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Author(s)	Hasegawa, Masashi; Kida, Ikuhiro; Wada, Hiromi
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A volumetric analysis of the brain and hippocampus of rats rendered perinatal hypothyroid

Masashi Hasegawa [a, b]

Ikuhiro Kida [c]

Hiromi Wada [a]

Division of Psychology, Graduate School of Letters, Hokkaido University [a]

Japan Society for the Promotion of Science [b]

Integrated Neuroscience Research Team, Tokyo Institute of Psychiatry [c]

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Corresponding author: Masashi Hasegawa

Address: Division of Psychology, Graduate School of Letters, Hokkaido University, Kita10 Nishi7, Kita-ku, Sapporo, 060-0810, Hokkaido, JAPAN

Tel: +81-11-706-3321

Fax: +81-11-706-3321

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Abstract

The thyroid hormone is essential for the proper development of the central nervous system (CNS). Hormone deficiency during CNS development causes neurological abnormalities in the brain. The hippocampus is one of the brain regions vulnerable to hormone deficiency, and the volume of dentate gyrus and cornu ammonis are reduced by transient hypothyroidism during CNS development. However, it remains unclear whether transient hypothyroidism specifically reduces the whole hippocampal volume. In the present study, we used magnetic resonance imaging (MRI) to examine the effects of perinatal hypothyroidism on the ratio of hippocampal volume to brain volume as well as brain and hippocampal volumes overall. Perinatal hypothyroidism was induced by adding the anti-thyroid drug, methimazole, to the drinking water of pregnant dams from gestational day 15 to postnatal day 21. The MRI experiment was conducted when the rats were between 7 and 11 months old. The results showed reductions of the hippocampal and brain volume of the treated group. However, the ratio of hippocampal volume to brain volume was comparable between the control and treated groups. These results indicate that perinatal hypothyroidism minimizes the brain as a whole, but does not minimize the hippocampus in particular.

Introduction

The thyroid hormone is essential for the proper development of the mammalian central nervous

system (CNS). Hormone deficiency during the neonatal, perinatal, or early postnatal period causes neurological abnormalities, such as a reduction in dendritic elaborations, neurite outgrowth, synaptogenesis, and myelination as well as delayed cell differentiation and migration [5, 13, 15, 24, 26, 30]. The hippocampus is one of the brain regions vulnerable to the disruption of the thyroid hormone during CNS development. The number of cells and the area of the granular layer in the dentate gyrus (DG) of the hippocampus were reduced when a rat was treated with an anti-thyroid drug in the neonatal period [28]. This neonatal hypothyroidism also reduced the number of branching points of the apical and basal dendritic trees of the pyramidal cells in the cornu ammonis (CA) of the hippocampus [29]. These neurological abnormalities of hippocampal structures were irreversible even if thyroid hormone states were reverted to normal levels after CNS development, as shown by Madeira et. al. [17, 18]. The number of cells and the volume of the granular layer in the DG of an adult rat, postnatal day (PND) 180, were both reduced by transient postnatal hypothyroidism for a period of 30 days after birth [17]. Similarly, the volume and the number of cells of the pyramidal cell layer in CA1 were significantly reduced by the same transient postnatal hypothyroidism [18].

These studies showed that hypothyroidism during CNS development caused neurological abnormalities and reduced the volume of several regions in the hippocampus. However, it remains unclear whether transient hypothyroidism during CNS development specifically reduces the entire

hippocampal volume in the brain since few experiments have conducted volumetric analysis of the hippocampus of a rat rendered transient hypothyroid. Therefore, the present study used magnetic resonance imaging (MRI) to examine the effects of perinatal hypothyroidism on brain and hippocampal volume, as well as the ratio of hippocampal volume to brain volume, in order to clarify whether hippocampal volume was reduced specifically.

Materials and methods

Sixteen pregnant rats of the Wistar strain were purchased on gestational day (GD) 8. These animals were individually housed and randomly assigned to either the control or treated group (n=8, per group). The treated group was given the anti-thyroid drug, methimazole (MMI), at concentrations of 0.02% (w/v) to induce perinatal hypothyroidism. MMI was dissolved in distilled water and administered to the dams via drinking water from GD15 to PND21. MMI blocks the synthesis of thyroxine (T_4) and triiodothyronine (T_3) [6] and can cross the placenta readily, reaching fetal to maternal serum ratios of approximately 1:1 [20]. The dams of the control group did not receive any treatment. All litters were culled to 8 pups on PND7 with equal numbers of males and females in both groups where possible. Thus, rats were divided as control male (CM), treated male (TM), control female (CF), and treated female (TF). At PND21, the pups (i.e. CM and CF) to be tested were weaned and housed 2-3 per cage. The pups (TM and TF) were weaned on PND28 because

developmental retardations were observed. The 4 CM, 5 TM, 5 CF, and 5 TF rats were taken at random from 8 mothers of either the control or treated group for MRI experimentation. MRI experiments were conducted when the male rats were aged from 7 to 8 months and the female rats were aged from 9 to 11 months, respectively. We tested one control and one treated rat in the same day wherever possible so that the mean ages of the rats were balanced between groups of the same sex. Additionally, 4 male rats of the Wistar strain aged 13 weeks were purchased as a control weight-matched (CM-w) group to the TM group since there was a significant difference in body weight between the CM and the TM rats. The room temperature was maintained at 22±2°C and the relative humidity was 50±10% under a 12-h light/dark cycle (dark, 07:00-19:00 h). Animals had free access to food and water in their home cages. This research was carried out with the approval of the Center for Advanced Science and Technology (Hokkaido University). The environmental conditions complied with the Guide for the Care and Use of Laboratory Animals (Hokkaido University).

MRI experiments were performed on a 7 T horizontal-bore spectrometer (Varian Inc., CA, USA) with a 40 mm i.d. volume transmitter/receiver coil. The rats were initially anaesthetized by the inhalation of an air containing 4% isoflurane, followed by an i.p. injection of 1.25g/kg b.w. urethane. The rats were placed supinely and their heads were fixed using a wire tooth restrainer to prevent the rats from moving. Three mutually perpendicular slices were acquired through the brain as scout

images. Then, coronal images were taken with a spin echo sequence following the parameter; repetition time: 4000 ms, echo time: 20 ms, number of averages: 4, field of view: 40mm×40 mm, matrix size: 256×256, slice thickness: 0.8mm, slice gap: 0.2mm, number of slices: 30 slices. The acquired coronal images were perpendicular to a line connecting the superior end of the olfactory bulb with the superior end of the cerebellum (Fig. 1A).

For volumetric analysis, the hippocampus and brain were manually outlined using image analysis software, Image J (http://rsbweb.nih.gov/ij/). Hippocampal tissue borders were defined according to Wolf et. al. [31], and by referring to a standard rat brain atlas [25]. Five to six consecutive slices clearly showing the contrast of the hippocampus were used for volumetric analysis of the hippocampal volume（see, Fig. 1C and D). Twelve to fourteen consecutive slices were used for the volumetric analysis of the brain. The first slice included the olfactory bulb, the area of which was not more than 50% of the brain area of that slice. The slice prior to the opening of the cerebellum was defined as the last slice. Finally, the total areas of the hippocampus and brain were multiplied by inter-slice interval (0.8mm slice thickness + 0.2mm slice gap) for volume calculation. Only the last slice was multiplied by 0.8mm. The volume analysis (i.e. outline and calculation) was repeated twice and the hippocampal volume and brain volume were averaged for each animal. Then, the data of each group were averaged.

The body weight, hippocampal volume, brain volume, and the ratio of hippocampal volume to brain

volume were analyzed by a one-way ANOVA with MMI treatment as a between-subject factor. The CM and the CM-w groups were the age-matched and weight-matched groups for TM, respectively. The data of the CM, TM, and CM-w groups were analyzed together by ANOVA in order to decrease the possibility of a type I error. The data of male and female rats were analyzed separately. An α level of 0.05 was adopted. Post hoc tests were conducted using Ryan's method with an adjusted significance level.

Results

A one-way ANOVA for the body weight of male groups revealed the main effect of MMI treatment ($F(2,10)=37.17$, $p<0.001$). The body weight of the TM and CM-w rats was significantly less than the weight of the CM rats, and the weight of the TM and CM-w rats was comparable. The weight of the CF and TF rats did not differ significantly. For the volumetric data of the male groups, a one-way ANOVA revealed that there was a significant main effect of MMI treatment on the hippocampal volume ($F(2,10)=85.63$, $p<0.001$). The hippocampal volume of the TM rats was significantly reduced, compared to the volume of the CM and CM-w rats. In addition, it was found that MMI treatment had a significant main effect on brain volume ($F(2,10)=11.31$, $p<0.005$). The brain volume of the TM rats was significantly smaller than that of the CM rats. The brain volume of the TM rats was also reduced compared to that of the CM-w rats, although the difference in brain

volume between these groups did not reach the adjusted significant level (p=0.045 > nominal significant level=0.033). There was no significant difference in the ratio of hippocampal volume to brain volume for male groups. For the volumetric data of the female group, a one-way ANOVA revealed a significant main effect of MMI treatment on the hippocampal volume ($F(1,8)=14.51$, $p<0.01$). The hippocampal volume of the TF rats was significantly less than that of the CF rats. In addition, there was a significantly main effect of MMI treatment on brain volume ($F(1,8)=11.39$, $p<0.01$). The brain volume of the TF rats was significantly smaller than that of the CF rats. There was no significant difference for the ratio of hippocampal volume to brain volume in the female group as well as the male group. The body data and volumetric data of all groups are shown in Table 1 and Table 2.

Discussion

We found that perinatal hypothyroidism significantly reduced the brain volume as well as the hippocampal volume, but did not affect the ratio of hippocampal volume to brain volume in both male and female rats. These results indicate that perinatal hypothyroidism minimizes the brain including the hippocampus, but does not specifically minimize the hippocampus.

Comparing the volumetric data of the TM rats with that of the CM rats (the age-matched group), the hippocampal volume and the brain volume of the TM rats were smaller than those of the CM rats.

Similar results were obtained when comparing the volumetric data of the TM rats with that of the CM-w rats (the weight-matched group). It is assumed that the reduced brain and hippocampal volume of the TM rats would not be normal even if the body weight of the TM rats caught up with the weight of the CM rats because the brain and hippocampal volumes of the TM rats were smaller than that of the CM-w rats. Similar to the results of the male groups, the brain and hippocampal volume of the TF rats were smaller than that of the CF rats. These results are in line with the findings of Madeira et al. [17-19]. They demonstrated that transient postnatal hypothyroidism induced by subcutaneous injection of the anti-thyroid drug, propylthiouracil (PTU), for 30 days after birth reduced the weight of both the brain and hippocampus. Although there are qualitative differences between the reduction of the volume and the weight, our results and their findings indicate that the transient hypothyroidism during the CNS development minimizes the brain and hippocampus.

The thyroid hormone plays several critical roles in the proper development and maturation of the CNS, and thyroid hormone insufficiency affects neuronal cell migration and the formation of the cerebral cortex cytoarchitecture. Heterotopic neurons were found in the somatosensory cortex and several hippocampal regions of the progeny of the dams under the low iodine diet condition, which led to insufficient thyroid hormone production [4, 16]. The reduction of the thickness of the strata radiatum of CA1 in the hippocampus was also observed in the progeny of the low iodine group [16]. These neurodevelopmental alterations occurred even if the period of thyroid hormone insufficiency

was very restricted. The treatment of the MMI (0.02%, through drinking water) with the dams for only 3 days, from GD12 to GD15, also caused neuronal heterotopia in the neocortex and hippocampus of the pups of the treated dams [1]. These neruodevelopmental alterations could be caused by the impaired development of radial glia. Thyroid hormone insufficiency also affected the development of the radial glia, which is important for neuronal cell migration in the cerebral cortex [27]. The prenatal or perinatal hypothyroidism delayed the development of the radial glia in the hippocampus and neocortex [21, 22]. The mature radial glial cell fiber was reduced in the hippocampus of the rat fetus under hypothyroid conditions at GD21 [21], and delayed developmental patterns of the radial glia were observed in the neocortex of the hypothyroid rats from GD21 to PND10 [22]. Thus, the impaired development of the radial glia could be related to the abnormal neuronal migration and cerebral cortex cytoarchitecture. It could be possible that these altered neurodevelopments lead to the reduction of the volume of the brain and hippocampus observed in the present study.

Additionally, the defective myelination caused by the hypothyroidism could be one of the factors that reduced the brain volume. Although the number of axon itself was not reduced, the prenatal and chronic postnatal hypothyroidism heavily reduced the number of myelinated axons in the anterior commissure and corpus callosum; reductions of 66% and 76%, respectively, were observed as compared to the control group. [3]. In fact, the number of myelinated axons could affect the size of

the corpus callosum [14]. Also, the change in white matter volume could contribute to the change in whole brain volume in humans [7]. In the present study, it is not clear whether the volume of white matter was reduced or not. However, taking these findings into consideration, the defective myelination might lead to a reduction of brain volume.

Contrary to our results, Farahvar et al. [8, 9] documented a significant recovery of the hippocampal structure of rats with transient postnatal hypothyroidism induced by PTU (0.1% in dam's drinking water from birth until PND25). The total surface area of the hippocampus and the hippocampal laminar volume were significantly reduced when a treated rat was under hypothyroid conditions at PND25. However, the area and volume measurements became comparable to those of the control group at PND90 after a rehabilitation period. In the present study, the hippocampal volume as well as the brain volume of the treated groups did not revert to normal levels even after the treated rats were given a long rehabilitation period from the perinatal hypothyroidism. Although the causes of this discrepancy regarding the hippocampal volume were not clear, the duration of the hypothyroid condition could have contributed to the discrepancy. While we administered MMI to the treated rats from GD15 to PND21, Farahvar et al. administered PTU to the treated rats form birth to PND 25. Each hippocampal region has its own developmental schedule, and the neurogenesis of the hippocampus occurred during the prenatal period with the exception of DG [2]. Therefore, the effects of the perinatal hypothyroidism on the hippocampus might be more severe than those of

postnatal hypothyroidism, and perinatal hypothyroidism could cause an irreversible reduction of hippocampal volume. Nonetheless, further studies are needed to clarify the causes of the discrepancy.

In summary, the present study revealed that perinatal hypothyroidism irreversibly reduces the brain, including the hippocampus, but does not affect hippocampal volume specifically. Although no specific volume reduction of the hippocampus was observed in the present study, it has been demonstrated that transient hypothyroidism during CNS development caused structural and functional alterations in the hippocampus. The synaptic system of mossy fiber to CA3 was altered by the transient postnatal hypothyroidism [19]. The perinatal hypothyroidism also irreversibly impaired the synaptic transmission and plasticity in CA1 [10] and DG [11]. Since the hippocampus plays important roles in learning and memory, these structural and functional alterations would subsequently impair cognitive functions. In fact, recent behavioral and electrophysiological studies have shown that the altered synaptic transmission and plasticity caused by transient hypothyroidism were associated with spatial leaning deficits [12, 23]. Since we did not conduct behavioral experiments, it is not clear whether the volume reduction of the hippocampus observed in our study would affect cognitive functions that require normal hippocampal functions. Thus, future study is needed to elucidate the relationship between the volume reduction of the hippocampus caused by the transient hypothyroidism and cognitive functions.

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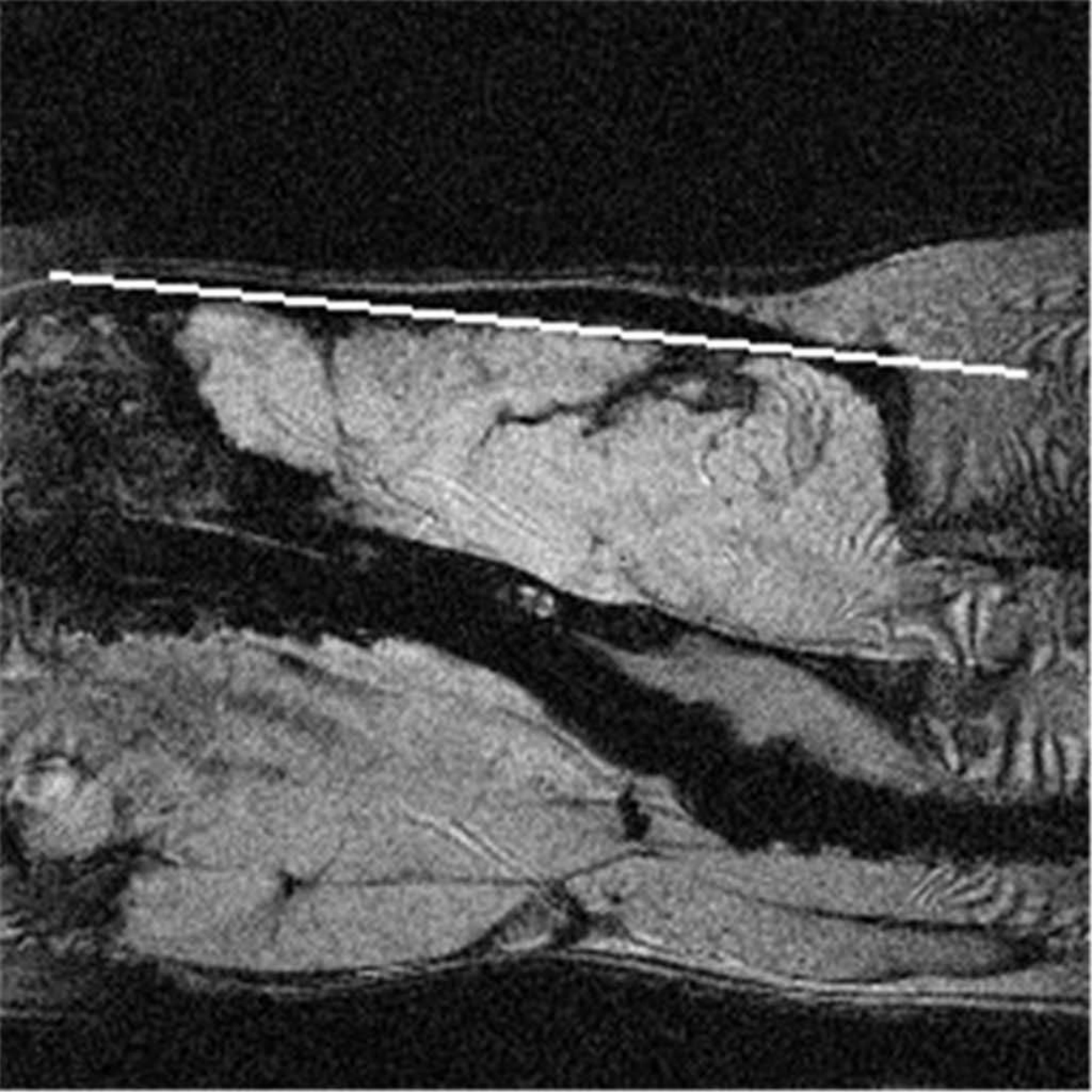
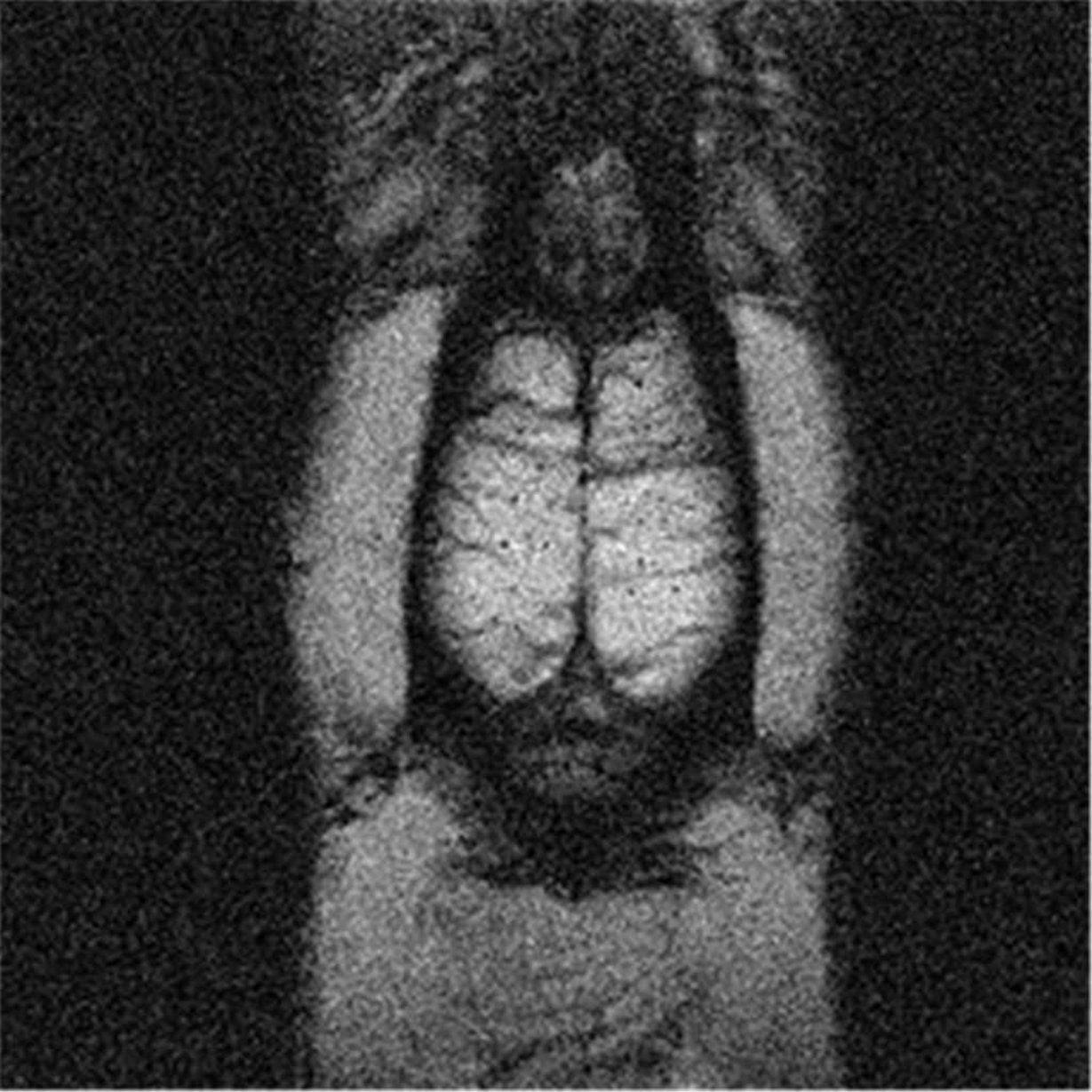
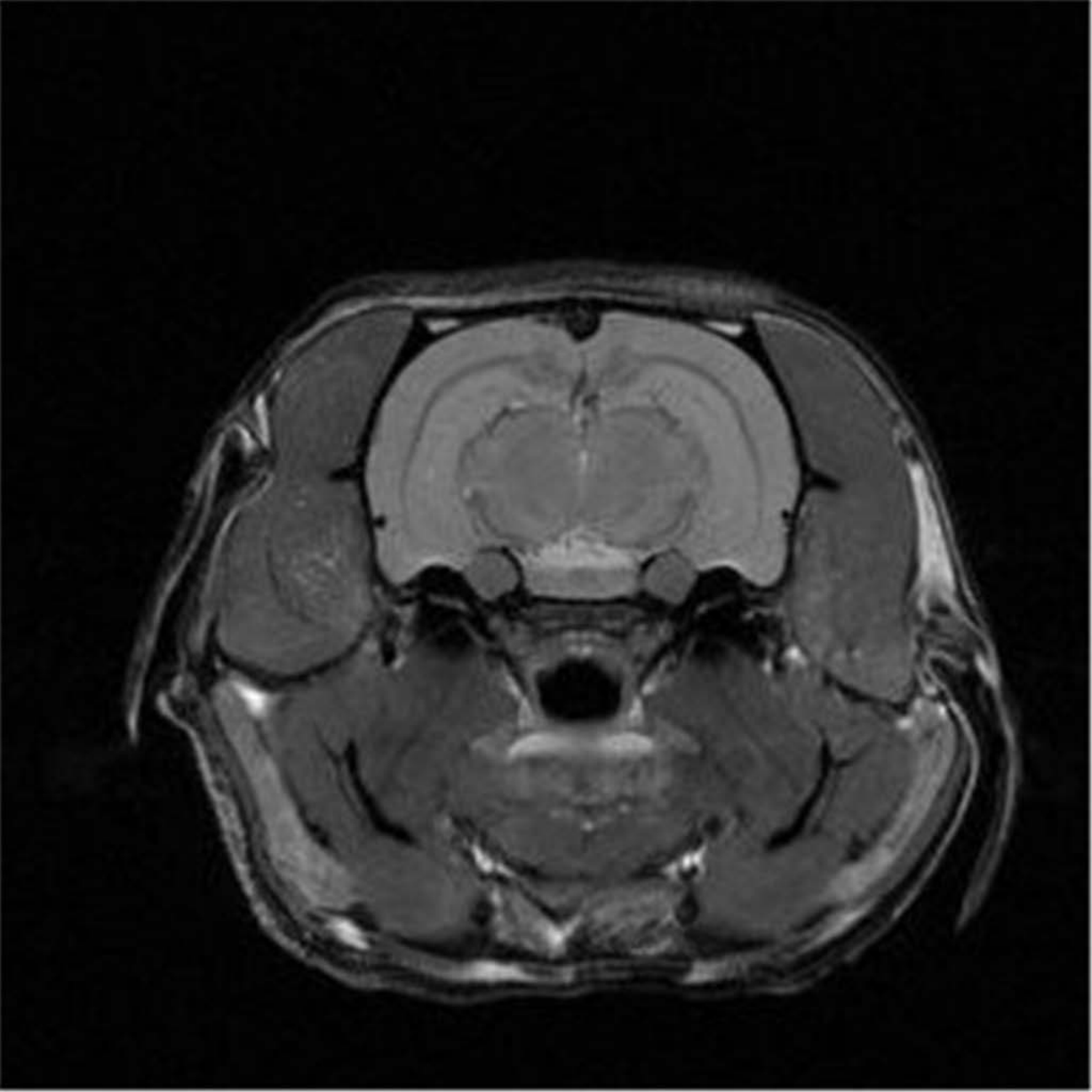
Legend

Fig.1. The scout brain images and the images for volumetric analysis. The line connecting the

superior end of the olfactory bulb with the superior end of the cerebellum (white line) was drawn in the sagittal brain image (A). The horizontal brain image (B) was acquired along the white line in (A). Finally, the coronal images such as (C) for the volumetric analysis were acquired using the horizontal image. The magnified image of (C) is represented in image (D), and the white line in (D) delineated the left hippocampus. These brain images were derived from a CM rat.







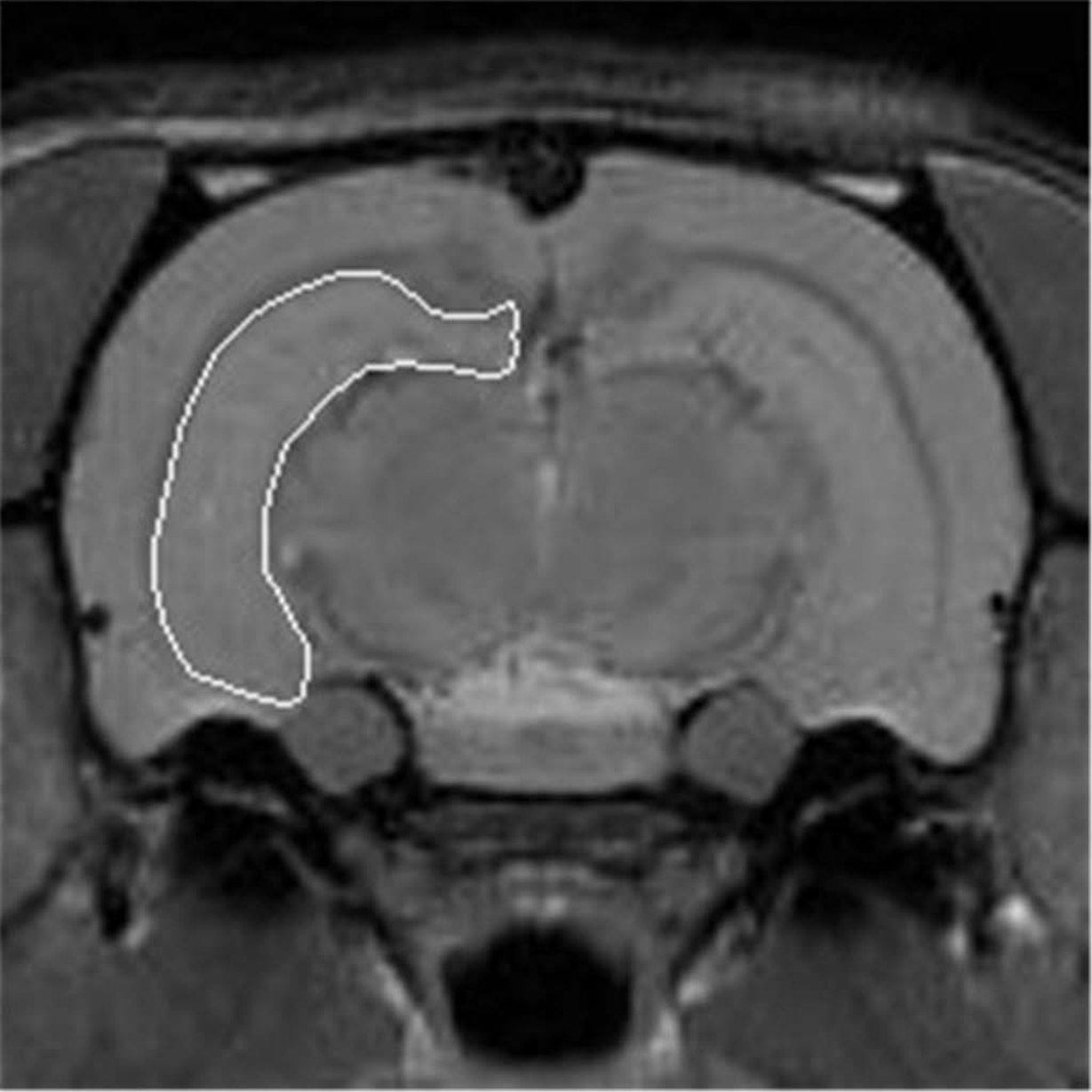


Table.1 The body weight and volumetric data for male groups

	CM	TM	CM-w
Body weights (g)	453 ± 18	377 ± 12 [a]	381 ± 2
Hippocampal volume (mm^3)	93 ± 1	79 ± 2 [a, b]	84 ± 1
Brain volume (mm^3)	1413 ± 45	1236 ± 40 [a]	1322 ± 61
Hippocampus/brain volume (%)	6.6 ± 0.2	6.4 ± 0.2	6.4 ± 0.3

Values are mean ± S.D.

[a] Significant differences between CM and TM rats after post hoc comparison.

[b] Significant differences between CM-w and TM rats after post hoc comparison.

Table.2 The body weight and volumetric data for female groups

	CF	TF
Body weights (g)	264 ± 13	252 ± 14
Hippocampal volume (mm^3)	75 ± 3	68 ± 2[a]
Brain volume (mm^3)	1205 ± 50	1102 ± 34 [a]
Hippocampus/brain volume (%)	6.3 ± 0.4	6.2 ± 0.1

Values are mean ± S.D.

[a] Significant differences between CF and TF rats after post hoc comparison.