Published in final edited form as:

Neuroscience. 2019 February 01; 398: 55–63. doi:10.1016/j.neuroscience.2018.11.040.

Chronic intermittent hypoxia induces robust astrogliosis in an Alzheimer's disease-relevant mouse model

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Abstract

Sleep disturbances are a common early symptom of neurodegenerative diseases, including Alzheimer's disease (AD) and other age-related dementias, and emerging evidence suggests that poor sleep may be an important contributor to development of amyloid pathology. Of the causes of sleep disturbances, it is estimated that 10% - 20% of adults in the United States have sleep-disordered breathing (SDB) disorder, with obstructive sleep apnea accounting for the majority of the SDB cases. The clinical and epidemiological data clearly support a link between sleep apnea and AD; yet, almost no experimental research is available exploring the mechanisms associated with this correlative link. Therefore, we exposed an AD-relevant mouse model (APP/PS1 KI) to chronic intermittent hypoxia (an experimental model of sleep apnea) to begin to describe one of the potential mechanisms by which SDB could increase the risk of dementia. Previous studies have found that astrogliosis is a contributor to neuropathology in models of chronic intermittent hypoxia (IH) and AD; therefore, we hypothesized that a reactive astrocyte response might be a contributing mechanism in the neuroinflammation associated with sleep apnea. To test this hypothesis, 10-11-month-old wild type (WT) and APP/PS1 KI mice were exposed to 10 hours of IH, daily for four weeks. At the end of four weeks brains were analyzed from amyloid burden and astrogliosis. No effect was found for chronic IH exposure on amyloid-beta levels or plaque load in the APP/PS1 KI mice. A significant increase in GFAP staining was found in the APP/PS1 KI mice following chronic IH exposure, but not in the WT mice. Profiling of genes associated with different phenotypes of astrocyte activation identified GFAP, CXCL10, and Ggt1 as significant responses activated in the APP/PS1 KI mice exposed to chronic IH.

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Authors' contributions: TM, AL, WJA, and ADB designed the research studies. TM, KR, DNL, EH, KJR, and ADB performed the experiments. TM, and ADB drafted the manuscript with the assistance of the other authors, who read and approved the final manuscript.

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Author Disclosure Statement: No competing financial interests exist.

Keywords

sleep-disordered breathing; sleep apnea; astrocytes; neuroinflammation; Alzheimer's disease

Introduction

Sleep disturbances are an early symptom of a neurodegenerative disease, and maybe a contributing factor in the development of neurodegeneration, including Alzheimer's disease (AD) (Daulatzai, 2015; Guarnieri and Sorbi, 2015; Jha et al., 2018; Peers et al., 2009). For instance, clinical and epidemiological data support a link between sleep-disordered breathing and age-related cognitive decline and dementia (Abner et al., 2015; Beebe and Gozal, 2002; Chen et al., 2016; Gelber et al., 2015; Lucey and Holtzman, 2015; Osorio et al., 2015). In the United States, the prevalence of sleep-disordered breathing affects an estimated 10-20% of the adult population and is even greater in the aged population (30-80%) (Osorio et al., 2015; Roepke and Ancoli-Israel, 2010). Postmortem investigation of patients with sleep apnea found increased microinfarcts, neuroinflammation and neuronal loss compared to the patients who did not have a recorded history of sleep apnea (Gelber et al., 2015).

There has been very little experimental research directed at understanding the mechanism associated with this correlative link between sleep-disordered breathing and the increased risk of dementia. A key component observed in both neurodegenerative diseases and sleep apnea is neuroinflammation (Daulatzai, 2015). Neuroinflammation has been seen in people with sleep apnea (Gozal, 2009; Gozal and Kheirandish-Gozal, 2008; Gozal et al., 2009; Rosenzweig et al., 2015), and in experimental models of the disease (Sapin et al., 2015; Smith et al., 2013). Accumulating experimental evidence supports the role of chronic neuroinflammation induced by sleep apnea as a potential mechanism by which sleep apnea contributes to the increased risk of developing a neurodegenerative disease (Kheirandish et al., 2005; Zhang et al., 2010; Zhang et al., 2012; Zhang et al., 2013; Zhu et al., 2012). Recent evidence has suggested that astrocytes may be critically involved in the detrimental aspects of neuroinflammation, and a new nomenclature has been developed defining astrocyte responses as A1-type or A2-type (Clarke et al., 2018; Liddelow and Barres, 2017; Liddelow et al., 2017; Yun et al., 2018). We hypothesized that a reactive astrocyte response might be a contributing mechanism in the neuroinflammation associated with sleep apnea.

Chronic intermittent hypoxia (IH) exposure given during the sleep period of the circadian cycle is a well-established model of sleep apnea (Li et al., 2003; Moreno-Indias et al., 2015; Row et al., 2003; Xu et al., 2004). We have used this model of sleep apnea to mechanistically investigate the correlative link between sleep problems and earlier onset of dementia. In particular, we exposed an APP/PS1 humanized knock-in mouse model to IH and evaluated reactive astrocyte response. Mice were exposed to IH for 10 hours a day, for a total of 4 weeks. Our results showed that IH caused robust astrogliosis in the cortex of APP/PS1 KI mice and an increase in both A1-type and A2-type astrocyte associated markers. We found no effect of IH on amyloid plaque load in the neocortex of mice exposed to 4 weeks of IH.

Materials and Methods

Animals

Adult mice (10-11 months old) APP^{NLh/NLh} × PS-1^{P264L/264L} (APP/PS-1 KI) (Flood et al., 2002) (n=16; 7 F and 9 M) a double gene-targeted knock-in mouse on a C57BL/6J background and C57BL/6J (n=22; 10 F and 12 M, from Jackson) males and females were used in this experiment. Mice were assigned randomly to groups before the start of the experiment. Animals were housed in a controlled humidity and temperature environment and 12/12-h light/dark cycle with free access to food and water. Body weight was measured once a week during the experiment. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Kentucky, and that were conducted in accordance with the standards of proper experimentation in the Guide for the Care and Use of Laboratory Animals and ARRIVE guidelines.

Experimental design

Animals were randomly divided in control (sham) and intermittent hypoxia (IH) groups and placed in two identical commercially designed chambers (Fig. 1A) (BioSpherix Oxycycler A84 unit, W76.2 × D50.8 × H50.8 cm, Bioinstruments, Redfield, NY) that were able to accommodate up to 7 cages with a maximum of 5 mice in each cage. The two chambers were connected to supplies of pure O₂ and N₂ that were used to produce changes in O₂ concentration and regulated by the BioSpherix computer software. During the exposure periods, the concentration of O₂ was cyclically reduced from 21% to 10% in the IH group. The mice were left in the chambers for a total of 4 weeks and exposed to intermittent hypoxia (IH group) or normoxic condition (sham group) for a total of 10 hours a day (7 am-5 pm) Fig. 1A, followed by a 21% oxygen concentration between 5pm and 7 am. Sodasorb CO₂ absorbent (Grace Materials Technologies, Discovery Sciences, Chicago, IL, USA) was used during the entire duration of the experiment to remove excess of CO₂ in the chamber, silicagel orange (Fisher Scientific, cat #AC392030050) nontoxic grade was used for drying purposes. Cages were changed weekly after 5 pm and the weight of the mice was recorded. Room and chamber temperature and humidity were recorded as well (average temperature: IH = 20.1°C ± 0.38°C; Sham = 20.6°C ± 0.47°C; housing room = 20.5°C ± 0.42°C; mean ± SD) (average humidity: IH = 39.3% ± 14.7%; Sham = 63.9% ± 11.3%; housing room = 38.6% ± 10.2%; mean ± SD).

The IH protocol (Fig. 1B) consists of 4-minute cycles of 2 min of hypoxia (10% O₂) and 2 min of normoxia (21 % O₂). Hypoxia is initiated by injecting N₂ into the chamber, resulting in a low point of 10 % O₂ in 2 min, then O₂ is injected over 2 min, resulting in 21% O₂ within 2 min. Each cycle lasted 4 min, for a total of 15 cycles/hour and 150 cycles/day. Sham mice were exposed to normoxic gas in a chamber identical to the IH group. For the remaining 14 hours, O₂ concentration was kept at 21% in both IH and sham group.

Immunohistochemical and Biochemical analysis

Mice were deeply anesthetized with 5% isoflurane and then transcranial perfused with ice-cold PBS for 5 min. The brains were rapidly removed and dissected. Left hemibrain was fixed in 4 % PFA and used for immunohistochemistry (IHC), the right was dissected and

flash frozen for biochemical analysis. IHC staining was done following established methods, and quantified using the Aperio ScanScope XT digital slidescanner or Zesis AxioScan Z. 1 and HALO software (Indica labs) or Aperio ImageScope software positive pixel count algorithm (version 9), as previously described (Bachstetter et al., 2012; Bachstetter et al., 2013). Primary antibodies used included mouse biotin anti- β -Amyloid, 1-16 antibody (6E10 A β) (Covance cat#39340-200; (1:3000) Biolegend, San Diego, CA) and rabbit anti-glial fibrillary acid protein (GFAP) (Dako Cat#Z0334; (1:10000); Dako, Glostrup, Denmark). GFAP stained tissue was double labeled with 1% Thioflavin S (1326-12-1, Sigma Aldrich). A β 1-40 and A β 1-42 were measured by V-Plex ELISA from Meso Scale Discovery (MSD) according to the manufacturer's instructions as we have previously described (Bachstetter et al., 2012). Transcriptional changes in astrocyte were measured in cortex homogenates using a customized low-density TaqMan gene expression array card according to the manufacturer's instructions as previously described (Webster et al., 2015). The following TaqMan Assays were included on the array card: Amigo2-Mm00662105_s1, B3gnt5-Mm01952370_u1, C3-Mm01232779_m1, Cd109-Mm00462151_m1, Cd44-Mm01277161_m1, Clcf1-Mm01236492_m1, Clu-Mm01197002_m1, Cxcl10-Mm00445235_m1, Emp1-Mm00515678_m1, Fbln5-Mm00488601_m1, Fkbp5-Mm00487406_m1, Gbp2-Mm00494576_g1, Gfap-Mm01253033_m1, Ggt1-Mm01333302_m1, H2-T23-Mm00439246_g1, Iigp1-Mm00649928_s1, Lcn2-Mm01324470_m1, Psmb8-Mm00440207_m1, Ptgs2-Mm00478374_m1, Ptx3-Mm00477268_m1, S100a10-Mm00501458_g1, Serpina3n-Mm00776439_m1, Serping1-Mm00437835_m1, Slc10a6-Mm00512730_m1, Sphk1-Mm00448841_g1, Stat3-Mm01219775_m1, Steap4-Mm00475405_m1, Tgm1-Mm00498375_m1, Timp1-Mm01341361_m1, Tm4sf1-Mm00447009_m1, Vim-Mm01333430_m1. All endpoint analyses were conducted on coded samples, that were randomized, and the individuals involved in the endpoint analysis were blind to the experimental groups of the samples. All samples were processed and analyzed together in one batch for each endpoint.

Statistics

JMP Software version 12.1.0 (SAS Institute, Cary, NC, USA) was used for statistical analysis. Graphs were generated using GraphPad Prism version 7.0. Differences between mean were considered statistically significant at $\alpha=0.05$. The normality assumption was assessed using the Shapiro-Wilk test, and heterogeneity of variances was assessed with Levene's test. When the assumptions for parametric tests were violated the data was either transformed, or nonparametric tests were used. Changes in body weight between sham and IH were compared using an unpaired t-test. The effect of IH exposure on amyloid load in the APP/PS1 KI mice was compared using an unpaired t-test. A two-way ANOVA was used to determine the effect of genotype and IH exposure for changes in GFAP staining in the cortex. Differences in GFAP staining between groups was compared using a Tukey-Kramer test. Response screening in JMP was used to identify genes that had a statistically significant difference between groups (WT + sham, WT + IH, KI + sham, KI + IH) following false discovery rate (FDR) correction. Differences in gene expression changes between groups were compared using a Tukey-Kramer test. Values are expressed as mean $\pm$ SEM unless otherwise noted. Scatter plots represent individual mice.

Results

Two identical chambers were used to expose wild-type (WT) and APP/PS1 KI (KI) mice to normoxic or to intermittent hypoxia, which is an experimental model of the hypoxic component of sleep apnea (Fig. 1). Overall, no adverse events, such as increased aggression or mortality, occurred over the 4 weeks of chronic IH exposure. Following 4 weeks of chronic IH exposure, a decrease in body weight was found for WT ($t(20) = 2.70$, $p = 0.014$) and KI mice ($t(14) = 1.78$, $p = 0.097$) compared to the sham matched controls (Fig. 1C), which is consistent with previous studies (Carreras et al., 2012; Thomas et al., 2017; Wang et al., 2018). The change in body weight that occurred over the course of the experiment was not sufficient to warrant premature stoppage of any of the mice from the experiment.

Chronic Intermittent hypoxia exposure did not change amyloid plaque load

The APP/PS1 KI mice begin to deposit amyloid plaques at 9 months of age (Flood et al., 2002; Murphy et al., 2007). By 11-12 months of age amyloid plaques are evident in the neocortex and to a lesser extent in the hippocampus (Bachstetter et al., 2012). Therefore, to determine if chronic IH affected amyloid plaque burden histological quantification was evaluated in multiple brain regions using 6E10 antibody. In the WT mice no 6E10+ amyloid plaques were observed. Cerebral amyloid angiopathy was not observed. As shown in Figure 2A, amyloid plaques were seen, as expected, in the APP/PS1 KI mice. Quantification of the amyloid load using digital neuropathology (Fig. 2B) was done to determine the area of the neocortex, corpus callosum, subiculum, CA1/2, dentate gyrus, and thalamus that was 6E10+. Exposure to 4 weeks of IH did not increase amyloid plaque burden in the KI mice compared to the KI + sham mice (Fig. 2C). To determine whether determine if chronic IH affected levels or distribution of A β , we prepared PBS soluble, and FA soluble fractions of cortex and measured A β 40 and A β 42 by quantitative A β ELISA. Exposure to 4 weeks of IH had no effect on A β 40 (Fig. 2D) or A β 42 levels (Fig. 2E).

Chronic Intermittent hypoxia exposure caused robust astrogliosis

GFAP immunohistochemical staining was used to measure reactive astrocytes in the cortex. As shown in Figure 3A, GFAP staining was sparingly found in the cortex of WT + sham mice. Quantitatively, digital neuropathological assessment of GFAP staining was completed in the neocortex, corpus callosum, CA1/2, dentate gyrus, thalamus, and striatum as shown in the heatmap (Fig. 3B). The greatest change in GFAP staining by genotype and IH exposure was found in the neocortex. In the neocortex found an overall main effect by two-way ANOVA ($F_{3,36}=58.17$; $p<0.0001$), as well as a significant effect of genotype ($p<0.0001$), IH exposure ($p=0.0006$), and interaction of IH and genotype ($p=0.0253$) (Fig. 3C)

Qualitatively, in the WT + IH mice, areas of strong GFAP staining can be observed along blood vessels (Fig. 4). In the KI + Sham injured mice, clusters of GFAP staining can be seen throughout the cortex, most likely corresponding to regions of amyloid plaques. In the KI + IH mice, a substantial increase in GFAP staining was found throughout the cortex. In addition to amyloid plaque associated astrogliosis. A strong reactive GFAP staining was seen around blood vessels in the KI + IH mice. To quantify GFAP staining that appeared to be associated with blood vessels, regions of interest were generated using HALO software

(Indica Labs) around GFAP labeled vasculature profiles, and percentage of the neocortex that was positive of blood vessel GFAP staining was quantified. The KI + IH mice were found to have the greatest area of GFAP labeled vasculature profiles in the neocortex; however, we did not find a statistical difference between genotypes or IH exposure (Fig. 4B). Next, we sought to determine if chronic IH exposure increased the density of plaque-associated astrocytes. Tissue sections were double labeled with GFAP and Thioflavin S, and a 200µm radius circle centered on thioflavin S positive staining was generated using HALO software (Indica Labs) (Fig. 5A). Area quantification of GFAP staining in that plaque-associated region of interest found no effect of IH exposure (Fig. 5B). These results indicate that while chronic IH exposure qualitatively appeared to be most associated with blood vessels, the increase in GFAP staining seen following IH exposure was likely an increase in diffuse reactive astrocytes, and not explicitly reactive astrocytes associated with blood vessels or plaques.

Chronic Intermittent hypoxia exposure increased genes associated with A1-type and A2-type astrocytes

Using the recently identified genes that have been shown to be selectively upregulated following either endotoxin or an ischemic injury in astrocytes (Clarke et al., 2018; Liddelow and Barres, 2017; Liddelow et al., 2017; Yun et al., 2018), we developed a custom TaqMan low-density array to determine if those genes were responsive to chronic IH exposure. As shown in the heat map, a pattern of increased expression of pan reactive, A1-type and A2-type genes was found in the KI + IH exposed mice (Fig. 6A). Following FDR correction, five genes were found to have a significant overall effect, including: GFAP ($p=0.00003$), CXCL10 ($p=0.001$), B3gnt5 ($p=0.0043$), Tgm1 ($p=0.0043$), Ggta1 ($p=0.0115$). In agreement with the histological results, we found an additive effect of IH exposure and genotype on GFAP gene expression, with the KI+IH mice having the highest level of GFAP gene expression (Fig. 6B). CXCL10 was a second gene previously classified as a pan reactive astrocyte marker that showed a potential synergistic effect on IH and genotype. Similarly, Ggta1, an A1-type marker, was found to be significantly elevated in the KI + IH mice compared to all other groups. Interestingly, the ischemic injury associated gene, Tgm1, was found to be increased by IH in WT mice compared to WT + sham mice. However, there was not an additive effect of IH in the KI mice compared to KI + sham mice on Tgm1 expression in the cortex. Finally, a genotype effect was seen for B3gnt5 gene expression in the cortex with the KI mice showing less B3gnt5 compared to the WT mice, but there was no effect of IH exposure.

Discussion

We report here four key findings that highlight an underappreciated role of chronic IH in driving reactive astrogliosis in the context of AD-relevant amyloidosis. First, four weeks of chronic IH, with 150 cycles per day of O₂ reaching 10% saturation for a few seconds produced robust astrogliosis in the cortex of aged APP/PS1 KI mice. Interestingly, the same IH exposure in age-matched WT mice had only marginal effects on astrocytes. Both WT and APP/PS1 KI mice were found to have a similar change in body weight. Second, in WT mice in the limited areas where increased astrogliosis was seen it was qualitatively associated with

blood vessels. Similar reactive astrogliosis was seen around blood vessels in APP/PS1 KI mice exposed to chronic IH; however, the area of astrogliosis around the blood vessels was disproportionately larger in the KI + IH mice compared to the WT mice exposed to chronic IH. Third, profiling of pan, A1-type, and A2-type astrocyte associated markers found that the reactive astrocyte response seen in the KI + IH mice was statistically associated with pan reactive and A1-type markers. In contrast, in WT mice chronic IH exposure selectively increased A2-type astrocyte markers. A2-type astrocyte markers were identified as genes that increased in astrocytes in response to ischemic injury, while A1-type astrocyte markers were defined as genes that were increased in astrocyte in response to LPS endotoxin challenge (Liddel et al., 2017). The KI + IH mice were found to have an upregulation of the A1-type astrocyte markers, suggesting that an interaction between the A β and the IH drives a similar subset of genes as endotoxemia. The implications for changes in the profile of astrocyte associated markers warrants further mechanistic investigation. Finally, we found that four weeks of chronic IH exposure did not increase amyloid plaque load. Overall, our results indicate that chronic IH exposure can drive reactive astrogliosis, but only if the brain is already fragile due to preexisting neuropathology.

A major goal of our study was to develop an animal model to explore how sleep-disordered breathing may contribute to AD-relevant neuropathology. To this end, we used a well-characterized mutant mouse model of amyloidosis. The APP/PS1 KI has a progressive amyloid deposition starting at six months of age that increases linearly over time (Flood et al., 2002; Murphy et al., 2007). A strength of the KI mice is that the endogenous mouse promoter drives the APP and PS1 mutations, and thus maintain a physiologically relevant expression of the APP and PS1 genes. We believe that it is important to consider the limitations of the model systems used in our study when interpreting our findings. A limitation of the APP/PS1 KI model is that it does not have tau pathology or neuronal loss, and thus we were not able to model how sleep-disordered breathing might influence these pathologies. Second, the chronic IH model, used in our study, is a well-established experimental model of sleep apnea (Li et al., 2003; Moreno-Indias et al., 2015; Row et al., 2003; Xu et al., 2004). The chronic IH model simulates the hypoxia-reoxygenation events experienced by patients with sleep apnea. However, the pattern of O₂ changes induced in the IH model does not accurately reflect the amplitude and periodicity of those experienced by patients with sleep apnea. The gas exchange in the chamber was a rate-limiting step in both amplitude and periodicity of the O₂ changes. While, we could have adjusted the computer program set points to induce a more variable pattern of O₂ changes, as seen in people with sleep apnea, we chose to use the more established pattern of IH exposure, as previously published (Li et al., 2003; Moreno-Indias et al., 2015; Row et al., 2003; Xu et al., 2004). Third, hypoxic events could be either neuroprotective or neurotoxic depending on the severity, duration, and frequency of the hypoxia (Beebe and Gozal, 2002; Boroujerdi and Milner, 2015; Kim et al., 2015; Rosenzweig et al., 2015). Previous studies have shown that the model of chronic IH used in our experiments can contribute to neuronal injury (Gozal et al., 2001; Gozal et al., 2003; Smith et al., 2013); but there may be a component of the chronic IH that may also be neuroprotective (Satriotomo et al., 2016).

Growing evidence suggests that chronic IH exposure can accelerates not only amyloid pathology but also tau pathology in an AD mouse model (Gao et al., 2013; Li et al., 2009;

Liu et al., 2016; Sun et al., 2006; Yagishita et al., 2017). We found that four weeks of chronic IH exposure did not increase amyloid plaque load. In agreement with our results, Shiota et al. (Shiota et al., 2013) demonstrated that eight weeks of IH exposure in an AD-relevant mouse model was not associated with worsening amyloid plaque deposition in the brain. In contrast to our results, some studies have reported that IH exposure increased amyloid pathology (Gao et al., 2013; Li et al., 2009; Liu et al., 2016; Yagishita et al., 2017). The protocol used in our study is fundamentally different from the groups that saw an increase in amyloid plaque load. For example, to induce hypoxia, some studies used a sealed 125ml jar (Gao et al., 2013; Li et al., 2009), a hypobaric chamber (Liu et al., 2016), or much stronger hypoxia exposure (Yagishita et al., 2017). Moreover, our study was the only one to use mice with a physiological relevant expression pattern of the APP and PS1 genes. Therefore, differences in amyloid pathology could also be a result of the transgenic overexpression patterns used in the studies that found changes in amyloid pathology.

Previous studies have shown that IH exposure in wild-type rodents could induce reactive gliosis (Gozal et al., 2001; Wang et al., 2018; Xavier Aviles-Reyes et al., 2010), and that even one day of IH exposure was sufficient to induce gliosis (Xavier Aviles-Reyes et al., 2010). Our results are in contrast to these studies, as we found that four weeks of IH exposure produced very limited astrogliosis around a subset of blood vessels in WT mice. Our observations suggest that other comorbid pathologies are necessary to see the reactive astrocyte response.

In summation, our results also suggest that the otherwise healthy brain is likely resilient to a short period of IH exposure, but the brain may have less resilience to IH exposure if amyloid pathology is present. In particular, the presence of amyloid pathology can have an additive effect with IH exposure on reactive gliosis. Future studies are warranted to determine mechanistically the detrimental versus protective aspects of the astrocyte response to IH exposure in AD-relevant mouse models.

Acknowledgements:

The corresponding author, Adam Bachstetter, PhD, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Research reported in this publication was supported by National Institutes of Health under award numbers P30 AG028383 and R00 AG044445 (ADB). The content is solely the responsibility of the authors and does not represent the official views of the funding organizations.

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Figure 1: Experimental overview.

(A) Aged matched WT or APP/PS1 KI (KI) mice were exposed to intermittent hypoxic (IH) or normoxic (sham) conditions by altering the injection of oxygen or nitrogen into a BioSpherix Oxycycler chamber. (B) A representative trace from the BioSpherix Oxycycler, show the program used for adjusting the O₂ levels in the chamber in gray dashed line. The actual O₂ saturation as monitored by the BioSpherix Oxycycler chamber is shown in the solid magenta line. (C) Changes in body weight were seen after 4 weeks of exposure to chronic IH exposure. Each circle represents an individual animal.

Figure 2: 6E10+ amyloid plaque load in the cortex.

(A) Example photomicrographs of 6E10 immunohistochemical staining in the cortex in 11-12 month old APP/PS1 KI (KI) mice following 28 days of chronic intermittent hypoxia exposure (IH). **(B)** Example of the computer-generated markup created by the positive pixel algorithm demonstrating the ability of the algorithm to accurately detect the immunostaining. Colors, yellow < orange < red, indicate staining intensities low < medium < high, respectively. **(C)** Quantification of the 6E10+ staining found that IH exposure did not increase histological amyloid plaque load in the neocortex, corpus callosum, subiculum, CA1/2, dentate gyrus, and thalamus. Quantitative Aβ ELISA of PBS soluble and FA soluble found no effect of IH exposure on Aβ40 **(D)** or Aβ42 in the neocortex **(E)**. Each circle represents an individual animal.

Figure 3. Chronic intermittent hypoxia exposure (IH) cause robust astrogliosis.

(A) Low power photomicrographs show GFAP staining. Arrows indicate area shown in Figure 4. (B) A survey of changes in GFAP staining is shown by the heat map across multiple brain region. (C) Quantification of the GFAP staining in the cortex shows the effect of genotype, and the additive effect of IH in the context of the APP/PS1 KI genotype. ** p<0.01 compared to KI + sham. *** p<0.0001 compared to WT + sham. Each circle represents an individual animal.

Figure 4. Effects of chronic intermittent hypoxia exposure (IH) on GFAP labeled vasculature profiles

(**A**) high power photomicrographs show GFAP staining in the cortex from areas identified in Figure 3. (**B**) Quantification of the area of GFAP staining in the cortex associated with vasculature profiles found no statistical differences between genotype, or IH exposure. Each circle represents an individual animal.

Figure 5. Effects of chronic intermittent hypoxia exposure (IH) on plaque-associated astrocytes. (A) A Representative example of the region of interest that was generated around thioflavin S, which was used to quantify the area of GFAP staining that was associated within a 200µm radius from the center of a plaque (B). Each circle represents an individual animal.

Figure 6. Gene expression changes in the cortex of associated reactive astrocyte markers.

(A). Heat map illustrates changes in the pan reactive, A1-type, and A1-type genes in the cortex of mice following 4 weeks of sham or chronic intermittent hypoxia (IH) exposure.

The asterisk identifies genes that were statistically significantly different following FDR correction and shown in the scatter plot (B). Each circle represents an individual animal.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to WT + sham. § $p < 0.05$, §§ $p < 0.01$ compared to

WT + IH. ‡ $p < 0.05$, ‡‡ $p < 0.01$ compared to KI + sham.