Treatment of Alzheimer's Disease with Anti-Homocysteic acid Antibody (Homocysteic acid induces Alzheimer's pathology; memory impairment and neurodegeneration. Amyloid accelerates HA toxicity)

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Abstract

Homocysteic acid (HA) may play an important role in Alzhiemer disease (AD) as we previously reported that HA induced accumulation of intraneuronal Aβ42. In this study, we first analyzed HA levels in a mouse model of AD. 4-month old pre-pathologic 3xTg-AD mice exhibited higher levels of HA in the hippocampus as compared to age-matched nontransgenic, suggesting that HA accumulation may precede both Aβ and tau pathologies. To further determine the pathogenic role of HA in AD, we treated young 3xTg-AD mice with vitamin B6-deficient food for 3 weeks to induce the production of HA in the brain. Concominantly, mice received either saline or anti-HA antibody intraventricularly using a guide cannula every 3 days. Mice received anti-HA antibody significantly rescued cognitive impairment induced by vitamin B6 deficiency. Pathologically, 3-week treatment with vitamin B-6 deficient food resulted in strong neurodegeneration in the hippocampal CA1 zone and decreased hippocampal volume. In contrast, anti-HA antibody treatment attenuated these pathological changes. Taken together, we conclude that increased brain HA triggers memory impairment whose condition was deteriorated by amyloid and subsequent neurodegeneration and reduction of neurogenesis. Our results indicate a pathogenic role of HA in AD.

Introduction

Amyloid plaques and neurofibrillary tangles are the two pathophysiological hallmarks of Alzheimer's disease (AD). Intracellular amyloid beta 42 is increasingly recognized as an early pathological trigger that can lead to amyloid plaques and even induce neurofibrillary tangles. We previously found that homocysteic acid (HA) induces intracellular accumulation of amyloid beta 42 and the production of α-synuclein in the presence of methionine. It is known that α-synuclein facilitates the formation of tau aggregates. Consequently, HA affects the two pathophysiological hallmarks of AD and may be involved in its etiology.

Previous studies have reported that psychological stress accelerates Alzheimer's disease (AD); however, recently it has been established that stress directly induces pathological changes in this disease (1, 2). The underlying mechanism, however, is unclear. Hasegawa *et al.* found that homocysteic acid (HA) induced amyloid beta 42 accumulation in neuronal cells, resulting in cell dysfunction and finally cell death (3). HA is also known as an NMDA receptor agonist (4), and is released from astrocytes by the activation of beta-adrenergic receptors under mental stress (5).

HA can therefore induce AD. The 3xTg-AD mouse model developed by Oddo et al (6) showed memory impairment at an early age because of amyloid beta accumulation in neuronal cells (7). We observed increased level of HA in the hippocampus of 3xTg-AD homozygous mice compared with that of 4-month-old nontrangenic mice, when the former showed accumulation of amyloid beta in neuron. We then confirmed the etiological effect of HA on the disease process of AD in 3xTg-AD hemizygous mice. Hemizygous mice were used instead of homozygous mice because they have reduced transgenic gene expression for amyloid precursor protein (APP), presenilin, and Tau compared with homozygous mice. Disease pathogenesis does not easily occur in hemizygous mice through the action of certain etiological agents such as APP, presenilin and Tau compared with homozygous mice. We expected that if HA were a true etiological agent of AD, it would accelerate memory impairment in 3xTg-AD hemizygous mice and that administering anti-HA antibodies would inhibit this acceleration.

Methods

*3xTg-AD mice**

The mouse germline used in this study was kindly gifted by Professor F. M. Laferla (University of Calfornia, Irvine). Housing environment (12h/12h light/dark cycle) was germ-free clean room. 3xTg-AD hemizygous male mice (5 and 7 month-old) were 7 mice used. Also Non-Tg mice were 4 mice used. The 3xTg-AD mice develop both plaque and tangle pathology in AD-relevant brain regions. The 3xTg-AD mice develop extracellular Aß deposits prior to tangle formation, consistent with the amyloid cascade hypothesis. Despite equivalent overexpression of the human ßAPP and human tau transgenes, Aß deposition develops prior to the tangle pathology, consistent with the amyloid cascade hypothesis. In addition, these mice exhibit deficits in synaptic plasticity, including long-term potentiation (LTP) that occurs prior to extracellular Aß deposition and tau pathology, but is associated with intracellular Aß immunoreactivity. These studies support the view that synaptic dysfunction is a proximal defect in the pathobiology of AD, preceding extracellular plaque formation and neurofibrillary pathology. As these 3xTg-AD mice phenocopy critical aspects of AD neuropathology, this model will be useful in pre-clinical intervention trials, particularly because the efficacy of anti-AD compounds in mitigating the neurodegenerative effects mediated by both signature lesions can be evaluated.

Vitamin B6-deficient food

Vitamin B6-deficient food was purchased from Kyudo Ltd. Nutrient composition has been described further in the study.

Anti-HA antibody

Anti-HA antibodies were purchased from MoBiTec Co. (Germany). Polyclonal antisera were raised in rabbits after immunization with a glutaraldehyde-containing HA conjugate, following which antibody specificity was determined by performing ELISA with competition experiments involving HA-G-BSA (compound cross-reactivity ratio 1:1), cysteine-G-BSA (1:85), and homocysteine-G-BSA (1:231)

Morris water maze test

The apparatus used for all Morris water maze tasks comprised a circular aluminum tank (1.5 m in diameter) painted white and filled with water maintained at 26–29°C. The maze was located in a room containing several simple, visual extramaze cues. To reduce stress, mice were placed on a platform in both the hidden and cued versions of the task for 10 s prior to the first training trial.

Spatial reference Morris water maze training

Mice were trained to swim to a 14-cm circular clear Plexiglas platform placed 1.5 cm beneath the water surface that was invisible to the mice while swimming. The platform location was randomly selected for each mouse, but was kept constant for that mouse throughout the training period. In each trial, the mouse was placed in the tank at one of the four designated start points in a pseudorandom order. Mice were allowed to search for and escape to the submerged platform. If a mouse failed to find the platform within 60 s, it was manually guided to it and allowed to remain there for 10 s. Then, each mouse was placed in a holding cage under a warming lamp for 25 s until the start of the next trial. To ensure that memory differences were not due to the lack of task learning, the mice were trained for four trials a day for as many days as required to meet the criterion, which was defined as <20-s mean escape latency before the first probe trial was run. To prevent overtraining, probe trials were run for each group as soon as they met the group criterion and stopped after all the groups met the criterion. Retention of spatial training was assessed 1.5 and 24 h after the last training trial. Both probe trials consisted of a 60-s free swim in the pool without the platform. Mice were monitored by a camera mounted on the ceiling directly above the pool, and all trials were stored on videotape (burnt onto a DVD.)or subsequent analysis. The parameters measured during the probe trial comprised (1) initial latency time to reach the platform,

Immunohistochemistry

Mice were sacrificed by CO_2 asphyxiation, and the brains were rapidly removed and fixed for 48 h in 4% paraformaldehyde. Sections (50-μm thick) were processed for free-floating immunohistochemistry as previously described (6). Anti-amyloid beta (6E10), anti-APP (22C11), and amyloid beta (40/42)-specific antibodies were applied overnight at 4°C. Sections were developed with diaminobenzidine (Vector Laboratories)

substrate using the avidin–biotin–horseradish peroxidase system (Vector Laboratories). Quantification of amyloid beta was performed as described previously (8). Mice were excluded from the antibody group analysis (behavior and histology) if the cannulae were found to be placed incorrectly. To obtain the percentage difference between the antibody- and PBS-treated tissues (controls), we applied the following formula: number of pixels in the antibody-treated hippocampus − number of pixels in the PBS-treated hippocampus/number of pixels in PBS-treated hippocampus. The number of pixels calculated in each case is the sum of five readings per mouse averaged across the entire group.

HA level measurement

HA was extracted from mouse brain (hippocampus and cortex) with an acid of trichloroacetic acid. Brain samples were prepared by a modification of the method of Reed and Bellerche (8). Brains (1.50–2.00 g) were isolated from 4-month-old 3xTg-AD homozygous male mice. The mice were killed by rapid decapitation and their brains were quickly excised and placed on an ice-cold Petri dish. For the gradient high-performance liquid chromatography (HPLC) method, tissue samples were weighed and homogenized using a sonicator for 10 s in ice in 4 ml of ice-cold 10% (w/v) trichloroacetic acid per 100 mg tissue (wet weight). HA (4 μg) was added as the internal standard. For isocratic HPLC, tissue samples were divided into six aliquots. The samples were homogenized as described above. The homogenates for isocratic or gradient HPLC were left on ice for 1 h and centrifuged at 20,000 ×*g* for 25 min. The supernatant was washed five times with an equal volume of diethyl ether and the aqueous phase was maintained. Residual ether was evaporated under nitrogen at room temperature for 5 min. Immediately thereafter, 20 μL was injected into the HPLC system.

Ventricular cannula

The mice were anesthetized, 2-mm-wide incisions were made in the left hemisphere, and a guide cannula was inserted into the left ventricular space using a Teflon tube (1 mm in diameter). This operation did not impair learning and memory performance, and the abilities of the operated mice were similar to those of mice that did not undergo surgery.

Plasma catecholamine levels

Plasma was collected from the eyelid arteries. under anesthesia, and catecholamine levels were measured by HPLC.

Membrane APP measurement

Rat embryonic neuronal cells were cultured using the method described in (3) and membrane APP was detected using the c-terminal antibody of Sigma A8717. Homocysteic acid was added to the culture medium and incubation was performed for 48 h.

Results

HA levels in mouse model brain

We measured HA levels in the brains of 4-month-old 3xTg-AD-homozygous mice; at this age, mice display intracellular accumulation of amyloid beta in the brain regions affected by AD. This accumulation also appears to be associated with the early memory deficit exhibited by these mice (7). As shown in Table 1, HA levels in the 3xTg-AD mice were clearly higher than those in control nontransgenic mice. This finding, in combination with those from our *in vitro* studies, indicates that HA may modulate the pathology of AD. To confirm our hypothesis, we fed 3xTg-AD-h and nontransgenic mice with vitamin B6-deficient food because B6 deficiency has been reported to increase HA levels in these mice (9).

Anxiety reaction

At 3 weeks of vitamin B6-deficient feeding, both 3xTg-AD-h and nontransgenic mice showed a strong anxiety reaction (see DVD). That is, they preferred dark areas, showed lower locomotor activity, and moved along the edge of the cage in the open field. In contrast, the experimental group treated with anti-HA antibodies did not show any anxiety reaction (i.e., they preferred bright areas, showed high locomotor activity, and moved to the center, not along the edge of the cage in the open field). This anxiety behavior was confirmed by determining the plasma catecholamine levels. Transgenic control and nontransgenic mice showed higher levels of catecholamine than experimental transgenic mice (Fig. 1). Hemizygous mice aged 7 months showed strong freezing behavior and occasional seizures, but experimental mice of the same age did not show any of the behavior problems that were evident in the control group (see DVD).

Memory experiment

We next evaluated the hippocampus-dependent memory by Morris water maze. 5-month old 3xTg-AD mice fed with vitamin B6 deficient diet showed a significant memory impairment compared to 2-month old normal mice (Fig. 2). Notably, co-treatment with anti-HA antibody significantly rescued vitamin B6 deficient-induced memory impairment (Fig. 2). However, the nontransgenic and transgenic control groups showed memory impairment.

This result indicates that HA can induce memory impairment. Memory impairment induced by HA was greater in the presence of amyloid beta, because transgenic control mice, in whom there was amyloid beta production and accumulation (see below), showed the worst memory impairment of the three groups (Fig. 2a). Amyloid alone did not induce memory impairment, despite the presence of higher level of both amyloid beta 40 and 42 in the transgenic 5-month-old mice (hemizygous +B6 deficient + anti-HA antibody) compared with nontransgeneic mice (10). Moreover, transgenic experimental mice showed better memory performance than nontransgenic mice.
We also confirmed that HA induced memory impairment. As shown in Fig. 2b, C57BL mice showed memory impairment according with B6 deficient food feeding time. And final complete memory impairment mice which were fed B6 deficient food for 3 months could recover their impairment by the addition of anti-HA antibody, indicating that these memory impairment was induced by HA. The thing that should be paid attention is the time which needed to become memory impairment. In the case of C57BL, the time needed to show memory impairment was 3 months, but 3xTg-AD case, the time was 3 weeks. In other words, HA toxicity was strengthend by the presence of amyloid.

Pathological change

Figure 3 demonstrates the immunohistochemical staining for amyloid beta in the amygdala, cortical, and hippocampal cells of transgenic control and transgenic experimental mice. Numerous stained neurons were seen in transgenic control mice, indicating amyloid beta accumulation in these neurons. In contrast, transgenic experimental mice did not show any stained neurons in the hippocampus and showed fewer stained neurons in the amygdala and cortex compared with transgenic control mice, indicating inhibition of amyloid beta accumulation. We did not observe any amyloid beta accumulation in the neurons of nontransgenic and transgenic control mice fed normal food (data not shown).

Since we observed a preventive effect of anti-HA antibody on the acceleration of pathogenesis of AD in 3xTg-AD mice induced by vitamin B6-deficient food, we looked for a curative effect of anti-HA antibody on the established pathological changes in 3xTg-AD mice with AD.

3xTg-AD hemizyous 7-month-old male mice were fed B6-deficient food for 3 weeks

(control), and anti-HA antibody (5 μl) was injected into their ventricular space every 3 days using guide cannulae. After 3 weeks, we measured memory performance in the Morris water maze tasks following which we evaluated the hematoxylin–eosin and immunohistochemical staining of the brain specimens.

The results are shown in Figure 4. Control mice showed poor memory performance but experimental mice injected with anti-HA antibody showed strong recovery of performance after 2 days of training, indicating good memory performance.

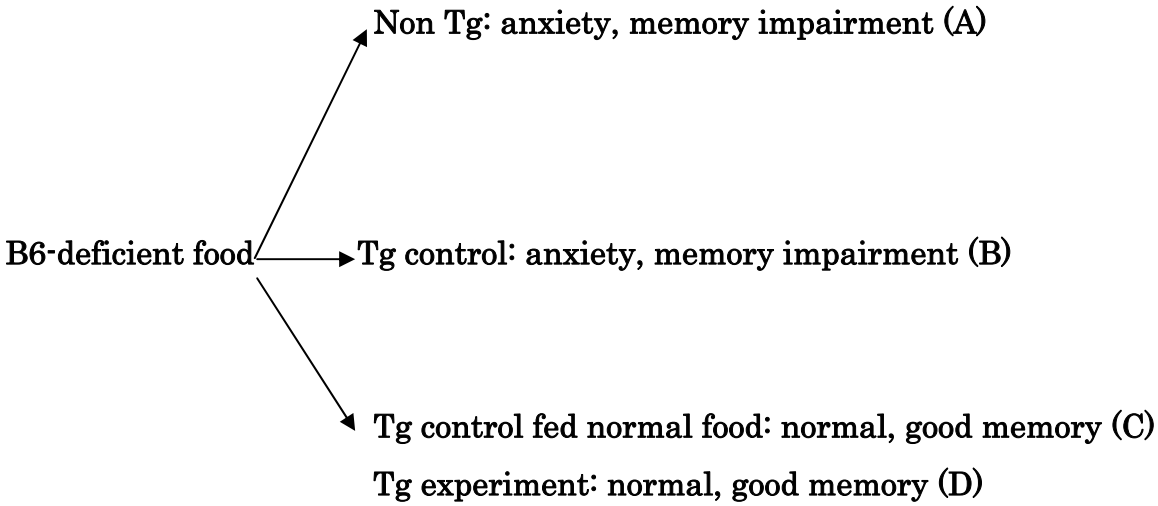
Consistent with the results of the memory performance task, the hippocampi of control mice showed a marked deficiency of neuronal cells in the CA1 region (as indicated by hematoxylin–eosin and especially immunohistochemical staining). A marked loss of cells was observed in control mice (indicated by the ellipses in Fig. 5) compared with experimental mice. In addition, many macrophages appeared in the CA1 area of control mice compared with the experimental group (indicated by the arrow in Fig. 5b). Fig. 6 showed that control mice accumulated amyloid beta into neuronal cells in cortex (circled line), but experimental mice accumulated less amyloid beta than that of control.

Figures 5 illustrate that B6-deficient food induced strong neurodegeneration in the hippocampal CA1 region and anti-HA antibody treatment induced marked recovery of both neurogenesis and memory performance. Hippcampal volume was decreased in control mice, but it recovered in the experimental group (Table 2).

Neuronal death induced by HA

HA induced the neurodegenerative cell loss in CA1 zone (Fig. 5), we would like to confirm this HA effect in vitro system. Rat embryonic brain neurons were cultured according to the method (3), HA showed the neurodegenerative effect compared with that of homocysteine. Cleary shown in Fig. 7, HA showed strong neurodegenerative effect compared with that of homocysteine.

Discussion



Tg: Transgenic

Figure

A and B illustrate that vitamin B6-deficient food induced an anxiety reaction and D illustrates that HA did the same. A and B also illustrate that vitamin B6-deficient food accelerated cognitive (memory) impairment in AD mice. It can be deduced that B6-deficient food itself induced memory impairment in nontransgenic mice. Additionally, it can be inferred that transgenic control mice themselves functioned normally, as on being fed normal food they displayed normal memory performance. B6-deficient food induces an increase in homocysteine, homocysteine sulfinic acid, and homocysteic acid levels (9). Recently, the *New England Journal of Medicine* reported that lowering the level of homocysteine alone does not suppress cognitive decline in elderly people, and that treating homocysteine levels should not be focused upon. Homocysteine sulfinic acid is formed by the peroxidation of homocysteine (12) following which the latter does not play a role in the pathogenesis of AD, indicating that homocysteine sulfinic acid does not contribute to the disease pathogenesis of AD.

Finally, because HA is formed from homocysteine and methionine (13), it can possibly accelerate the pathogenesis of AD. The results of our transgenic experiments confirmed that anti-HA antibody suppressed memory impairment induced by B6-deficient food. This hypothesis is supported by our data suggesting that higher levels of HA are present in 3xTg-AD-h compared with nontransgenic mice (Table 1).

Figure 2 shows that memory impairment induced by HA was stronger in the presence of amyloid beta. However, amyloid beta itself does not induce memory impairment, because transgenic experimental mice, who had higher levels of amyloid

beta 40 and 42 (10), displayed better memory performance than nontransgenic mice. Recently, it has been reported that amyloid oligomers induce LTP suppression through the action of NMDA (14); this finding supports our observations.

Our observations indicate that anti-HA antibody can bring about normal recovery in AD. It can therefore be concluded that HA is a true etiological agent for AD.

Our hypothesis that HA accelerated the pathogenesis of AD was confirmed by the immunohistochemical observations. Amyloid beta accumulated in hippocampal neurons in the transgenic control group but not in the transgenic experimental group.

The curative effect of anti-HA antibody was so strong that a substantive recovery in the memory performance tasks was observed. This recovery was attributed to neurogenesis in the hippocampus brought about by anti-HA antibodies. This is the first study reporting such a phenomenon.

We observed that neuronal cell death was induced by HA (Fig. 7) which also destroyed the membrane APP (Fig. 8). APP has been reported to affect neurogenesis (16), therefore, anti-HA antibodies can alleviate the destructive effect of HA on APP and can consequently induce neurogenesis.

HA induced memory impairment in our study, and this impairment was greater in the presence of amyloid beta (Fig. 2a,b), i.e., even if amyloid beta were to be decreased, HA would still be present in the affected brain tissue. Hence, amyloid vaccination could not restore the cognitive function despite reduced amyloid levels (17). Our findings on anti-HA antibody treatment in transgenic mice show that the toxicity of HA were decreased and neurodegeneration was inhibited following which hippocampus volume increased, suggesting that the neurogenesis might be occurred. (Figs. 5 and 6 and Table 2). This led to recovery of memory performance to a normal state. Recently, it has been reported that NMDA antagonists such as MK-801 induce neurogenesis in the dentate gyrus of the hippocampus (16) and that HA is an agonist of the NMDA receptor (4). Thus, our observation that anti-HA antibodies induced neurogenesis is feasible and is consistent with the previous study (18).

Imagawa *et al* reported a case of treatment-influenced recovery of familial AD in Japan

The author prescribed vitamin B6, coenzyme Q10, and iron citric acid supplements

to two sisters and noted marked recovery in both (19).

In contrast, mice that were fed B6-deficient food showed increased levels of HA, which then induced strong neurodegeneration in the presence of amyloid beta, because HA induced amyloid beta accumulation in neuronal cells (Fig. 6) (3).

In the above-mentioned study (19), the authors suggested that recovery from familial AD occurred because treatment with vitamin B6 decreased HA levels in the patients. Unfortunately, this treatment did not prevent cognitive decline after 2 years of treatment, which may have been related to the fact that HA can be produced in two ways. One is via the homocysteine pathway, which can be inhibited by vitamin B6; the other is via the methionine pathway, which cannot be inhibited by vitamin B6. In any event, anti-HA antibody can decrease the toxicity of HA and can consequently induce marked recovery of cognitive ability in the mouse model.

In conclusion, (1) HA can accelerate pathological changes in AD; (2) HA toxicity was decreased by anti-HA antibody, which induced strong neurogenesis in the hippocampus, consequently resulting in marked recovery of memory performance; and (3) HA induced early pathological changes in normal mice, i.e., memory impairment. (Fig. 2b). According to Koch's postulates (15), these three points indicate that HA is a true etiological agent of AD.

Our hypothesis is also supported by the observation that anti-HA antibodies induced marked recovery in 7-month-old hemizygous mice (see immunohistochemical observations in Figures 5 and 6 and the behavioral observations included in the DVD). This is the first study to demonstrate marked recovery from AD induced by treatment with anti-HA antibodies. Our findings prove the strong curative effect of anti-HA antibody treatment and support the idea that HA is a true etiological agent and an accelerator in the pathogenesis of AD.

In addition to our discussion mentioned above, one more thing should be paid attention. From our experiment, amyloid hypothesis is not in central dogma of Alzheimer's disease. From Fig. 2a, 2b, it can be clarified that amyloid accerelated HA toxicity and amyloid itself has no effect on memory impairment. In other words, amyloid toxicity in Alzheimer's disease is not central dogma.

Alzheimer's pathology is composed two phenomena. One is memory impairment and the other is neurodegeneration. HA showed these two phenomena (Fig.2a,2b and Fig. 7) And recent unsatisfied reports, amyloid treated therapy faild in clinical trail.

That　is, amyloid level decreased , but cognitive ability became worse.

From these reports and our observations, it can conclude that amyloid hypothesis is not central dogma of Alzheimer's disease and HA can be one of true etiological compounds.

Acknowledgment

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Figure legends

Fig. 1 Plasma levels of catecholamines in nontransgenic (NonTg), transgenic (Tg) control, and experimental mice. a, adrenaline; b, noradrenaline; c, dopamine. The figure shows average data for three mice

Fig. 2a Long-term memory test in the Morris water maze. Nontransgenic mice had an average score of three mice each day. Hemizygous transgenic control (hemizygous + B6 deficeint) mice had an average score of three transgenic control mice each day. Transgenic experimental mice (hemizygous +B6 deficient + anti-HA antibody) had an average score of three transgenic experimental mice each day.

Fig. 2b C57BL mice memory impairment induced by HA
C57BL mice were 5 month-old male mice. Mice were fed B6 deficient food for 1.5 months and 3 months. Morris water maze test was conducted at 1.5 months and 3 months. Each 5 mice were used memory test. After 3 months, memory impairment mice were added anti-HA antibody according to same method of 3xTg-AD. After one month, memory test was conducted.

Fig. 3 Immunohistochemical observations of amygdalar, cortical, and hippocampal neurons. Anti-HA antibody was diluted 100-fold. Immunohistochemical observations were made thrice, and each observation gave the same result. Five hemizygous transgenic 5-month-old mice and 10 homozygous transgenic 3-month-old mice were fed B6-deficient food for 3 weeks. Transgenic experimental mice were injected with anti-HA antibody every 3 days. For details, see Methods. CTL, transgenic control mice; Ex, transgenic experimental mice.

Fig. 4 Curative effect of anti-HA antibody as shown by long-term memory performance. Seven-month-old male 3xTg-AD-h mice fed B6-deficient food for 3 weeks served as the control. Experimental mice were treated with anti-HA antibody every 3 days; the antibody (100-fold dilution) was injected into the brain as described in Methods. The figure shows average data for five male mice. To demonstrate the strong curative effect of the antibody, we show for comparison the results for 2-month-old hemizygous male mice, whose memory performance was normal.

Fig. 5 (a) Hematoxylin–eosin staining of cortical and hippocampal neurons in control

and experimental groups. Mice were the same as those shown in Fig. 4. (b) Magnification of (a) showing macrophages in the CA1 area of the hippocampus of control and experimental mice.

Fig. 6 Immunohistochemical staining of cortical and hippocampal neurons in control and experimental mice. Staining was performed as described in the Methods section.

Fig. 7 Comparison of neuronal cell death between mice treated with homocysteine and those treated with homocysteic acid. Cells were cultured using the method described in (3). Neuronal death was measured by the MTT method (3).

Fig. 8 Western blot analysis of APP with c-terminal antibody of Sigma A8717. Rat embryonic neuronal cells were cultured using the method described in (3) and membrane APP was detected using the c-terminal antibody of Sigma A8717. Homocysteic acid was added to the culture medium and incubation was performed for 48 h.

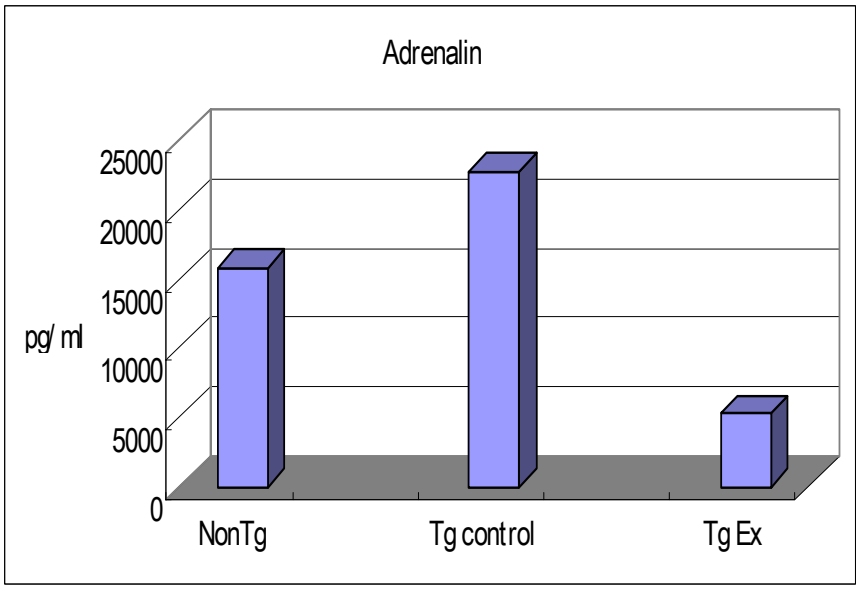


Fig. 1a Plasma Adrenalin

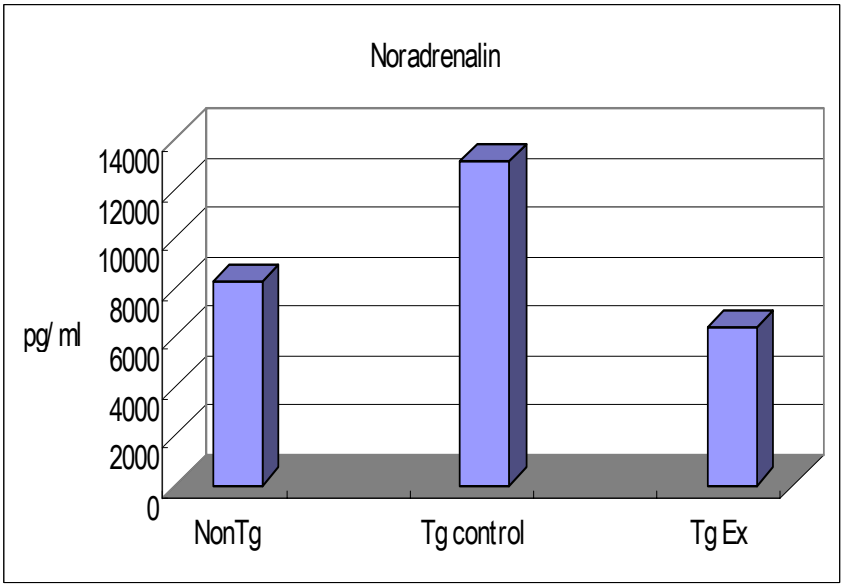


Fig. 1b
Plasma Noradrenalin

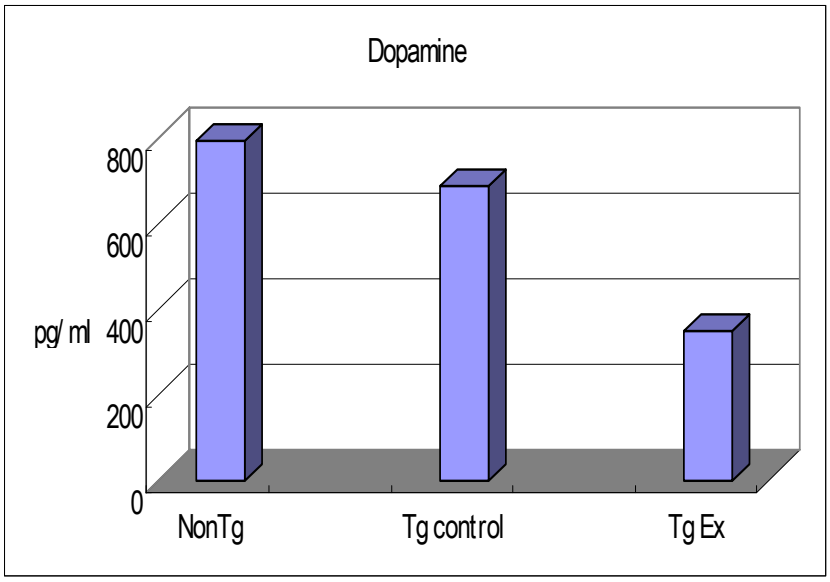
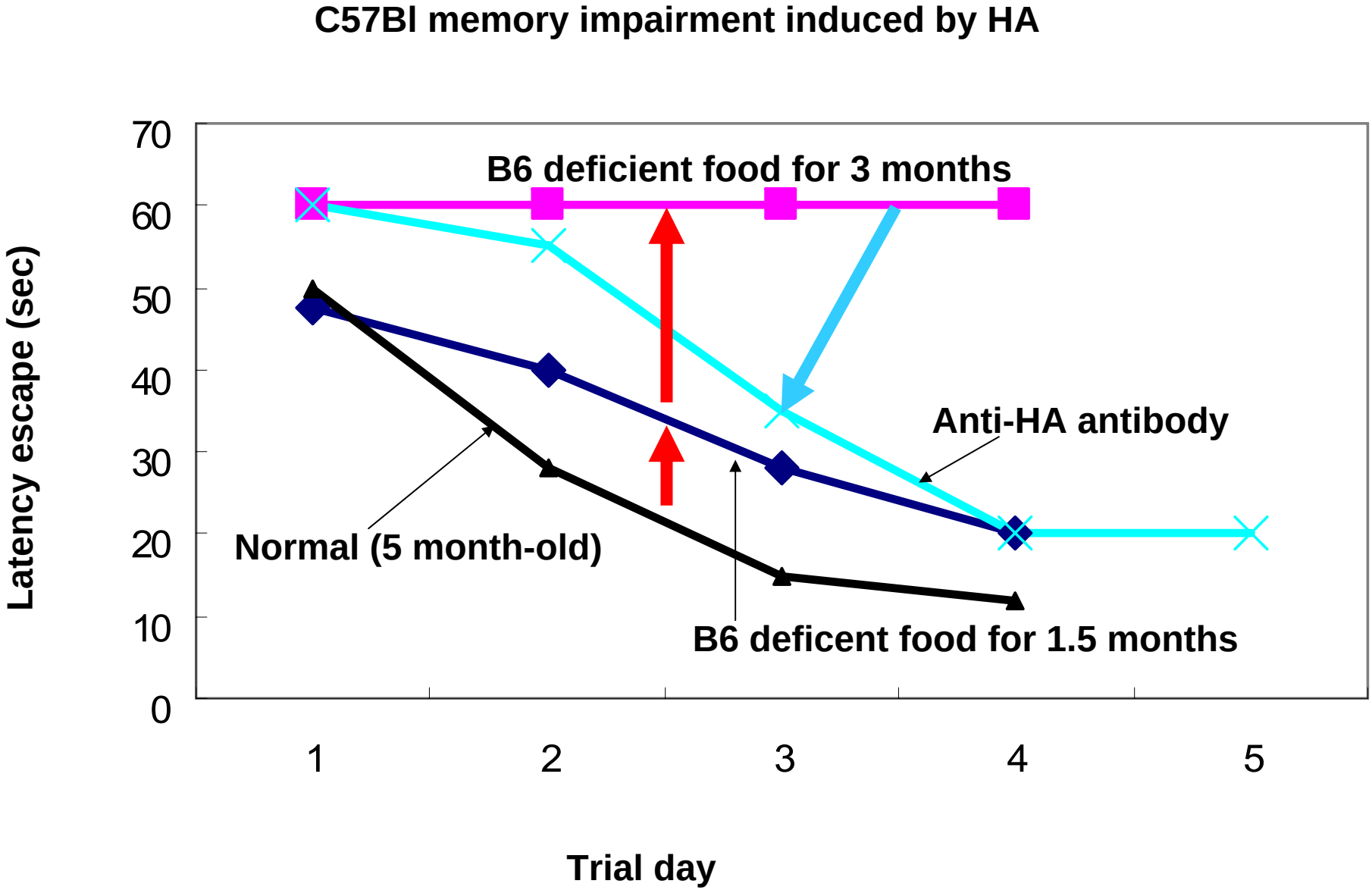
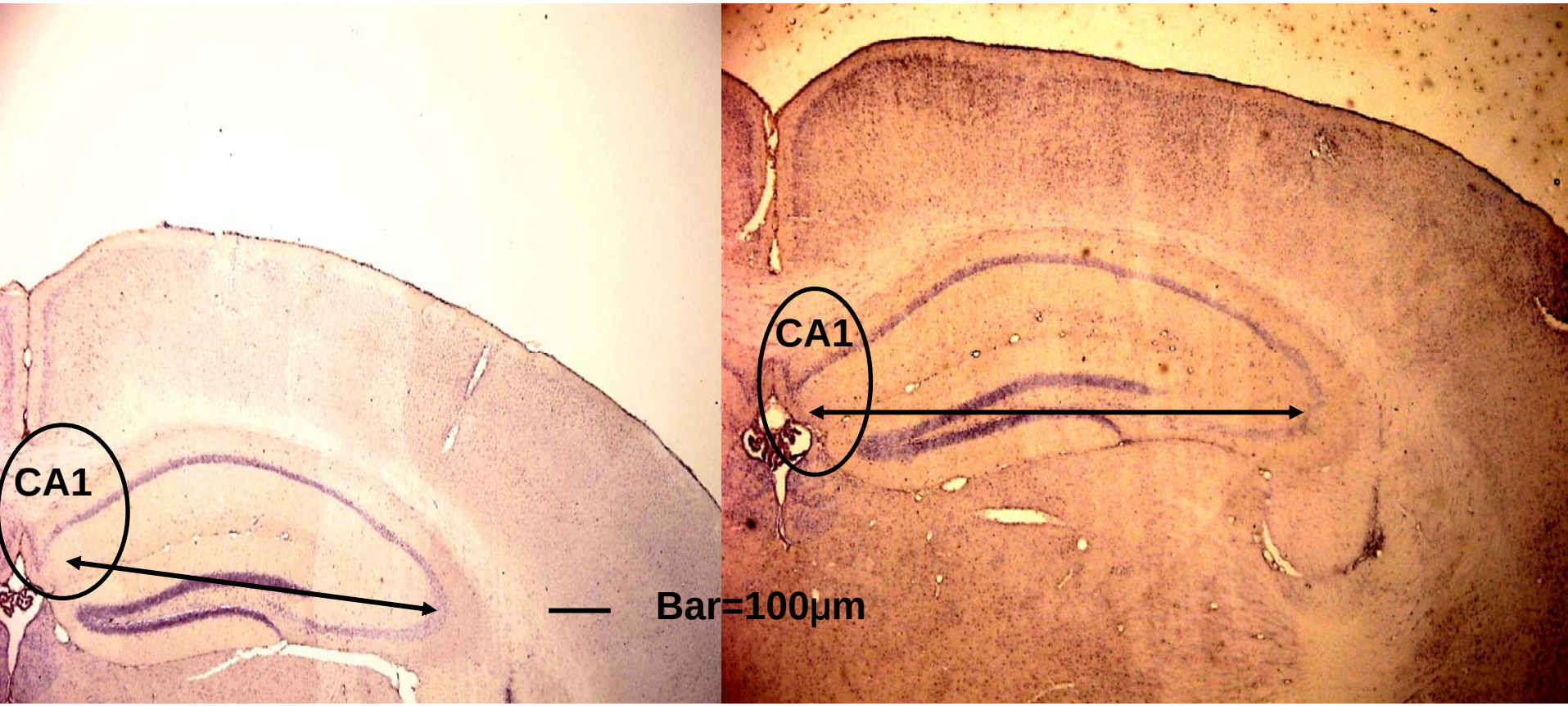


Fig. 1c
Plasma Dopamin


C57Bl memory impairment induced by HA
Latency escape (sec)
70
60
50
40
30
20
10
0
1
2
3
4
5
Trial day
B6 deficient food for 3 months
Anti-HA antibody
Normal (5 month-old)
B6 deficent food for 1.5 months

Hematoxylin Staining



CT: Male 7 month-old+ B6 defcient

EX: Male 7 month-old+ B6 defcient
+ Anti-HA antibody

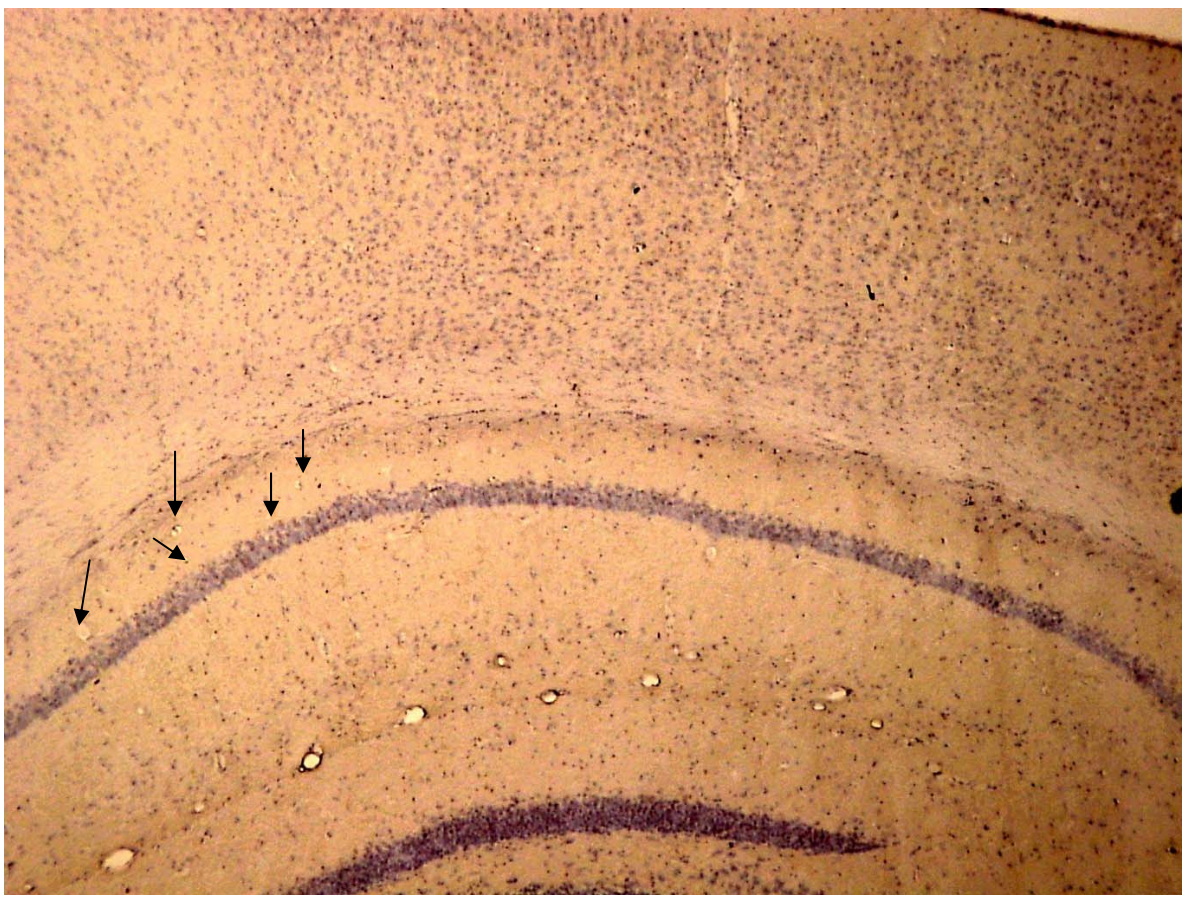
Hippocampus major axis (cm)

						Average
Control	9	8.8	7.8	9	8.2	8.6 ± 0.5
						P<0.01
Experiment	9.6	10.1	9.6	10.3		9.9 ± 0.4

Control: 7 month-old hemizygous + B6 deficient

Experiment: 7 month-old hemizygous male +B6 deficient + anti-HA antibody

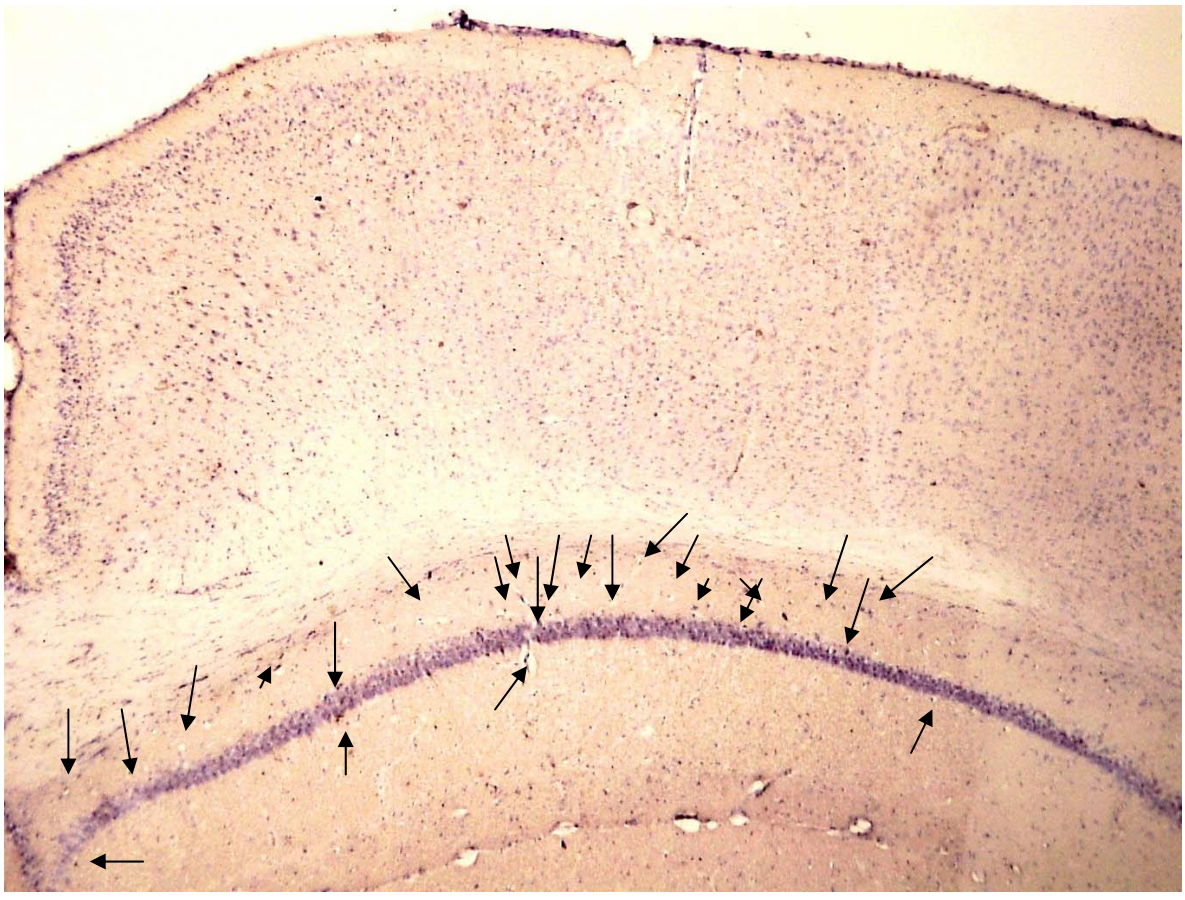
5 different sections were observed and measured its major axis. Average (n=2) is shown.



7 month-old +B6 deficient　Male +Anti-HA antibody

Bar=100μm

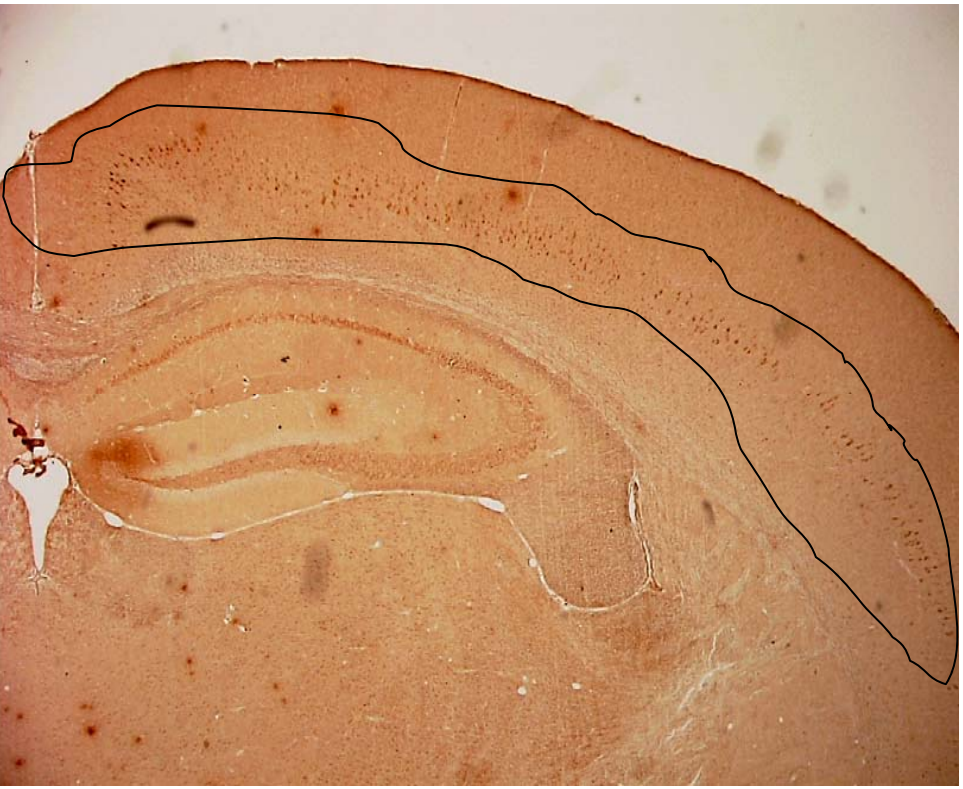
Fig.5b(1)



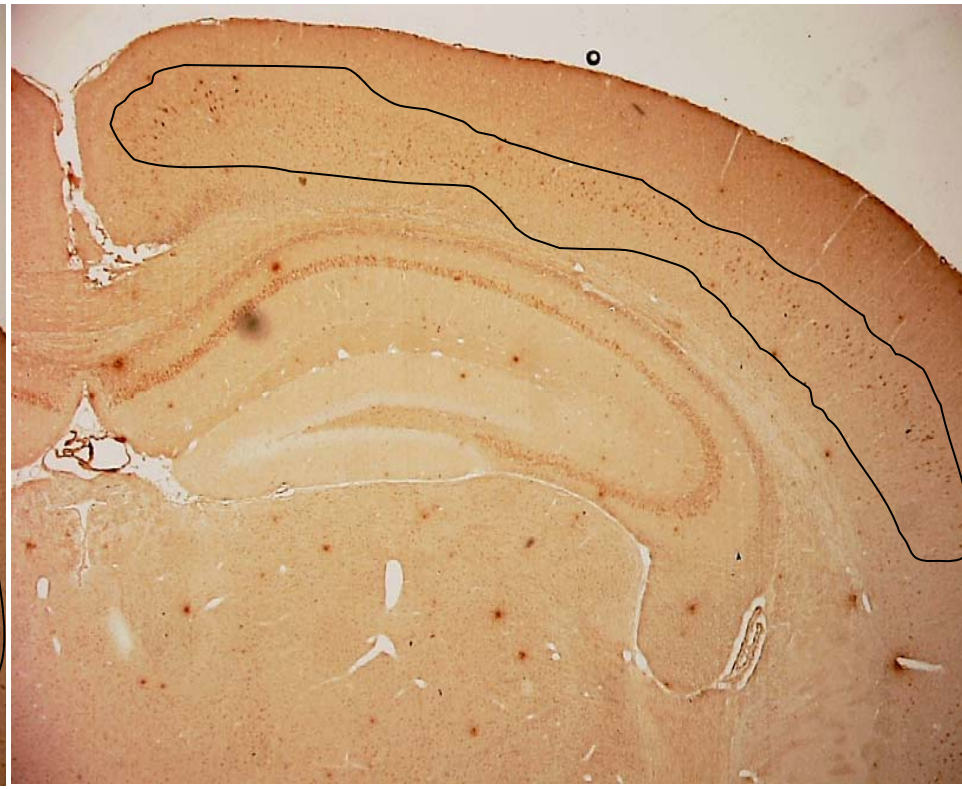
7 month-old +B6 deficient Male

Fig.5b(2)　Macrophage in CA1 area　Arrow indicates marcorphage

β-Amyloid immunohistochemical staining



7 month-old Male +B6 deficient



7 month-old Male +B6 deficient
+ Anti-HA Antibody

Comparison of Neural Impairment between Homocysteine and Homocysteic acid

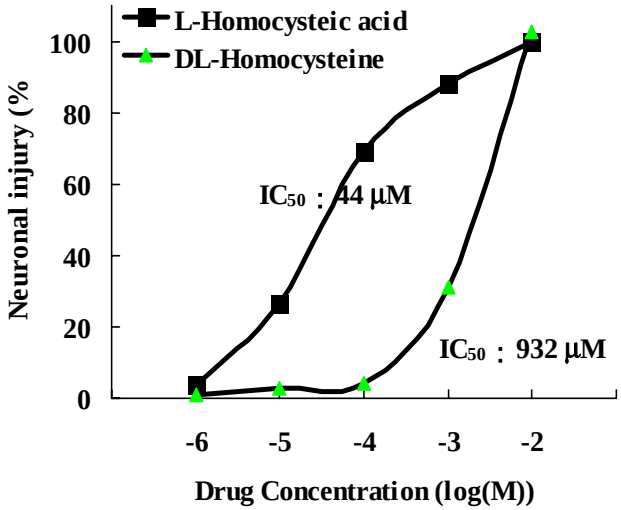


Fig. 7

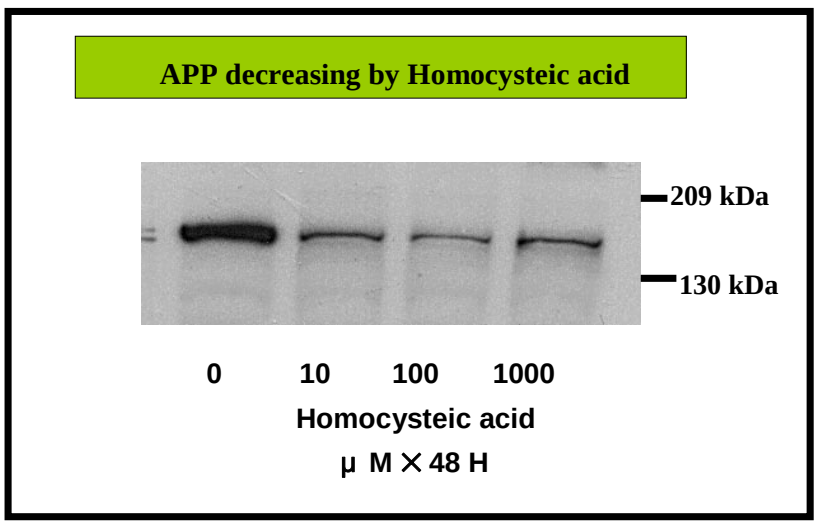

APP decreasing by Homocysteic acid
209 kDa
130 kDa
0
10
100
1000
Homocysteic acid
μ M × 48 H

Fig. 8