Pathological Effects of Obstructive Apneas During the Sleep Cycle in an Animal Model of Cerebral Small Vessel Disease

Eric E. Lloyd, David J. Durgan, Sharyl R. Martini, Robert M. Bryan

Abstract—We tested the hypothesis that apneas during the sleep cycle exacerbate hypertension and accelerate changes that occur with cerebral small vessel disease. Obstructive sleep apnea was modeled by intermittent inflations of a chronically implanted tracheal balloon to occlude the airway during the sleep cycle (termed OSA) in spontaneously hypertensive stroke-prone (SHRSP) rats, a model of cerebral small vessel disease. SHRSP rats and their parent strain, Wistar Kyoto (WKY) rats, were exposed to OSA for 2 weeks (from 9 to 11 or from 18 to 20 weeks). At 9 weeks, hypertension was developing in the SHRSP rats and was firmly established by 18 weeks. OSA exposure increased systolic blood pressure in SHRSP rats by \(\approx 30\) mmHg in both age groups compared with shams that were surgically prepared but not exposed to OSA \((P<0.05)\). OSA exposure also increased systolic blood pressure in WKY rats by 20 and 37 mmHg at 11 and 20 weeks, respectively \((P<0.05)\). OSA exposure in SHRSP rats compromised blood–brain barrier integrity in white matter at both 9 and 20 weeks of age when compared with SHRSP sham rats \((P<0.05)\). Microglia were activated in SHRSP rats exposed to OSA but not in sham rats at 11 weeks \((P<0.05)\). At 20 weeks, microglia were activated in sham SHRSP rats \((P<0.05)\) compared with WKY sham rats and were not further activated by OSA. Neither was blood–brain barrier integrity altered nor microglia activated in any of the WKY groups. We conclude that OSA accelerates the onset of the cerebral pathologies associated with cerebral small vessel disease in SHRSP, but not WKY, rats. (Hypertension. 2015;66:00-00. DOI: 10.1161/HYPERTENSIONAHA.115.05764.) • Online Data Supplement

Key Words: blood-brain barrier • cerebral small vessel disease • obstructive sleep apnea • stroke-prone spontaneously hypertensive rats • vascular cognitive impairment

Obstructive sleep apnea (OSA) is a pathological condition in which the upper airway collapses during sleep to partially (hypopnea) or completely (apnea) restrict the movement of air into the lungs during inhalation.\(^1\) With the onset of hypopnea or apnea, the ventilatory effort increases, producing arousal to a lighter stage of sleep where a patent airway can be reestablished.\(^2\) In extreme cases, the airway can repeatedly collapse throughout sleep at rates exceeding 100 apneas per hour. Each episode of apnea produces progressive hypoxia, progressive hypercapnia, arousal from sleep, and a negative intrathoracic pressure as the breathing effort continues against a closed airway.\(^1\)

OSA is an independent risk factor or is closely associated with several cardiovascular (including cerebrovascular) diseases.\(^1,3-9\) However, the role of OSA in the development of one cerebrovascular disease, cerebral small vessel disease (CSVD), has not been extensively studied. CSVD is characterized by pathology of the small penetrating vessels supplying subcortical structures, leading to blood–brain barrier (BBB) disruption, neuroinflammation, lacunar infarcts, microbleeds, and cognitive dysfunction.\(^10-13\) Although studies have not directly focused on OSA as a potential contributor to CSVD development or progression per se, several studies have attempted to determine whether OSA is associated with CSVD-like injuries to the brain, that is, brain injuries that can, but not necessarily, result from CSVD.\(^2\) We tested the hypothesis that apneas during the sleep cycle exacerbate hypertension and accelerate pathological changes that occur with CSVD. We used a model of OSA, where a balloon, chronically implanted in the trachea, was remotely inflated to produce 60 apneas per hour (considered severe in humans) for 8 hours of the sleep cycle.\(^2,21\) We evaluated the rats for CSVD-related pathological changes, including increases in systolic blood pressure, disruption of the BBB, neuroinflammation, white matter integrity, and cognitive deficits. We show that OSA in SHRSP rats leads to exacerbated hypertension, decreased BBB integrity, microgli activation, and cognitive impairment.

Received April 30, 2015; first decision May 18, 2015; revision accepted July 20, 2015.
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The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.115.05764/-/DC1.

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Hypertension is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.115.05764
Methods
A complete description of the methods can be found in the online-only Data Supplement. All studies were approved by the Institutional Animal Care and Use Committee at the Baylor College of Medicine. Male inbred SHRSP and Wistar Kyoto (WKY) rats were maintained on a regular rodent chow diet and housed in a satellite facility with 12-hour light (0600–1800 h) and 12-hour dark (1800 to 0600 h) cycle.

At either 8 or 17 weeks of age, rats were prepared with a chronically implanted intratracheal balloon that could be remotely inflated to occlude the trachea to model OSA.24,25 One week was allowed for recovery from the surgical procedure. Rats were subjected to repeated apneas (60 apneas per hour for 8 hours during the sleep cycle, with each apnea lasting 10 s). The repeated apneas continued during the sleep cycle for 2 weeks. Sham rats were instrumented with the balloon implants, but did not undergo any apneas.

Systolic blood pressure was measured using tail-cuff plethysmography. BBB integrity was assessed by the extravasation of Evans blue dye after an IV injection and by extravasation of immunoglobulin G (IgG), a protein found only in plasma when the BBB is intact. Astrocytes were visualized with an antibody directed against GFAP, and microglia were visualized using an antibody directed against Iba-1. Morphometric analysis was used to determine the activation state of microglia as previously described.26 The Kluver Barrera method was used to visualize white matter from brain sections. The novel object recognition test27,28 was used to determine working memory by measuring the ability of each rat to discriminate between a novel object and an object previously presented to the rat.

Parametric data are expressed as mean±SEM, and nonparametric data are presented using box and whisker plots with median values. Differences between groups were determined using 2-way repeated measures ANOVA or ANOVA on ranks followed by a Holm–Sidak test when appropriate. Differences were considered as statistically significant if P≤0.05.

Results
Figure 1 shows systolic blood pressures for SHRSP and WKY rats before and after 2 weeks of OSA or sham. Systolic blood pressure was significantly greater in the SHRSP rats at all times compared with the WKY rats. Hypertension was still developing in the SHRSP rats at 9 weeks (Figure 1A), as noted in the SHRSP shams, but had plateaued at 18 weeks of age (Figure 1B; also see Figure S1 in the online-only Data Supplement). In both strains and at both ages, OSA significantly increased systolic blood pressure. Systolic blood pressure increased by 20 and 37 mm Hg at 11 and 20 weeks in the WKY rats after OSA when compared with the corresponding WKY sham rats, respectively. In the SHRSP rats, systolic blood pressures were 28 and 32 mm Hg greater in OSA rats compared with shams at 11 and 20 weeks, respectively. Thus, OSA increased systolic blood pressure in both strains in both age groups.

Weight changes for all groups of rats are shown in Figure S2. BBB permeability as measured by Evans blue extravasation after an IV infusion is shown in Figure 2. Evans blue extravasation was significantly increased after OSA in white matter at 11 weeks in the SHRSP rats compared with the sham rats (Figure 2B). Although not statistically significant, the data suggest a potential increase in extravasation at 20 weeks in white and gray matter in SHRSP rats undergoing OSA (P=0.14 and 0.08, respectively). There were no significant differences in Evans blue extravasation in WKY rats as a result of OSA in either age groups (Figure 2A).

Figure S3 shows extravasation of IgG from arterioles in OSA and sham rats for both strains and at both ages. Note the IgG (green fluorescence) surrounding the arterioles in the SHRSP rat after OSA at 11 weeks, with even greater extravasation shown in the same group at 20 weeks of age. Figure 3 summarizes the results for IgG extravasation. No IgG extravasation occurred in SHRSP sham rats at 11 weeks of age or in WKY rats in any of the groups. Although not statistically significant, there is a suggestion for BBB disruption in SHRSP rats after OSA at 11 weeks of age (Figure S3; Figure 3). By 20 weeks of age, the SHRSP rats were showing BBB disruption in the sham group, and OSA increased this even further. Figure S4 shows microglia (green) at 11 weeks of age. Microglia activation was assessed using a categorical scale from 1 to 4, where 1 was unactivated microglia and 4 was highly activated microglia.26 Figure 4 shows a boxplot summary of the results for microglia activation. In the cingulate gyrus, microglia activation was significantly greater in the SHRSP sham rats at 11 weeks compared with WKY sham rats at the same age. The microglia were further activated, but not significantly, by OSA in the SHRSP rats at 11 weeks. Microglia were significantly activated by OSA in the corpus callosum and external capsule at 11 weeks in the SHRSP rats.
CSVD,12,13,17,29 we tested the hypothesis that apneas during the sleep cycle has similarities to the risk factors associated with CSVD. The specific neuropathological changes that occurred with the aging process from 11 to 20 weeks in SHRSP sham rats (ie, not undergoing apneas). Consistent with our hypothesis, we demonstrated that both aging and OSA in SHRSP rats increased systolic blood pressure (Figure S1; Figure 1), accelerated breakdown of the BBB (Figures 2 and 3; Figure S3), and activated microglia (Figure 4; Figure S4). At 20 weeks of age, OSA further damaged the BBB and further increased systolic blood pressure. Microglia of 20 week SHRSP sham rats were already activated and were not further activated by OSA (Figure 4). Additionally, we observed that SHRSP rats exposed to OSA had cognitive deficits that were not observed in sham rats at either age (Figure 5). Thus, cognitive changes occurring with OSA in SHRSP rats exceeded any cognitive changes, if they occurred, between 9 and 20 weeks of age in sham SHRSP rats. The above changes resulting from OSA in SHRSP rats are consistent with our hypothesized changes.

We did not observe damage to deep brain structures, including disorganization or disarray of white matter tracts, white matter loss, microinfarcts, microbleeds, or astrogliosis, in any SHRSP rats, regardless of age or treatment (Figures S5–S7). As the complications of CSVD progress in SHRSP rats or in humans, white matter is damaged and ultimately lost.12,13,23,29 Histologically, white matter tracks can be seen to be disturbed, and myelin basic protein, a major constituent of white matter, can be significantly decreased.

SHRSP rats on a conventional diet begin to develop subcortical lesions and loss of white matter starting around 20 weeks of age or older depending on the colony or laboratory conducting the studies.23 However, when placed on a high Na+/low K+ diet, 1% NaCl in drinking water, and perfusion deficit produced by carotid artery ligation, the disease process can be dramatically accelerated to the point where subcortical lesions and loss of white matter occur by 20 weeks.23,29

Discussion

OSA initiates a pathological cascade that consists of sympathetic activation, inflammation, oxidative stress, endothelial dysfunction, and metabolic disorders.1 Given that this cascade has similarities to the risk factors associated with CSVD,12,13,17,29 we tested the hypothesis that apneas during the sleep cycle exacerbate hypertension and accelerate pathological changes that occur with CSVD. The specific neuropathological changes included neuroinflammation, BBB integrity, white matter integrity, and cognitive function. If our hypothesis is valid, then 2 weeks of OSA (9–11 weeks) in 11-week-old SHRSP rats should produce similar pathological changes that occurred with the aging process from 11 to 20 weeks in SHRSP sham rats (ie, not undergoing apneas). Consistent with our hypothesis, we demonstrated that both aging and OSA in SHRSP rats increased systolic blood pressure (Figure S1; Figure 1), accelerated breakdown of the BBB (Figures 2 and 3; Figure S3), and activated microglia (Figure 4; Figure S4). At 20 weeks of age, OSA further damaged the BBB and further increased systolic blood pressure. Microglia of 20 week SHRSP sham rats were already activated and were not further activated by OSA (Figure 4). Additionally, we observed that SHRSP rats exposed to OSA had cognitive deficits that were not observed in sham rats at either age (Figure 5). Thus, cognitive changes occurring with OSA in SHRSP rats exceeded any cognitive changes, if they occurred, between 9 and 20 weeks of age in sham SHRSP rats. The above changes resulting from OSA in SHRSP rats are consistent with our hypothesized changes.

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Figure 5. Novel object recognition, a measure of working memory, in the spontaneously hypertensive stroke-prone (SHRSP) sham and SHRSP obstructive sleep apnea (OSA) rats at 11 and 20 weeks of age. Rats were subjected to a training session, where 2 identical objects were placed in front of the rats for 3 minutes. Time spent at each object was recorded. The 2 objects were subsequently removed, and 1 minute later, the rats were subjected to a working session, where an object with identical characteristics to the original objects used in the training session and one novel object were placed in front of the rats. Time spent at each object was again recorded. With normal working memory, rats tend to spend more time exploring the novel object as indicated by a positive discrimination ratio.

We were expecting OSA to accelerate the disease process in SHRSP rats (especially at 20 weeks of age) to a similar degree. However, our expectations that subcortical lesions, including disorganization and loss of white matter, would result from OSA did not materialize (Figures S5 and S7A), except in rats which were salt-loaded (Figure S7B). We speculate that OSA, without salt loading, was not sufficiently severe or sufficiently prolonged to produce deep brain lesions or white matter damage. Salt loading seemed to provide the additional stress necessary to produce damage to subcortical structures.

In WKY rats, the parent strain for the SHRSP, blood pressures increased in both age groups as a result of OSA exposure (Figure 1); however, no damage to the BBB or activation of microglia was observed (Figures 2–4). Unfortunately, the WKY rats would not explore and, as a result, could not be used in the novel object recognition test. We note that when Long–Evans rats were exposed to OSA using the same model, we did not observe any increase in blood pressure even when the OSA was extended from 2 weeks to 2 months (Durgan et al.24, Crossland et al.25 and unpublished observations). Two apparently normal strains of rats, WKY and Long–Evans, had different blood pressure responses when exposed to OSA.

When taking the results as a whole, OSA in SHRSP rats, an animal model for CSVD, accelerated the onset of the cerebral pathologies and exacerbated pathological changes associated with CSVD. Several of our end points for this study, including loss of BBB integrity and neuroinflammation, are considered events leading to lesions in subcortical regions of the brain in CSVD.23,30,31

In this study, we did not attempt to delineate the exact role of the relative increase in blood pressure stemming from OSA as it related to BBB damage and microglia activation. We note that hypertension is an integral part of CSVD in many individuals and may constitute a particular inheritable form of CSVD.12,32,33

Perspective

Our study provides strong evidence that OSA, at least in an animal model, accelerates the disease process involving CSVD. Our data also suggests that relatively short periods of OSA can exacerbate and accelerate the onset of CSVD. Although translating 2 weeks of OSA in a rat model to an equivalent time in humans is impractical, the results do point to the idea that OSA, even for short periods of time, could accelerate or exacerbate the effects of CSVD, especially in those individuals with associated risk factors. The most effective treatment for OSA is continuous positive airway pressure, which provides pressure by a mask placed over the nose or nose and mouth to prevent the airway from collapsing. If continuous positive airway pressure is an effective treatment for prevention of CSVD and allows reversal of CSVD, then early diagnoses and treatment of OSA is extremely important. One encouraging study, although limited in size, reported that cognitive impairments and white matter damage in individuals having OSA could be reversed after 1 year of continuous positive airway pressure treatment.15

Given that there are few studies dealing with the potential risks of OSA on the development and acceleration of CSVD and that OSA may be a potential modifiable risk factor or treatment for CSVD, this area deserves more in-depth studies.

Sources of Funding

This study was funded by Grant 5R01NS080554-1 to R.M. Bryan.

Disclosures

None.

References


Novelty and Significance

**What Is New?**

- This study demonstrates that obstructive sleep apnea (OSA) accelerates the pathological consequences of cerebral small vessel disease (CSVD).
- Only 2 weeks of OSA, a relatively short period of time, was needed to significantly increase systolic blood pressure, produce significant damage to the blood–brain barrier, enhance microglia activation, and produce cognitive dysfunction in spontaneously hypertensive stroke-prone rats, a strain that recapitulates the pathological changes occurring with human CSVD.
- OSA was modeled in the spontaneously hypertensive stroke-prone rats and their parent strain, Wistar Kyoto rats, by remotely inflating a balloon, chronically implanted in the trachea, during 8 hours of the sleep cycle each day.

**What Is Relevant?**

- The exact cause and effect relationship between OSA and cardiovascular diseases has been difficult to fully understand in humans because of the heterogeneity of the study population in terms of confounding comorbidities, genetic makeup, age, life styles, and sex.
- Animal models of OSA, where the confounding comorbidities can be more tightly controlled, have been instrumental in overcoming the heterogeneity of human subjects being studied.
- Although OSA seems to be an independent risk factor for stroke and hypertension, its relationship to other cardiovascular diseases, such as CSVD, is less clear.
- We used an animal model to demonstrate that OSA accelerates the disease process involving CSVD, and only relatively short periods of time, where apneas occur during the sleep cycle, are needed to exacerbate and accelerate the progression of CSVD.

**Summary**

OSA accelerates the onset of the cerebral pathologies associated with CSVD in spontaneously hypertensive stroke-prone rats, a model for human CSVD. Our study provides strong evidence that OSA accelerates the disease process involving CSVD, and only relatively short periods of OSA are needed. In humans, early diagnosis and treatment for OSA is especially important in individuals having CSVD.
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Hypertension. published online August 10, 2015;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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http://hyper.ahajournals.org/content/early/2015/08/10/HYPERTENSIONAHA.115.05764

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PATHOLOGICAL EFFECTS OF OBSTRUCTIVE APNEAS DURING THE SLEEP CYCLE IN AN ANIMAL MODEL OF CEREBRAL SMALL VESSEL DISEASE

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Short title: Apneas During the Sleep Cycle in SHRSP Rats

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Methods

All studies were approved by the Institutional Animal Care and Use Committee at the Baylor College of Medicine. Male inbred SHRSP (strain code 324, subline A3NCrl) and WKY rats (strain code 008, subline NCrl) were obtained through Charles River and maintained on a regular rodent chow and acidified water ad libitum and housed in a satellite facility with 12-hours light (0600 to 1800 h): dark (1800 to 0600 h).

**OSA model:** At either 8 or 17 weeks of age, rats were surgically prepared with an anesthetic cocktail consisting of Ketamine 37.5 mg/mL, Xylazine 1.9 mg/mL, and Acepromazine 0.7 mg/mL (1 μL/g body weight). An intra-tracheal balloon occluder was implanted in each rat. Tubing connected to the balloon was tunneled under the skin to the back of the neck where it exited (2; 3). The tubing was passed through a metal spring harnessed to the rat at one end and a swivel on the top of the cage at the other. After passing out of the cage, the tubing was connected to a computer-controlled pressure system to inflate the tracheal balloon. Each rat received subdermal injections of an analgesic (5 mg/kg Ketoprofen) and an antibiotic (5 mg/kg Baytril) for three consecutive days. One week after surgery rats were randomly assigned to an OSA or sham-OSA group.

For the OSA group, the tracheal balloon was repeatedly inflated to occlude the trachea (2; 3). Apneas (10 seconds each) averaged 60 inflations per hour (equivalent to severe OSA in humans) for eight hours (8 AM to 4 PM) each day during the sleep cycle for two weeks. In the initial study, a single 10 second episode of apnea increased PCO$_2$ by ~ 8 mm Hg, decreased pH by ~ 0.08 units, and produced ~12% O$_2$ – hemoglobin desaturation (2). Sham OSA rats were instrumented with the balloon implant and tether system but did not undergo any apneas.

**Tail cuff plethysmography** (Harvard Apparatus) was used to measure systolic blood pressure between 10 AM to 3 PM, before and following 14 days of OSA or sham-OSA. A minimum of 4 consecutive pressure readings were averaged for each measurement period.

**Novel Object Recognition** was performed according to previously published reports (1; 4). In a 3-min “training” session, each rat was exposed to a pair of identical objects (A1 and A2) in a test chamber with time spent at each object A1 and A2 measured. One minute later in a “test” session, each rat was exposed for three minutes to an object identical to the original objects (A3) along with a novel object (B). Discrimination ratios in the “training” and “working” sessions were calculated as (T$_{A1}$-T$_{A2}$)/(T$_{A1}$+T$_{A2}$) and (T$_{B}$-T$_{A3}$)/(T$_{B}$+T$_{A3}$) where T= the time spent at a particular object.

**BBB integrity and tissue processing:** Rats were anesthetized as described above and 2% Evans blue dye (0.4 ml/kg) was injected into the femoral vein. Following circulation of the dye for 1 hour, the rat was perfused with 120 ml chilled 0.9% NaCl at a pressure of 50 mmHg through a hypodermic needle inserted into the left ventricle. The thoracic aorta was clamped to maximize perfusion of the brain and the right atria was punctured to allow blood and perfusate to exit the circulatory system. The brain was removed from the skull, placed in a chilled slicing matrix and sectioned into two 3-mm thick coronal sections at stereotactic coordinates +3mm to bregma (rostral section), and bregma to -3mm (caudal section). Each section was cut in the sagittal plane along the midline to
create symmetrical halves corresponding to the left and right hemispheres. Grey matter, white matter, and striatum were dissected from the rostral and caudal sections of one hemisphere, placed in pre-weighed tubes, and flash frozen. The remaining section from the opposite hemisphere was fixed overnight in 10% neutral-buffered formalin and paraffin embedded for histological processing.

For Evans blue studies, frozen tissue samples were homogenized in 50% trichloroacetic acid in saline and centrifuged at 10k rpm for 20 min. Evans blue concentrations were calculated in supernatant samples from the absorbance using a plate reader at 620 nm. Sample absorbances were measured against a standard curve of Evans blue dye in saline (0–4 µg/ml).

**Histology and immunofluorescence:** Tissues embedded in paraffin were sectioned at 5 µm. After dewaxing, sections were stained with Klüver-Barrera stain. For immunofluorescence, slides were dewaxed, hydrated, and incubated in PBS for 5 min. For IgG immunofluorescence (a protein found only in plasma when the BBB is intact), slides were blocked for 1 hour with 4% normal goat serum in phosphate buffered saline (PBS) followed by incubation with 1:500 FITC-goat anti-rat IgG (ABCAM) for 1 hour at room temperature in block solution. Slides were rinsed three times in 0.1% BSA in PBS for 5 min and stained with DAPI (0.1 µg/ml in PBS). For GFAP (astrocyte marker, Sigma) and Iba-1 (microglia marker, Wako) immunofluorescence, slides were additionally subjected to heat-induced epitope retrieval for 20 min at 95-100°C using citrate buffer (10 mM anhydrous citric acid, 0.05% tween 20, pH 6.0). Slides were rinsed in PBS and blocked with 4% normal goat serum in PBS. The sections were incubated with primary antibodies (1:500) at room temperature for 1 hour, rinsed 3 times with PBS 0.1% BSA, followed by incubation with Alexa-488 goat anti-rabbit (Invitrogen) secondary antibody (1:500), rinsed, and coverslipped. Microscopy images were electronically stored and analyzed using Adobe Photoshop CS3 Enhanced in a blinded fashion.

**Microglia activation:** Morphometric analysis included 4 discrete categories of activation with the following criteria (5): 1) Inactive- highly ramified processes and an oval cell body, 2) Slightly activated- ramified processes and an oval cell body, 3) Activated- slightly ramified processes with a slightly oval cell body, and 4) Highly activated, non-ramified processes and a circular (amoeboid) cell body.

**Western Blot:** Brain samples were minced on ice and homogenized in buffer containing 1% SDS (Bio-Rad), and protease inhibitor cocktail (Complete Mini, Roche). Samples were boiled for 15 min and centrifuged at 15,000 g for 15 min. Protein was analyzed in the supernatant using the DC protein assay (Bio-Rad). Samples (5 µg protein / lane) were size fractionated by electrophoresis for 1 hour using 90 V at room temperature with a 12% tris-glycine gel. Proteins were transferred to a PVDF membrane. The blots were blocked with PBS containing 5% nonfat dry milk with 0.1% Tween 20 for 1 h at room temperature and subsequently probed with 10 µg/ml using an antibody directed against myelin basic protein antibody (Sigma M3821), a marker for white matter, in blocking solution. Membranes were washed (3 times for 20 min each wash) in PBS and probed with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:10,000 dilution; Pierce) for 1 hour at room temperature. Blots were incubated in chemiluminescent substrate (Femto kit, Pierce) for 5 min at room temperature and exposed to film (Hyperfilm ECL, Amersham Biosciences).
Reference List


Figure S1. Systolic blood pressures in naïve SHRSP and WKY rats during aging. The ages when the rats were exposed to OSA are indicated by the grey boxes.
Figure S2. Weights of rat strains at different ages with OSA and sham OSA. *p<0.01 compared to corresponding sham.
Figure S3. IgG extravasation (green) into the perivascular space of arterioles after OSA or sham in SHRSP and WKY rats at 11 and 20 weeks of age. Scale bar = 15 μm.
Figure S4. Microglia from external capsule in SHRSP sham, SHRSP OSA, WKY sham, and WKY OSA at 11 weeks of age. Green: Iba-1 (marker for microglia), blue: DAPI. Scale bar = 15 μm. Microglia activation was assessed using a categorical scale from 1 to 4 where 1 is unactivated and 4 is highly activated. In the images, WKYs were scored as 1, SHRSP sham was scored as 2, and SHRSP OSA was scored as 4.
Figure S5. (A) Myelin basic protein-1 quantitation by Western blot showing expression of isoforms (21 and 18 kDa) in SHRSP and WKY OSA and sham OSA rats at 11 and 20 weeks of age. (B) Western blots of myelin basic protein-1 in SHRSP OSA and sham OSA at 11 and 20 weeks of age.
Figure S6. (A) GFAP quantitation of white matter regions in SHRSP and WKY rats (by pixel intensity) at 20 weeks of age. (B) GFAP (green) fluorescence indicates astrocytes in external capsule of SHRSP OSA and sham OSA rats. Blue = DAPI fluorescence for nuclei. Bar = 50 μm.
Figure S7. Kluver Barrera staining of white matter in the external capsule. (A) Representative images (20X) show integrity in SHRSP and WKY rats after OSA at 11 and 20 weeks of age. (B) In order to obtain a positive control for white matter damage, we added 1% NaCl to the drinking water of SHRSP rats from age 12 to 17 weeks with OSA occurring from 18 to 20 weeks. * indicates myelin bundle decompression and myelin fiber disarray; note the less intense staining. Bar = 100 µm.