

Stability of the synaptic structure in the hippocampus of BALB/c mice with allergic rhinitis

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Abstract

Objective: The aim of this study was to determine whether allergic rhinitis can induce structural changes in the synapse formation in the hippocampus of BALB/c mice immunocytochemically.

Methods: Allergic rhinitis was induced in mice by two intra-peritoneal injections of ovalbumin administered with a one-week interval. After two weeks, the sensitised mice were challenged with an intra-nasal injection of ovalbumin for two weeks. To analyse the hippocampal synaptic structures, sections were immunostained with antibodies against glutamic acid decarboxylase 65 and glutamic acid decarboxylase 67 (for γ -aminobutyric acid-ergic terminals), synaptophysin (for glutamatergic and γ -aminobutyric acid-ergic terminals) and spinophilin (for dendritic spines). The number of nasal rubbing movements was significantly greater in the allergic rhinitis mice than in the control mice. However, the expression patterns of the four above-mentioned synaptic markers in the hippocampus showed no detectable difference between the allergic rhinitis and control mice.

Results and Conclusion: These data indicate that the synaptic structure in the hippocampus might remain unaltered in allergic rhinitis patients.

Key words: Spine; Hippocampus; Allergic Rhinitis; Immunohistochemistry

Introduction

Chronic diseases in childhood can lead to learning disorders during school life. Allergic rhinitis is one of the important impairments that occur in childhood, because it causes nasal obstruction-induced disturbed breathing. In addition, nasal symptoms, including a sensation of itch and rubbing of the nose, sneezing, rhinorrhoea, and congestion reduce health-related quality of life by causing loss of sleep and limiting the activity of the patient.¹ Allergic rhinitis is believed to cause learning disorders by causing reduced attention, disturbed night sleep, consequent daytime sleepiness, etc.² Various treatments are administered to ameliorate allergic rhinitis and its symptoms; anti-histamine therapy is the most commonly used treatment. Clinical trials with anti-histamines have shown that 36–80 per cent of patients experience good to excellent symptom relief (approximately twice of that noted in the placebo group) in seasonal allergic rhinitis and 55–86 per cent experience moderate to excellent symptom relief in perennial allergic rhinitis.³ However, the possibility of learning disability due to the sleepiness induced as a side effect of the anti-histamines remains a concern. Ketotifen, an anti-histamine, reduces the accuracy of preschool children to perform

working memory tasks.⁴ However, the mechanism underlying the learning disability induced in patients with allergic rhinitis remains unclear.

The hippocampus, an important region in the brain, is responsible for learning and memory. It is reportedly associated with cognitive mapping, detection of spatial configuration, and contextual learning and declarative memory.⁵ Furthermore, synapse formation in the hippocampus might be closely related to learning and memory. Significant age-related impairments in hippocampus-dependent learning have been assessed using water maze and fear-conditioning tests.⁶ Yang *et al.*⁷ reported that learning and novel sensory experience lead to spine formation and elimination by a protracted process in mice, and that the extent of spine remodeling correlates with the behavioural improvement observed after learning.

Despite these studies, it is still not known whether allergic rhinitis can induce anatomical changes during synapse formation in the hippocampus. Therefore, in this study, we examined the possible alterations in the expression patterns of synapse-related molecules in the hippocampus of mice models of allergic rhinitis using the following markers: glutamic acid decarboxylase (GAD) 65 and 67, synaptophysin and spinophilin.

Our results indicate that the synaptic structure present in the hippocampus may remain unaltered in the allergic rhinitis patients.

Materials and methods

Twelve seven-week-old virgin female BALB/c mice (Japan Crea Inc., Hamamatsu, Japan) were housed in separate standard cages. They were bred under the specific pathogen-free conditions at our Institute and were allowed to feed and drink *ad libitum*. On day 0, six mice were intra-peritoneally sensitised with 30 µg ovalbumin (OVA) diluted in 100 µl of alum solution. This injection was repeated on day 7. On the other hand, the control (non-sensitised) mice were intra-peritoneally injected with phosphate-buffered saline mixed with 100 µl of alum solution on day 0 and the injection was repeated on day 7. On days 24, 25, 26, 27, 28, 31, 32, 33, 34 and 35 after the first sensitisation, the OVA-sensitised mice were challenged with an intra-nasal injection of 10 µl of 10 µg/ml OVA solution prepared in phosphate-buffered saline and administered into both the nostrils. The control mice were challenged with an intra-nasal injection of 10 µl of phosphate-buffered saline into both the nostrils. After the last challenge, the mice were placed in the observation cages, and the number of sneezing attacks and nasal rubbing movements were counted for 10 minutes by the method proposed by Sugimoto *et al.*⁸ On day 36, three mice from each group were anaesthetised with ether and transcardial perfusion was performed with 4 per cent paraformaldehyde. The brains of these mice were removed and soaked overnight at 4°C in the same solution. Serial 25-µm-thick coronal sections were cut on a vibratome (VT1000S; Leica Microsystems, Wetzlar, Germany); these sections were blocked overnight at 4°C in 1 per cent bovine serum albumin

(BSA) prepared in phosphate-buffered saline containing 0.3 per cent Triton X-100 and 0.05 per cent sodium azide. The blocked sections were incubated for 3 days at 4°C with the following primary antibodies raised in different species: mouse monoclonal anti-synaptophysin (1:20 000; a gift from Dr Fujita), mouse monoclonal anti-GAD65 (1:100 000; MAB5406; Chemicon, Temecula, California, USA), rabbit polyclonal anti-spinophilin (1:10 000; a gift from Dr Greengard) and rabbit polyclonal anti-GAD67 (1:5000; G5038; Sigma-Aldrich, St. Louis, Missouri, USA). These sections were washed with phosphate-buffered saline and then incubated at 4°C with a mixture of fluorescein isothiocyanate-conjugated donkey anti-rabbit immunoglobulin (Ig) G antibody (1:250; Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania, USA) and cyanine 3-conjugated donkey anti-mouse IgG antibody (1:500; Jackson ImmunoResearch Laboratories) for 3 h. Finally, these sections were mounted in Vectashield (Vector Laboratories, Peterborough, UK) and examined under a confocal laser-scanning microscope (TCS-SP2; Leica Microsystems). All these experiments were performed in accordance with the Guidelines for Animal Experiments practiced at our Institute and the Law (No. 105) and Notification (No. 6) of the Government of Japan. We assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals (A22-143-0 and A26-148-0).

Results

The observed nasal symptoms are shown in Figure 1. The number of sneezing attacks and nasal rubbing movements were higher in the allergic rhinitis group than in the control group ($p < 0.1$ and < 0.05 ,

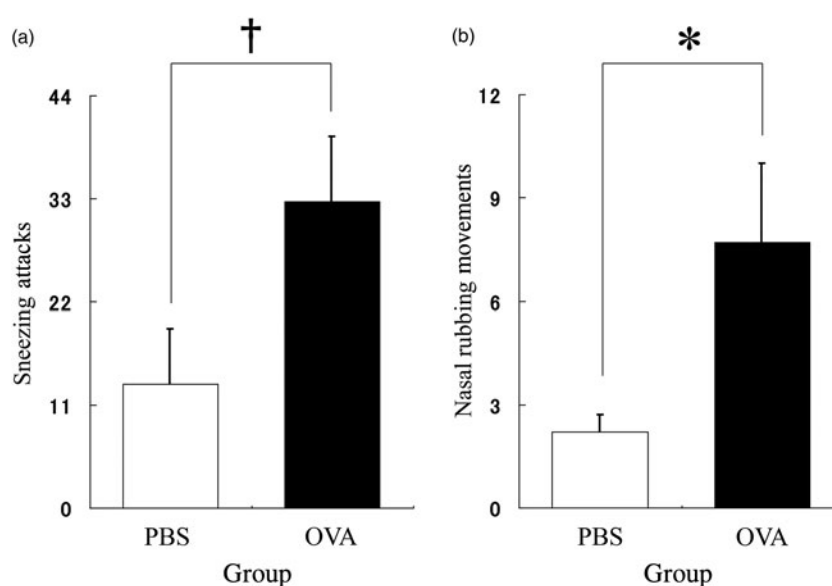


FIG. 1

Behavioural assessment of allergic response. (a) The number of sneezing attacks was greater in the ovalbumin (OVA) group (black column) than in the phosphate-buffered saline (PBS) group (white column). (b) The number of nasal rubbing movements was greater in the OVA group (black column) than in the phosphate-buffered saline group (white column). ($n = 6$ in each group) † $p < 0.1$; * $p < 0.05$.

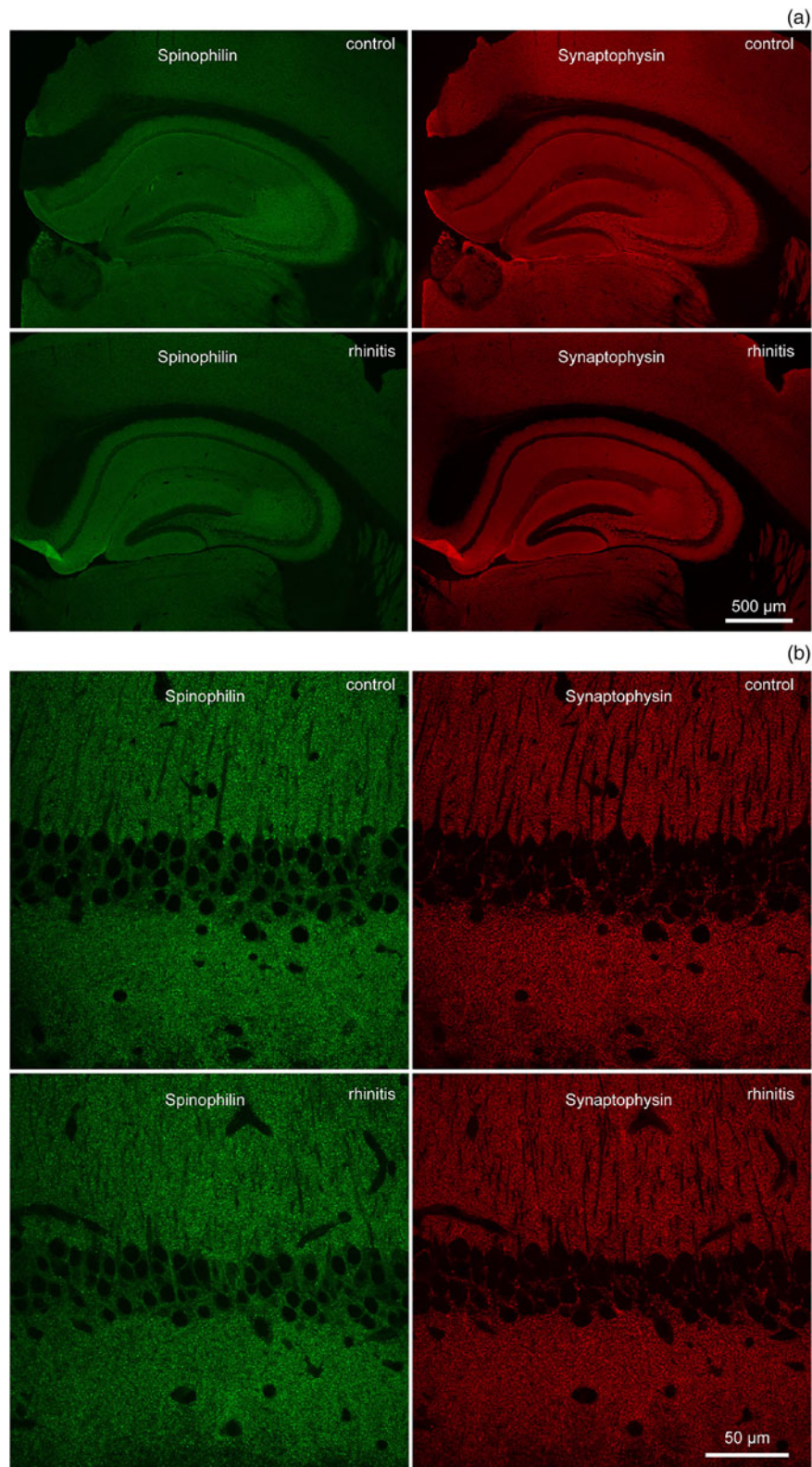


FIG. 2

Representative patterns of expression of spinophilin and synaptophysin in the hippocampus of control and rhinitis animals. (a) There are no apparent differences in immunolabelling for spinophilin and synaptophysin in the whole area of the hippocampus. (b) Higher magnification ($\times 400$) images of the cornu ammonis 1 area of the hippocampus immunostained for spinophilin and synaptophysin.

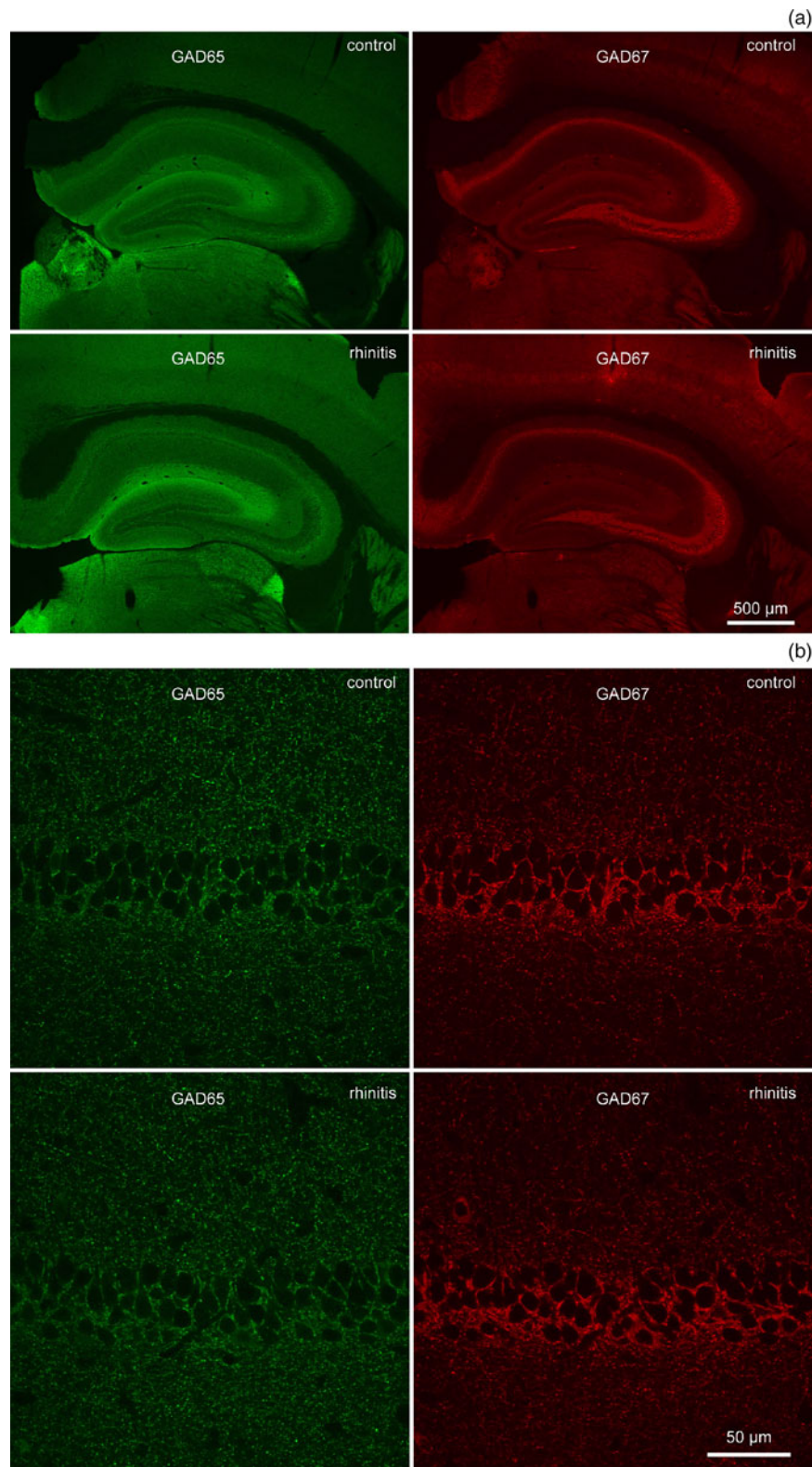


FIG. 3

Representative patterns of expression of glutamic acid decarboxylase (GAD) 65 and GAD67 in the hippocampus of control and rhinitis animals. (a) There are no apparent differences in immunolabelling for GAD65 and GAD67 in the whole area of the hippocampus. (b) Higher magnification ($\times 400$) images of the cornu ammonis 1 area of the hippocampus immunostained for GAD65 and GAD67.

respectively). The results indicate that an animal model of allergic rhinitis was successfully developed.

Immunofluorescence double labelling for synaptophysin and spinophilin showed no differences in the

expression patterns between the AR and control groups (Figure 2). Similarly, we did not observe any difference in the distribution patterns of GAD65 and GAD67 between the two groups (Figure 3).

Discussion

Our study provides the first evidence that allergic rhinitis may not alter the synaptic structures in the hippocampus, at least during a two-week exposure time. Neuroimmunomodulation plays a crucial role in homeostasis because the central nervous system is closely related to the immune system. Several evidences have validated the effect of allergy on brain function. Costa-Pinto *et al.* reported that OVA-immunised mice entered the open arm in lesser time than non-immunised mice in the elevated plus-maze test, and that the number of Fos-immunoreactive neurons in the paraventricular nucleus and the serum corticosterone levels were higher in OVA-immunised mice.⁹ Tonelli *et al.* showed that in an open field test, the total distance moved in the allergic mice was the same as that moved in the control mice, and that the duration of social contact in the allergic mice was shorter than that observed in the control mice.¹⁰ The evidence suggests that the allergic response induces anxiety, social withdrawal, and hyperactivity of the hypothalamic–pituitary–adrenal axis. To the best of our knowledge, no morphological or physiological research of the brain has been conducted thus far to show the effect of allergic response on cognitive functions such as learning or memory.

Synaptic reconstruction is thought to be a critical morphological change occurring in the brain during learning. The spine apparatus, which is considered a post-synaptic structure, is closely associated with learning and memory. Deller *et al.* used the radial arm maze test to show that absence of the spine apparatus decreased the ability of mice to acquire spatial learning; they also showed a decrease in the long-term potentiation of the field excitatory post-synaptic potential in synaptopodin-deficient mice.¹¹ Another group of researchers showed that the administration of oestradiol benzoate improved spatial learning and increased the level of hippocampal synaptophysin.¹² These findings led us to hypothesise that learning impairment in patients with allergic rhinitis is due to changes in the synapse formation in the hippocampus. However, the absence of changes in the levels of synaptophysin and spinophilin refuted this hypothesis.

Allergic response is one of the commonly known stressors that affect a living body, especially the hippocampal morphology. Exposure to elevated serum levels of corticosterones and prolonged stress induced by restraint and immobilisation induces the shrinkage of apical dendrites of cornu ammonis 1 and 3 (CA1 and CA3, respectively) neurons, and reduces the spine number.¹³ An increase in the corticosterone levels in animals with allergic rhinitis may alter the synaptic structure in the hippocampus. However, in our study, we found that the synaptic structure in the hippocampus was stable in allergic rhinitis mice. However, further studies are required to elucidate these discrepancies.

The hippocampal γ -aminobutyric acid-ergic (GABAergic) interneurons play a crucial role in

regulating the activity of the principal neurons. This regulation mainly consists of three processes.¹⁴ First, the activation of post-synaptic GABA receptors produces direct hyperpolarising or shunting effects and then counteracts the membrane depolarisation induced by excitatory inputs. Second, GABA receptor-mediated synaptic responses directly block the action potential firing. Finally, the hippocampal interneurons lead to the synchronisation of the firing of principal neurons during network oscillations. The pharmacological blockade of cholinergic systems, which disrupts the low-frequency theta rhythm of the oscillations in the hippocampus, exerts delayed effect on the acquisition rate of the behavioural conditioned response.¹⁵ In our study, the intra-nasal challenge with OVA did not affect the immunoreactivity of GAD65/67, thereby proving that the hippocampal GABAergic interneurons are not responsible for the learning impairment in allergic rhinitis patients.

- **This is a research article describing stability of the synaptic structure in the hippocampus of BALB/c mice with allergic rhinitis**
- **Immunofluorescence double labelling for synaptophysin and spinophilin showed no differences in the expression patterns**
- **We did not observe any difference in the distribution patterns of GAD65 and GAD67**
- **The present study reports that allergic rhinitis may not alter the synaptic structure in the hippocampus, at least during a short-term exposure**

An increase has been observed in the already high prevalence rate of allergic rhinitis across the world.^{16–18} Marshall *et al.* showed that during ragweed seasons, allergic patients experience a subtle decrease in their cognitive processing; some patients also express difficulties in working memory.¹⁹ Moreover, Shaaban *et al.* reported that the adjusted relative risk for asthma was 1.63 for atopy alone, 2.71 for non-allergic rhinitis and 3.53 for allergic rhinitis, and that only allergic rhinitis with sensitisation to mites was associated with an increased risk of asthma; this risk was independent of sensitisation to other allergens.²⁰ Therefore it is very important to take strong countermeasures against allergic rhinitis. In this study, intra-nasal short-term exposure to the allergen did not produce an abnormal change in the synaptic structure of the hippocampus. There is a high possibility that allergic rhinitis induced learning impairment is independent of structural changes in the brain; instead, it could be based on the subsequent effects of allergic rhinitis such as reduced attention and sleepiness during the daytime. These factors indicate that adequate treatment of allergic rhinitis can relieve patients and prevent subsequent

complications. The present study reports that allergic rhinitis may not alter the synaptic structure in the hippocampus, at least during a short-term exposure. Active treatment of allergic rhinitis in childhood may be recommended in order to prevent learning disorders.

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