

B6 deficient feeding or homocysteic acid induces the earlier Alzheimer's pathological change in normal C57BL male mice

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Abstract

It is the first report that the earlier Alzheimer's pathological changes can be induced in normal C57BL mice, by B6 deficient feeding for 3 month, and this pathological changes were completely inhibited by anti-homocysteic acid antibody. According to Koch's postulate, if a pathogen of Alzheimer's disease is administrated to the normal animal, we would observe the Alzheimer's pathology in the normal animal. We actually have observed this pathology in normal C57BL male mice.

Text

Recently our research team has found (1) that anti-homocysteic acid antibody could show the strong cure and preventive effect on 3xTg-AD mice with normal feeding or with vitamin B6 deficient feeding. Homocysteic acid (HA) was already shown to have a neurodegenerative toxicity and its toxicity was increased by the presence of amyloid (2). Our anti-HA antibody can recognize the receptor-bound HA type, not the free HA. And HA is well known as an agonist of glutamic acid receptor such as NMDA and metabotropic receptor (3). Our findings show that the pathological process of 3xTg-AD mice was induced by glutamic acid receptor-bound HA. However it has not been clarified that our findings will be satisfied in well-known sporadic Alzheimer's disease, because 3xTg-AD mice is a model for familiar Alzheimer's disease, not a model for the sporadic Alzheimer's disease. Here we show that vitamin B6 deficient feeding in normal C57BL mice whose age was 4 and 9 month-old showed the earlier Alzheimer's pathological changes such as memory impairment and amyloid accumulation into neurons. And these earlier Alzheimer's pathological process were completely inhibited and recovered to normal cognitive ability such as good memory formation by anti-HA antibody. These our findings have submitted the very important dogma for Alzheimer's pathological process. That is, HA is one of some real pathogens of Alzheimer's disease according to Koch's postulate. Now we will explain it. According to Koch's postulate, (a)the pathogen will be found in disease organ. (b) If this pathogen can be taken off from the disease animal, the disease can be cured. (c) The pathogen will be added to normal sensitive animal, we can observe the same disease pathological change in normal sensitive animal.

Now HA is the real pathogen of Alzheimer's disease according to above Koch's postulate.

- (a)We can observe the high level of HA in model mice, when the memory impairment started and amyloid accumulation into neuron was observed in all area of amygdale, cortex and hippocampus.
- (b)We can observe the cure effect of anti-HA antibody or anti-HA vaccine in model mice with normal feeding or with B6 deficient feeding.

(c) We can observe that normal C57BL mice (9 month-old is sensitive for Alzheimer's disease) with B6 deficient food for 3 months showed the memory impairment and also amyloid beta accumulation into neuron. And these pathological change was completely inhibited by anti-HA antibody.

(d) In case of (c), normal young mice (4 month-old and final 7 month-old) did show the same pathological change as shown in normal old mice (9 month-old and final 12 month-old), which suggests HA is one of some real pathogens of Alzheimer's disease regardless of the absence or presence of amyloid, because young C57BL mice did not show any amyloid presence. We confirmed with amyloid antibody of 4G8 with immunohistochemical staining.

In human case, some papers reported that down syndrome patient showed an abnormal vitamin B6 metabolism, lower level of B6 compared with normal control (4-7). It is well-known that down syndrome patient will proceed 100% Alzheimer's disease. Then our observation that B6 deficient feeding induced normal C57BL mice to have cognitive and memory impairment, whose impairment are related to Alzheimer's disease from the observation of down syndrome. And B6 deficient induces the pre-Alzheimer's cognitive and memory impairment of human case also.

B6 deficiency in down syndrome is explained by genetic abnormality. It is well-known that vitamin B6 is a cofactor for cystathionine beta-synthase (CBS) (8) and CBS level in down syndrome is higher than that of normal by 1.5 times, then B6 deficiency is appeared by genetic abnormality. And our unpublished observation showed HA is produced by CBS. Then down syndrome shows essentially HA abnormal increased level. Why does down syndrome proceed to 100% Alzheimer's pathology? Because abnormal higher level of amyloid (APP gene is located in 21 chromosome) and also abnormal higher level of HA work together to induce Alzheimer's pathology, whose situation is completely same as that of 3xTg-AD mice (APP transgenic and higher level of HA which was reported in our previous paper (1)).

Now from these our findings, it shows that HA is a real pathogen in Alzheimer's disease.

In case of (d), amyloid synthesis is a one of ageing factors and amyloid modulates NMDA receptor (8). And HA is well known as an agonist of NMDA and metabotropic receptors. Then it is suggested that amyloid modulates NMDA receptor and HA bound to amyloid-modulated NMDA receptor, which induced amyloid accumulation into neurons and consequently induces memory impairment and proceeded to neurodegeneration (9).

Why did we use B6 deficient feeding in our experiment?

It is best way to circulate homocysteic acid itself in model mice, but homocysteic acid may induce the seizure of model mice. So since we had to increase HA level and circulate constantly in mice brain, we used the B6 deficient feeding which was reported to increase HA level (10).

It is the first observation that HA induced the earlier Alzheimer's pathological change in normal C57BL mice. C57BL mice is normal mice which can not increase amyloid level. These mice showed Alzheimer's pathological change induced by HA. It is not

clarified why these mice accumulated amyloid into neuron with B6 deficient feeding. However regardless of the absence or presence of amyloid, HA itself did induce the memory impairment or the cognitive impairment and also induced the neurodegeneration.

In conclusion, our findings present that HA is one of some real pathogens of Alzheimer's disease according to Koch's postulate regardless of the absence or presence of amyloid.

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Figure legends

Fig. 1: C57BL mice memory impairment induced by HA

C57BL mice were 9 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. One group (n=5) was measured at 1.5 month. The other group (n=5) was measured at 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 3xTgAD. After one month, memory test was conducted. Figure shows the average of 5 mice performance.

Fig. 2a C57BL mice (9 month-old) + B6 deficient for 3 months

Immunohistochemical staining of amyloid beta with 4G8. Control with no antibody showed the accumulation of amyloid beta into neuron in amygdala and cortex area. Antibody treatment showed no accumulation of amyloid beta.

Fig. 2b C57BL mice (9 month-old) + B6 deficient (3 months) + Anti-HA antibody

Same as that of Fig.2a

Fig. 3 C57BL mice (12 month-old) with normal feeding

Same as that of Fig. 2a

Fig. 4a C57BL male mice (4 month-old) behavior trace record.

Mice were put into new 50 cm x 50 cm box type cage. Their behavior were recorded with DVD.

Control: C57BL male mice +B6 deficient food for 3 months.

Anti-HA antibody: C57BL male mice +B6 deficient food for 3 months+anti-HA antibody
Antibody treatment was same as described our manuscript (3) after B6 deficient feeding for 2 months. For one month, antibody treatment was conducted.

Fig. 4b Morris-water maze test of C57BL mice

Figure shows the average of 3 male mice. Control and antibody treatment were same as that of Fig. 4a.

C57Bl memory impairment induced by HA

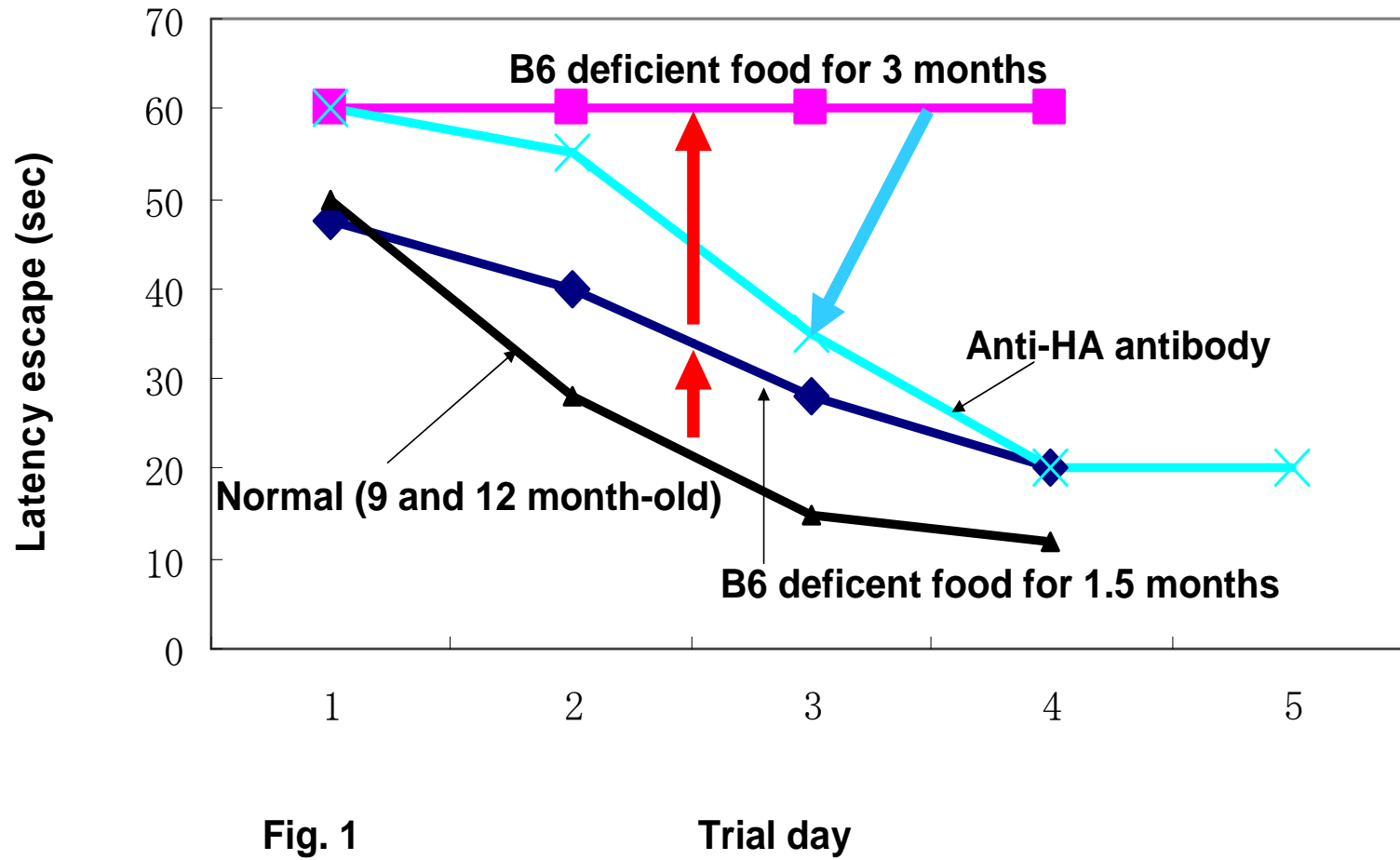
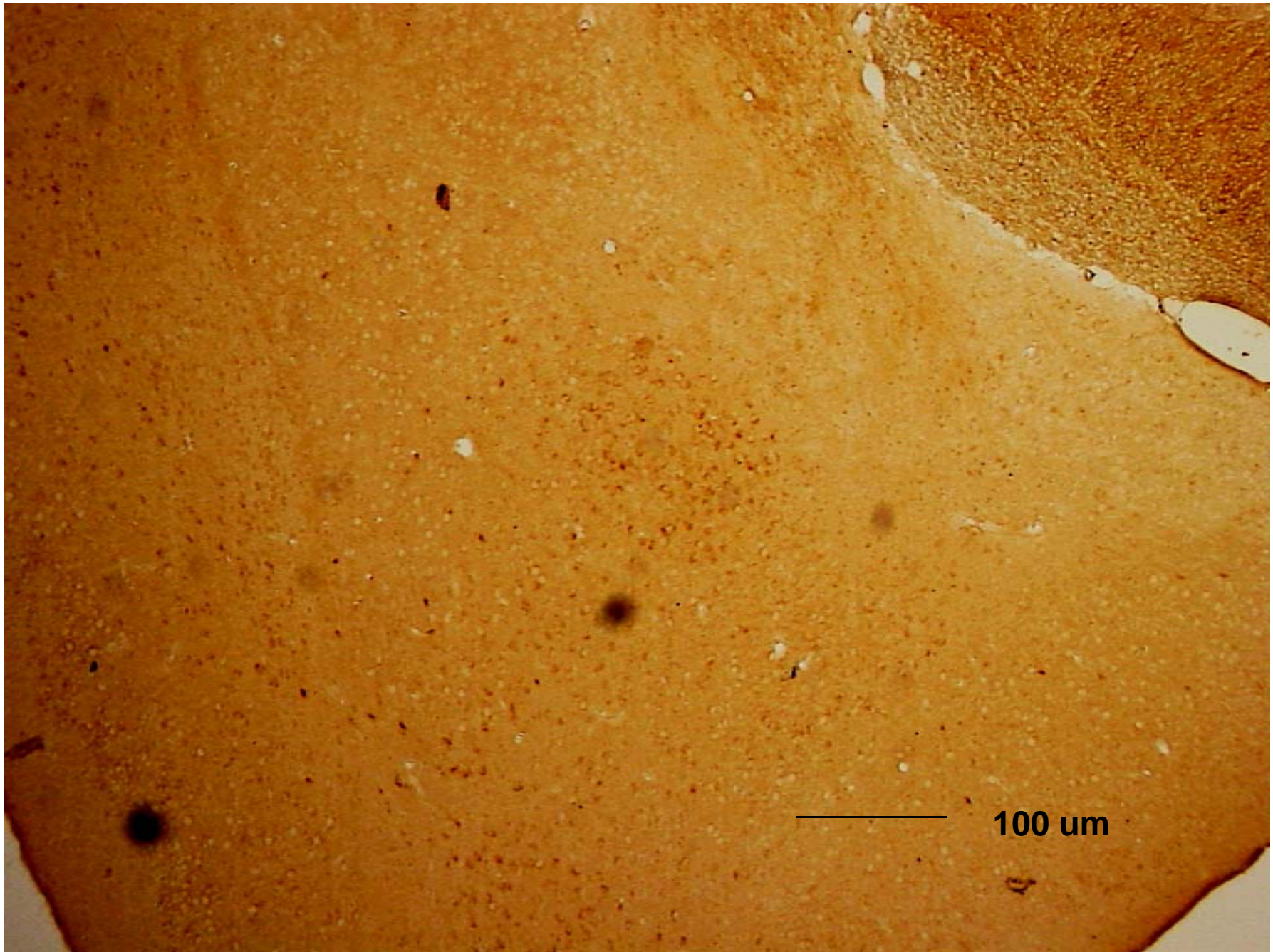


Fig. 1

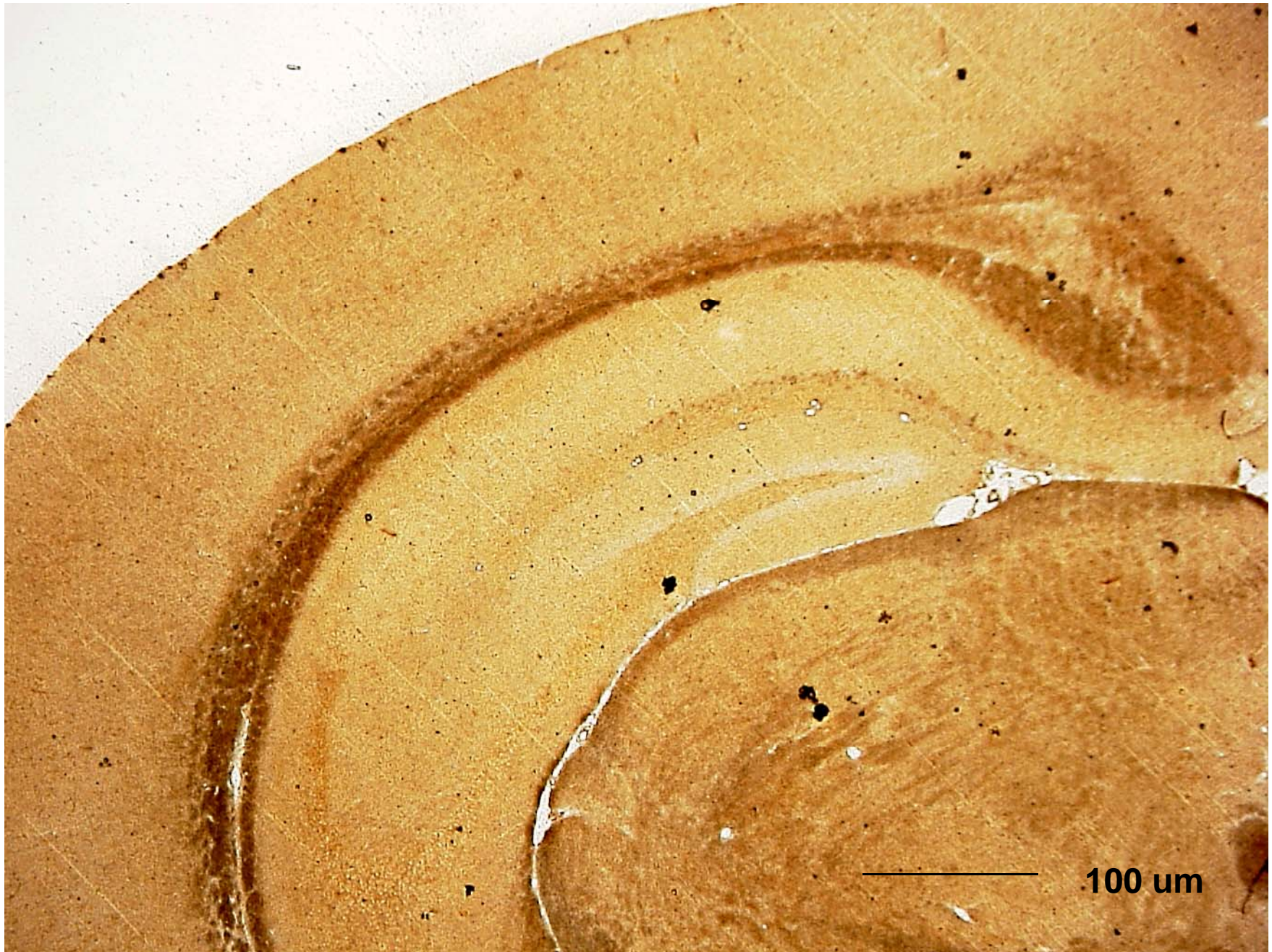
Trial day



C57BL mice (9 month-old) + B6 deficient for 3 months

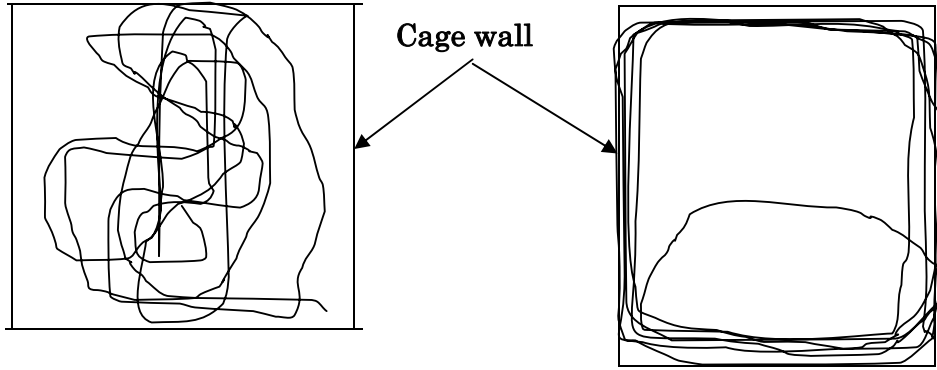


C57BL mice (9 month-old) + B6 deficient (3 months) + Anti-HA antibody



C57BL mice (12 month-old) with normal food, control

C57BL behavior trace record in width space cage



Control

Anti-HA antibody treatment

Control: no anxiety = cognitive ability impairment

Anti-HA antibody treatment = anxiety

Young C57BL (4 month-old male+B6 deficient feeding for 3 months)

