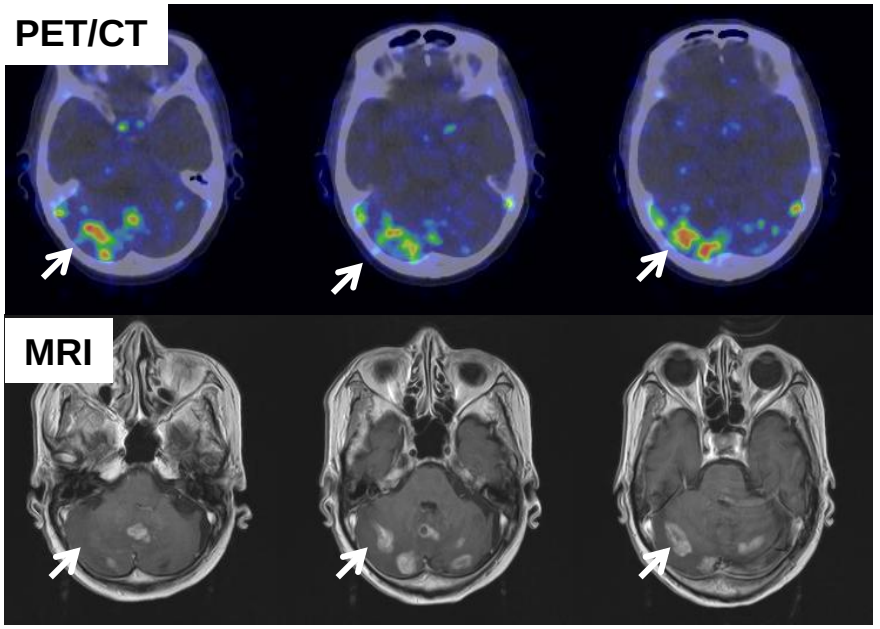


Sagittal



Metastatic cancer lesion (Brain)

Case 4



All images at 48 h postinjection

Tamura et al. *J Nucl Med* 2013; 54:1869–1875