Cerebral neuronal dysfunction associated with human prion diseases

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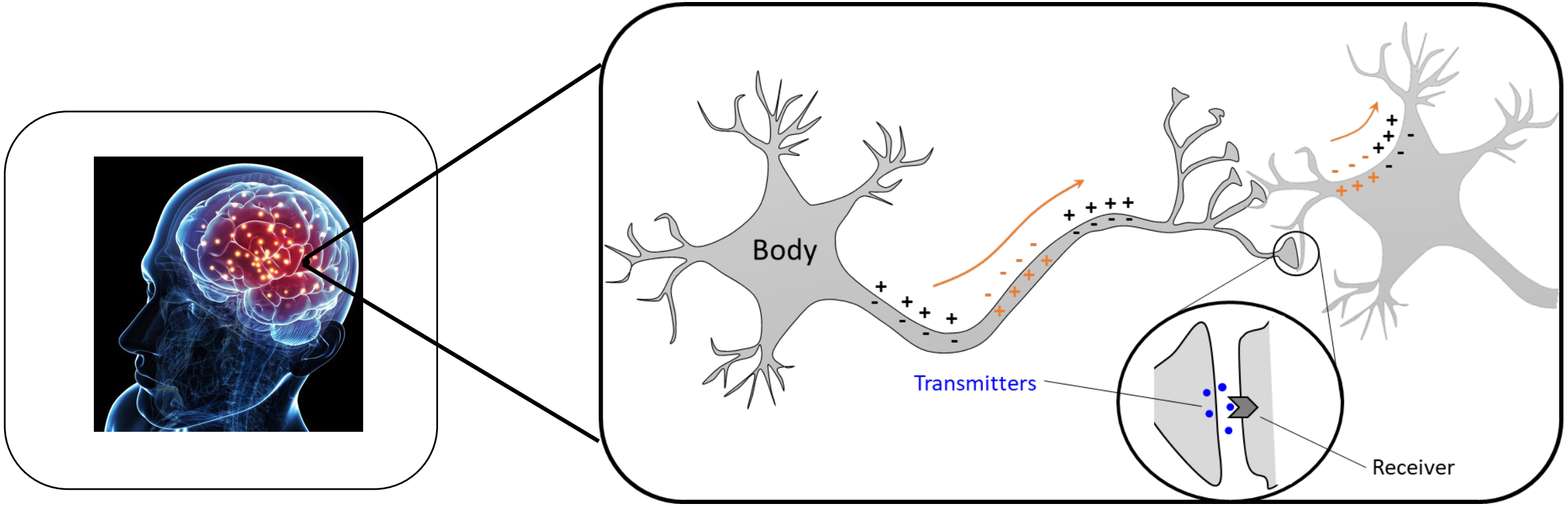
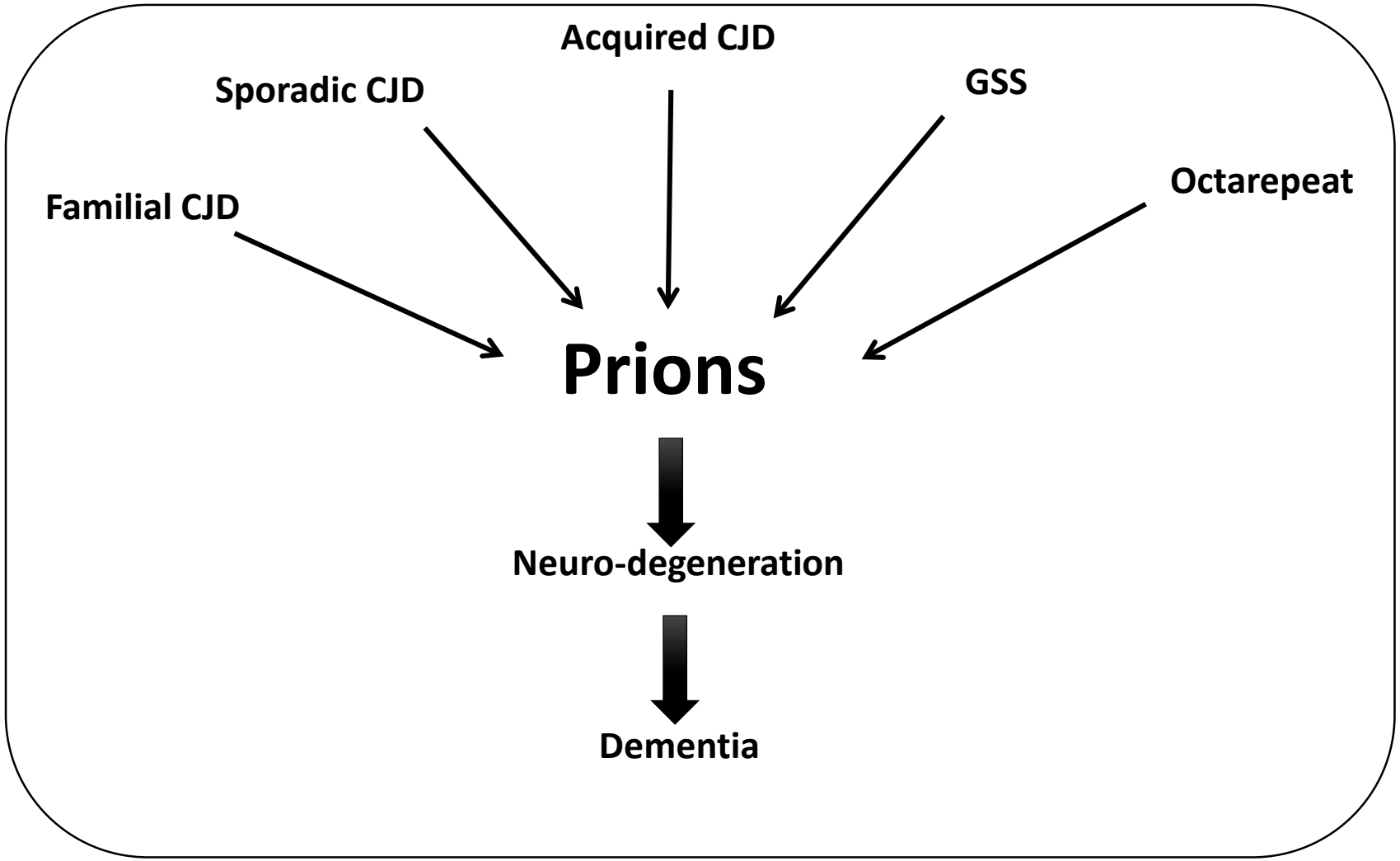
Your brain is made up of billions of **interconnected** nerve cells called “Neurons”



- Neurons are **electrical wires** of our brain – It can propagate and transmit electrical current
- Electrical signaling can be transmitted between neurons that are in close proximity by releasing and receiving transmitters (chemicals). The transmission site is called the **synapse**.
- Increased transmission of electrical signaling between neurons is associated with memory formation and learning
- Dysfunction of neuronal electrical transmission is associated with **dementia**

Prion disease causes severe dysfunction and degeneration of neurons



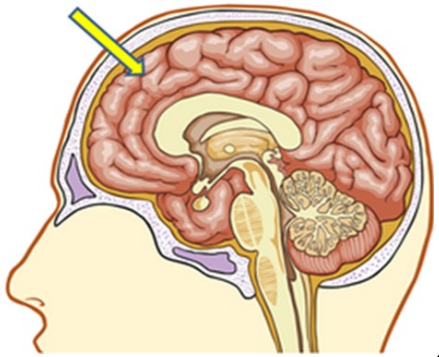
All prion diseases are associated with the production abnormal prion proteins (**Prions**).

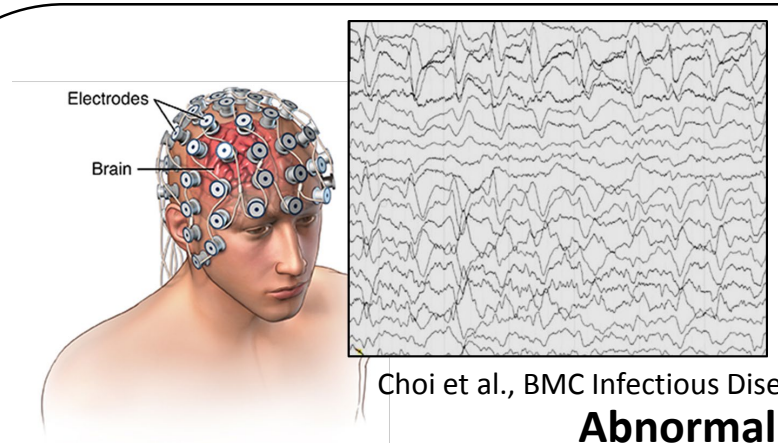
Dysfunction of neuronal <u>electrical transmission</u> is associated with **dementia**

The main question of our study is: **What causes dementia in prion disease?**

Cerebral cortex, a region of the brain that is commonly affected in prion diseases

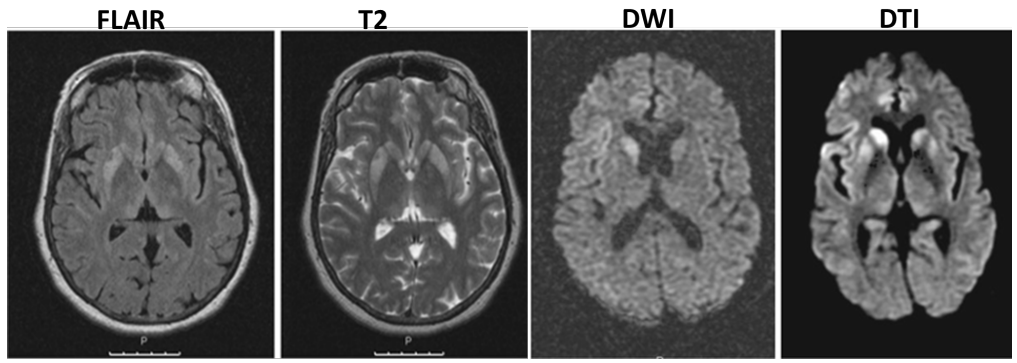
Cerebral Cortex





Choi et al., BMC Infectious Diseases, 2009)

Abnormal EEG



(Appel et al., J. Neurology, 2012)

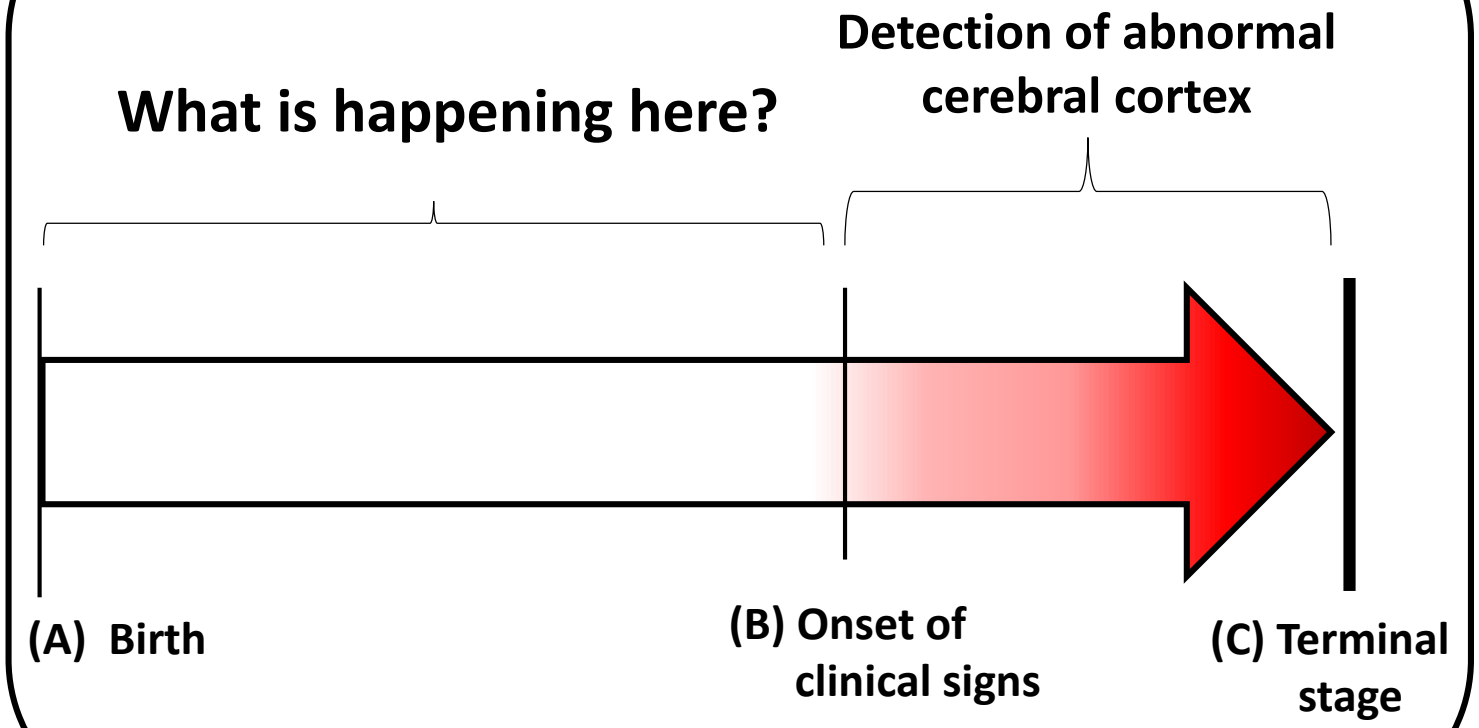
Abnormal MRI

Cerebral cortex plays important roles in our cognition and behaviour

- Meaning that the neuronal activity in the cerebral cortex is essential for memory formation, learning, etc.

In prion diseases, activity of the cerebral cortex is commonly affected, which is usually detected as abnormal EEG and MRI.

Prion disease progression



(A)-(C): Lifetime of prion disease Patient

Abnormal cerebral cortex is usually detected following the clinical onset, correlating with the:

i. cognitive and behavioral impairment
ii. Increased accumulation of prions in the nerve cells (at the terminal stage)

In human prion diseases, any **pathological event prior to the clinical onset is largely unknown**.

Obstacles:

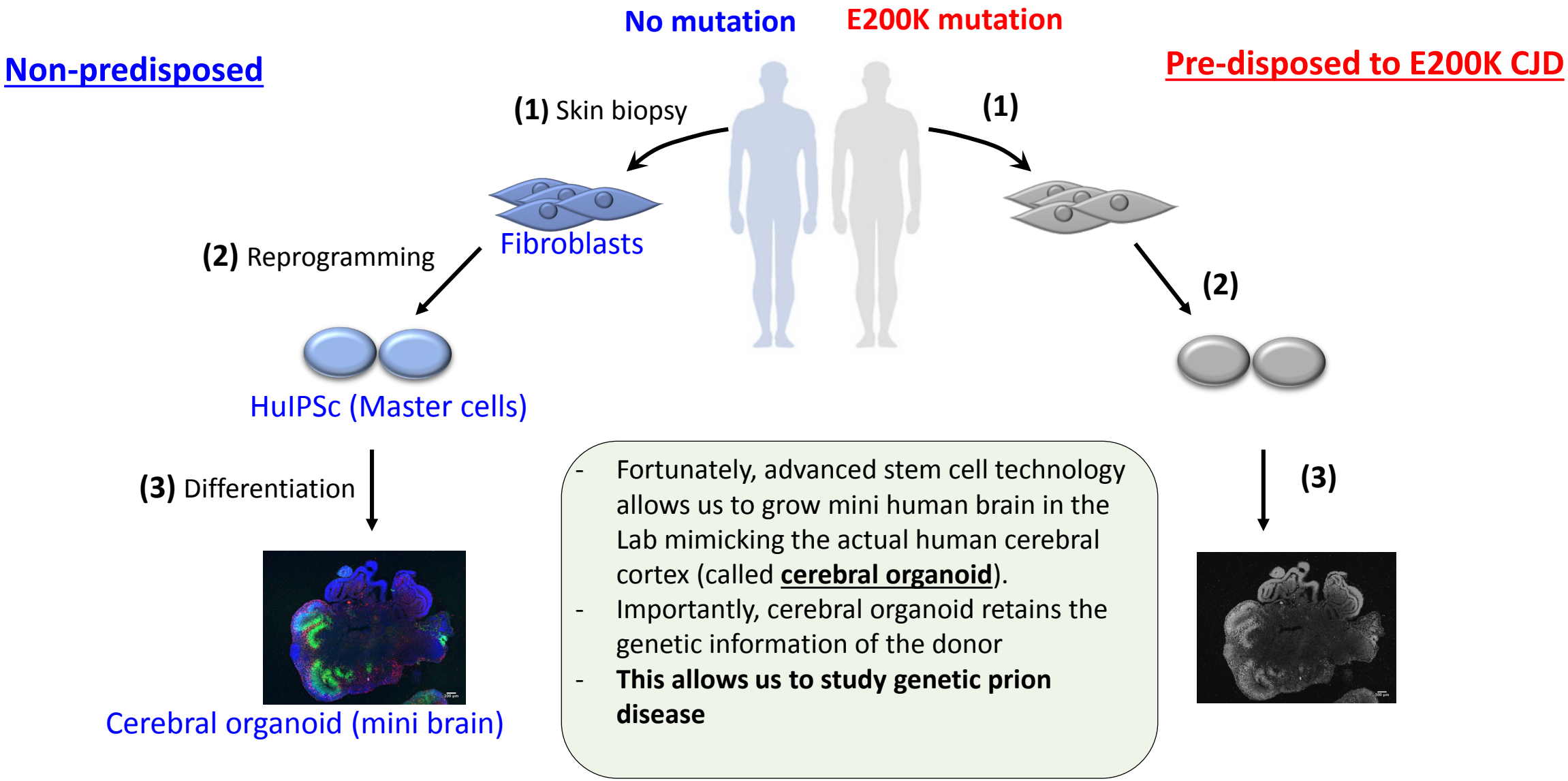
i. We cannot experiment on human
ii. If we use post-mortem brain tissue, they are already at clinical disease

Genetic mutation

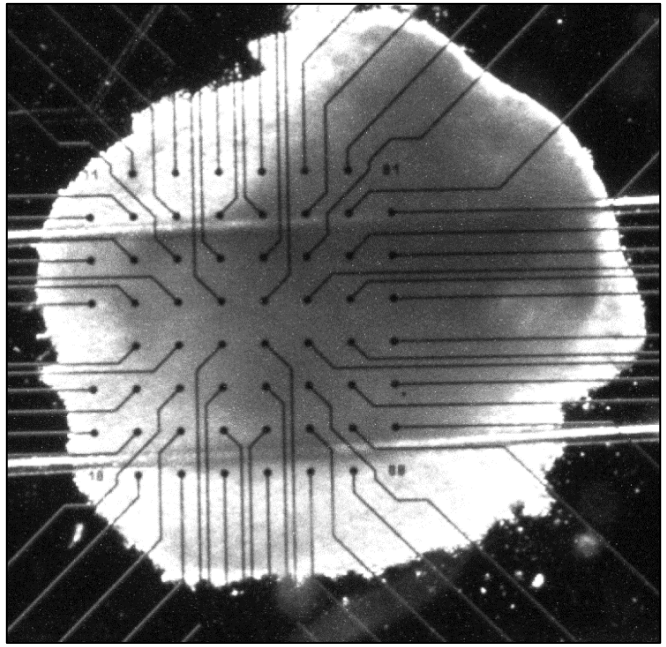
AIM # 1:

To investigate the influence of genetic mutations that **pre-dispose** individuals to prion disease on **neuronal electrical signaling** and health

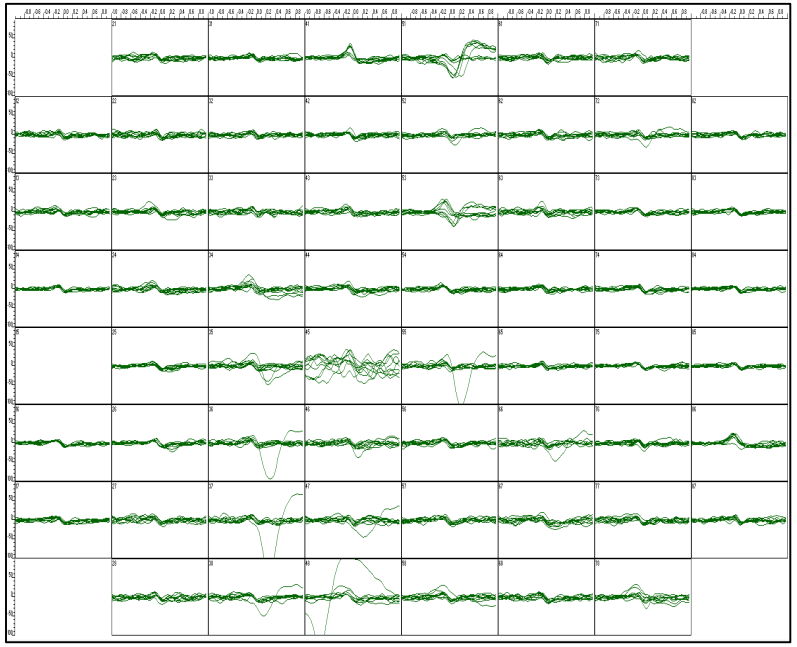
Mini human brain can now be grown in the Lab!



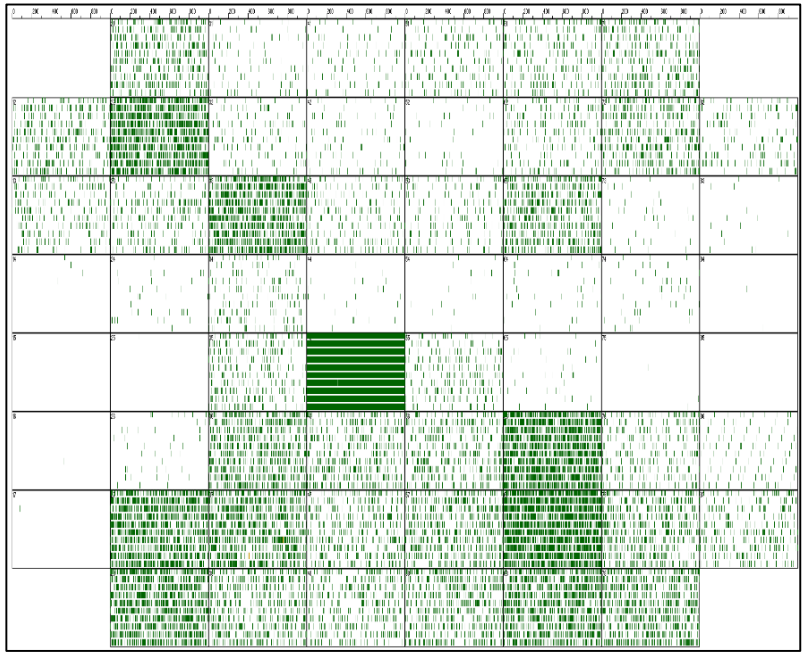
Both non-predisposed and pre-disposed organoids **generate neuronal activity associated with memory and learning**



- A slice of cerebral organoid sitting on micro-electrodes (black dots)



- The **spikes** are neuronal electrical signaling called **action potentials** detected by the micro-electrodes (each box is an electrode)



- Density of green dots in each box (electrode) measures **how frequent neurons generate action potentials**
- Green boxes that are next to each other indicate **neuronal network connectivity** (via **synaptic transmission**), a physiological correlate of memory formation and learning.

How to detect neuronal dysfunctions associated with the E200K mutation

We stimulated neuronal activity by treating organoids with a chemical stimulant called Glycine (a neuro-transmitter).

Neuronal activity normally responds to glycine in a dose-dependent manner.

Dysfunction of neuronal activity is usually not detectable in response to lower or optimal doses of glycine

High doses of glycine can over activate neuronal activity, which can indicate the ability of neurons to withstand stressful conditions

Potential results: - Based on "the higher you climb, the harder you fall"

i. Susceptible neurons (to high doses of glycine) = Normal

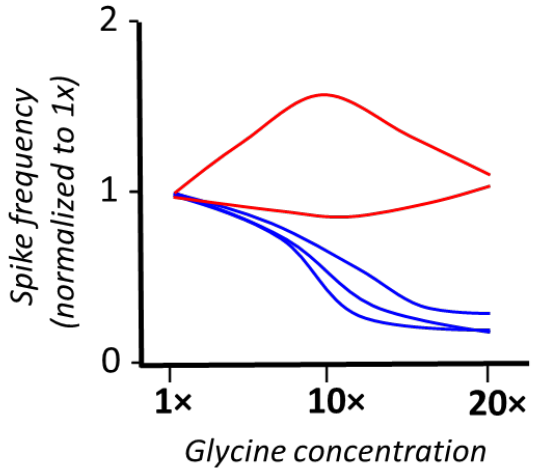
- Overactivation of neuronal activity causes fatigue and excitotoxicity (cellular dysfunction that can cause cell death)

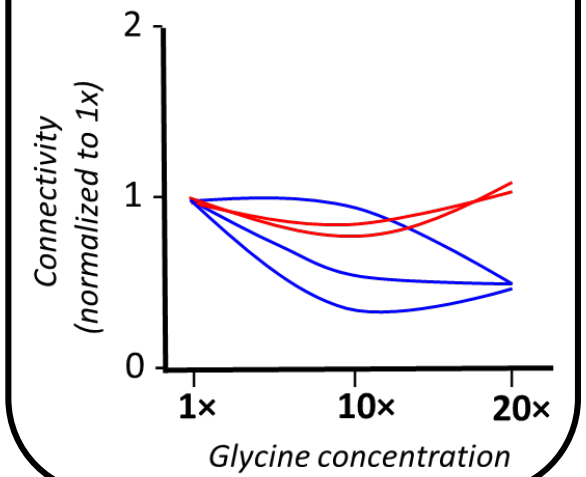
ii. Tough neurons (not disrupted by high doses of glycine) = abnormal

- Overactivation of neuronal activity do not cause fatigue and excitotoxicity.

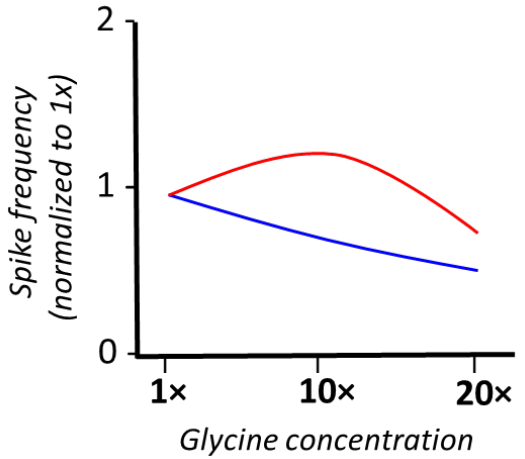
Pre-disposed organoids have higher tolerance to fatigue/excitotoxicity than non-predisposed organoids

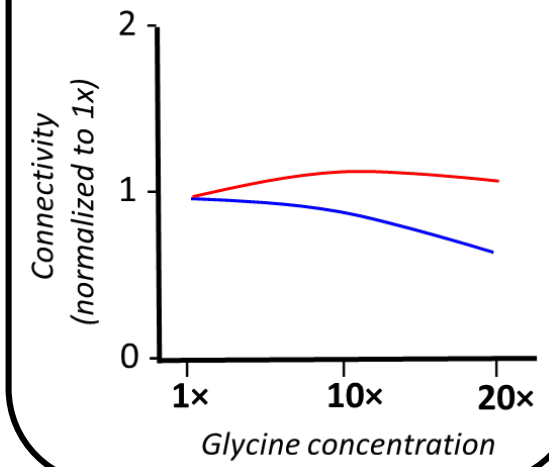
3 months old





6 months old





10+ months old (Future direction)

Non-predisposed

Pre-disposed

- **3 Glycine doses:** 1x, 10x, and 20x
- **3 time-points**: 3, 6, and 10+ months old.
- 2 lines of pre-disposed organoids from 2 carriers of E200K
- 3 lines of non-predisposed organoids from 3 individuals without the mutation.

Results:

Normal neuronal activity:

-**Reduced neuronal activity** (spike frequency and connectivity) of non-predisposed organoids in response to 10x and 20x glycine

Abnormal neuronal activity:

- **Enhanced or unchanged neuronal activity** (spike frequency and connectivity) of pre-disposed organoids in response to 10x and 20x glycine. Similar results at 6 months

Sporadic CJD (Future direction)

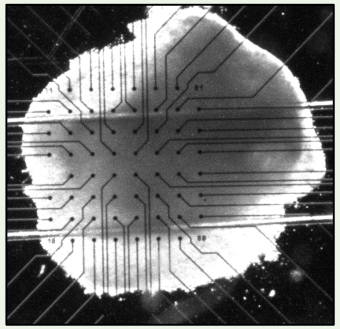
AIM # 2:

To identify the changes in neuronal electrical signaling that are induced by exposure to human prions

Our recent report:

- Organoids expressing normal prion protein could faithfully develop pathology of prion diseases following exposure to sporadic prions (Groveman et al., Acta. Neuropathological Comm., 2019)

Future study:



+ Prions from sporadic CJD → **Assess neuronal electrical signaling over time**

Future directions

AIM # 3:

To determine whether proposed anti-prion compounds can reverse or prevent prion-induced changes in neuronal signaling and identify new pathway targets for therapeutics

- Upon the completion of aim 1 and 2, we will have identified the molecular mechanism associated with neuronal dysfunctions in the cerebral cortex during the disease progression, which can be used as a target for therapeutic drug development

Summary

(1) We have found that both non-predisposed and pre-disposed organoids are capable of generating neuronal activity associated with memory and learning.

- Neurons are interconnected, allowing them to communicate in a network, a neuro-physiological correlate of memory formation and learning.

(2) We have found that the mechanism by which neurons generate and transmit electrical signaling is abnormal in cerebral organoids from individuals who are pre-disposed to E200K CJD.

- Pre-disposed cerebral organoids **have higher tolerance to fatigue/excitotoxicity** than organoids that do not have the mutation – “the higher you climb, the harder you fall”

(3) We are now trying to identify the exact molecular mechanism associated with the abnormal neuronal activity of pre-disposed organoids.

(4) We are currently assessing the electrical signaling property of cerebral organoids (with no mutation) following exposure to prions from sporadic CJD.

(5) We are hoping to determine some molecular mechanisms of abnormal neuronal activity in prion diseases, which can be used as targets for therapeutic drug development.

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