Received: 8 September 2017 | Revised: 28 December 2017 | Accepted: 2 January 2018

DOI: 10.1002/jnr.24216

RESEARCH ARTICLE

WILEY

Nasal obstruction during adolescence induces memory/learning impairments associated with BDNF/TrkB signaling pathway hypofunction and high corticosterone levels

Takuya Ogawa[1] | Hidemasa Okihara[1] | Satoshi Kokai[1] | Yasunori Abe[1] | Uchima Koecklin Karin Harumi[1] | Mio Makiguchi[1] | Chiho Kato[1] | Tadachika Yabushita[1] | Makoto Michikawa[2] | Takashi Ono[1]

[1]Department of Orthodontic Science, Oral Health Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

[2]Department of Biochemistry, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan

Correspondence
Hidemasa Okihara, Department of Orthodontic Science, Oral Health Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan.
E-mail: hideorts@tmd.ac.jp

Funding Information
This research was sponsored by the Ministry of Education, Culture, Sports, Science, and Technology of Japan under contract grant number 15K11339.

Abstract

The hippocampus is an important brain region involved in memory and learning. Brain-derived neurotrophic factor (BDNF), tyrosine kinase receptor B (TrkB), and phospho-p44/p42 mitogen-activated protein kinase (MAPK) are known to contribute to hippocampal memory/learning. The present study aimed to clarify the effects of nasal obstruction during the growth period on memory/learning in an animal model, using combined behavioral, biochemical, and histological approaches. Male BALB/C mice underwent unilateral nasal obstruction (UNO) by cauterization at 8 days of age and were subjected to Y-maze and passive avoidance tests at 15 weeks of age. The serum corticosterone levels were measured using an enzyme-linked immunosorbent assay, and brain tissues were subjected to hematoxylin-eosin staining and histological analysis or homogenization and Western blot analysis. Compared with control mice, UNO mice had lower blood oxygen saturation levels and exhibited apparent memory/learning impairments during behavioral testing. Additionally, the UNO group had higher hippocampal BDNF levels and serum corticosterone levels, lower hippocampal TrkB and phospho-p44/p42 MAPK levels, and reduced neuron numbers relative to controls. Our findings suggest that UNO during adolescence affects the hippocampus and causes memory/learning impairments.

KEYWORDS

BDNF, corticosterone, hippocampus, memory/learning impairment, SpO_2

1 | INTRODUCTION

Nasal breathing can be obstructed by structural and functional problems in the nose and other regions, including deviation of the nasal septum, adenoid hypertrophy, allergic rhinitis, and sinusitis (Bucca et al., 1995; Craig, Teets, Lehman, Chinchilli, & Zwillich, 1998; Gross, 1974). Nasal respiration disorders have been reported to affect the whole body through effects such as cephalalgia, feebleness, sleep disorders, and hypersomnolence (Bhattacharyya et al., 2012; Camelo-Nunes & Sole, 2010; Castro, Marinho, & Cavalcante, 2013). Furthermore, previous studies have shown that mouth breathing, a consequence of nasal congestion, is an important factor in dental caries and periodontal disease (Emslie, Massler, & Zwemer, 1952; Lite et al., 1949).

Significance

Recent reports have linked nasal obstruction to poor academic ability during adolescence. However, the mechanism underlying memory/learning impairments is poorly understood. Here, we demonstrate the effects of nasal obstruction during the growth period on memory/learning in male BALB/C mice, using behavioral, biochemical, and histological approaches. This is the first study to clarify the relevance of nasal obstruction to memory/learning function in an animal model. Further studies are needed to clarify the underlying mechanisms and provide a rationale for treating nasal obstruction in younger humans to prevent memory/learning impairments.

During preadolescence and adolescence, mouth breathing causes a range of craniofacial and occlusal problems, including an open bite, maxillary protrusion, and lateral cross-bite (Harai, Redlich, Miri, Hamud, & Gross, 2010; Pirilä-Parkkinen et al., 2009), and masticatory stimulation via the periodontal ligament and temporomandibular joint are known to affect the structure and function of the central nervous system (CNS) (Okihara et al., 2014). A recent study found that children who do not use mouth breathing exhibit better reading comprehension, arithmetic skills, and working memory function when compared with children who use mouth breathing (Kuroishi et al., 2015). Moreover, adenoidal hypertrophy and allergic rhinitis were found to lead to poor academic ability (Fensterseifer, Carpes, Weckx, & Martha, 2013; Walker et al., 2007) and high stress levels (Kim, Kim, Park, Kim, & Choi, 2017) during adolescence. Furthermore, a previous study showed that stress induces hippocampal atrophy and memory loss (Sapolsky, 2000). These findings suggest that nasal obstruction induces memory/learning impairments and increases stress. Biochemical studies in rats have suggested an association of nasal obstruction with peripheral structural and functional changes (e.g., maturation of the jaw-opening reflex and tongue protrusive forces) (Funaki, Hiranuma, Shibata, Kokai, & Ono, 2014; Uchima Koecklin et al., 2015). However, the effects of nasal obstruction during growth on the development of the CNS and, consequently, memory/learning remain unknown.

In the brain, the hippocampus is an essential region in terms of memory and learning abilities. In previous studies, patients with hippocampal damage exhibited noticeable deficits in memory function (Eichenbaum, 2015; Zola-Morgan, Squire, & Amaral, 1986). Peripheral stimulation, such as stress and hypoxia, leads to structural changes in the hippocampus and thus affects memory and learning (Kempermann, Kuhn, & Gage, 1997). Brain-derived neurotrophic factor (BDNF) also plays an important role in changing the hippocampal structure (Thoenen, 2000). BDNF regulates the connectivity of neurons in the developing and adult CNS and peripheral nervous system and promotes neuronal differentiation and survival (Barde, 1994; Lewin & Barde, 1996). BDNF signals through its receptor, tyrosine kinase receptor B (TrkB), to contribute to neuronal development, synaptic function, and memory/learning (Thoenen, 1995). BDNF/TrkB signaling subsequently activates the mitogen-activated protein kinase (MAPK) pathway (Reichardt, 2006), which has previously been shown to be necessary for memory and learning (Sweatt, 2001), by inducing the phosphorylation of p44/42 MAPK. Accordingly, the level of phosphorylated (phospho)-p44/p42 MAPK can be used as an indicator of BDNF/TrkB signaling activity (Alonso, Vianna, Izquierdo, & Medina, 2002).

Several studies have investigated whether changes in the oral cavity affect the hippocampal structure and function and, in turn, memory and learning. A study by Okihara and coworkers (2014) indicated that in mice, decreased mastication during the growth period increased the BDNF levels and decreased the TrkB and phospho-p44/p42 MAPK levels in the hippocampus and impaired memory and learning ability. Therefore, BDNF, TrkB, and phospho-p44/p42 MAPK are closely associated with memory and learning ability. Several other studies have reported that changes in the oral environment, such as masticatory dysfunction, induce memory and learning impairments (Kubo et al., 2007; Yamazaki, Wakabayashi, Kobayashi, & Suzuki, 2008). Furthermore, several studies have confirmed that masticatory dysfunction leads to decreased numbers of neuron cells (Okihara et al., 2014; Tsutsui et al., 2007). Neuronal nuclei (NeuN), an antigen expressed specifically by neuronal nuclei, has recently been used frequently as a neuronal marker. Immunohistochemical analysis of the expression of NeuN in the hippocampus has demonstrated that restraint stress induced the loss of neurons and impaired memory/learning (Lee et al., 2017).

Similarly, stress is well known to affect the hippocampus and memory/learning ability. Previous studies have shown that stress elevates corticosterone levels in the blood and impairs memory and learning (de Quervain, Roozendaal, & McGaugh, 1998; Song, Che, Min-Wei, Murakami, & Matsumoto, 2006). Nevertheless, the possible associations among nasal obstruction, stress, and memory and learning ability have not been assessed.

Therefore, the present study aimed to clarify the effects of nasal obstruction during adolescence on memory and learning in a mouse model, using behavioral, biochemical, and histological approaches.

2 | MATERIALS AND METHODS

All experiments described herein were approved by the Institutional Animal Care and Use Committee (protocols 0170338A and A2017-286A) and performed in accordance with the Animal Care Standards of Tokyo Medical and Dental University.

2.1 | Animals

Six-day-old male BALB/C mice were obtained from Sankyo Laboratory Service (Tokyo, Japan) and randomly divided into control (CONT; $n = 9$) and unilateral nasal obstruction (UNO; $n = 9$) groups. At 8 days of age, all mouse pups were anesthetized via hypothermia (10 min at −18 °C), and those in the UNO group underwent left-sided nasal obstruction via selective cauterization of the left external nostril, the simplest and most common procedure used to induce nasal obstruction in neonatal animals (Padzys, Thornton, Martrette, & Trabalon, 2011). In particular, the tissue surrounding the left external nostril was burned by placing a surgical cauterizing instrument (1 mm in diameter) on the nostril to occlude the nostril orifice without causing mechanical or chemical damage to the olfactory mucosa (Funaki et al., 2014). After cauterization, the nostril was coated with 3% chlortetracycline (Aureomycin Ointment; Pola Pharma, Tokyo, Japan) to prevent infection. The pups were kept warm (37 °C) for 30 min and then returned to their dams. Mice in the CONT group underwent a sham operation in which the cauterizing instrument was placed approximately 1–2 mm above the left nostril. The body weights of the mice were measured throughout the experimental period.

2.2 | Blood oxygen saturation levels

In isoflurane-anesthetized rats, blood oxygen saturation (SpO_2) was recorded using a pulse oximeter (MouseOx Plus; STARR Life Sciences

Corp., Oakmont, PA) and collar clip sensors. The sensors were placed dorsolaterally, with the tips situated near the carotid arteries. SpO_2 signals were sampled at 1 Hz and averaged over 40–50 s of data (Bavis et al., 2014).

2.3 | Behavioral tests

We used the Y-maze and passive avoidance tests to evaluate memory and learning abilities in 15-week-old mice. The Y-maze apparatus comprised three arms (300 mm × 60 mm × 150 mm) that were separated by 120° angles and randomly assigned designations of "A," "B," and "C." The mice were allowed to freely explore the three arms for 8 min. The numbers of arm entries and of triads were recorded to calculate the percentage of alternations in the maze. The percent alternation was calculated as the ratio of the actual-to-possible alternations (defined as the total number of arm entries minus 2) multiplied by 100, as shown in the following equation:

$$\textit{Percent alternation}\,(\%) = [(\textit{number of alternations})/(\textit{total arm entries} - 2)] \times 100$$

An entry into an arm was recorded as the placement of all four limbs within the arm (Holcomb et al., 1999).

The passive avoidance test apparatus comprised an illuminated compartment (100 mm × 100 mm × 145 mm) with a hole through which the mice entered a dark compartment (180 mm × 180 mm × 145 mm) with a grid on the floor. The two compartments were separated by a guillotine door. On the first day, the mice were habituated in the illuminated and dark compartments for 300 s. On the second day, the mice were placed into the light compartment with the guillotine door open, and the time required to enter the dark compartment, where the mice received an electrical stimulation (0.3 mA, 5 s), was measured. The time limit to enter the dark compartment was set at 300 s. Twenty hours later, the time to enter the dark compartment was again measured and compared with the previously recorded time to evaluate the memory and learning abilities of the mice (Yamagata et al., 2009).

2.4 | Sample collection

After the passive avoidance test, the mice were perfused with phosphate-buffered saline under isoflurane anesthesia. The brains were dissected out and divided into right and left hemispheres. The right hemispheres were separated into the cerebral cortex and hippocampus, which were frozen immediately and stored at −80 °C.

2.5 | Western blot analysis

The protein levels of BDNF, TrkB, and phospho-p44/p42 MAPK were measured in right hippocampal samples using Western blotting (Okihara et al., 2014). The wet weight of each hippocampus was measured, and the tissue was homogenized in 20 volumes of 20 mM Tris-buffer containing a protease inhibitor cocktail (Sigma, St. Louis, MO) and 5 mM ethylenediaminetetraacetic acid in a handheld homogenizer (Microtec, Chiba, Japan) while on ice. The samples were then centrifuged at 100,000*g* for 20 min at 4 °C. A 10-μl aliquot of the supernatant was removed and used to determine the total protein concentration with a Micro BCA Protein Assay Reagent kit (Pierce, Rockford, IL). A one-tenth volume of trichloroacetic acid was added to the remaining supernatant to precipitate the proteins. The samples were incubated on ice for 30 min and centrifuged at 15,000*g* for 30 min at 4 °C. Sample buffer was then added to the protein precipitate (20 μl of sample buffer/40 μg of protein), and the samples were frozen at −80 °C until analysis.

The samples were separated on 15% (for BDNF) or 10% (for TrkB and phospho-p44/p42 MAPK) sodium dodecyl sulfate–polyacrylamide gels at 20 mA for 45 min. The separated proteins were transferred to polyvinylidene fluoride membranes using transfer buffer (25 mM Tris, 192 mM glycine, 10% [v/v methanol]) at 200 mA for 45 min. The membranes were then blocked for 1 hr at room temperature (~24 °C) in 0.05% Tris-buffered saline-Tween 20 (TBS-T; 50 mM Tris, pH 7.4, 133 mM NaCl) containing 2.5% (w/v) skim milk powder. TBS-T was also used to wash the membranes. The membranes were incubated overnight at 4 °C with antibodies specific for BDNF (product number EPR1292, 1:1,000 dilution; Abcam, Cambridge, UK), TrkB (sc-12, 1:200; Santa Cruz Biotechnology, Dallas, TX), phospho-p44/42 MAPK (9101, 1:1,000; Cell Signaling Technology, Danvers, MA), and NeuN (12943, 1:1,000; Cell Signaling Technology). Antibodies specific for β-actin (clone C4, sc-47778; 1:200 dilution; Santa Cruz Biotechnology) and pan-ERK (610124, 1:1,000; BD Biosciences, San Jose, CA) were used as loading controls. After washing, the membrane was incubated with horseradish peroxidase–conjugated anti-rabbit (BDNF, TrkB, p44/42) or anti-mouse secondary antibodies (pan-ERK, actin) diluted in TBS-T with 2.5% skimmed milk for 1 hr at room temperature. Regarding target protein detection, an ECL Prime Western Blotting Detection system (GE Healthcare, Tokyo, Japan) was used to visualize the protein bands, and ImageJ software (National Institutes of Health, Bethesda, MD) was used to measure the integrated optical densities of the bands.

2.6 | Histological analysis

The left hemisphere of the brain was fixed with 4% paraformaldehyde, embedded in paraffin, and cut into 3.0-μm-thick coronal sections. On the basis of a previous study (Tsutsui et al., 2007), the sections were selected from −1.46 mm to −2.30 mm relative to bregma. The tissue sections were deparaffinized, and hematoxylin and eosin (HE) stain was applied to the sections. For cell counting, we selected the same location and arrangement of cells in the CA1 and CA3 regions (Kirino, Tamura, & Sano, 1986; Okihara et al., 2014), counted the numbers of neuronal cells present in each area, and calculated the cell number/40,000 μm^2. We used 1 section per animal for cell-counting analyses, which were performed by a single blind examiner who counted each section 3 times.

2.7 | Corticosterone enzyme-linked immunosorbent assay

We performed cardiocentesis during the perfusion of the mice, and collected blood samples in the evening. Blood samples were centrifuged

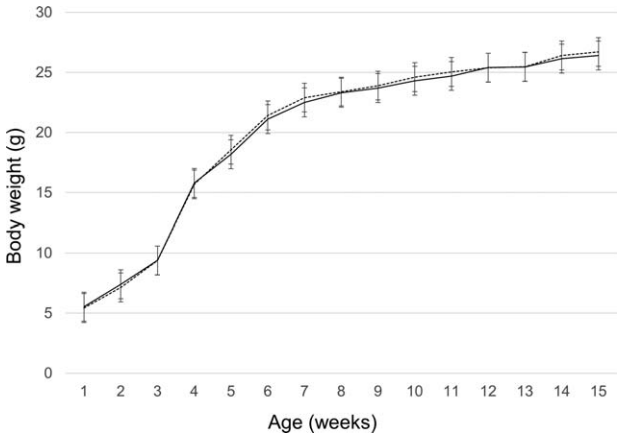

FIGURE 1 Mean body weights throughout the experimental period. There was no significant difference in weight between the age-matched UNO and the CONT groups of male BALB/C mice. CONT, control; UNO, unilateral nasal obstruction

at 1,000 rpm for 2 min at 4 °C to separate the serum. These samples were stored at −80 °C until the corticosterone assay was performed. Serum corticosterone levels were measured using an enzyme-linked immunosorbent assay kit (Yanaihara Institute Inc., Shizuoka, Japan) according to the manufacturer's instructions.

2.8 | Statistical analysis

Quantitative results are expressed as mean ± standard deviation and median values as appropriate. Intergroup comparisons of data were made using the Wilcoxon signed-rank test and Mann–Whitney *U* test. SPSS software (SPSS, Inc., Chicago, IL) was used for the statistical analyses. A p value $< .05$ was considered to indicate a significant difference.

3 | RESULTS

3.1 | Body weight

The mean body weights of all mice increased throughout the experimental period. There was no significant difference in this parameter between the age-matched UNO and CONT groups (Figure 1).

3.2 | SpO_2 levels

In the UNO group, the SpO_2 level at 15 weeks of age was significantly lower than that of the CONT group (96.02% ± 0.86% vs. 98.04% ± 0.30%, respectively, $p < .05$, median difference: 1.90; Figure 2).

3.3 | Y-maze test

The total arm entries were 29.86 ± 9.86 in the CONT group and 26.75 ± 9.72 in the UNO group. Here, the percentage of alternations made by the UNO group was significantly lower than that of the CONT group (46.05% ± 9.64% vs. 71.87% ± 4.83%, $p < .05$, median difference: 29.59; Figure 3).

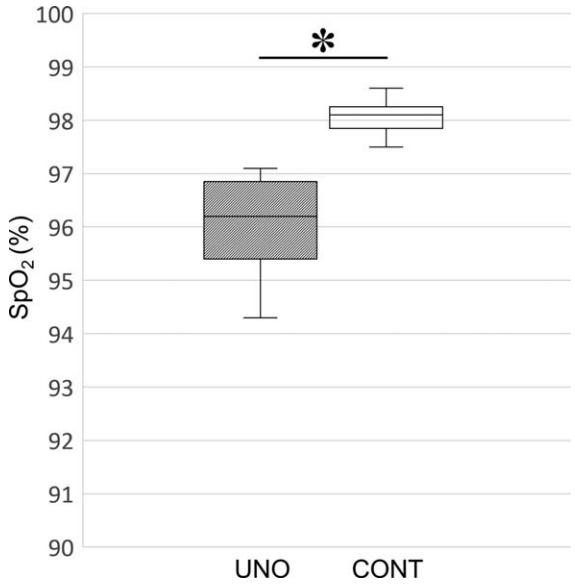

FIGURE 2 Mean SpO_2 levels at 15 weeks of age. The SpO_2 levels at 15 weeks of age were significantly lower in the UNO group than in the CONT group (96.02% ± 0.86% vs. 98.04% ± 0.30%, median difference: 1.90). *$p < .05$. Box edges represent the upper and lower quantiles, with the median values shown by the middle line in each box. The whiskers represent the maxima and minima. CONT, control; SpO_2, blood oxygenation saturation; UNO, unilateral nasal obstruction

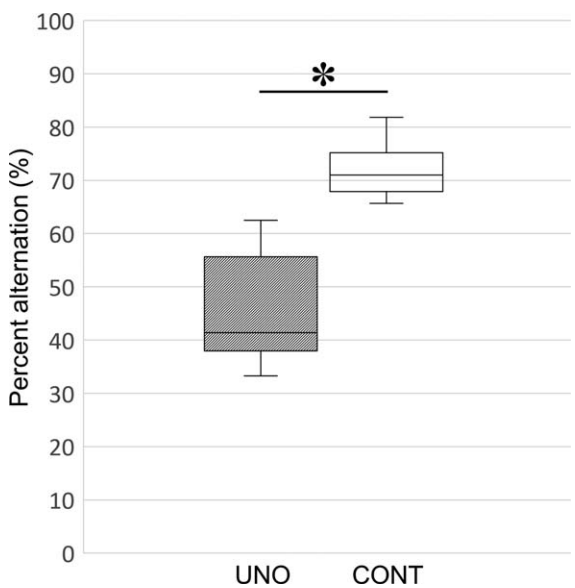

FIGURE 3 Mean percentage of alternations in the Y-maze test. The percentage of alternations in the UNO group was significantly lower than that of the CONT group (46.05% ± 9.64% vs. 71.87% ± 4.83%, median difference: 29.59). *$p < .05$. The numbers of total arm entries were 29.86 ± 9.26 in the CONT group and 26.75 ± 9.72 in the UNO group. Box edges represent the upper and lower quantiles with the median values shown by the middle line in each box. The whiskers represent the maxima and minima. CONT, control; UNO, unilateral nasal obstruction

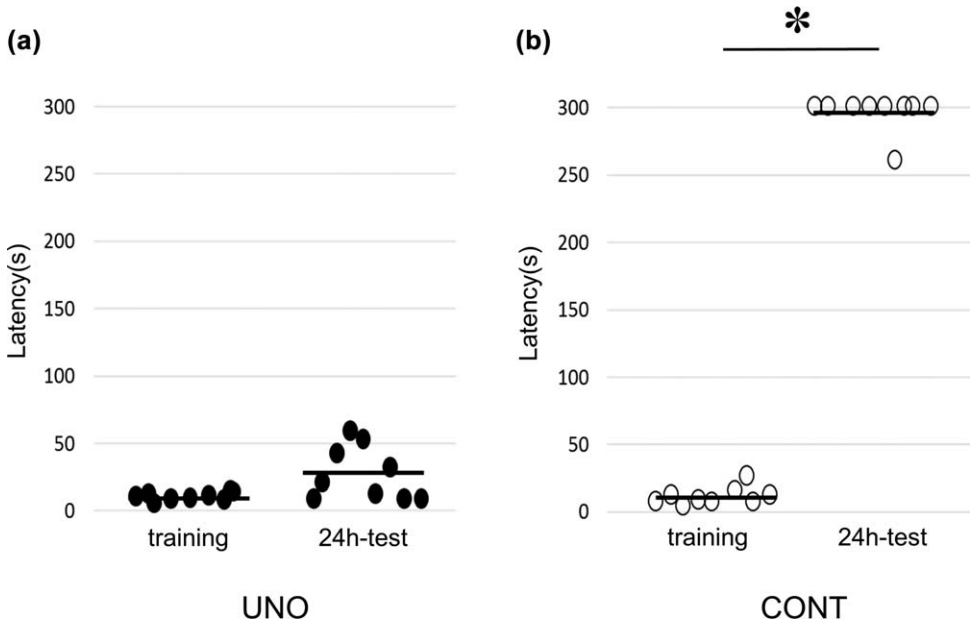

FIGURE 4 Latency in the passive avoidance test in the UNO (a) and CONT groups (b). There was no significant difference in the latency periods between the training and 24-hr tests in the UNO group (a). The latency period during training was significantly longer than that during the 24-hr test in the CONT group (b). $^*p < .05$. CONT, control; UNO, unilateral nasal obstruction

3.4 | Passive avoidance test

In the UNO group, the latency periods did not differ significantly between the training and 24-hr tests (median difference: 16.2; Figure 4a). By contrast, in the CONT group, the latency in the 24-hr test was significantly longer than that in the training test ($p < .05$, median difference: 291.7; Figure 4b).

3.5 | Western blot

In the hippocampus, the BDNF protein levels were significantly higher in the UNO group than in the CONT group (0.87 ± 0.14 vs. 0.52 ± 0.07, $p < .05$, median difference: 0.30; Figure 5a). By contrast, both the hippocampal TrkB protein levels (0.17 ± 0.04 vs. 0.31 ± 0.05, $p < .05$, median difference: 0.17; Figure 5b) and phospho-p44/p42 MAPK levels (0.26 ± 0.05 vs. 0.53 ± 0.07, $p < .05$, median difference: 0.27; Figure 5c) were significantly lower in the UNO group than in the CONT group.

3.6 | Histology

When the numbers of nerve cells were counted in the CA1 and CA3 regions of the hippocampus, we found that these values were significantly lower in the UNO group than in the CONT group (CA1: 46.4 ± 3.7 vs. 57.2 ± 3.0, median difference: 12.0; CA3: 47.4 ± 3.2 vs. 61.2 ± 3.8, median difference: 13.0; $p < .05$ for both; Figure 6).

3.7 | Serum corticosterone measurement

The serum corticosterone levels were significantly higher in the UNO group than in the CONT group (12.50 ± 2.74 vs. 7.64 ± 2.40 ng/ml, $p < .05$, median difference: 5.74; Figure 7).

4 | DISCUSSION

In this study, we investigated the effects of UNO during the growth period on the memory and learning abilities of male BALB/C mice. Our findings suggest that UNO during the growth period affects memory and learning.

Many studies have examined the effects of nasal respiratory disorders on morphological and functional changes in the craniofacial region in humans. Nasal congestion may lead to craniofacial changes, including mouth breathing, maximum protrusion, and adenoid facies (Harari et al., 2010; Pirilä-Parkkinen et al., 2009). Indeed, children with adenoid hypertrophy, allergic rhinitis, and oral respiration caused by nasal congestion exhibit deficits in their comprehensive, mathematical, and academic abilities and working memory compared with healthy children (Fensterseifer et al., 2013; Kuroishi et al., 2015; Walker et al., 2007). However, few studies have examined changes in memory and learning ability related to nasal obstruction. To our knowledge, the present study is the first to examine the mechanisms underlying the relationship between a unilateral nasal respiratory disorder and memory and learning ability.

Gozal, Daniel, & Dohanich (2001) reported that intermittent hypoxia (IH) causes neuronal apoptosis in the hippocampus, and a loss of hippocampal neurons is known to induce memory and learning deficits (Rosenzweig & Barnes, 2003). A previous study observed a decreased SpO_2 in an IH model compared with CONT animals (Newhouse et al., 2017). Similarly, we observed significant reductions in the SpO_2 level and the numbers of neurons in the hippocampal CA1 and CA3 regions in the UNO group relative to the CONT group at 15 weeks of age. These results suggest that UNO decreases the SpO_2 and induces a loss of hippocampal neurons, ultimately leading to memory

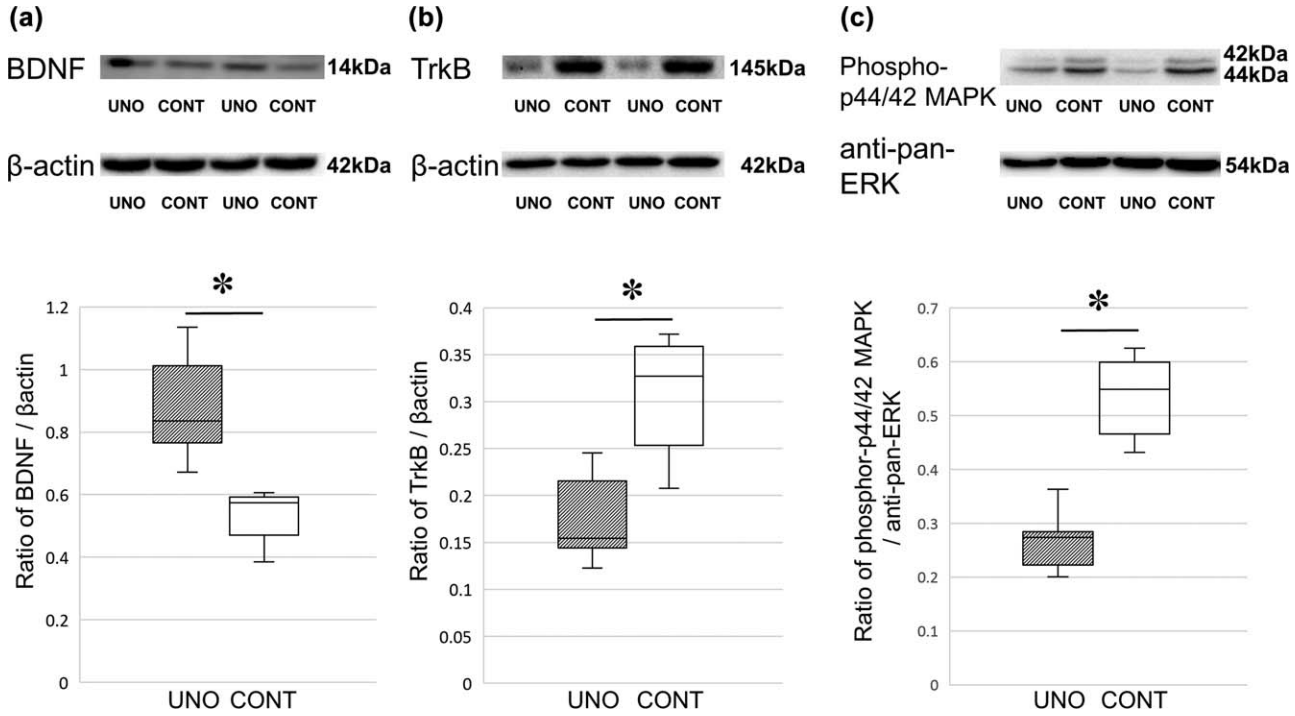

FIGURE 5 Protein levels of BDNF (a), TrkB (b), and phospho-p44/42 (c) in the hippocampus. The hippocampal levels of BDNF were significantly higher in the UNO group than in the CONT group (0.87 ± 0.14 vs. 0.52 ± 0.07, median difference: 0.30) (a). The hippocampal levels of TrkB were significantly lower in the UNO group than in the CONT group (0.17 ± 0.04 vs. 0.31 ± 0.05, median difference: 0.17) (b). The hippocampal levels of phospho-p44/42 MAPK were significantly lower in the UNO group than in the CONT group (0.26 ± 0.05 vs. 0.53 ± 0.07, median difference: 0.27) (c). $^*p < .05$. Box edges represent the upper and lower quantiles, with the median values shown by the middle line in each box. The whiskers represent the maxima and minima. BDNF, brain-derived neurotrophic factor; CONT, control; TrkB, tyrosine kinase B; UNO, unilateral nasal obstruction

and learning impairments. However, most previous studies have examined SpO_2 levels in IH models. Accordingly, further studies are needed to determine the mechanism linking nasal obstruction with memory and learning.

In our study, UNO led to decreases in the numbers of cells in the hippocampal CA1 and CA3 regions. We note that although HE is a nonspecific cellular stain, the majority of the cells in the hippocampus are neurons and glial cells; in particular, approximately 40% of cells in the CA1 and CA3 regions are neurons (Simić, Kostović, Winblad, & Bogdanović, 1997). Although the number of stained cells was not equal to the number of neurons, unlike glial cells, neurons have a large cytoplasm, which allows the distinction of the two cell types. It is likely that the reduced cell numbers represent a UNO-induced loss of hippocampal neurons.

We also found that UNO induced changes in BDNF/TrkB signaling, as indicated by the phospho-p44/p42 MAPK levels. Significantly higher hippocampal levels of BDNF protein were observed in the UNO group relative to the CONT group, whereas the former group exhibited significantly lower levels of TrkB and phospho-p44/p42 MAPK relative to the latter group. Both BDNF and TrkB are necessary for neuronal development and maintenance, as well as memory and learning (Klein, Parada, Coulier, & Barbacid, 1989; Klein et al., 1993), and impairments in BDNF/TrkB signaling cause memory and learning deficits (Okihara et al., 2014). The binding of BDNF to TrkB induces the phosphorylation of p44/p42 MAPK, which is also important for the maintenance of memory and learning (Gooney, Shaw, Kelly, O'Mara, & Lynch, 2002). Our study findings of impaired BDNF/TrkB signaling and decreased p44/p42 MAPK phosphorylation suggest that long-term memory and learning impairments are more likely to occur in the UNO group than in the CONT group. A previous human study found that hypoxia induced BDNF overexpression in the blood (Helan et al., 2014), and a significant positive correlation between cortical and serum BDNF levels has been identified (Karege, Schwald, & Cisse, 2002). In addition, BDNF protein overexpression has been linked with memory and learning impairments in mice (Papaleo et al., 2011). Moreover, an infusion of BDNF into the rat hippocampus was shown to cause the downregulation of TrkB (Frank, Ventimiglia, Anderson, Lindsay, & Rudge, 1996), and decreased TrkB expression has been shown to induce declines in long-term memory (Minichiello et al., 1999). A previous study suggested that occlusal hypofunction induces BDNF overexpression by decreasing TrkB expression (Okihara et al., 2014). However, researchers have demonstrated that repetitive stress induces high corticosterone levels and reduces the expression of BDNF mRNA (Smith, Makino, Kvetnansky, & Post, 1995). In our study, we observed higher BDNF protein levels consequent to nasal

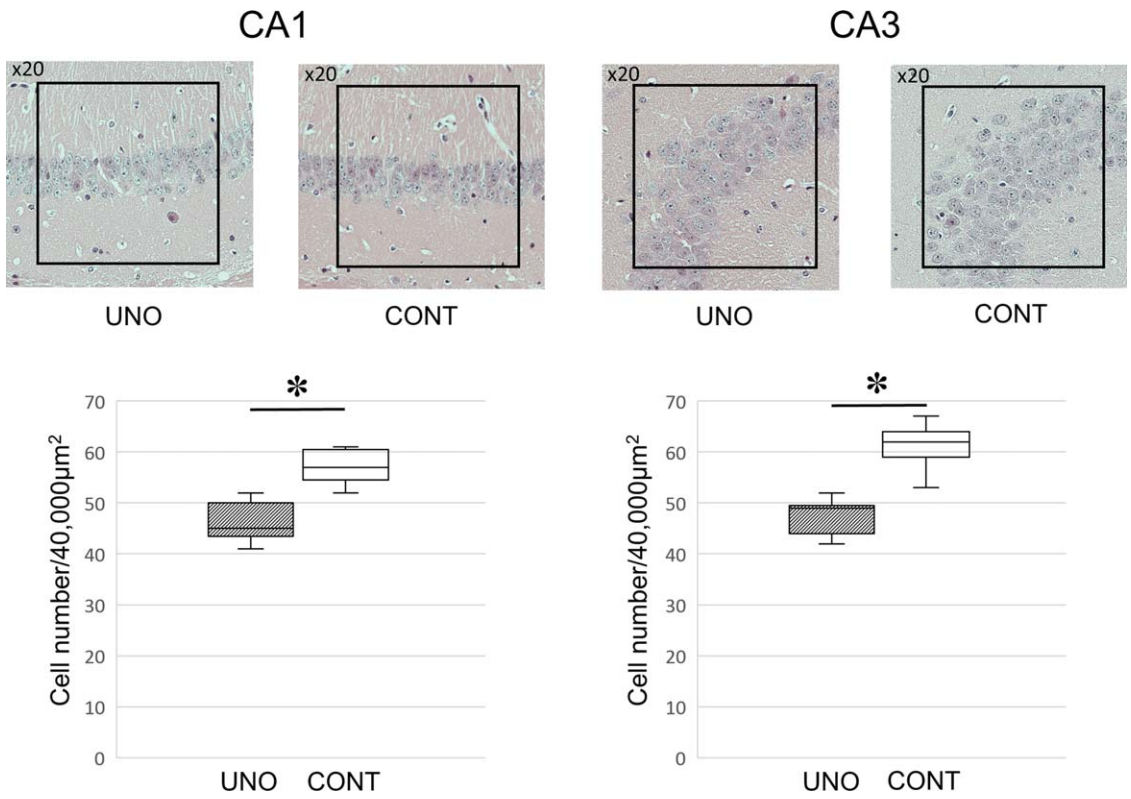

FIGURE 6 Nerve cell numbers in the CA1 and CA3 regions of the hippocampus. The figure magnification is 20-fold. We counted the number of neurons in the area surrounded by a square (40.000 μm). The numbers of nerve cells in the CA1 and CA3 regions of the hippocampus were significantly lower in the UNO group than in the CONT group (CA1: 46.4 ± 3.7 vs. 57.2 ± 3.0, $p < .05$, median difference: 12.0; CA3: 47.4 ± 3.2 vs. 61.2 ± 3.8, $p < .05$, median difference: 13.0). Box edges represent the upper and lower quantiles, with the median values shown by the middle line in each box. The whiskers represent the maxima and minima. CONT, control; UNO, unilateral nasal obstruction

obstruction. These results suggest that nasal obstruction leads to higher corticosterone levels and increases the expression of BDNF protein, while decreasing the expression of BDNF mRNA in a negative-feedback manner. Further studies of BDNF and TrkB mRNA expression are needed to clarify these alterations in BDNF/TrkB signaling.

Our findings suggest that UNO leads to a reduction in SpO_2, overexpression of BDNF, and downregulation of TrkB. Accordingly, reduced signaling downstream of the BDNF/TrkB axis decreases the phosphorylation of p44/42 MAPK. As mentioned above, our findings suggest that these changes in protein expression and signaling induce memory and learning impairments. Similarly, another study showed that impaired BDNF/TrkB signaling induced a loss of nerve cells (Numakawa et al., 2010). Furthermore, we did not observe a significant difference in hippocampal NeuN protein levels between the UNO and CONT groups (data not shown). It has been shown that there is no difference between two groups in the number of neurons of the whole hippocampus. However, the number of neurons in the CA1 and CA3 regions in the UNO group was decreased in comparison with the CONT group in our results. It was likely difficult to detect a difference in Western blot because the ratio of CA1 and CA3 neurons to the number of neurons in the whole hippocampus was small. To be precise,

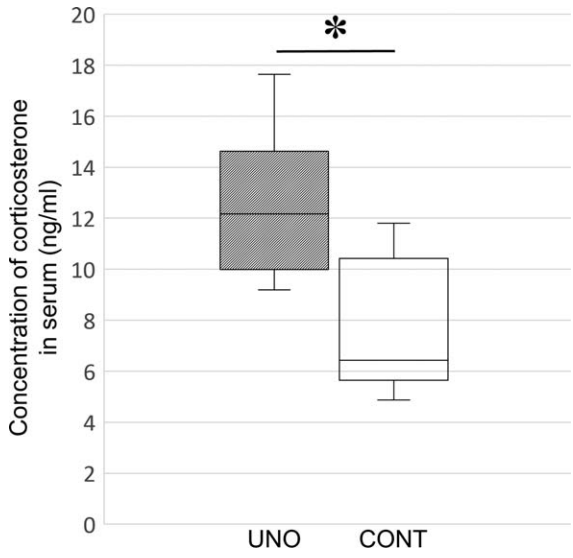

FIGURE 7 Blood corticosterone levels in 15-week-old mice. The blood corticosterone levels were significantly higher in the UNO group than in the CONT group (12.50 ± 2.74 vs. 7.64 ± 2.40 ng/ml, median difference: 5.74,). $^*p < .05$. Box edges represent the upper and lower quantiles, with the median values shown by the middle line in each box. The whiskers represent the maxima and minima. CONT, control; UNO, unilateral nasal obstruction

the immunostaining of NeuN is necessary; unfortunately, we have already used all the samples of sections for data analysis. Therefore, further studies are needed to clarify this. Thus, the present results suggest that impaired BDNF/TrkB signaling may induce memory and learning impairments via neuronal loss.

Corticosterone is a corticosteroid-type steroid hormone synthesized in the adrenal cortex. In humans, corticosterone is a weakly potent glucocorticoid and mineralocorticoid. However, in rodents, corticosterone is the main glucocorticoid involved in the regulation of energy metabolism, immune reactions, and stress responses (Koolhaas et al., 1999). In an animal model, stress was found to enhance the secretion of corticosterone in the blood (Diorio, Viau, & Meaney, 1996). Elevated corticosterone levels have been linked to a loss of nerve cells in the hippocampus, resulting in memory and learning impairments (Song et al., 2006). The present results show that the serum corticosterone concentrations in the UNO group were significantly higher than those in the CONT group. The UNO and CONT groups differed only in the presence or absence of UNO. Therefore, UNO itself appears to increase stress and promote the increased secretion of corticosterone, followed by a loss of hippocampal neurons and memory and learning impairments.

The present study is the first to demonstrate the effects of nasal obstruction during adolescence on learning and memory ability. The decrease in SpO_2 and/or increase in stress induced by UNO altered BDNF/TrkB signaling and consequently decreased phospho-p44/42 MAPK levels, leading to a loss of nerve cells in the hippocampus and the deterioration of memory and learning abilities. However, although the aforementioned pathway is the most plausible with regard to the memory and learning impairments induced by UNO, further studies are needed to fully understand the mechanisms underlying such impairments.

ACKNOWLEDGMENTS

The authors thank Dr. Jin-ichi Ito and Dr. John Cha-Gyun for providing technical support during the Western blotting experiments.

CONFLICT OF INTEREST

None of the authors have any competing or conflicting interests related to this manuscript.

AUTHOR CONTRIBUTIONS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the analysis. The following authors were responsible for various areas of the study: Conceptualization and Methodology: T.Ogawa, H.O, S. K, T.Y. Investigation: T.Ogawa, H.O, Y.A, U K. KH, M.Makiguchi. Formal analysis: T.Ogawa, H.O, S.K, C.K, T.Y, M.Michikawa, T.Ono. Writing - original draft: T.Ogawa, H.O. Writing - review & editing: M.Michikawa, T.Ono. Funding acquisition: H.Okihara, T.Yabushita. Supervision: T.Ono.

ORCID

Hidemasa Okihara http://orcid.org/0000-0002-3762-2597

REFERENCES

Alonso, M., Vianna, M. R., Izquierdo, I., & Medina, J. H. (2002). Signaling mechanisms mediating BDNF modulation of memory formation in vivo in the hippocampus. *Cellular and Molecular Neurobiology*, *22*, 663–674.

Barde, Y. A. (1994). Neurotrophins: A family of proteins supporting the survival of neurons. *Progress in Clinical and Biological Research*, *390*, 45–56.

Bavis, R. W., vanHeerden, E. S., Brackett, D. G., Harmeling, L. H., Johnson, S. M., Blegen, H. J., ... Fallon, S. C. (2014). Postnatal development of eupneic ventilation and metabolism in rats chronically exposed to moderate hyperoxia. *Respiratory Physiology & Neurobiology*, *198*, 1–12.

Bhattacharyya, N. (2012). Sleep and health implications of snoring: A populational analysis. *Laryngoscope*, *125*, 2413–2416.

Bucca, C., Rolla, G., Scappaticci, E., Chiampo, F., Bugiani, M., Magnano, M., & D'Alberto, M. (1995). Extrathoracic and intrathoracic airway responsiveness in sinusitis. *Journal of Allergy and Clinical Immunology*, *95*, 52–59.

Camelo-Nunes, I. C., & Sole, D. (2010). Allergic rhinitis: Indicators of quality of life. *Jornal Brasileiro de Pneumologia*, *36*, 124–133.

Castro, T. M., Marinho, D. R., & Cavalcante, C. C. (2013). The impact of environmental factors on quality of life and symptoms of children with allergic rhinitis. *Brazilian Journal of Otorhinolaryngology*, *79*, 569–574.

Craig, T. J., Teets, S., Lehman, E. B., Chinchilli, V. M., & Zwillich, C. (1998). Nasal congestion secondary to allergic rhinitis as a cause of sleep disturbance and daytime fatigue and the response to topical nasal corticosteroids. *Journal of Allergy and Clinical Immunology*, *101*, 633–637.

de Quervain, D. J., Roozendaal, B., & McGaugh, J. L. (1998). Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature*, *20*, 787–790.

Diorio, D., Viau, V., & Meaney, M. J. (1993). The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *Journal of Neuroscience*, *13*, 3839–3847.

Eichenbaum, H. (2015). The hippocampus as a cognitive map of social space. *Neuron*, *87*, 9–11.

Emslie, R. D., Massler, M., & Zwemer, J. D. (1952). Mouth breathing. I. Etiology and effects; a review. *Journal of the American Dental Association*, *44*, 506.

Fensterseifer, G. S., Carpes, O., Weckx, L. L., & Martha, V. F. (2013). Mouth breathing in children with learning disorders. *Brazilian Journal of Otorhinolaryngology*, *79*, 620–624.

Frank, L., Ventimiglia, R., Anderson, K., Lindsay, R. M., & Rudge, J. S. (1996). BDNF down-regulates neurotrophin responsiveness, TrkB protein and TrkB mRNA levels in cultured rat hippocampal neurons. *European Journal of Neuroscience*, *8*, 1220–1230.

Funaki, Y., Hiranuma, M., Shibata, M., Kokai, S., & Ono, T. (2014). Effects of nasal obstruction on maturation of the jaw-opening reflex in growing rats. *Archives of Oral Biology*, *59*, 530–538.

Gooney, M., Shaw, K., Kelly, A., O'Mara, S. M., & Lynch, M. A. (2002). Longterm potentiation and spatial learning are associated with increased phosphorylation of TrkB and extracellular signal-regulated kinase (ERK) in the dentate gyrus: Evidence for a role for brain-derived neurotrophic factor. *Behavioral Neuroscience*, *116*, 455–463.

Gozal, D., Daniel, J. M., & Dohanich, G. P. (2001). Behavioral and anatomical correlates of chronic episodic hypoxia during sleep in the rat. *Journal of Neuroscience*, *1*, 2442–2450.

Gross, R. B. (1974). Growth variations associated with induced nasal obstruction in the albino rat. *Angle Orthodontist*, *44*, 39–42.

Harari, D., Redlich, M., Miri, S., Hamud, T., & Gross, M. (2010). The effect of mouth breathing versus nasal breathing on dentofacial and craniofacial development in orthodontic patients. *Laryngoscope*, *120*, 2089–2093.

Helan, M., Helan, M., Aravamudan, B., Hartman, W. R., Thompson, M. A., Johnson, B. D., … Prakash, Y. S. (2014). BDNF secretion by human pulmonary artery endothelial cells in response to hypoxia. *Journal of Molecular and Cellular Cardiology*, *68*, 89–97.

Holcomb, L. A., Gordon, M. N., Jantzen, P., Hsiao, K., Duff, K., & Morgan, D. (1999). Behavioral changes in transgenic mice expressing both amyloid precursor protein and presenilin-1 mutations: Lack of association with amyloid deposits. *Behavior Genetics*, *29*, 177–185.

Karege, F., Schwald, M., & Cisse, M. (2002). Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. *Neuroscience Letters*, *328*, 261–264.

Kempermann, G., Kuhn, H. G., & Gage, F. H. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature*, *386*, 493–495.

Klein, R., Parada, L. F., Coulier, F., & Barbacid, M. (1989). TrkB, a novel tyrosine protein kinase receptor expressed during mouse neural development. *EMBO Journal*, *8*, 3701–3709.

Klein, R., Smeyne, R. J., Wurst, W., Long, L. K., Auerbach, B. A., Joyner, A. L., & Barbacid, M. (1993). Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. *Cell*, *75*, 113–122.

Kim, S. Y., Kim, M. S., Park, B., Kim, J. H., & Choi, H. G. (2017). Allergic rhinitis, atopic dermatitis, and asthma are associated with differences in school performance among Korean adolescents. *PLoS One*, *16*, e0171394.

Kirino, T., Tamura, A., & Sano, K. (1986). A reversible type of neuronal injury following ischemia in the gerbil hippocampus. *Stroke*, *17*, 455–459.

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., … Blokhuis, H. J. (1999). Coping styles in animals: Current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews*, *23*, 925–935.

Kubo, K. Y., Yamada, Y., Iinuma, M., Iwaku, F., Tamura, Y., Watanabe, K., … Onozuka, M. (2007). Occlusal disharmony induces spatial memory impairment and hippocampal neuron degeneration via stress in SAMP8 mice. *Neuroscience Letters*, *6*, 188–191.

Kuroishi, R. C., Garcia, R. B., Valera, F. C., Anselmo-Lima, W. T., & Fukuda, M. T. (2015). Deficits in working memory, reading comprehension and arithmetic skills in children with mouth breathing syndrome: Analytical cross-sectional study. *São Paulo Medical Journal*, *133*, 78–83.

Lee, J. S., Kim, H. G., Lee, H. W., Kim, W. Y., Ahn, Y. C., & Son, C. G. (2017). Pine needle extract prevents hippocampal memory impairment in acute restraint stress mouse model. *Journal of Ethnopharmacology*, *207*, 226–236.

Lewin, G. R., & Barde, Y. A. (1996). Physiology of the neurotrophins. *Annual Review of Neuroscience*, *19*, 289–317.

Lite, T. (1949). Mouth breathing, a contributing factor in the etiology of gingival irritation. *Journal of Dental Medicine*, *4*, 13.

Minichiello, L., Korte, M., Wolfer, D., Kühn, R., Unsicker, K., Cestari, V., … Klein, R. (1999). Essential role for TrkB receptors in hippocampus-mediated learning. *Neuron*, *24*, 401–414.

Newhouse, L. P., Joyner, M. J., Curry, T. B., Laurenti, M. C., Man, C. D., Cobelli, C., … Limberg, J. K. (2017). Three hours of intermittent hypoxia increases circulating glucose levels in healthy adults. *Physiological Reports*, *5*, e13106.

Numakawa, T., Suzuki, S., Kumamaru, E., Adachi, N., Richards, M., & Kunugi, H. (2010). BDNF function and intracellular signaling in neurons. *Histology and Histopathology*, *25*, 237–258.

Okihara, H., Ito, J., Kokai, S., Ishida, T., Hiranuma, M., Kato, C., … Michikawa, M. (2014). Liquid diet induces memory impairment accompanied by a decreased number of hippocampal neurons in mice. *Journal of Neuroscience Research*, *92*, 1010–1017.

Padzys, G. S., Thornton, S. N., Martrette, J. M., & Trabalon, M. (2011). Effects of short term forced oral breathing in rat pups on weight gain, hydration and stress. *Physiology & Behavior*, *102*, 175–180.

Papaleo, F., Silverman, J. L., Aney, J., Tian, Q., Barkan, C. L., Chadman, K. K., & Crawley, J. N. (2011). Working memory deficits, increased anxiety-like traits, and seizure susceptibility in BDNF overexpressing mice. *Learning & Memory*, *26*, 534–544.

Pirilä-Parkkinen, K., Pirttiniemi, P., Nieminen, P., Tolonen, U., Pelttari, U., & Löppönen, H. (2009). Dental arch morphology in children with sleep-disordered breathing. *European Journal of Orthodontics*, *31*, 160–167.

Reichardt, L. F. (2006). Neurotrophin-regulated signaling pathways. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *361*, 1545–1564.

Rosenzweig, E. S., & Barnes, C. A. (2003). Impact of aging on hippocampal function: Plasticity, network dynamics, and cognition. *Progress in Neurobiology*, *69*, 143–179.

Sapolsky, R. M. (2000). Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Archives of General Psychiatry*, *57*, 925–935.

Simić, G., Kostović, I., Winblad, B., & Bogdanović, N. (1997). Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. *Journal of Comparative Neurology*, *379*, 482–494.

Smith, M. A., Makino, S., Kvetnansky, R., & Post, R. M. (1995). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *Journal of Neuroscience*, *15*, 1768–1777.

Song, L., Che, W., Min-Wei, W., Murakami, Y., & Matsumoto, K. (2006). Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. *Pharmacology, Biochemistry, and Behavior*, *83*, 186–193.

Sweatt, J. D. (2001). The neuronal MAP kinase cascade: A biochemical signal integration system subserving synaptic plasticity and memory. *Journal of Neurochemistry*, *76*, 1–10.

Thoenen, H. (1995). Neurotrophins and neuronal plasticity. *Science*, *270*, 593–598.

Thoenen, H. (2000). Neurotrophins and activity-dependent plasticity. *Progress in Brain Research*, *128*, 183–191.

Tsutsui, K., Kaku, M., Motokawa, M., Tohma, Y., Kawata, T., Fujita, T., … Tanne, K. (2007). Influences of reduced masticatory sensory input from soft-diet feeding upon spatial memory/learning ability in mice. *Biomedical Research*, *28*, 1–7.

Uchima Koecklin, K. H., Kato, C., Funaki, Y., Hiranuma, M., Ishida, T., Fujita, K., … Ono, T. (2015). Effect of unilateral nasal obstruction on tongue protrusion forces in growing rats. *Journal of Applied Physiology*, *118*, 1128–1135.

Walker, S., Khan-Wasti, S., Fletcher, M., Cullinan, P., Harris, J., & Sheikh, A. (2007). Seasonal allergic rhinitis is associated with a detrimental effect on examination performance in United Kingdom teenagers: Case-control study. *Journal of Allergy and Clinical Immunology*, *120*, 381–387.

Yamagata, Y., Kobayashi, S., Umeda, T., Inoue, A., Sakagami, H., Fukaya, M., … Okabe, S. (2009). Kinase-dead knock-in mouse reveals an essential role of kinase activity of Ca21/calmodulin-dependent

protein kinase II alpha in dendritic spine enlargement, long-term potentiation, and learning. *Journal of Neuroscience*, *29*, 7607–7618.

Yamazaki, K., Wakabayashi, N., Kobayashi, T., & Suzuki, T. (2008). Effect of tooth loss on spatial memory and trkB-mRNA levels in rats. *Hippocampus*, *18*, 542–547.

Zola-Morgan, S., Squire, L. R., & Amaral, D. G. (1986). Human amnesia and the medial temporal region: Enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. *Journal of Neuroscience*, *6*, 2950–2967.

How to cite this article: Ogawa T, Okihara H, Kokai S, et al. Nasal obstruction during adolescence induces memory/learning impairments associated with BDNF/TrkB signaling pathway hypofunction and high corticosterone levels. *J Neuro Res*. 2018;96:1056–1065. https://doi.org/10.1002/jnr.24216