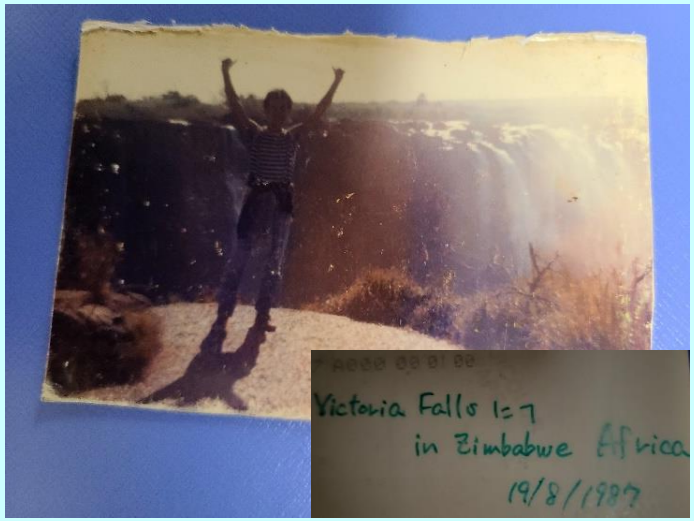
# Self Introduction



I' m sorry,
but this photo is over 35 years old…




**Yasushi Miyauchi**

Working Student
of Nagasaki University

**Background: Veterinarian,**
**Pathologist, Toxicologist**

**My job:**
**For around 30 years,**
**Developing new drugs of central nervous system mainly in pharmaceutical company**
**And**
**Conducting safety tests and risk assessment of medical drugs, device and chemical substances**

**For the past 2 years, I have been studying and researching in MPH course of Nagasaki Univ.**
**From this October, I will enroll in PhD course of Nagasaki Univ.**

# EXCITATORY AMINO ACIDS, POSSIBLE CAUSATIVE AGENTS OF NODDING SYNDROME AND NEW PATHOGENESIS

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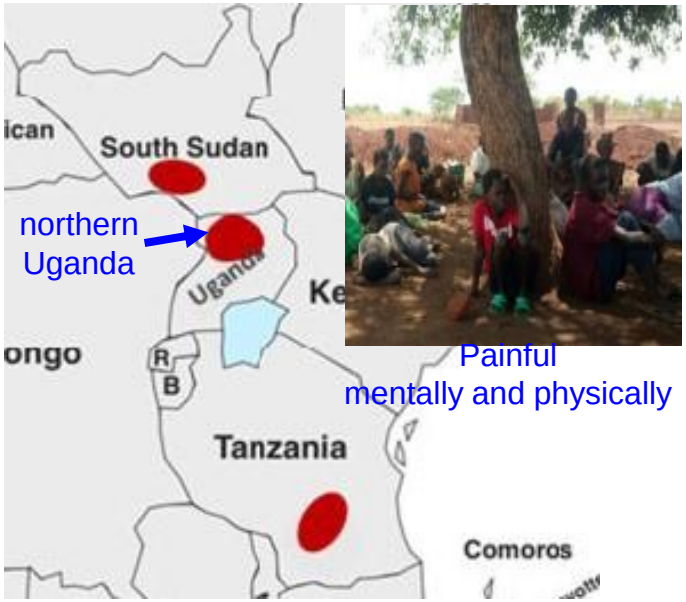



Geographical distribution of nodding syndrome in eastern Africa (Olum S, te al, 2020)



Painful mentally and physically

## About nodding syndrome (NS)

- One type of epilepsy characterized by nodding of the head
- Occurrence in children after 3 year of age
- Often in poverty areas, including refugee camps
- Relationship to onchocerciasis
- The cause still unknown

In this study
Excitatory Amino Acids such as, kainic acid, were examined as possible cause

# Objective



Excitatory amino acids as possible causative agents of nodding syndrome

Existing in nature ⇒ some as food


Kainic acid

?
In eastern Africa


Quisqualic acid


Ibotenic acid


Domoic acid

Inducing epilepsy in animals

✓ Experimental approach
- similarities of clinical symptoms and histological lesions on NS and kainic acid-treated rat

✓ Literature search approach
- kainic acid receptor agonists in eastern Africa as causative agents of NS
- Estimation of human toxicity; kainic acid receptor agonist

# Method



Research ethics;
✓ Animal Ethics Review by the Animal Experiment Committee of Kamakura Techno-Science, Inc

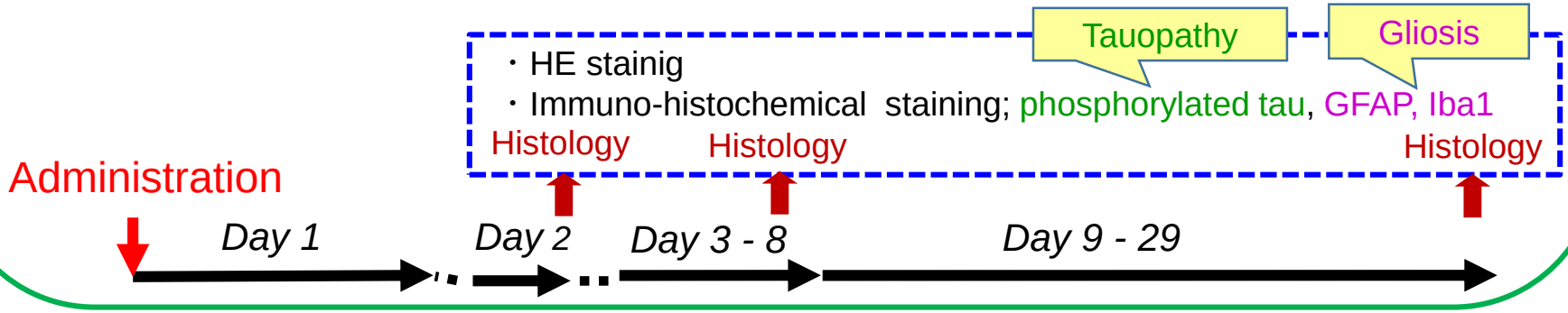
## Investigation of clinical symptoms and histological lesions in kainic acid-treated rat model

### Experiment 1; Acute phase

Administration: kainic acid; 10 mg/kg ip (n=6)
quisqualic acid; 10 mg/kg ip (n=6)
Clinical sign: Day 1 – Day 8
Histopathology in brain: Day 2 and Day 8

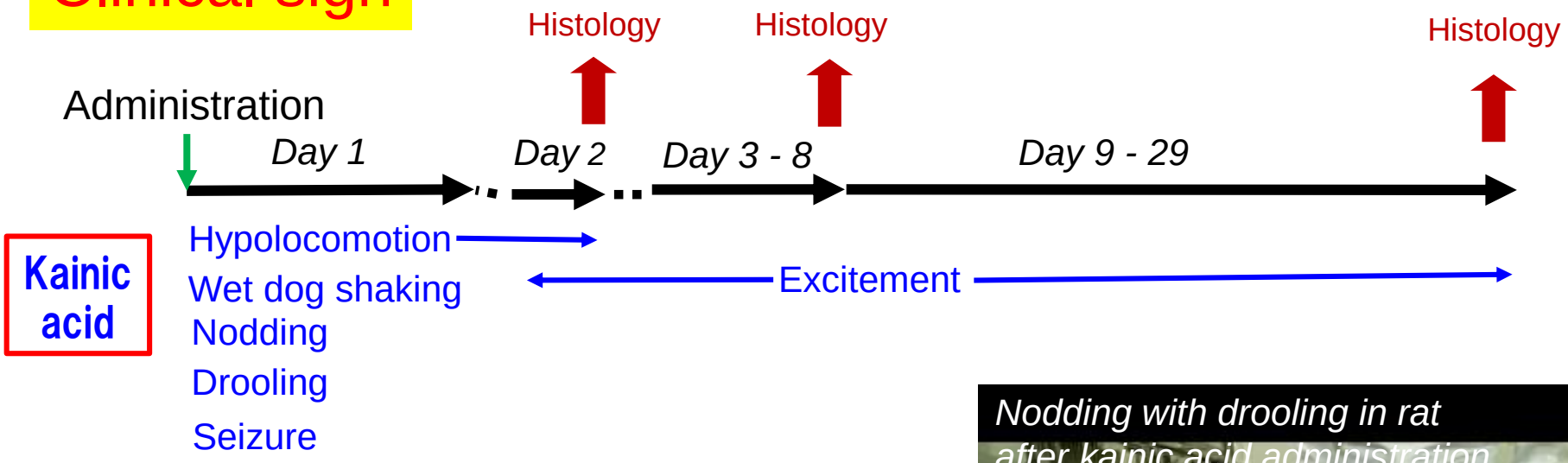
### Experiment 2; Chronic phase

Administration: kainic acid; 10 mg/kg ip (n=3)
saline; 10 ml/kg ip (n=3)
Clinical sign: Day 1 – Day 29
Histopathology in brain: Day 29

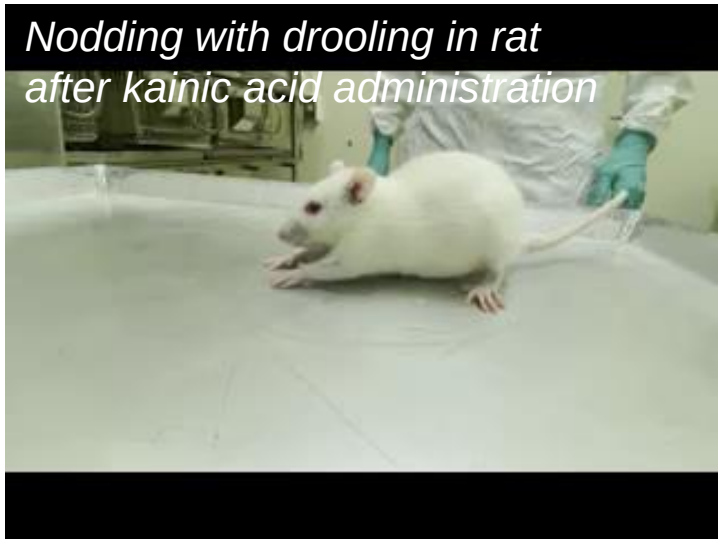


# Result; clinical sign

## Clinical sign




Kainic acid;
- ✓ Epileptic symptoms; seizure
  nodding with drooling
- ✓ Chronic symptoms; excitment





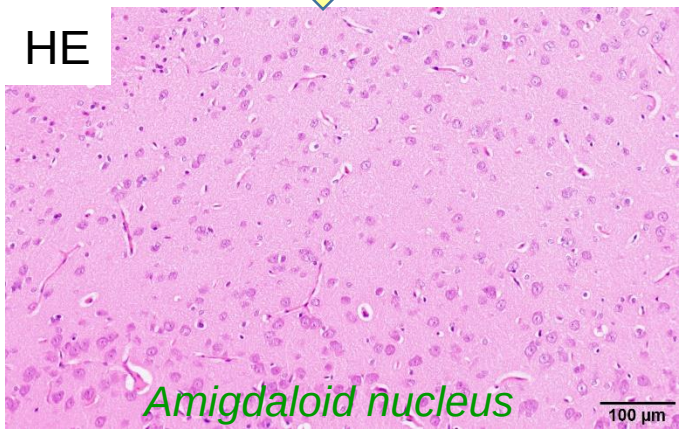
Quisqualic acid;
- ✓ hypolocomotion only

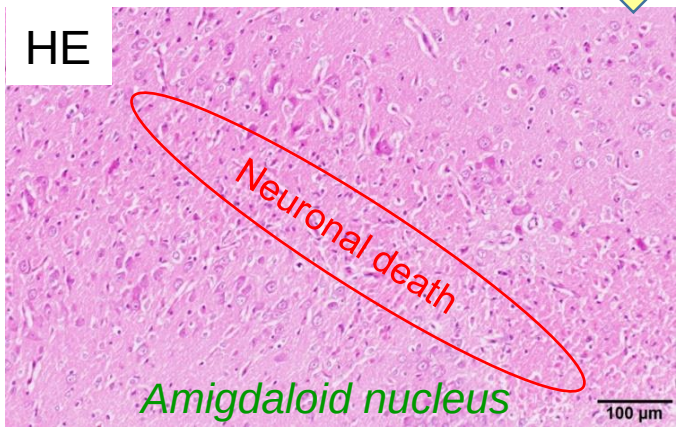
# Result; Histology



Saline; Day 29

Kainic acid; Day 29



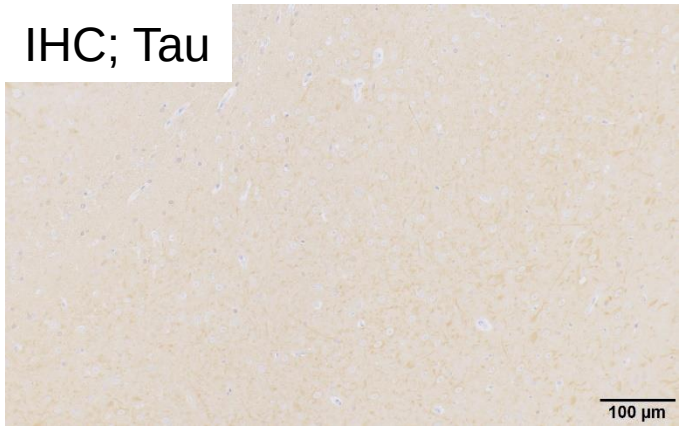



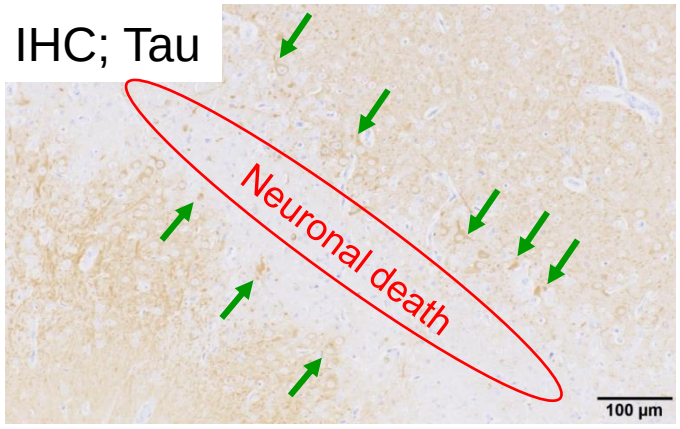

## Neuronal death

- Periform cortex
- Hippocampus
- Amygdaloid nucleus
- Thalamic nucleus

All bilateral

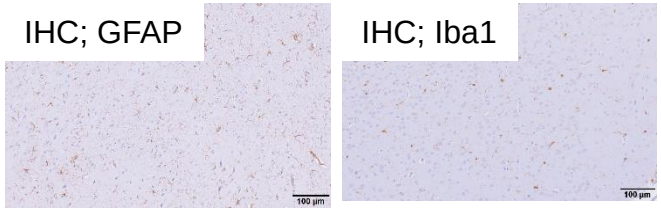
(No lesions with quisqualic acid)

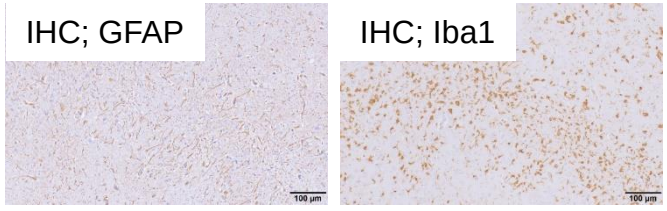



## Tauopathy

phosphorylated tau ↑
in neurons around regions of neuronal death
(green arrows)




## Gliosis

GFAP (+) astrocyte
Iba1 (+) microglia ↑

# The similarity of clinical symptoms and histological lesions between NS and kainic acid-treated rat model

kainic acid-treated rat 

◎ Epileptic symptoms; nodding with drooling and seizure

From the literature,

electroencephalography

✓ Spikes and slow waves in EEG; NS and this rat model

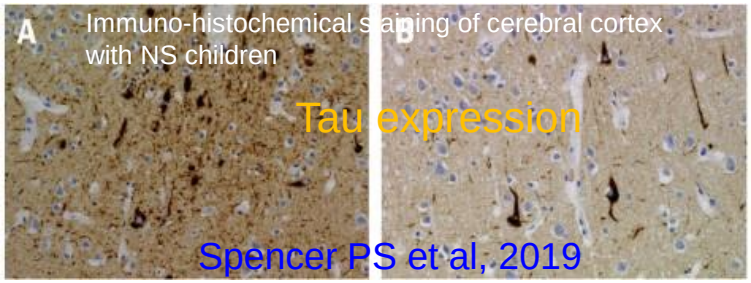
✓ A loss of neck muscle atonia; nodding symptoms in this rat model

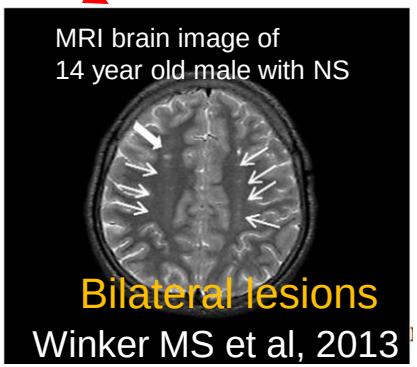
◎ Neuronal cell death in piriform cortex, hippocampus, amygdaloid nucleus and thalamic nucleus; bilateral with tauopathy and gliosis

From the literature,

✓ Neuronal cell death with gliosis; bilaterally with NS children

✓ Tauopathy; the topic of recent studies on NS



# From literature search

## Possible causative agent of NS in eastern Africa

### Kainic acid receptor agonist in moldy maize

*Ustilago maydis* in Sudan

Morchelo-d'Ragga PW et al, 2015

→ Tricholomic acid

Strong kainic acid receptor agonist

- ✓ *U. maydis;* a well-known fungal disease of maize
- ✓ Moldy maize: significantly consumed in the dietary history of NS
- ✓ Possibility of contamination of corn flour in emergency relief food

### Kainic acid receptor agonist in traditional medicine

*Valenrina officinalis*

https://ja.wikipedia.org

→ Valerenic acid

Not be confirmed in eastern Africa

*Digenea simplex*

https://www.zukan-bouz.com/syu

→ Kainic acid

Elimination of roundworms

# Estimation of human toxity

OAE 2023
Yasushi Miyauchi Sep. 21, 2023

## tricholomic acid, possible causative agent of nodding syndrome?

From this kainic acid-induced epilepsy model

Kainic acid; 10 mg/kg intraperitoneal (ip) dose in rat

↓ A coefficient of 5 in the risk assessment

Kainic acid; 2 mg/kg ip dose in human

↓ A coefficient of 10 in the risk assessment: Individual differences among humans

Kainic acid; 0.2 - 2 mg/kg ip dose in human

↓ ip= oral : amino acids are considered to be almost completely absorbed into the human

Kainic acid; 0.2 - 2 mg/kg oral dose in human

In humans,
the concentration of kainic acid that induces epileptic symptoms; 0.2 – 2 mg/kg

Taking this amount of *U. maydis*

↓

human toxicity at one time

Tricholomic acid in *U. maydis*

The content of tricholomic acid in dry maize infected with *U.maydis*: 0.36 mg per 1 g of dry maize

(Lizárrage-Guerra R et al, 1996)

In case of: Weight of a 5-year-old child; 18 kg
Eating amount of this maize this child; 30 g/day

In humans,
the exposure dose with this child [oral dose];
(0.36 mg/g × 30 g)/18 kg = 0.6 mg/kg

# Additional Discussion

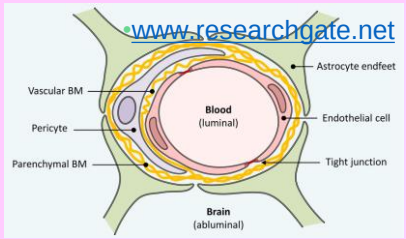
OAE 2023
Yasushi Miyauchi Sep. 21, 2023

## Blood-brain barrier (BBB) involvement regarding nodding syndrome

Even if the cause of NS is kainic acid, some questions remain

- ✓ Why does NS only happen to children?
- ✓ Why no NS in Mexico?
  (customary to take *U. maydis* as a delicacy)

Possible increased BBB permeability in NS children




Reports about the environment with NS children
The endemic regions of NS

- ✓ *Onchocerca* infected areas
- ✓ Refugee camps displaced by war or conflict

Hypothesis

In the pathogenesis of NS,
not only KA but also increased permeability of the BBB may be involved.

# Conclusion



- ✓ In kainic acid-treated rat epilepsy model, the similarity to NS was shown symptomatically and histologically
- ✓ Kainic acid and/or related compound are the causative agent of NS is suggested
- ✓ Particularly, tricholomic acid, a kainic acid receptor agonist, contained in moldy maize was found as possible candidate of the cause of NS.

Future plan

- Measurement of excitatory amino acids included in *U. maydis,* and cerebrospinal fluid and blood of NS children
- Study of central nervous toxicity in pregnancy rats with excitatory amino acid

## Acknowledgement

I'm very grateful Prof. Moji, teaching staffs, classmates including satellite students, the administrative staffs and colleagues at my work place.

## Reference

Miyauchi Y, Shiraishi A, Abe K, Sato Y, Kita K. Excitatory amino acids, possible causative agents of nodding syndrome in eastern Africa. J Tropical Med and Health. 2023; 51:30-36.

Olum S, Scolding P, Hardy C, Obol J, Scolding NJ. Nodding syndrome: a concise review. 2020; 2(1).

Spencer PS, Mazumder R, Palmer VS, Pollanen MS. Nodding syndrome phenotypes. Rev Neurol. 2019; 175:679-85.

Winkler AS, Fricdrich K, Velicheti S, Dharsee J, Konig R, Nassri A, Meindle M, et al. MRI findings in people with epilepsy and nodding syndrome in an area endemic for onchocerciasis: an observational study. Afr Health Sci. 2013; 13:529-40.

Marchelo-d'Ragga PW, Gama PBS, Misaka BC. First report of *Ustilago maydis* (Pers.) roussel the causal agent of smut of corn, *Zea mays* (L.) Pers. In South Sudan: A note. 2015; 3:353-55.

Lizárrage-Guerra R, López MG. Content of free amino acids in Huitlacoche (*Ustilago mydis*). J Agric Food Chem. 1996; 44:2556-9.

*Thank you for your attention*