

NAS Neuroscience Forum

The future of non-human primate research in neuroscience and brain disorders

October 4, 2018

Disease Modeling and Brain Mapping using genetically modified marmosets

Hideyuki Okano

Project Leader of Brain/MINDS

Dean and Professor,

Keio University Graduate School of Medicine

RIKEN Brain Science Institute



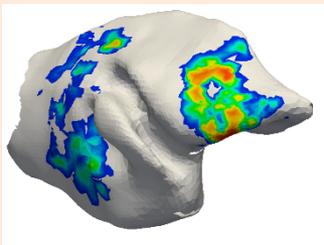
Brain/MINDS



Studying the neural networks controlling higher brain functions in the marmoset, to gain new insights into information processing and diseases of the human brain

Marmoset brain mapping

Hideyuki Okano: RIKEN/Keio



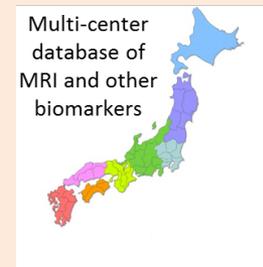
Innovative technology

Atsushi Miyawaki: RIKEN

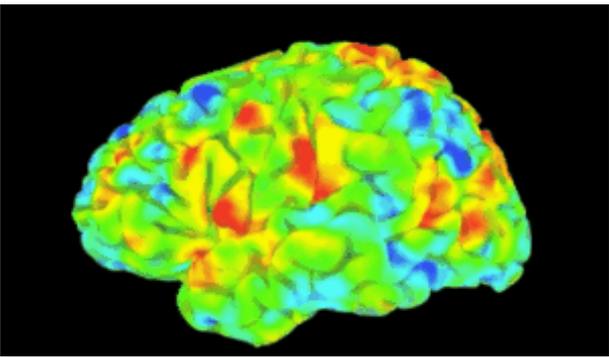


Disease biomarkers

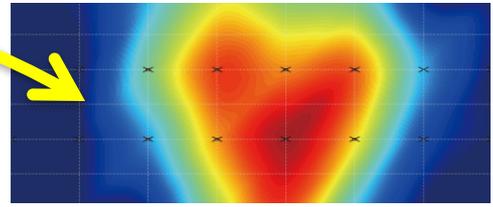
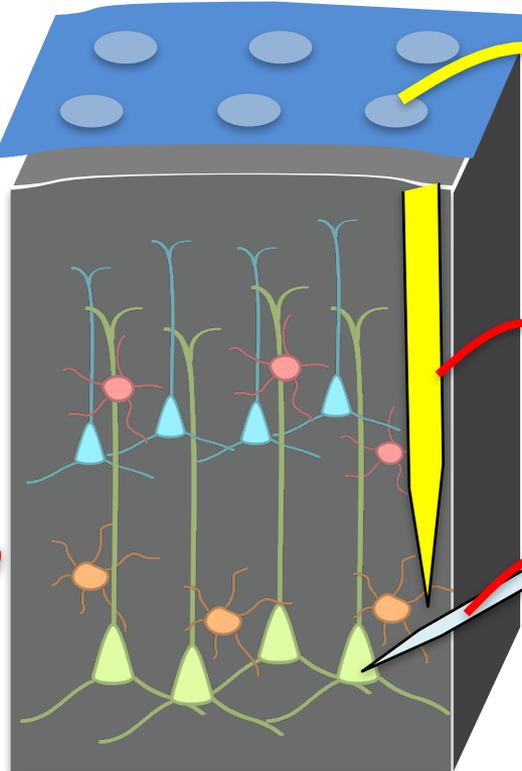
Kiyoto Kasai: University of Tokyo



Functional Brain Imaging



Whole brain



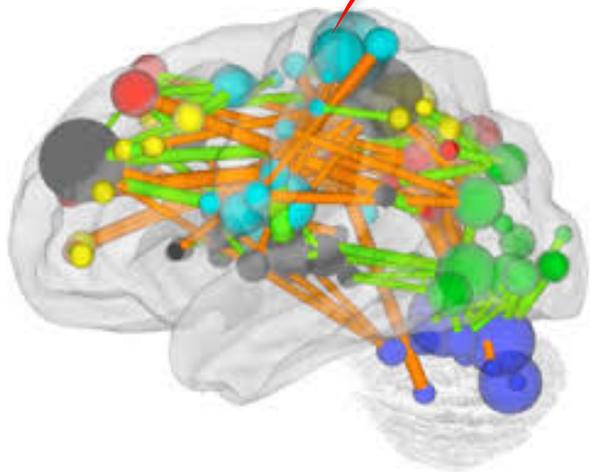
ECoG : Electrocorticography



LFP :Local field potential

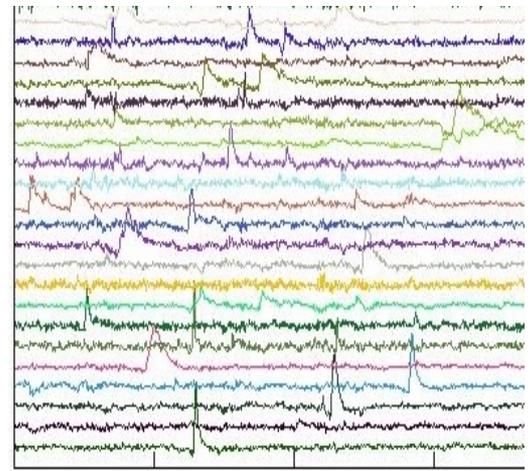


Intracellular recording



Macro circuit

Micro circuit



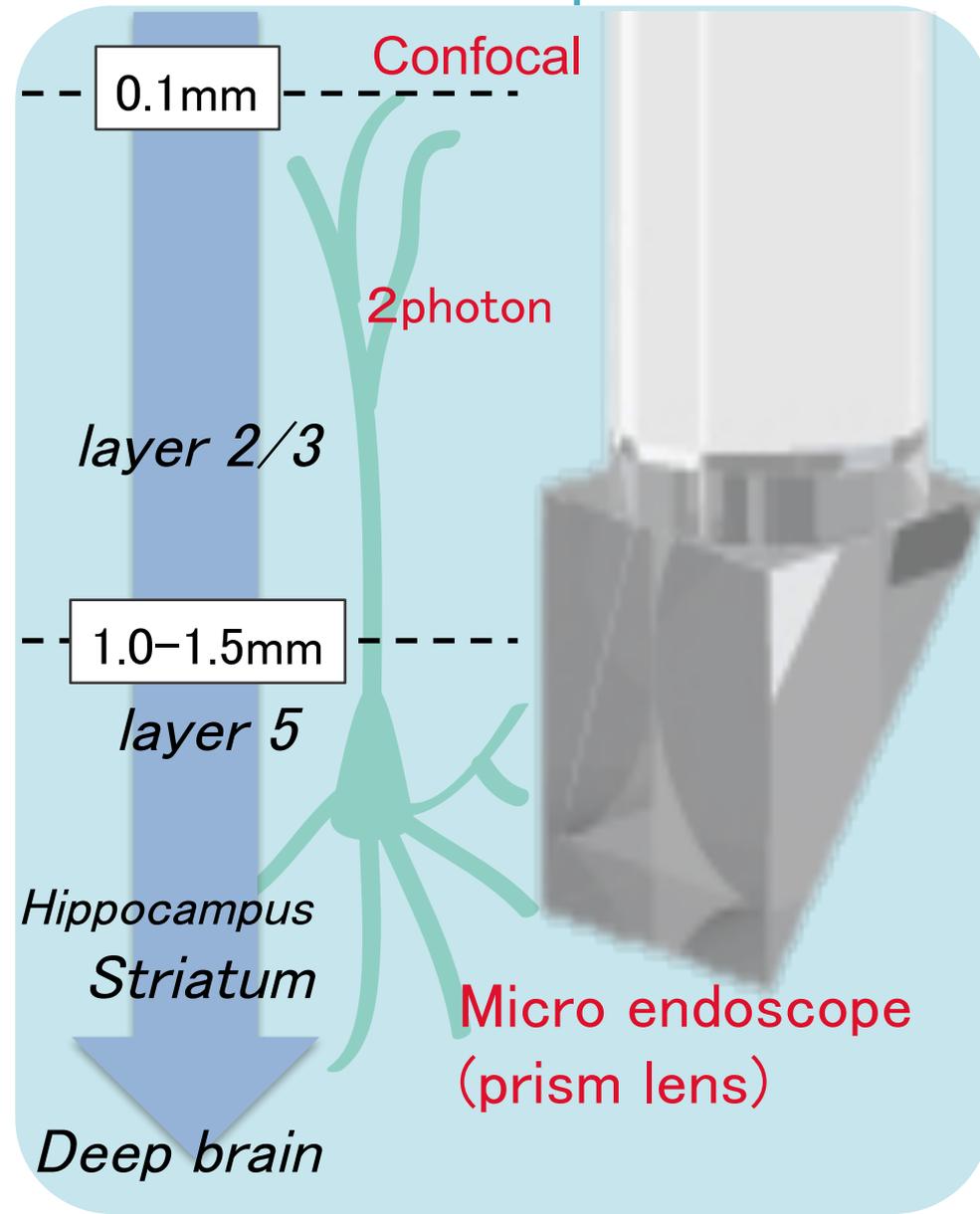
Calcium imaging

Cell resolution + large number of cells

nVista – New neuroimaging tool for deep brain –

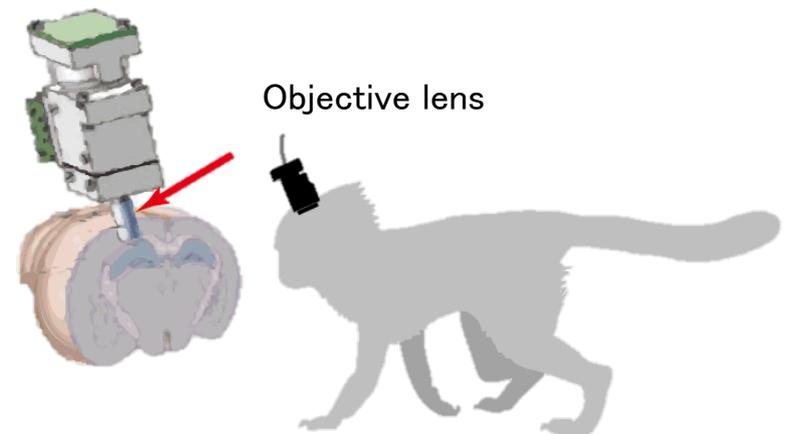
Ghosh et al., *Nat. Method* 2011

Tools to see deep brain



nVista

- Deep + Multi-layer
- 100–1000 neurons
- Long time
- Free moving

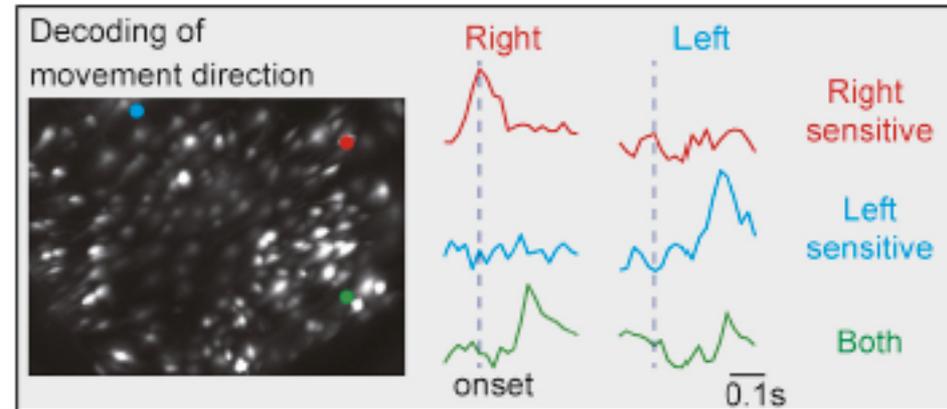
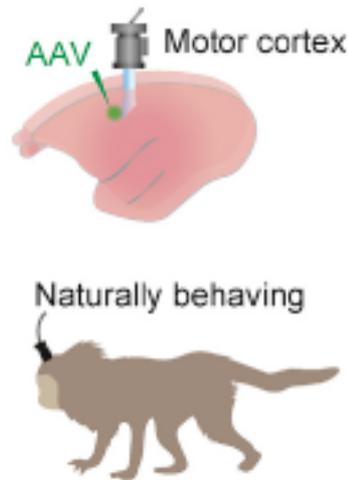
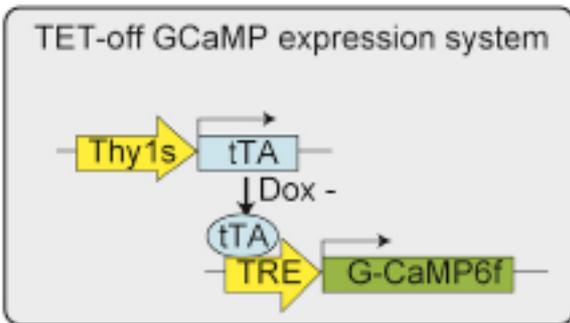


Monitoring the neuronal activity in Primary Motor Cortex and Decoding the Behavior

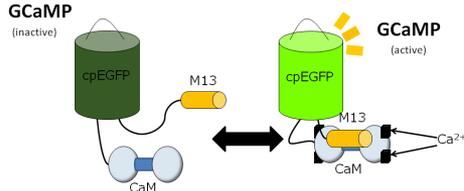
Cell Reports (2018) 24:2191-2195.

Calcium Transient Dynamics of Neural Ensembles in the Primary Motor Cortex of Naturally Behaving Monkeys

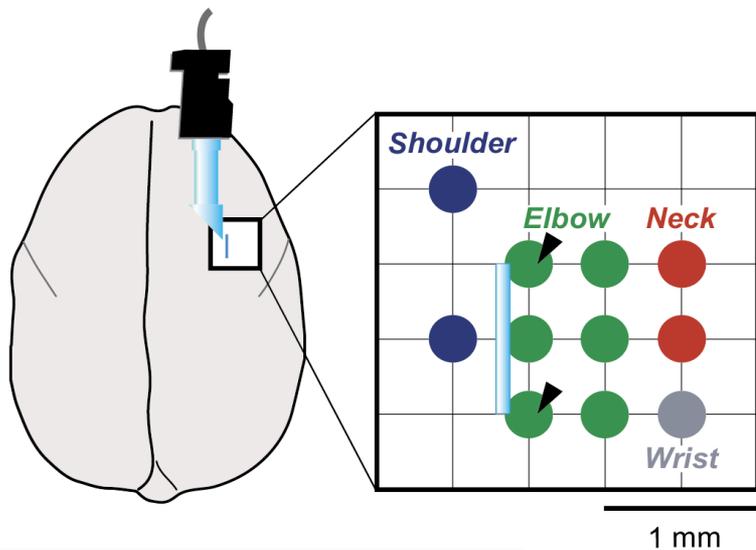
Takahiro Kondo,^{1,2,14} Risa Saito,^{3,14} Masaki Otaka,^{3,14} Kimika Yoshino-Saito,^{1,4} Akihiro Yamanaka,⁵ Tetsuo Yamamori,⁶ Akiya Watakabe,⁶ Hiroaki Mizukami,⁷ Mark J. Schnitzer,^{8,9,10} Kenji F. Tanaka,^{2,11} Junichi Ushiba,^{12,13,*} and Hideyuki Okano^{1,2,15,*}



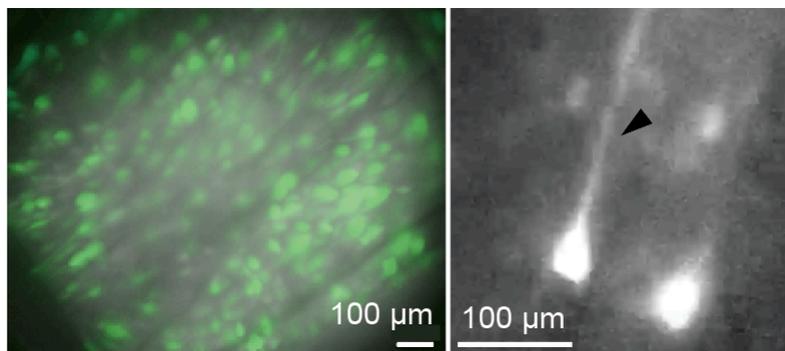
GCaMP: GFP-based Ca²⁺ probe



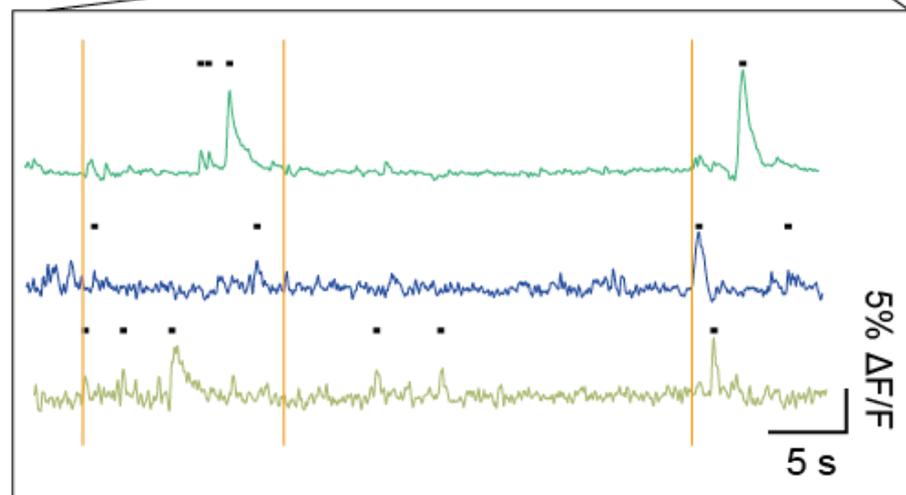
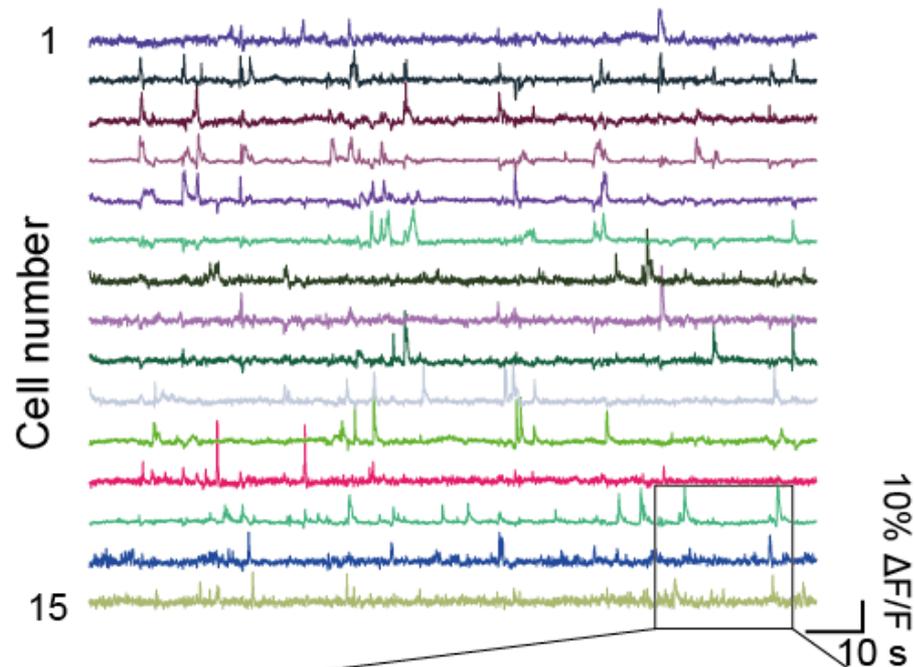
Ca²⁺ imaging with microendoscope in common marmoset M1



AAVs injection points



~200 neurons were detected



Ca²⁺ spiking activities and fluctuations

Ca imaging from Marmoset motor cortex during pellet reaching

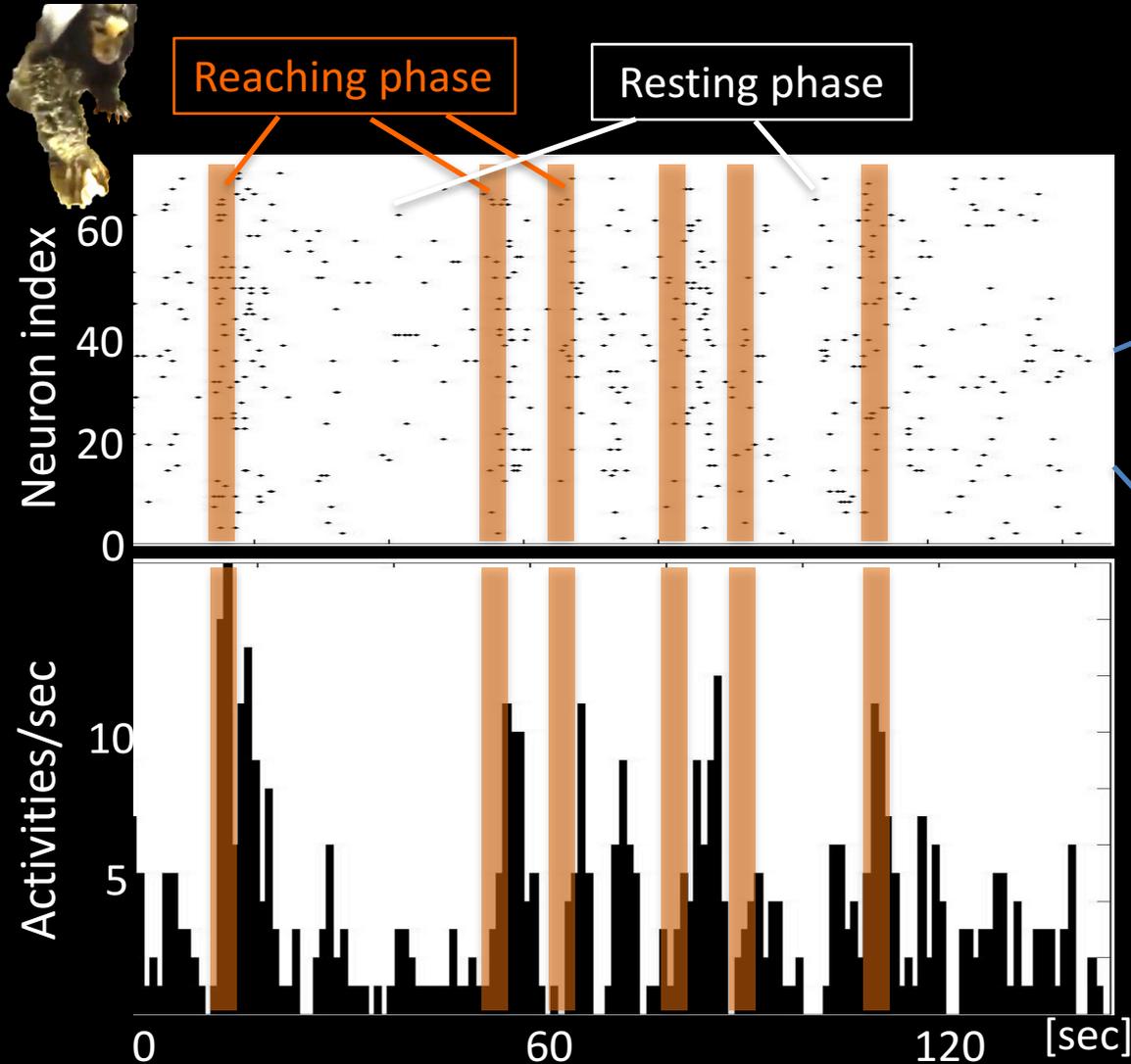
3 weeks after plantation (5wks after virus injection)

[from mamoset 3]

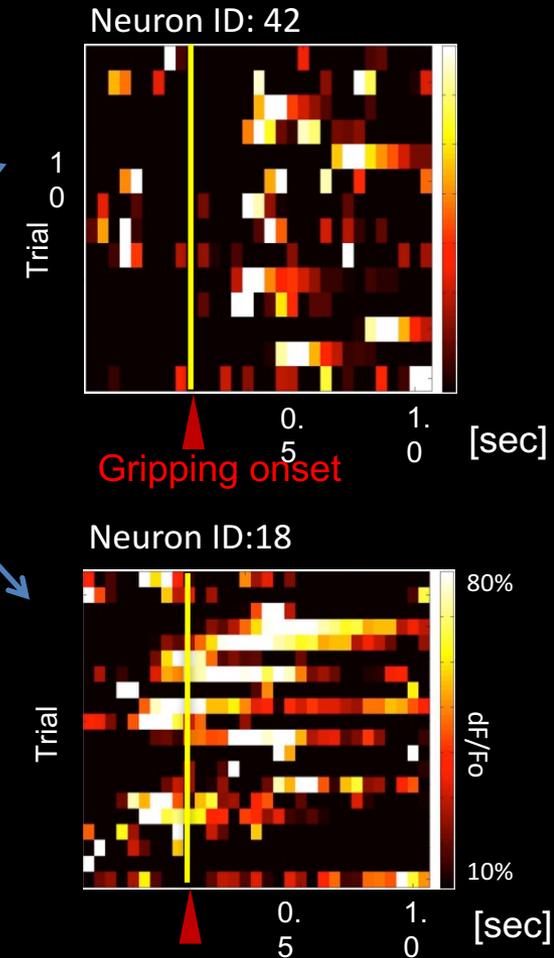


Neural activities between reaching and resting phase

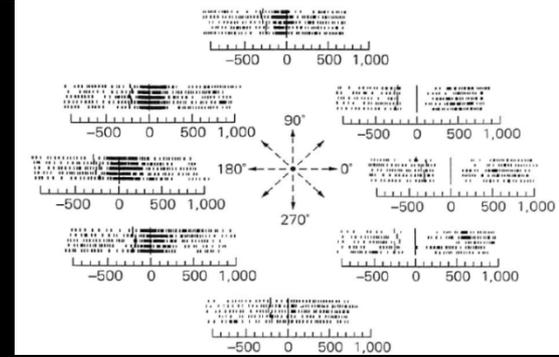
Time courses for all recorded neurons



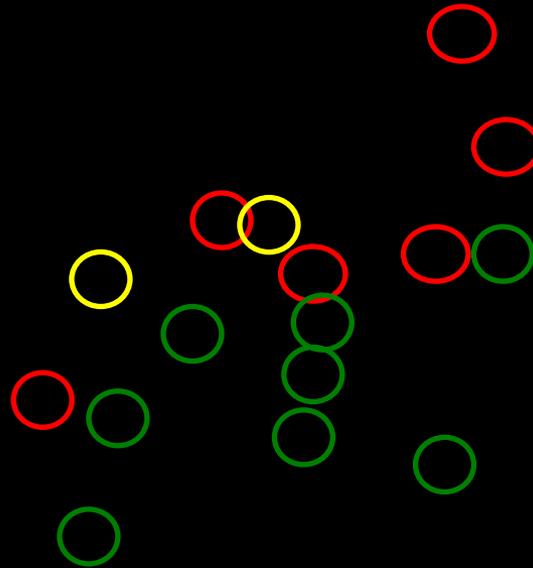
Individual trials for example neurons



Decoding the reaching direction with calcium imaging



The firing pattern of neurons which have PD (Georgopoulos *et al.*, 1982)



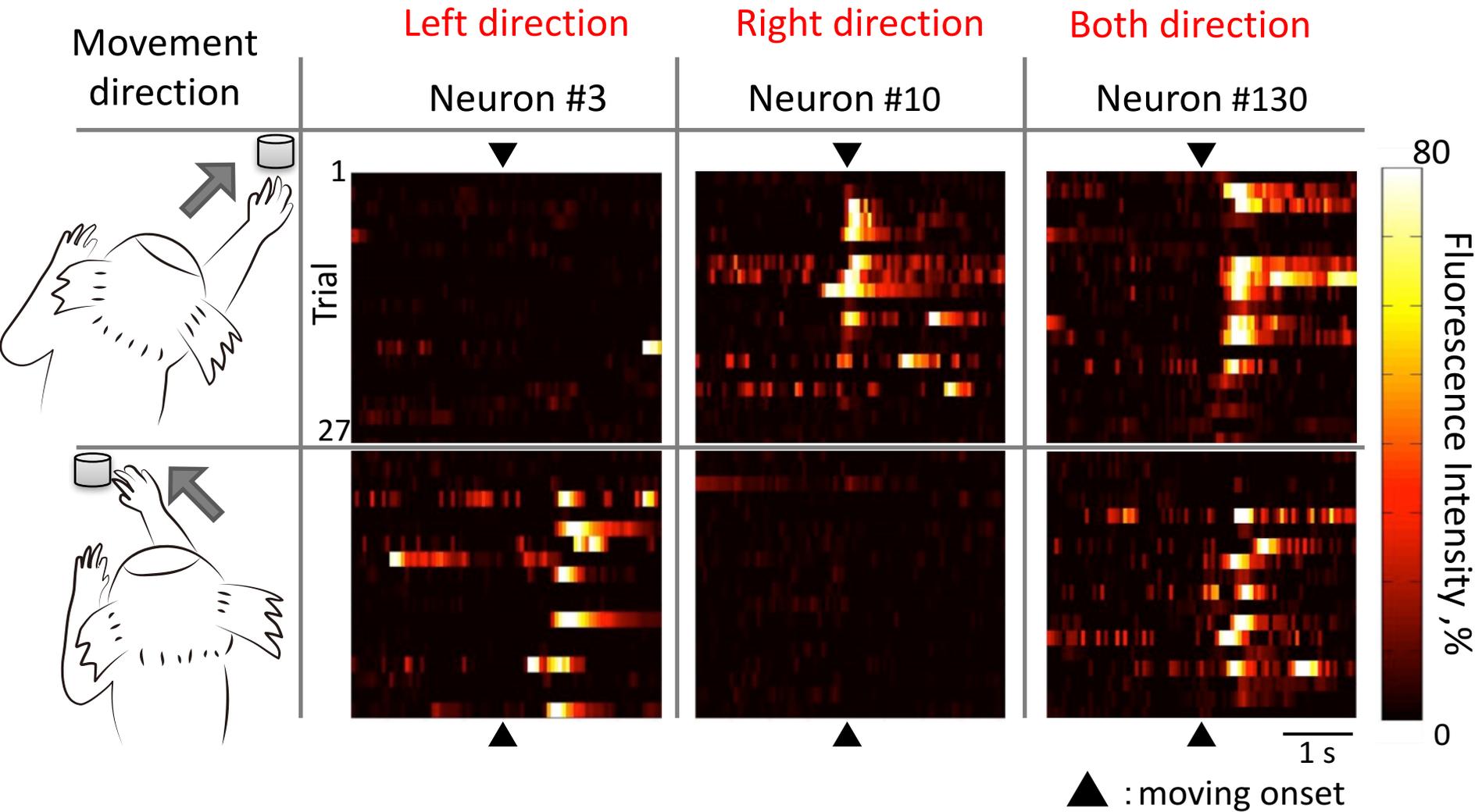
Two-way (Right, Left) reaching task

Neurons related to the right direction

Neurons related to the left direction

Neurons related to both right and left direction

Decoding of the reaching direction – right or left ?-

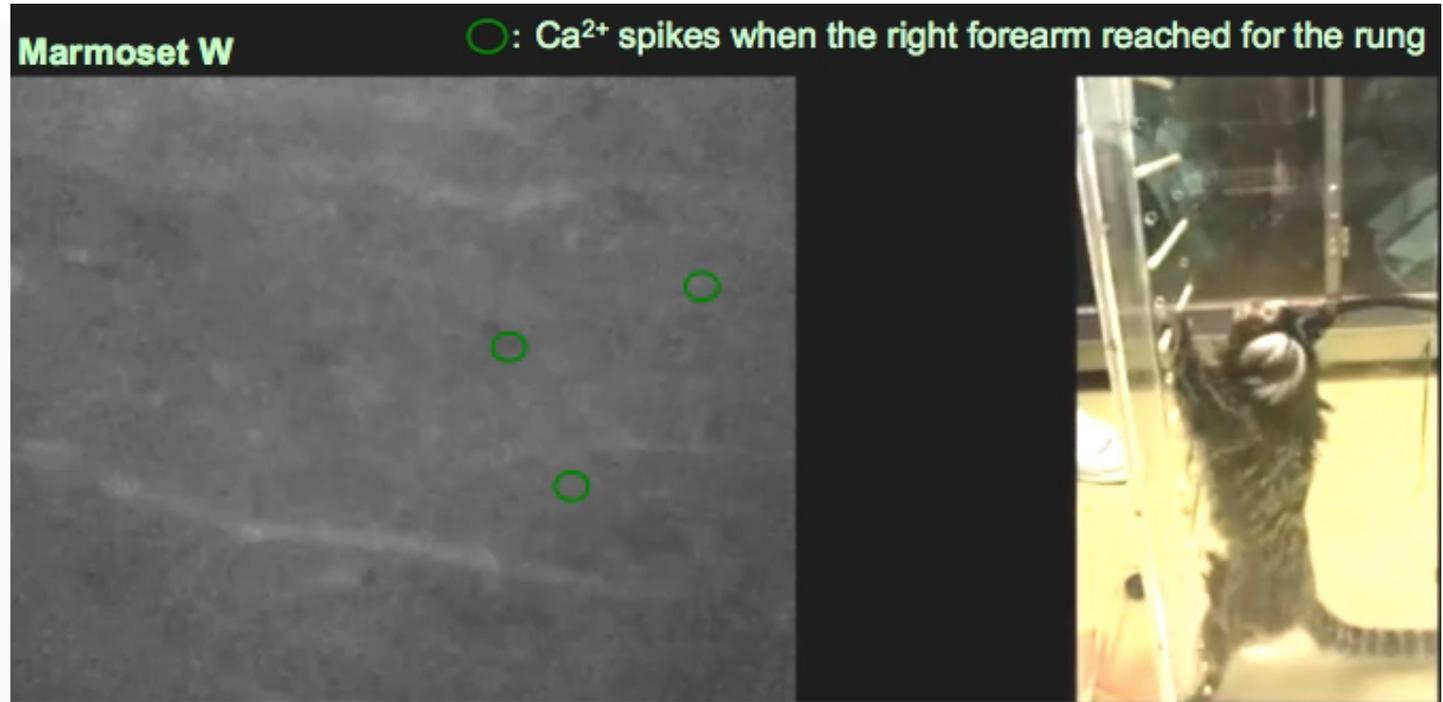
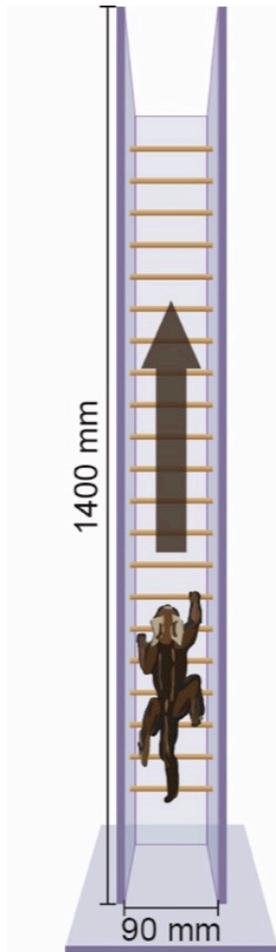


Reaching direction was accurately predicted from Ca^{2+} imaging data

Ca imaging from Marmoset motor cortex during Free Moving Marmoset

Ladder Climbing Behavior

Kondo et al., *Cell Reports* 2018



This technology using free moving marmoset will make it possible to dissect large-scale neural circuits during human-relevant behavior under natural conditions, enabling the study of complex behaviors, including social interaction fear, and anxiety and cognitive motor tasks. The combination of this technique with transgenic marmoset technologies has the potential to transform our understanding, diagnosis, and treatment of human brain diseases.

Future Issues of Imaging using Micro-Endoscope (nVista)

- Imaging deeper Brain Areas (Subcortical Areas including Basal Ganglia): Longer Prism lens
- Pathway-specific Imaging
- Cell Type Specific approaches using Cre Lines and in vivo Genome Editing
- Imaging more than two sites simultaneously.
- Imaging Disease Model Marmoset
- Combination with Optogenetics (Improvement of nVoke)
- Voltage Imaging

Imaging deeper Brain Areas (Subcortical Areas including Basal Ganglia)

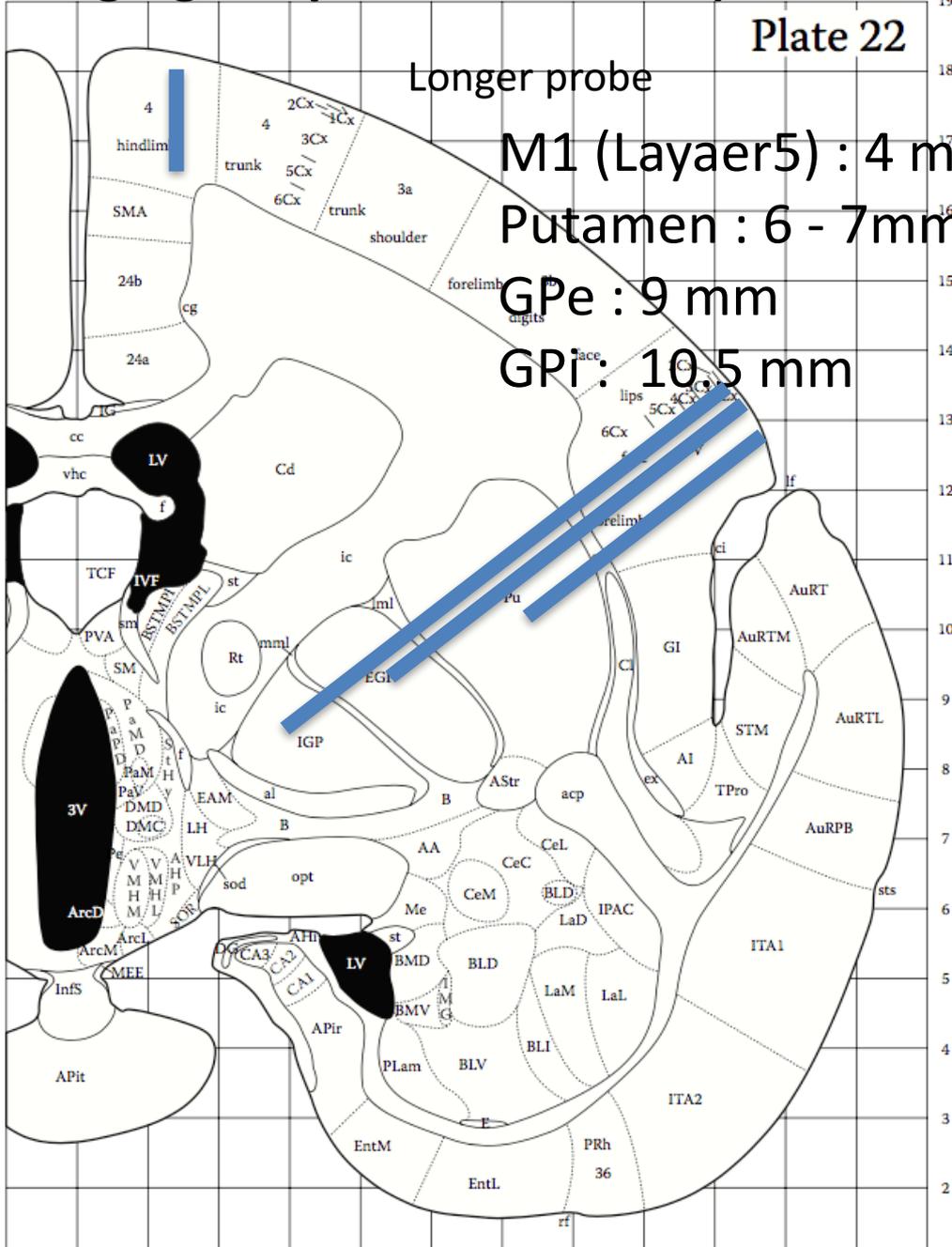


Plate 22

Longer probe

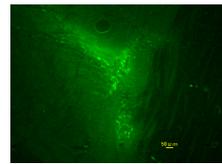
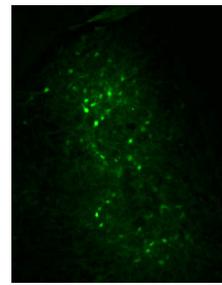
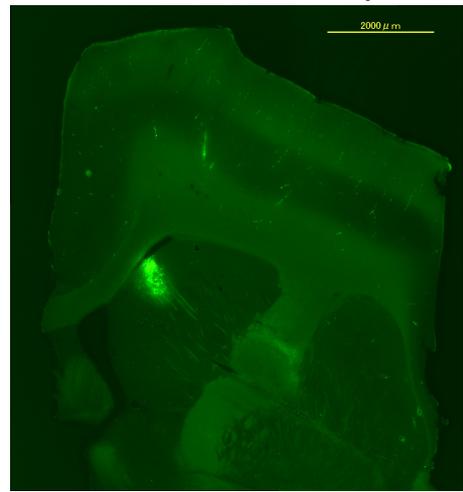
M1 (Layer 5) : 4 mm

Putamen : 6 - 7mm

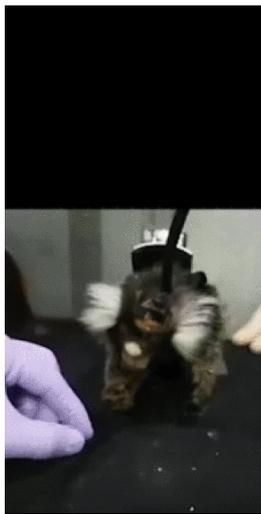
GPe : 9 mm

GPI : 10.5 mm

GCaMP expression at Str

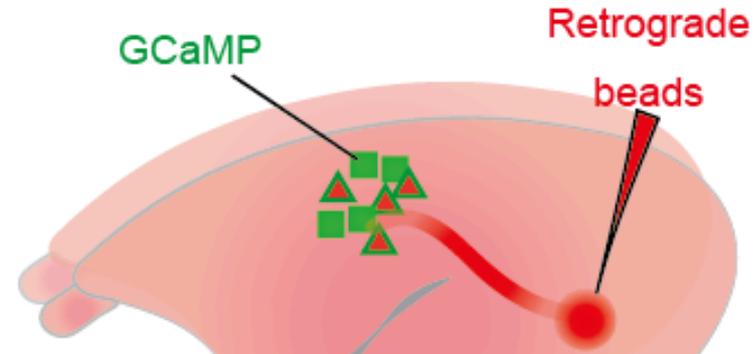


rAAV2/1-Thy1S-tTA (2.0×10^{11} vg/ml)
 rAAV2/1-TRE3-GCaMP6f (9.8×10^{11} vg/ml)



Pathway-specific Imaging

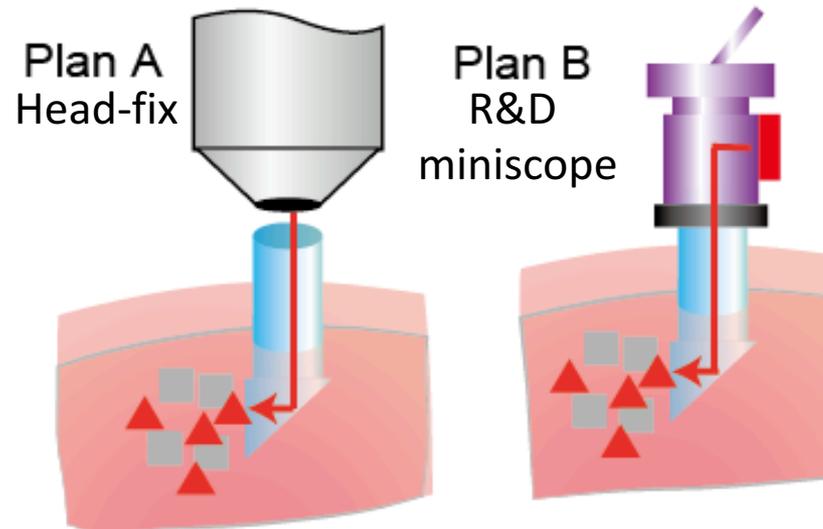
Retrograde labelling of pathway specific neuron



Striatum: AAV retro syn-mtTA

Motor cortex: AAV DIO TRE-GCaMP

Detection of red fluorescent signal

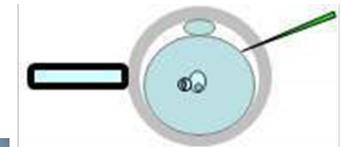


Modeling Psychiatric/Neural Diseases using GM marmosets

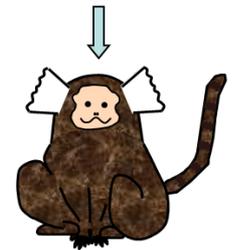


- Neurodegenerative Diseases
- Neurodevelopmental Diseases
- Psychiatric Diseases

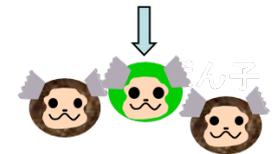
Marmosets can be genetically modified



Lentiviral vector injection



Embryo transfer to surrogate mother

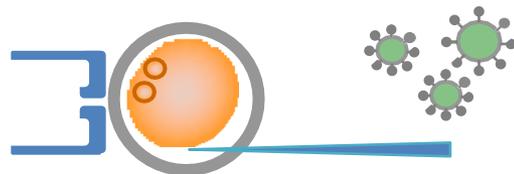
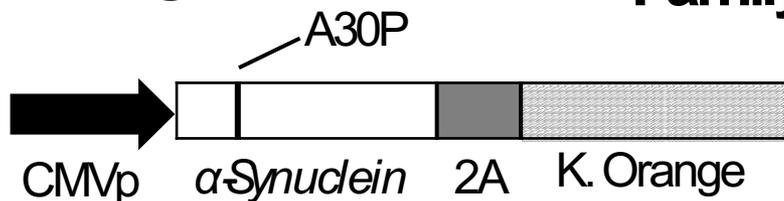


Transgenic marmoset

Sasaki et al., Nature, 2009
Sato et al., Cell Stem Cell, 2016

Generation of mutated α -Synuclein transgenic marmosetlines.

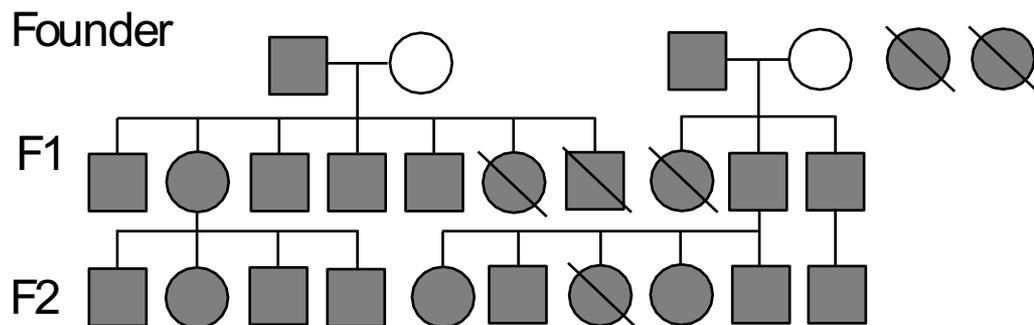
Transgene



Lentivirus
injection

early embryo

Family tree of Transgenic marmoset



Symptoms of Parkinson's Disease in human

extremely early stage

- sleep disturbance
(REM sleep behavior
disorder; RBD)

early stage

- tremor
- akinesia
- muscle rigidity
- gait disturbance

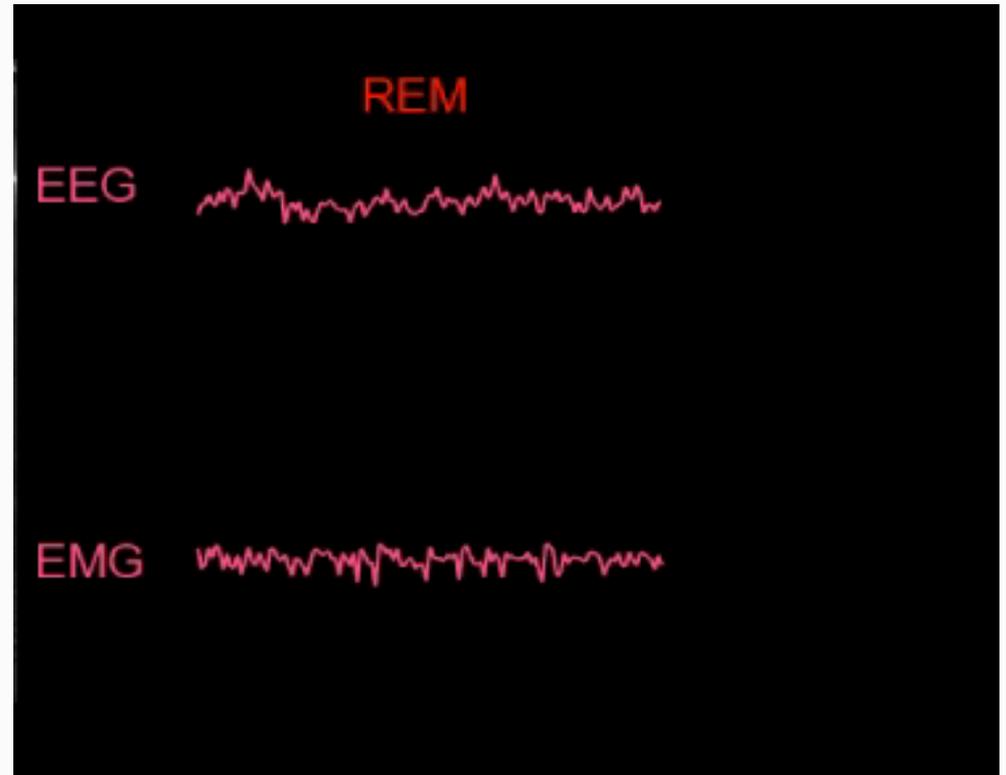
advanced stage

- dementia
- speech defect
- mind symptoms

Evaluation of sleep disorder in transgenic marmosets.

Measurement system

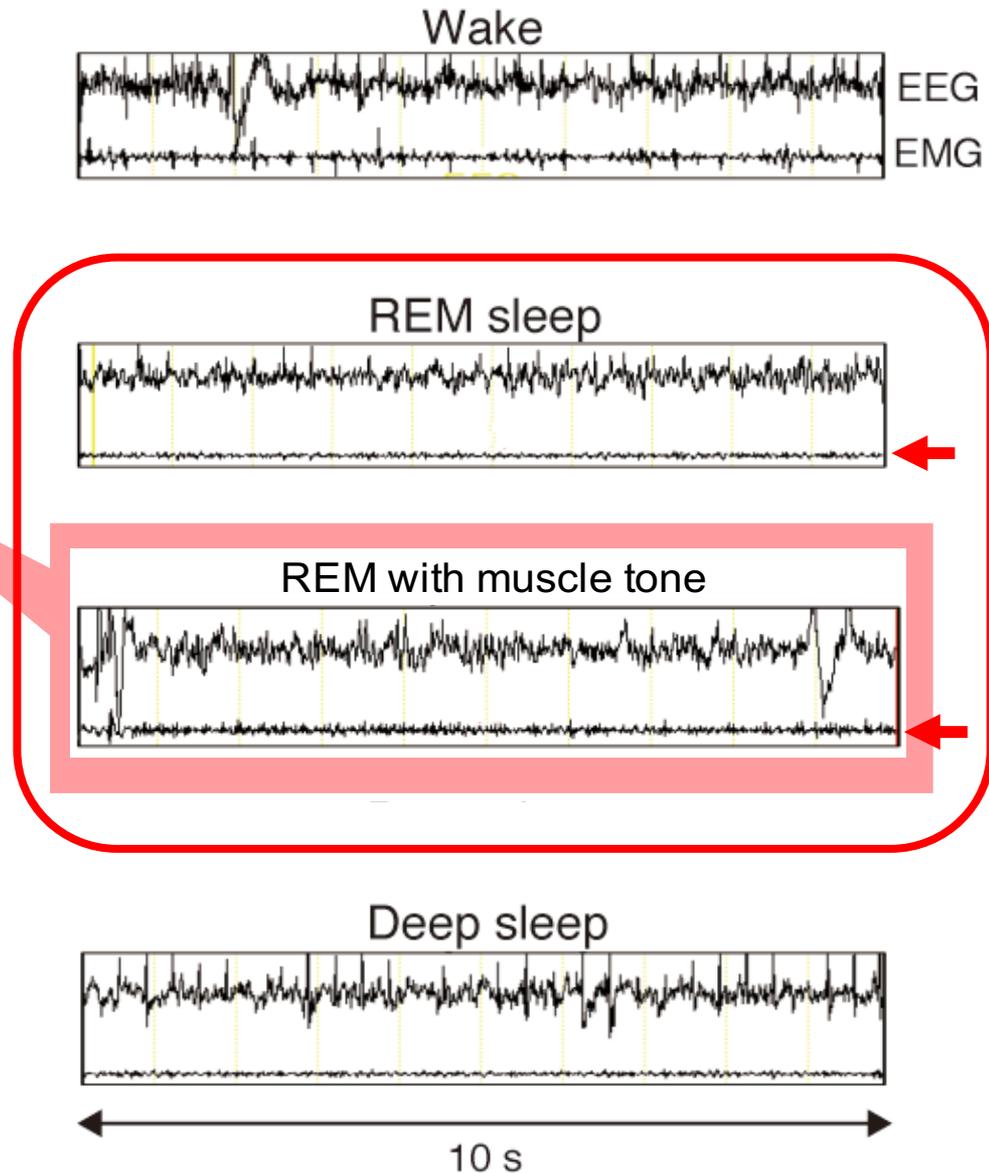
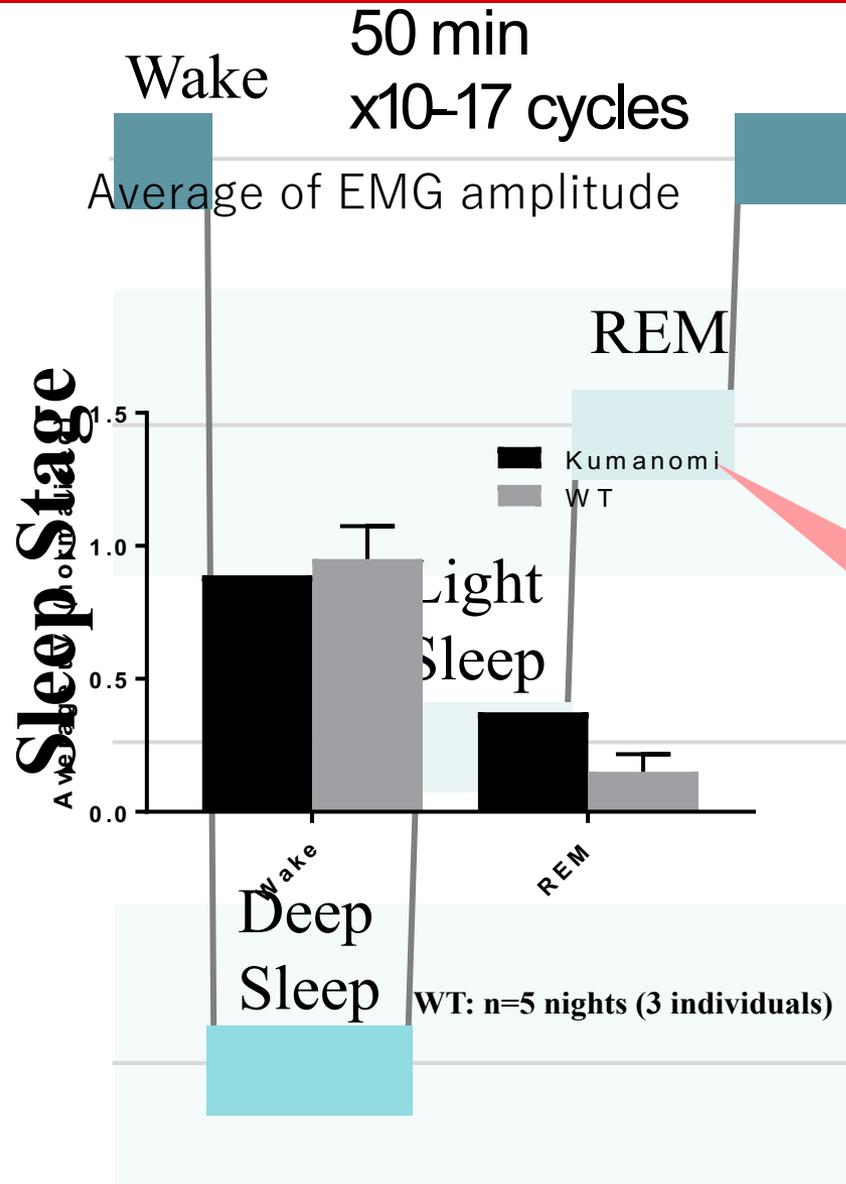
for simultaneous EEG/EMG recordings in marmosets



- Non-invasive (The scalp EEG)
- Simultaneous
- Wire-less

Dr. Sakaguchi (Tsukuba Univ.)
M.S. Kosugi (Keio Univ. Ushiba lab.)
Dr. Kondo (Keio Univ. Okano lab.)

REM sleep without atonia was observed in the Tg marmoset like PD patients.



The loss of dopamine transporter in the transgenic marmoset

Detected by dopamine transporter ligand [11C]-PE2I

Dr. Yokoyama (RIKEN CLST)

1.0Y

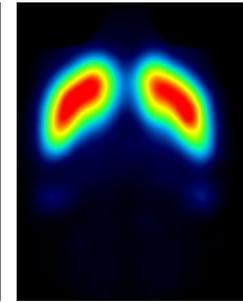
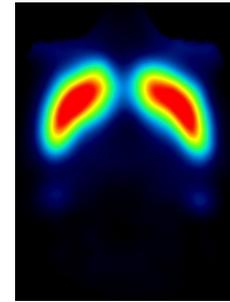
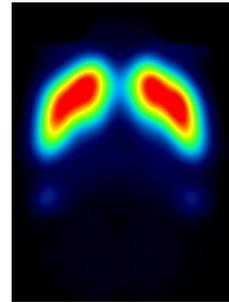
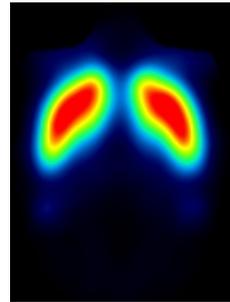
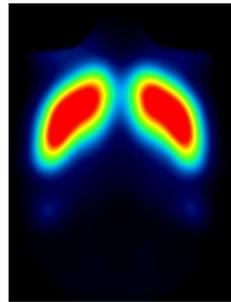
2.0Y

3.0Y

4.0Y

5.0Y

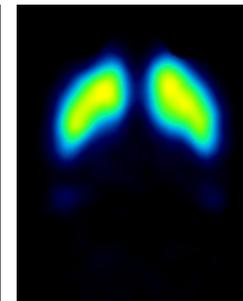
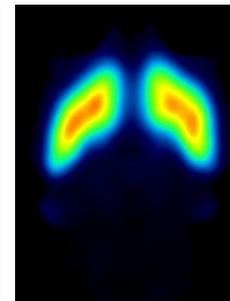
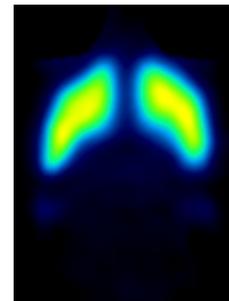
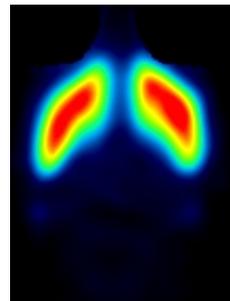
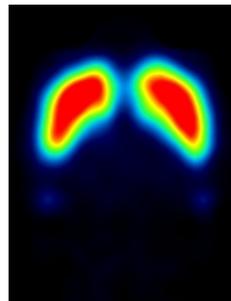
Control (average)



6
0
BPnd

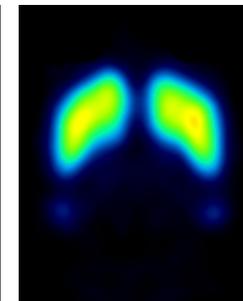
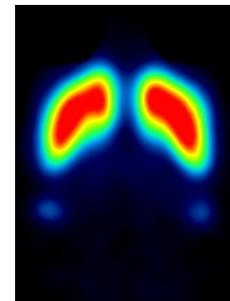
Tg 1 (Kumanomi)

Motor Symptom (+)
Sleep disorder (+)



Tg 2 (Himedaka)

Motor Symptom (+, Day-time activity ↓ ↓)
Sleep disorder (N.D.)



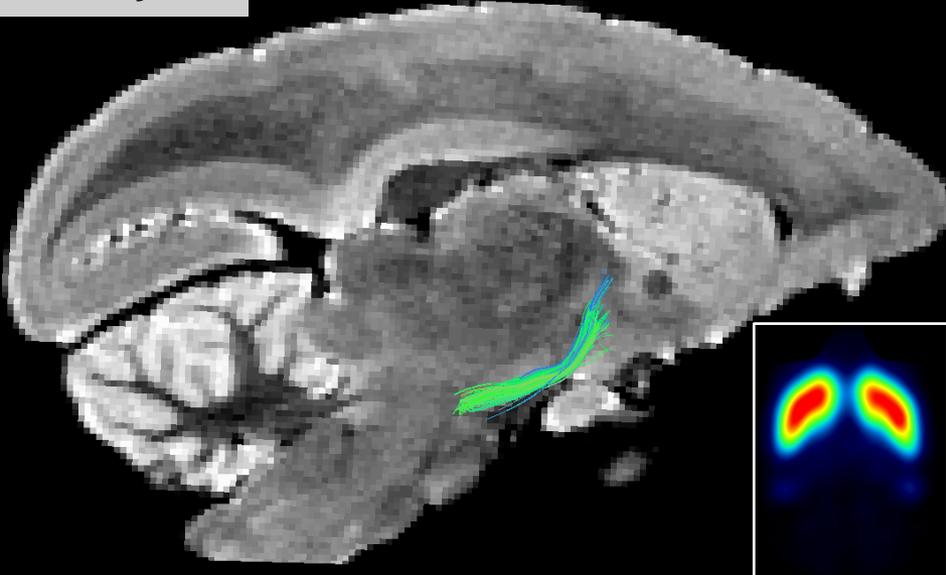
Several Tg marmosets showed a dramatic reduction of PET signal in both the putamen and the caudate.

MRI imaging in Tg marmoset (Himedaka) ex vivo study

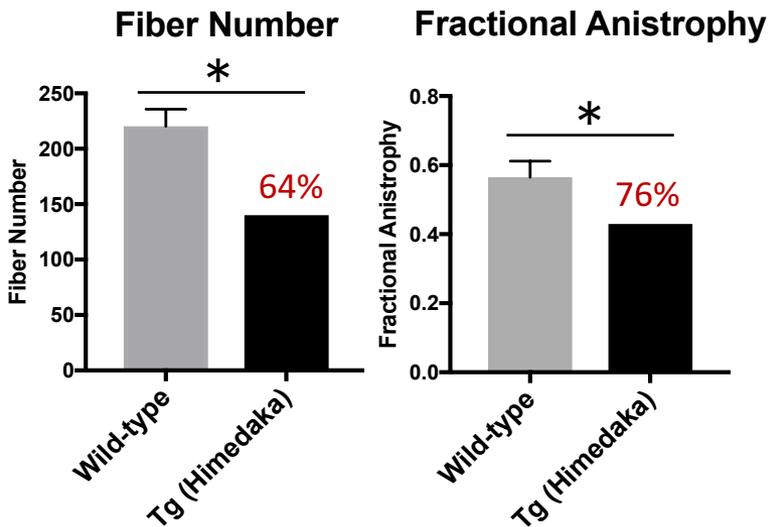
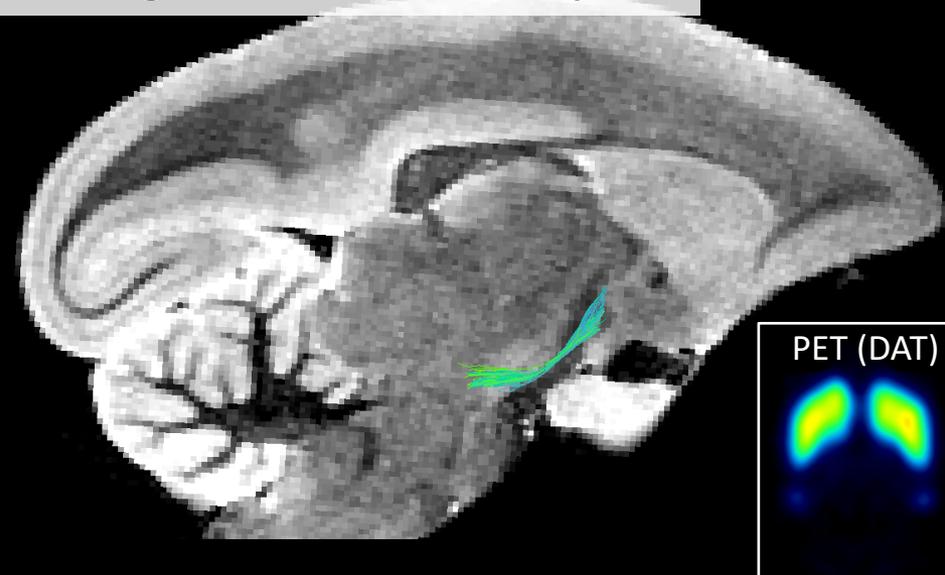
Imaging of nigro-striatal pathway

Dr. Hata (Riken Okano lab.)

WT 5y6m



Transgenic (Himedaka) 5y3m



<ex vivo MRI>

The number of nigral fibers project to striatum was low in Tg marmoset brain.



- consistent with the result of PET data (reduction of DAT)
- indicate the degeneration of DA neuron

A Resting tremor-like phenotype in Tg founder animal of 3 years old.



Symptoms of Parkinson's Disease in human

extremely early stage

- sleep disorder
- olfactory disturbance

early stage

- tremor (?)
- akinesia
- muscle rigidity
- gait disturbance

advanced stage

- dementia
- speech defect
- mind symptoms



Awake State



Under Anesthesia

PD-like gait disorder in Tg marmoset

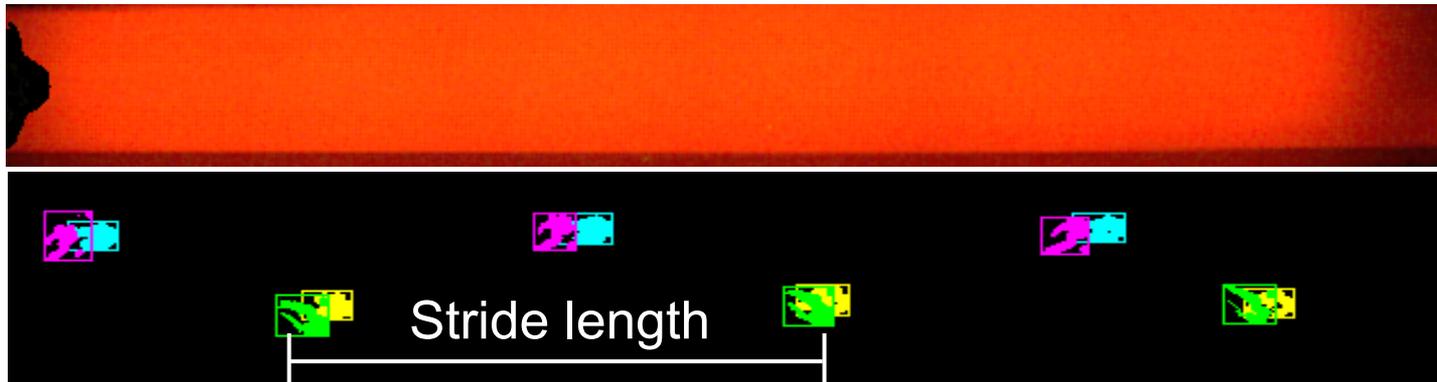
Catwalk System



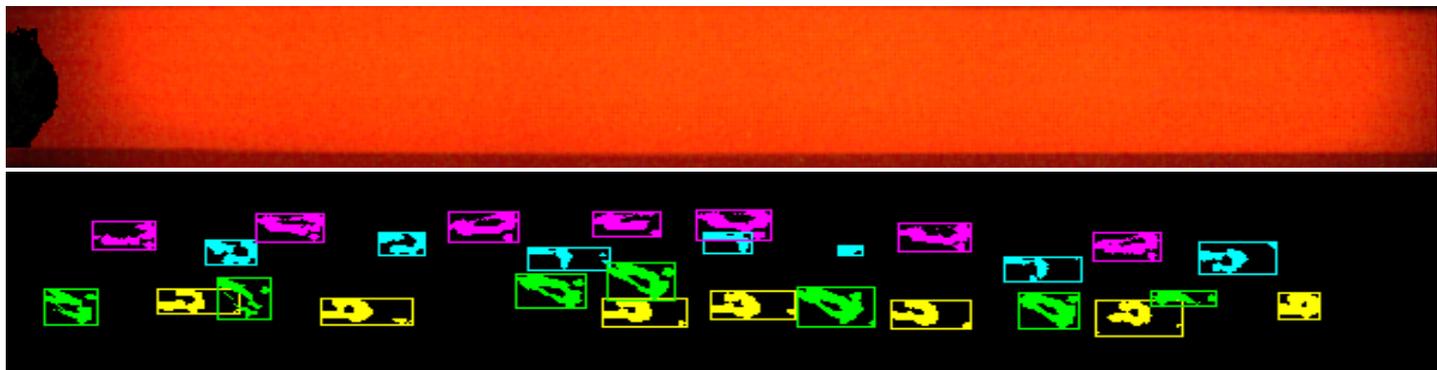
Cat Walk

Wild-type

Dr. Kondo (Dept. physiology)



Tg (755tgM)



The gait pattern was clearly disturbed in Kumanomi.

Is this gait disorder caused by the loss of dopamine?

Rescue experiment for Gait disorder



Rescue experiment

Tg (Kumanomi) pre L-dopa administration



Tg (Kumanomi) after L-dopa administration (30 min)



Future issues to be addressed using PD model transgenic marmoset

- What are the neuronal circuits damaged at the each clinical stage?
- Any relationship between the Pathogenic protein accumulation and each clinical symptom?
- Prediction of the Onset of each clinical symptom
- Development of the drugs to slow-down the Disease Progression as a Preemptive Approach

Development of marmoset disease models

Drug induce/surgical model

Lentiviral vector

Genome editing technique

Schizophrenia (PCP)

Parkinson's

Stroke

Parkinson's

Schizophrenia (SETD1A, Shank3)

Alzheimer

On going

Rett syndrome

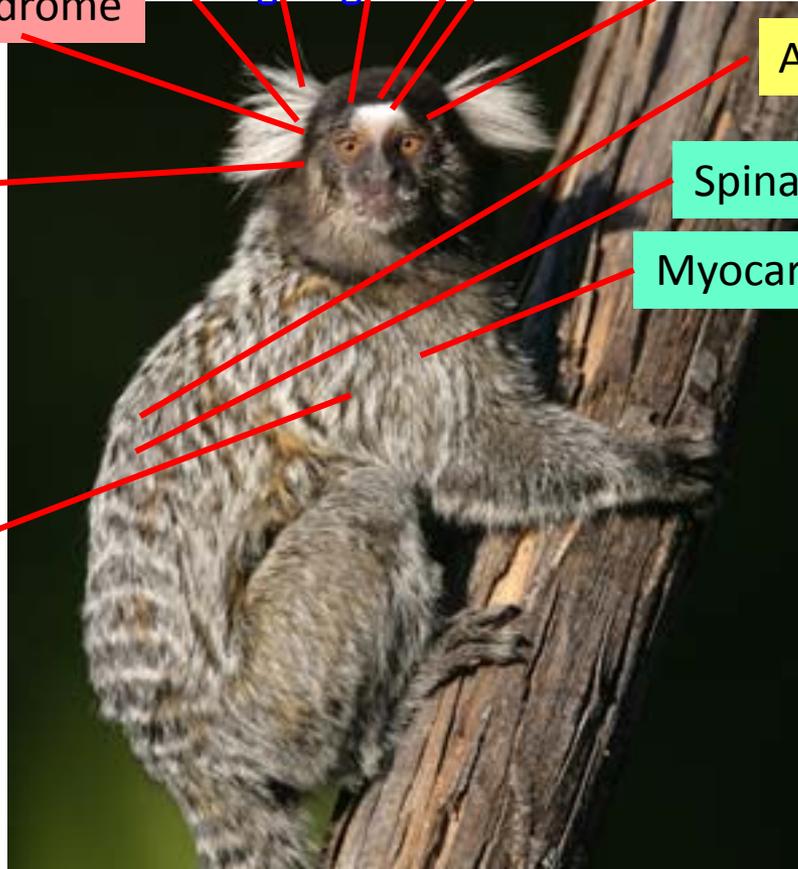
ALS

Alzheimer Disease
On going

Spinal cord injury

Myocardial infarction

Immunodeficiency



Thank you very much for your attention!



School of Medicine

KEIO
PHYSIOL

慶應義塾大学医学部生理学教室岡野研



Keio University/RIKEN

Reona Kobayashi
Takashiro Kondo
Ayako Murayama
Junichi Hata
Takakai Kaneko
Testu Yoshida
Noriyuki Kishi

CIEA

Erika Sasaki
Junko Okahara
Wakako Kumita
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Svante Paabo

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Tomomi Shimogori
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Tateo Yoshikawa
Atsushi Miyawaki

Osaka University

Hideki Mochizuki

Tsukuba University

Masanori Sakaguchi