

Regenerative Medicine in Alzheimer's Disease

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Regenerative Medicine in Alzheimer's Disease

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Introduction

Alois Alzheimer (1834 - 1915), a German psychiatrist, chanced to have a 51-year-old female psychiatric patient who developed strong delusion of persecution that her husband were having a love affair used to shout aloud, and turned violent. Dr. Alzheimer closely observed her and described in detail what he observed. As her diseases progressed, she lost memory at all, confused and disoriented four and half years later.

At autopsy, Dr. Alzheimer observed pronounced pathological changes in the cerebral cortex. He reported the case as a novel disorder in one of the German journals¹⁾ in 1907.

Recently, this Alzheimer's disease (AD) has turned to be one of the most shattering diseases throughout the industrial countries. AD is an intimidating disorder firstly because of its high prevalence as high as 80% of the senile dementia, secondly because of lack of established knowledge of etiology, prevention, and curing treatment, and thirdly because of its relentless progression by destroying humanly personality, and leading to eventual death.

By the way, we have long studies AD with XCT²⁻⁴⁾ for 30 years, and with MRI⁵⁾ for 10 years so as to discover the primary focus of AD in the brain.

Brain atrophy in AD is a dilatation of cerebral ventricles, especially when the lateral posterior horn is significantly dilated. Based on this, we have developed a new tomographic technique (Matsuzawa's tomographic method, patented) and finally succeeded in finding the primary focus. This primary focus is a specific destruction (deficit) of the amygdala and hippocampus in the limbic system occurring symmetrically on both sides. In general, there is a mechanism by which wounds of the living human body are healed. This mechanism involves neural stem cells which proliferate and differentiate into glial

cells and neural cells^{6,7}). The theory⁸) advanced by Cajal that the brain cells in the mature brain are not regenerated, but only reduced, has been the standard belief for as long as 80 years. This has now been proved to be incorrect. Regenerative medicine, in which Alzheimer's disease can be prevented and cured, is realized by inducing the healing capacity of neural stem cells through appropriate treatments.

1. Methods and Subjects

1-1. MRI

Development of Matsuzawa's tomographic method (Japanese patent number 4111826, European patent number 1344491).

The new tomography has been developed for carefully examining the cerebral limbic system of the cerebral parenchyma adjacent to the lateral posterior horn utilizing MRI (Fig. 1A, 1B and 1C).

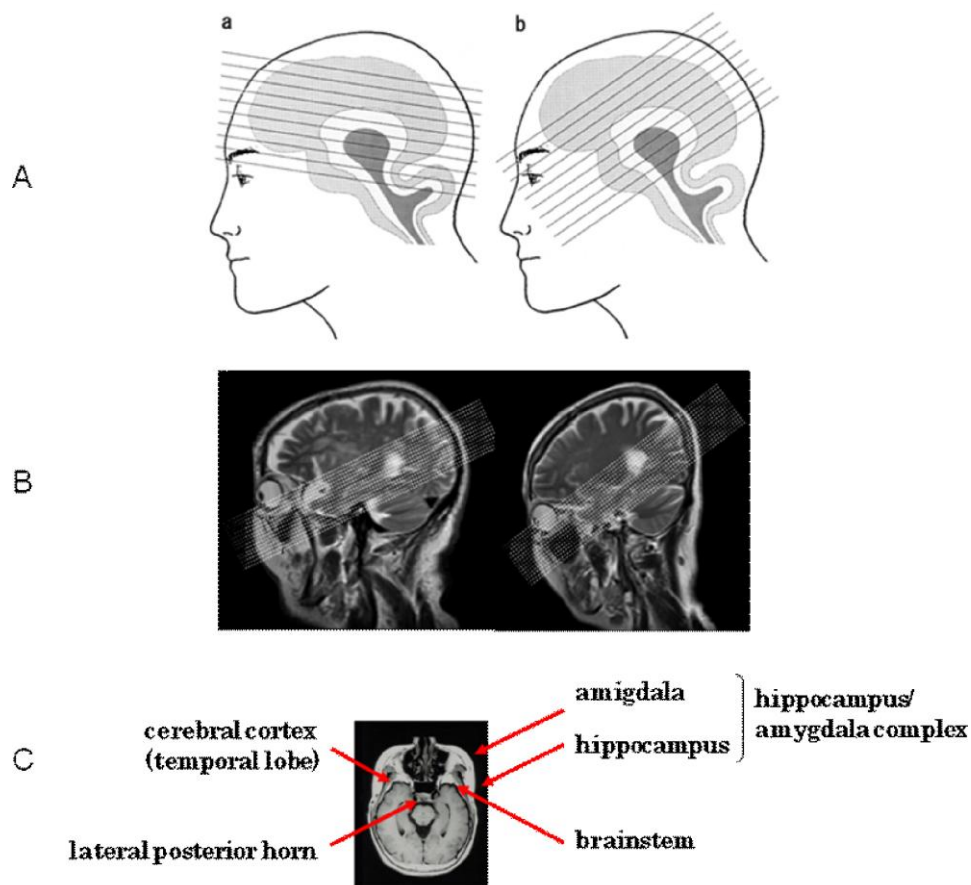


Figure 1. Matsuzawa's new tomographic method (A, B, C).

Figure 1A shows pattern diagrams of the conventional tomography and Matsuzawa's newly developed tomographic method (b). In the conventional tomography, several parallel sections were obtained above the line connecting the superior orbital margin and ear canal. As shown in Fig. 1B, a scout view is made via a T2-enhanced image for positioning, the lateral posterior horn outlined by the scout view is used as a reference line and several parallel cross-section diagrams are obtained above and below the reference line in the newly developed method. As shown in Fig. 1C, this method is capable of capturing a triple structure of the brain (including the brain stem, amygdala–hippocampal complex in limbic system and cerebral cortex) altogether on the same plane.

1.5 Tesler MRI

MRI Images were obtained with a 1.5 Tesla whole-body imager (Signa EXCITE XL; GE Medical Systems, Japan) by using a 8 channel Brain Array Coil. The Protocol included axial T2-weighted fast spin-echo images (4000/120 [repetition time msec / echo time msec], Echo Train Length; 16, Section Thickness; 5mm, Gap; 0mm, Field of View 24cm, Matrix; 320x320, NEX; 3). T1-weighted T1-Flair images (2400/24 [repetition time msec / echo time msec], Inversion Time; 1000msec, Echo Train Length; 6, GAP; 0mm, Field of View; 24cm, Matrix; 320x256).

3 Tesler MRI

MRI Images were obtained with a 3.0-Tesla whole-body imager (Signa EXCITE HD; GE Medical Systems, Japan) by using a 8 channel Brain Array Coil. The protocol included axial T2-weighted fast spin-echo images (4000/102 [repetition time msec / echo time msec]; Echo Train Length; 16, Section Thickness; 3mm, Gap; 0mm, Field of View; 24cm, Matrix; 512x320). T1-weighted T1-Flair images (2200/9.1 [repetition time msec / echo time msec] Inversion Time, 1000 msec, Section Thickness; 3mm, GAP; 0mm, Field of View; 24cm, Matrix; 320x256). 3D-SPGR (Spoiled Gradient Echo) images (11.8/5.2 [repetition time msec / echo time msec] Flip Angle; 10°, Slice Thickness; 1.4mm, Location per Slab; 60mm, Field of View; 24cm, Matrix; 320x256)

DWI (Diffusion Weighted Image)

Pulse Sequence; Spin Echo EPI; TR=5000msec, TE=75.9msec, Field of View; 24cm, Slice Thickness; 3.0mm (for 3Tesler) or 5.0mm n (for 1.5Tesler), Spacing; 0mm, Matrix; 256x128, NEX; 2 times, Imaging Option; ASSET (Asset Factor; 2.0).

In daily interventions, three types of images, including T1-weighted images (T1WI), T2-weighted images (T2WI) and diffusion-weighted images (DWI) are obtained by this method for each patient.

1-2. PET

- a. The subjects were scanned using an ECAT PT931/04-12 scanner (CTI Inc. Knoxville, TN, USA), and further subjects were administered placebo and scanned with the same equipment. The ECAT PT931/04-12 scanner has a spatial resolution of 7-8mm FWHM in the center of the field of view (FOV) and has a 5cm-long axial FOV that is not capable of covering the whole brain.
- b. Tracer: ^{18}F -FDG=2-deoxy-2- ^{18}F fluoro-D-glucose used as a trace^{9,10}.

1-3. Subjects

Normal volunteers

A total of 16 normal volunteers (males 8 and females 8) their age ranged from 13 to 89 were selected as a control group. Moreover, a total of 14 aged subjects (males 7 and females 7) their age ranged from 65 to 87 were added to the control group for the study on onset and progression of AD.

AD patients

- AD patients were distinguished from normal subjects by MMSE (minimetal state examination)¹¹) and MRI images, number of AD patients male 58 female 99, total 157, their age ranged from 65 to 83.
- Subjects for preventative treatment; patients of mixed type of mental disease depression and schizophrenia with hippocampus atrophy were selected to subjects for preventative treatments. Number of patients is 179 male and 277 female, total of 456, their age ranged from 14 to 85.

1-4. Diagram of regenerative medicine for prevention and treatment of AD (Fig. 2)

1-4-1. Onset

- 1) Heavy stress lowers the serotonin level, causes destructions of depression and makes a patient go into depression.
- 2) Total dopamine generates a toxicity that total dopamine originally has due to the decreased serotonin level, resulting in destructions of schizophrenia in the amygdala.

- In the normal state, the amount of total dopamine and serotonin is stable, owing to the negative feedback mechanism. Generated destructions demolish this stability mechanism, resulting in a discharge of total dopamine. The patient develops positive symptoms of schizophrenia. Likewise, serotonin is also discharged and the patient goes into a manic state. Bipolar depression is caused an abnormally high or low serotonin level. In AD, the destructions shown above and progressive atrophies of the hippocampi develop and progress to AD.

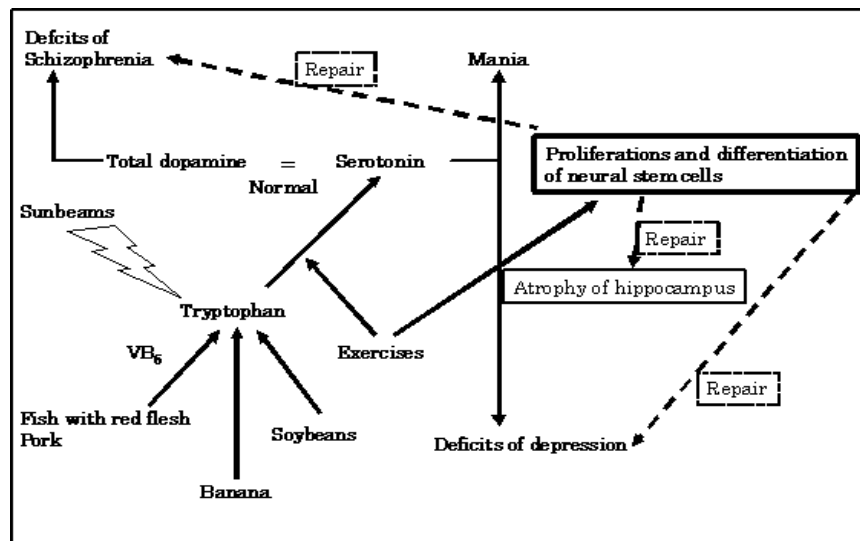


Figure 2. Diagram of regenerative medicine for prevention and treatment of AD.

1-4-2. Treatments

1) Pharmacologic treatment

Antidepressant drugs and antipsychotic drugs should be administered to patients at the same time without fail to normalize the amount of serotonin and total dopamine and stabilize the symptoms of patients. A minimum of necessary drugs should be used.

2) Alimentary therapy

Patients should eat foods that are rich in tryptophan, which is a precursor of serotonin (e.g., soybeans, bananas and lean fish). Patients should be advised to eat pork containing arachidonic acid, which is a component of the cell membrane.

3) Exercise therapy

This is an important therapy indispensable for increasing the serotonin level in the body and accelerating the proliferation and differentiation of neural stem cells.

4) Suggestion and persuasion therapy

It is important to explain the healing status of neural stem cells while showing images and to give an explanation that the disease can be completely healed to patients and family members.

2. Results

2-1. Primary foci of AD

All destructions (deficits) of Fig. 3b, c and d are observed in Alzheimer's disease patients. In other words, all of them are the primary focus of AD. MMSE (Mini Mental State Examination) is a mental test developed by Folstein et al. It is widely used throughout the world to discriminate AD patients from normal persons. AD is diagnosed if the MMSE score is 23 or less and atrophy of the hippocampus were recognized by MRI image.

Among mental disorders which are called by various names such as depression, schizophrenia, bipolar disorder, major depression, obsessional neurosis and social withdrawal, a patient always has both destructions b and c of depression and schizophrenia based on 3000 or more cases. All mental disorders are a mixed mental disorder caused by depression and schizophrenia. In the development of the mixed mental disorder,

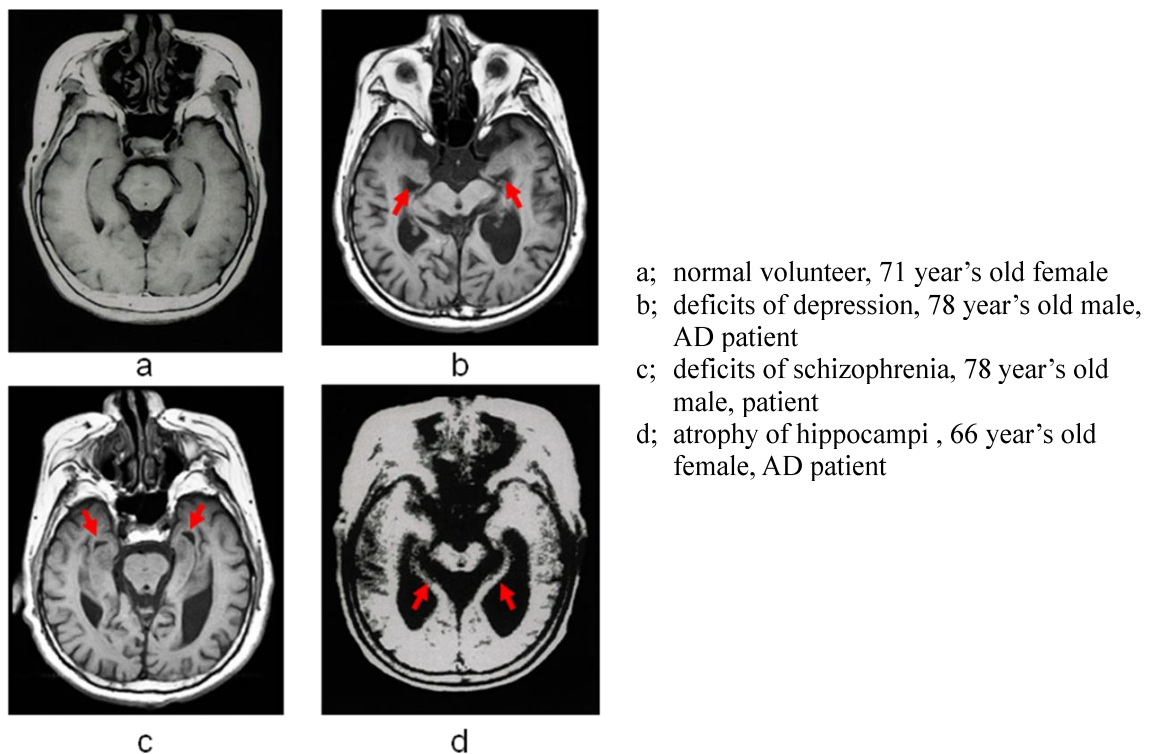


Figure 3. Primary foci of AD.

depression develops due to an abnormal reduction of serotonin and schizophrenia develops due to an abnormal increase of total dopamine. It seems that the mixed mental disorder consisting of depression and schizophrenia develops because the reduction and increase occur almost simultaneously.

The mixed mental disorder is roughly divided into the dementia type, social withdrawal type and affective disorder type. The dementia type is accompanied by an atrophied hippocampus. Dementia is sometimes observed in young people (i.e., presenile dementia). AD is dementia of the mixed mental disorder occurring in elderly people (65 years old or more), i.e., atrophied hippocampus type.

2-2. Onset and progression of AD

Figure 4 shows the onset and progression of AD. AD shows various symptoms accompanying its onset and progression. This is because AD is a progressive hippocampus atrophy type of mixed mental disorder consisting of depression and schizophrenia.

Photos on the left side of Fig. 4 show the progression of the atrophy of the hippocampus with MRI (T1WI). Photos on the right side of Fig. 4 show working conditions of the cerebral cortex with PET. Changes in glucose utilization imaged by PET were correlated with the atrophy of the hippocampus imaged by MRI.

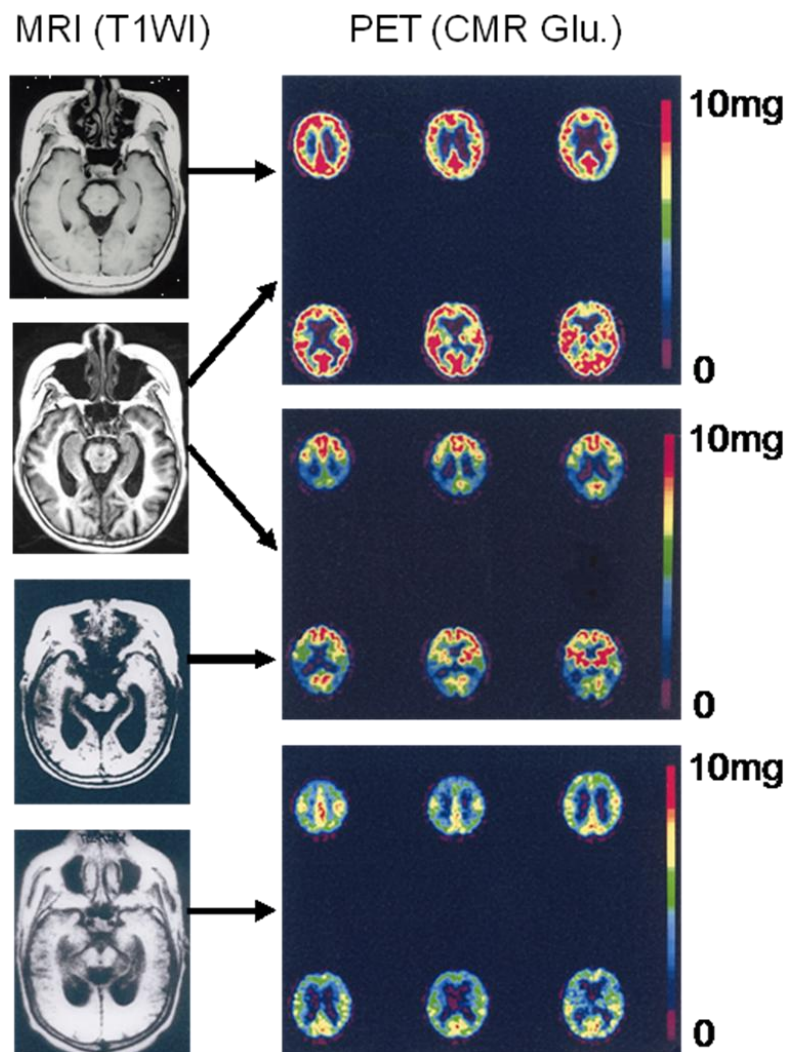


Figure 4. Onset and progression of AD.

The cerebral cortex is roughly divided into left and right hemispheres, and the frontal

and occipital lobe. They have different functions respectively. The primary area controlled by the brain stem, the brain of life, which works when humans look, listen and touch, and the association cortices of the higher-order brain function area controlled by the hippocampus, amygdala, ventral striatum, etc., (i.e., the brain of the mind) which works when humans think, are impressed, and have desire are mixed within the cerebral cortex.

Photos in Fig. 4 show the working states of the cerebral cortex sliced into six layers by height. The amount (mg/brain, 100 g/min) of glucose utilized by 100 g of the brain is indicated by color (0-10 mg) using a tracer (^{18}F FDG). The red and yellow show the regions of the brain which are functioning normally. The blue shows the regions of the brain whose function is decreased.

AD is a disease whose symptoms get worse and ultimately lead to death. Atrophy of the hippocampus progresses bilaterally. The destruction progresses to a certain level where both sides of the posterior half, i.e., six association cortices including the temporal, parietal and occipital lobes synchronously become impaired at once. This is the onset of AD.

In the early stage, patients sometimes develop dementia and sometimes return to a normal state, as if a light is turned on and off. This state is called intermittent dementia (early stage).

Dementia is fixed when the bilateral atrophy of the hippocampus further progresses (middle stage).

In the middle stage, patients have a high forgettery and loss faculty of orientation. They do not know who they are, where they are, and what they were doing at that time. Additionally, various symptoms such as depressive symptoms and delusions of persecution develop. In this stage, patients wander at day and night. Furthermore, bilateral atrophy of the hippocampus progresses and the hippocampus disappears. Then, the entire limbic systems in both sides disappears and are replaced by “big holes.” At this point in time, the prefrontal cortex and several motor association cortices also become impaired because the lesion expands to the ventral striatum. At this moment, all association cortices become impaired. Consequently, higher-order integrated functions of all association cortices, (e.g. memory, learning, cognition, thinking, motivation, creativity, and emotion) will be lost. Patients lose their humanities. However, the primary functions to sustain life (e.g. optical, auditory and tactile sensations, food appetite and the ability to excrete) remain almost intact and can survive (Late stage).

2-3 Pathology of AD

Figures 5A and B show findings of autopsies of patients who died from AD and other diseases. Figure 5A shows the brain of a patient who died due to AD (left) and the brain of a patient who died due to a different disease (right) seen from underneath. The author thanks Dr. Toshio Mizuno for his providing the photos.

Bilaterally symmetric holes (deficits) are observed in the left and right limbic system. This coincides with the findings of MRIs showing that AD is a disease causing deficits in the limbic system. Another important finding is that the cerebellum of patients who died of AD is hypertrophied (several times larger than that of patients who died of other diseases). This is supposed to be a compensatory hypertrophy to compensate for the dysfunction of cerebral cortices, suggesting that neural stem cells are proliferating and differentiating. Further research is required in this field.

Figure 5B is a photomicrograph of the remaining amygdala. The figure is a photomicrograph at a magnification of 200. The cells are dyed with a silver impregnation stain. The larger cells with projections are normal neural cells (B). They are gradually reduced in size (C) and fall to pieces. This kind of cell death is called cell suicide or apoptosis¹²).

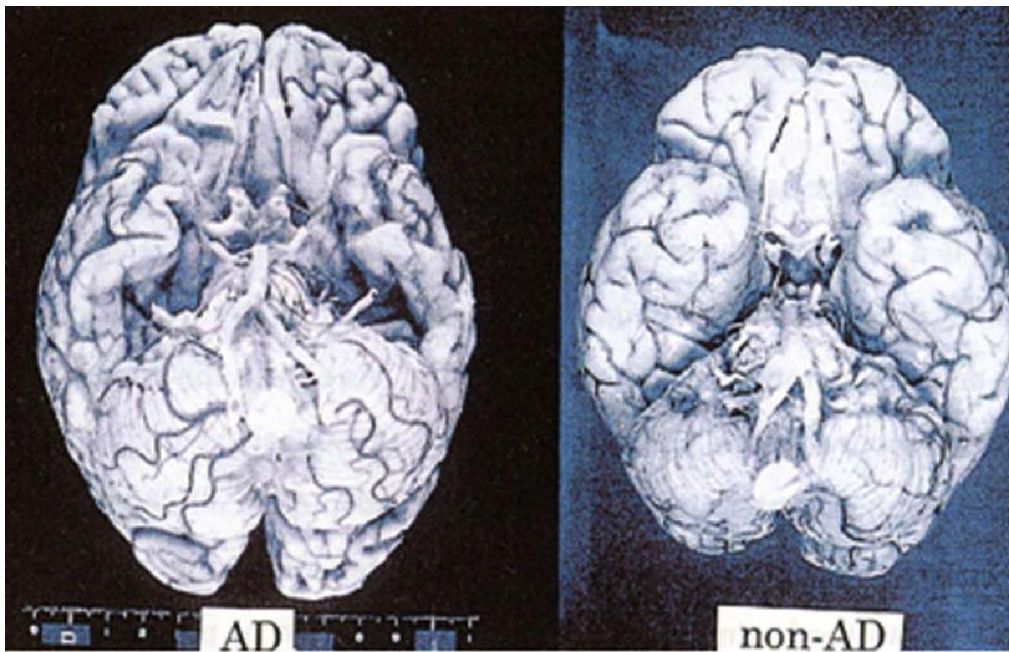
Many cells (D), like small lymphocytes, are observed along with senile pigment flecks (A). Most of them are macrophages (histiocytes). It is likely that they include killer T cells which trigger apoptosis.

2-4. Colony of neural stem cells in presenile dementia and AD

Figure 6A shows images of the brain of a 23-year-old male diagnosed with schizophrenia by a psychiatry shot by T1WI (upper row) and DWI (lower row) using Matsuzawa's tomographic method. Images of continuous sections were created from bottom to top (left to right in the figure) by a 3-Tesla MRI apparatus with slice thicknesses of 3 mm and no gap. T1WI (the first and second photos from the left) shows the destructions of schizophrenia half-healed through, treatments. The rightmost photo shows half-healed destructions of depression.

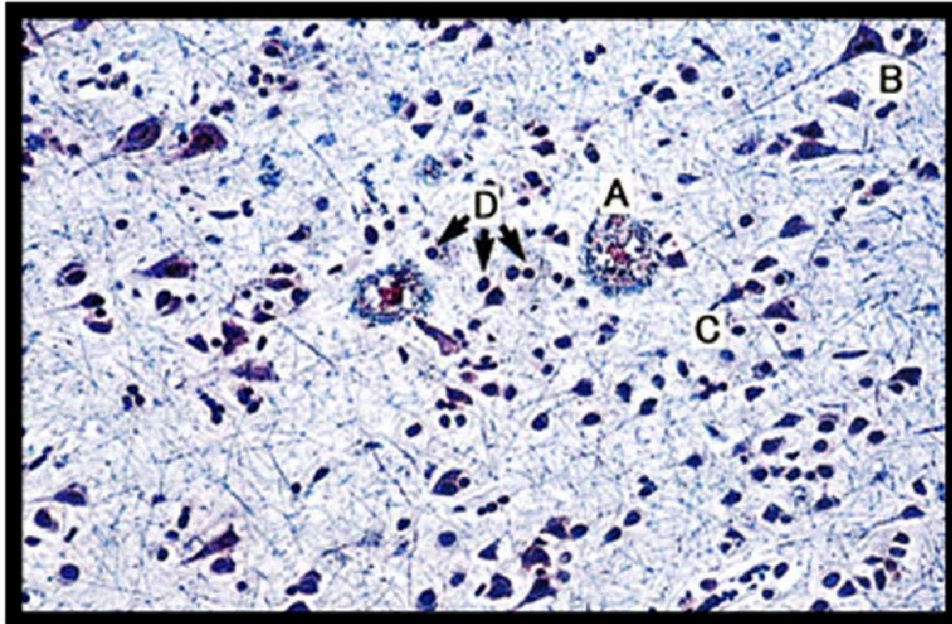
This patient is a psychiatric patient with schizophrenia mixed with depression. Based on more than 3000 cases diagnosed by Matsuzawa's tomographic method, all patients

Macroscopic findings



Symmetrical destruction of limbic system and enlargement of cerebellum in AD brain. This coincides with the MRI image.

Histological findings of the amygdala (silver stain)



- | | | | |
|---|------------------------------------|---|----------------------------|
| A | senile pigment flecks | B | neural cell (normal size) |
| C | neural cell dying due to apoptosis | D | macrophage + killer T cell |

Figure 5. Pathology of Alzheimer's disease.

with “diseases of the mind” are categorized into this mixed type. Not one patient who

developed only depression or only schizophrenia has been found. Actually, the disease which has been called by various names such as depression, schizophrenia, major depression, bipolar disorder and obsessional neurosis was actually this depression-schizophrenia mixed mental disorder.

Photos imaged by DWI in the lower row show a colony of neural stem cells (white areas indicated by arrows). Colonies of neural stem cells are observed all over, in areas such as the periphery of cerebral ventricles, hippocampus, amigdala and cerebral cortex. The reason why the author identifies them as colonies of neural stem cells will be explained later in this report.

Figure 6B shows images of a 69-year-old female diagnosed as having AD (MMSE=18). In this report, 65-year-old or older patients with degenerative dementia are called AD. These images are obtained by a 1.5-tesla MRI. T1WI images in the upper row show destructions for schizophrenia (1), destructions created by destructions of schizophrenia combined with atrophies of the hippocampi (2), and destructions of depression (3 and 4). In the DWI images in the lower row, many colonies of neural stem cells are observed all around (arrows) and this case may be cured through appropriate treatments.

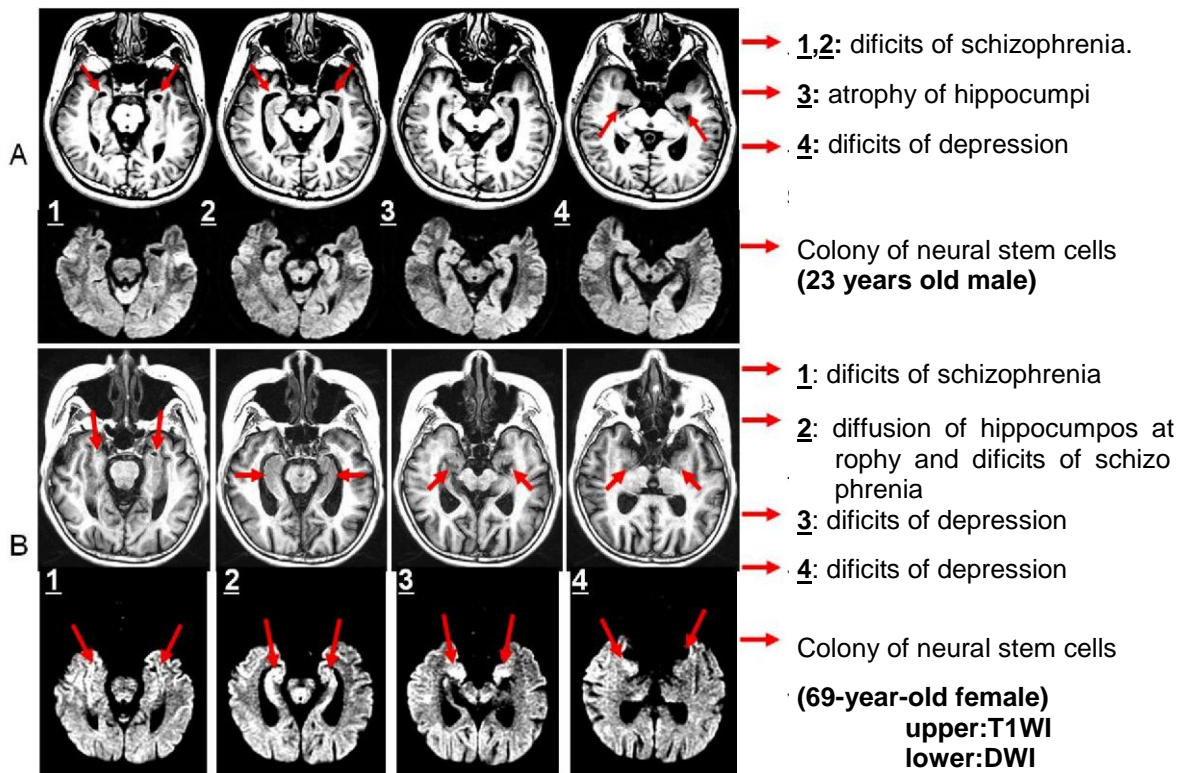


Figure 6. Colony of neural stem cells in senile dementia and AD.

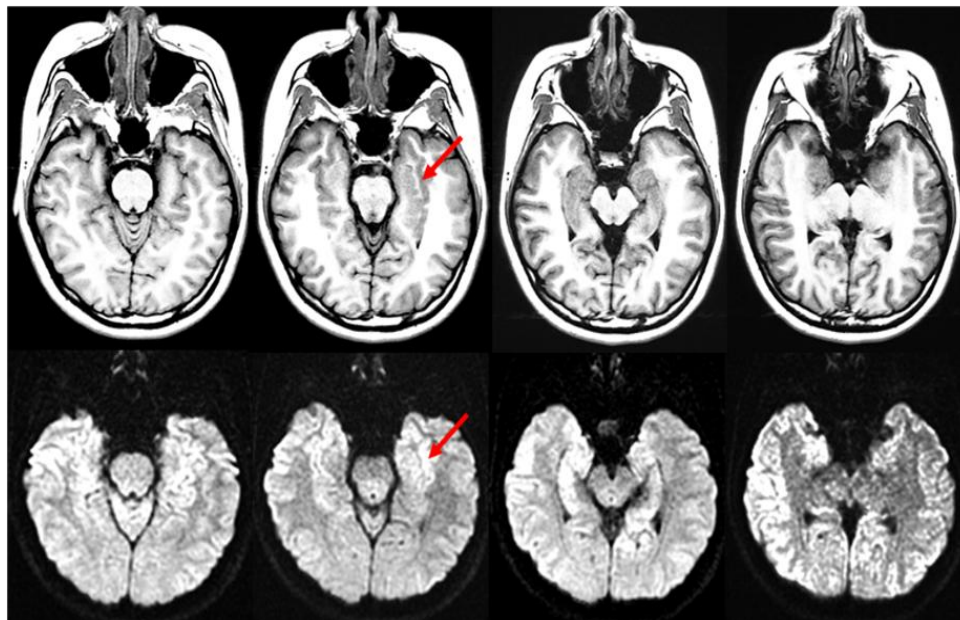
The number of colonies is smaller than the case of the 23-year-old male in Fig. 6A. The blood total dopamine of the patient was abnormally high, at 96 ng/ml (normal value: 0.5-6.2 ng/ml). Meanwhile, the blood serotonin was abnormally low, at 3 ng/ml (normal value: 53-200 ng/ml).

Mixed mental disorders which are “mental diseases” and Alzheimer’s disease develop due to an imbalance between total dopamine and serotonin. The details will be explained in a separate report.

2-5. Presenile dementia (Fig.7)

24-year-old female with mixed type of mental disorder visited us on October 5, 2005. She had a significant disorder of memory. She forgot things that had happened just recently and that she had eaten food immediately before. She visited us accompanied by her family. Her MMSE score was 19. She recovered significantly through therapies. Symptoms disappeared in October, 2006. She learned to be able to lead an active life and read books. Her MMSE recovered to 26. She is taking no medicine. T1WI and DWI images show that the destructions of schizophrenia and depression have been repaired and atrophies of the hippocampi have been improved completely.

2-6. Alzheimer’s diseases (AD)



→ **Upper (T1WI)** ; repair of hippocumps atrophy
Lower (DWI) ; colony of neural stem cells

Figure 7. Full cure case of senile dementia (24-year-old female).

Cure cases of AD

Full cure case (Fig. 8A)

A 78-year-old male visited us on November 22, 2004. He had symptoms in that he forgot things a lot, could not find his way home after going out, and could not read books or watch TV. He visited us accompanied by his wife. He responded relatively well to the treatments. T1WI images show the destructions of schizophrenia and the hippocampi repaired by neural stem cells. To our astonishment, the findings of T1WI on July 23, 2007 showed that colonies of neural stem cells had proliferated from the periphery of the hippocampi and had differentiated into cerebral cortex-like tissues. Neural stem cells are multipotent cells that can differentiate into any kind of cells as long as they are cells in the brain. In this case, MMSE was improved from 18 to 26.

Resistant case (Fig. 8B)

This is a treatment resistant case. A 79-year-old female with symptoms of completely forgetting things and loss of orientation visited us accompanied by her family. She did not respond to the treatments. Instead, she went from bad to worse. T1WI and T2WI images show significant bilateral atrophies of the amygdala and hippocampus. DWI shows few colonies of neural stem cells. MMSE was 10 or less.

Figure 8A. Full care case of AD (78-year-old male).

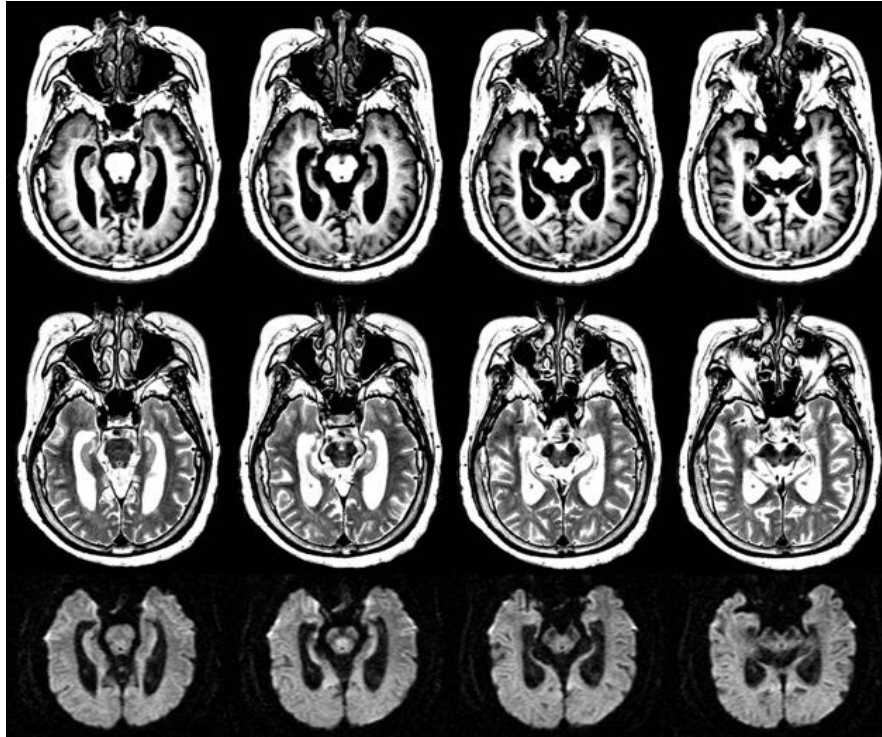


Figure 8B. Resistant case of AD (79-year-old female).

2-7. Combined results of regenerative medicine for prevention and treatment of AD

● Combined results

Figure 9 shows the combined results. Treatments were performed by dividing subjects into a would-be-AD group and Alzheimer's patients. The Would-be-AD group included 179 male and 277 female patients with atrophied hippocampi (total: 456) and mixed mental disorders who visited us over a period of three years (36 months). This cohort included all age groups from pre-teens to 80's.

The AD patient group patients were referred to our hospital by other medical institutes. They were positively diagnosed with AD by MRI and MMSE at our hospital. The group included 58 males and 99 females (total: 157) who were 65 years old or older. Both groups underwent the AD regenerative medicine treatment. The procedures were basically completely the same.

For results, "cure" and "convalescence" accounted for 89.4% in total of the would-be-AD (preventive care) group. It suggests that this method changes almost 90% of patients for the better and about half of them are cured. "Cure" and "convalescence" accounted for 74.6% in total of the AD group. This percentage is lower than that of the

preventive care group by 10% or more. This difference is caused by the difference in ages between the target groups, and ultimately by the difference in proliferations and differentiation of neural stem cells.

3. Discussion

1. It has become apparent that treatment results of regenerative medicine depend on the proliferation and differentiation potency of neural stem cells.
2. It is expected that iPS cells could be safely and effectively used for regenerative medicine in Alzheimer's disease in the near future.
3. For many ongoing studies on Alzheimer's disease, the following items may be commented on the results of Alzheimer's disease:
 - 1) Neurofibrillary degeneration (protein tau),
 - 2) Deposition of β amyloids,
 - 3) Decrease of acetylcholine, etc.

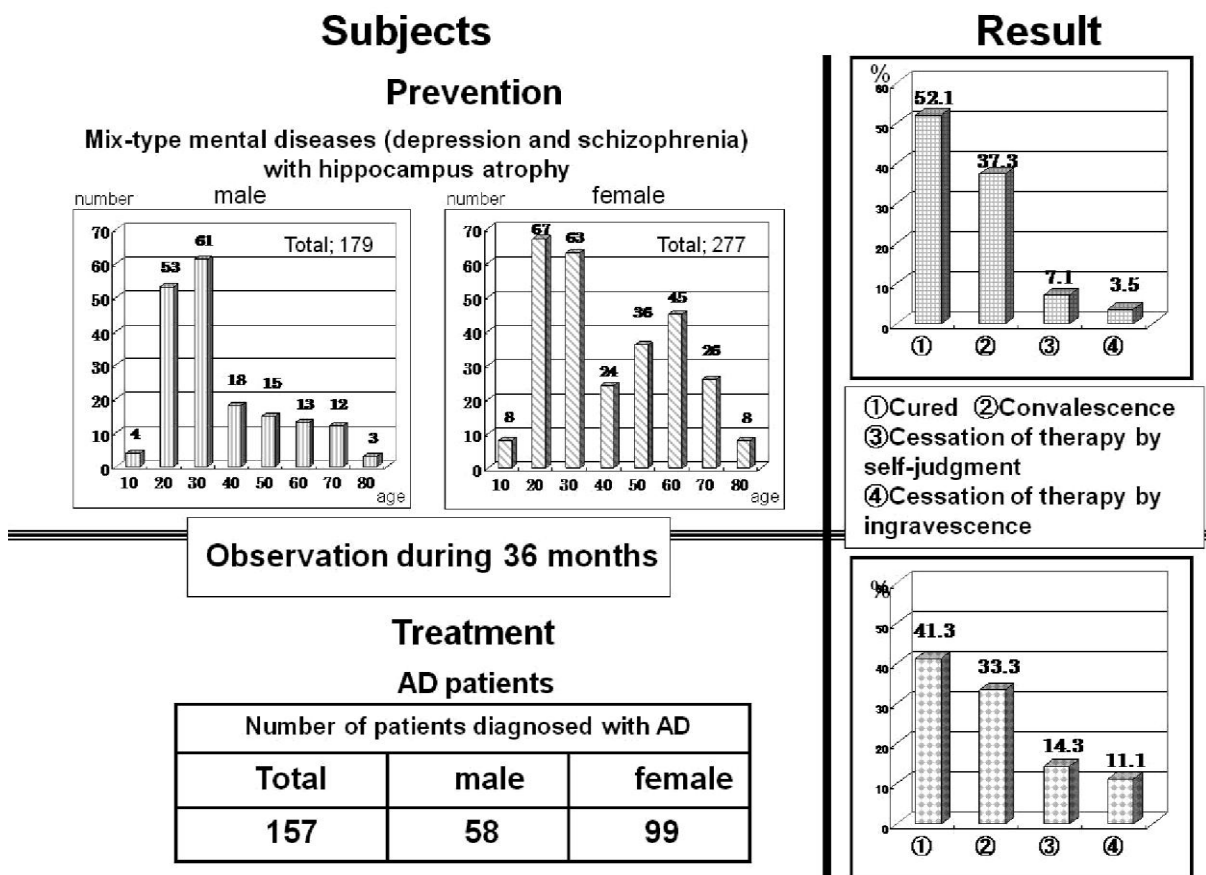


Figure 9. Combined results regenerative medicine in AD.

4. Conclusion

Alzheimer's disease is a disease in which a specific destruction (deficit) occurs both in the hippocampus and the amygdala in the limbic system symmetrically on both sides. In general, there is a mechanism by which wounds of the living human body are healed. This mechanism involves neural stem cells which proliferate and differentiate into glial cells and neural cells. Regenerative medicine, in which Alzheimer's disease can be prevented and cured, is realized by inducing the healing capacity of neural stem cells through appropriate treatments.

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